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Enzymatic Biocatalysis of Biomass from Aquatic Plant *Phragmite Karka* for Second-Generation Bioethanol Production

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Abstract

In the context of energy transition and the reduction of greenhouse gas emissions, the production of second generation bioethanol is also recognized as a promising way to reduce our dependence on fossil fuels. Then, the present studies aims to evaluate the enzymatic biocatalysis of biomass from aquatic plant *Phragmite karka* in the second-generation bioethanol production. Results obtained revealed a rapid decrease of °Brix during the fermentation of musts and underlined the efficacy of enzyme hydrolysis. The rate of sugar consumption by yeasts is between 32.43 and 70.27%.The yield of ethanol production of yeasts indicated that *Angel Brand Thermal-tolerant alcohol active dry yeast* was the best yeast strain for this fermentation. These findings underline the potential of *Phragmite karka* plant materials in the perspective of intensive production of second-generation bioethanol.

Keywords: *Phragmite karka;* Enzymatic hydrolysis; Bioconversion; Second-generation bioethanol; Benin.

1. Introduction

The use of fossil fuels is economically viable but unfortunately have a negative carbon footprint for the environment. Greenhouse gas emissions are attributable to the combustion of fossil fuels. They are the cause of several problems including climate change with obvious manifestations such as global warming, rising sea levels and the annual loss of ice mass from the ice sheet (in Antarctica). Face with the declining of fossil energy resources such as crude oil, and the many climatic disturbances, biofuels including bioethanol becomes an excellent substitute to gasoline which is the main fuel currently used in the field of transport [1]. Indeed, it is recognized that the use of pure bioethanol instead of gasoline allows a reduction in CO2 emissions in the order of 90% [2]. 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 [3]. According to Henry Ford [4], there is carburant in plants that can be obtained through fermentation. According to Rudolf [5], the use of vegetable fuel in engines is insignificant today. In addition, the interest in producing of biofluel also stems from the fact that it is a strategic energy substance whose use covers a wide range of industrial activities [6]. If developed countries are resolutely working on the energy transition, the situation of the developing countries in terms of energy remains worrying. For example, the West African Region, five years after the adoption of SDG 7 which consists of "guaranteeing access to reliable, sustainable and modern energy services for all", still has an access rate to electricity among the lowest. ECOWAS has therefore remained very sensitive to the SE4All initiative "Sustainable Energy for all", proclaimed by the General Secretary of the United Nations for the decade 2014-2024, with major objective, such as, to double the part of renewable energies. Four years from the end of the adoption of this initiative, the situation has not really changed and the member of countries of this Sub-Region, such as Benin, still have energy supply difficulties, despite their renewable energy potential. In Benin research and development programs relating to renewable energy sources are set up. Among these alternative energy sources, bioenergy in general and biofuels in particular, are the most targeted [7]. To achieve objectives, it is important to focus on the valorization of the country's biomass resources. In this contexte, Benin has a considerable hydrographic network and some of them are used for the informal trafficking of fossil hydrocarbons. Moreover, most waterways are enriched with organic substance which promotes the development of aquatic plants. These plants, real sources of

fermentable sugars, unfortunately cause eutrophication problems in waterways. They could therefore be potential sources of fermentable sugars and make a valuable contribution to Benin's energy policies. It is for these reasons that this work would like to evaluate the bioconversion potential of biomass from aquatic plant Phragmite karka in second-generation bioethanol production.

2. Material and Methods

2.1. Plant Collection

The stems of Phragmite karka was collected at Grand-popo (South of Benin). Once brought back to the laboratory, they were washed and dried at temperature of 25°C for three (03) months.

2.2. Pretreatment by Steam Explosion

Plants are cut, crushed and pretreated by steam explosion according to the method described by [8]. In fact, the ground vegetal material are soaked with distilled water (0.75 l.kg-1) and brought to a temperature of 270 °C in an electric saturated steam oven (Memmert brand 854 Schwabach) for one (1) hour.

2.3. Enzymatic Hydrolysis

The enzymatic hydrolysis of the pretreated ground material is carried out using Pectinase from Aspergillus niger at a enzyme concentration of 6g.kg-1. Incubation is carried out at a temperature of 50 ± 1°C for three (3) days. Periodic sampling followed by physicochemical analyses are allowed to follow the evolution of pH and total sugar production during the incubation period. After that, the plant material are pressed using a mechanical press equipped with a filter, in the presence of 0.5L of distilled water used as humectant. The collected extract is then sterilized at 121°C for 15 minutes.

2.4. Alcoholic Fermentation and Distillation

The three lyophilized strains of Saccharomyces cerevisiae, used for alcoholic fermentation, are purchased from the company "Angel Yeast CO., LTD". These are Angel Brand Thermal-tolerant alcohol active dry yeast (S1), Angel Brand Super alcohol active dry yeast (S2), Angel Super alcohol active dry yeast (S3). For the preparation of the fermentation musts, a preculture of the dry yeasts was carried out using peptone water (0.5g of dry yeast.ml-1) for 30 minutes at 25°C in order to revive the yeast cells. These revived yeasts are then used as a ferment in the preparation of the musts by using extract obtained from of Phragmite karka. For each strain of yeast, a concentrations of 5g.l-1 are used. The alcoholic fermentation is carried out in batch mode for four (4) days at a temperature of 29°C, and the some kinetic parameters (pH, Brix and Attenuation limit) are followed throughout the fermentation process. The pH is determined by direct measurement using the OHAUS ST10 brand portable pH meter, according to the method described by the NF ISO 1842 standards. The Brix degree is determined using a MISCO Palm brand portable refractometer Abbe 201, total sugar and Attenuation limit are determined by the method described by Kouwanou, *et al.* [8]. At the end of the fermentation process, the extraction of bioethanol was carried out by distillation using a column of vigreux QUICKFIT / FC3, and production yields (Rp) were determined.

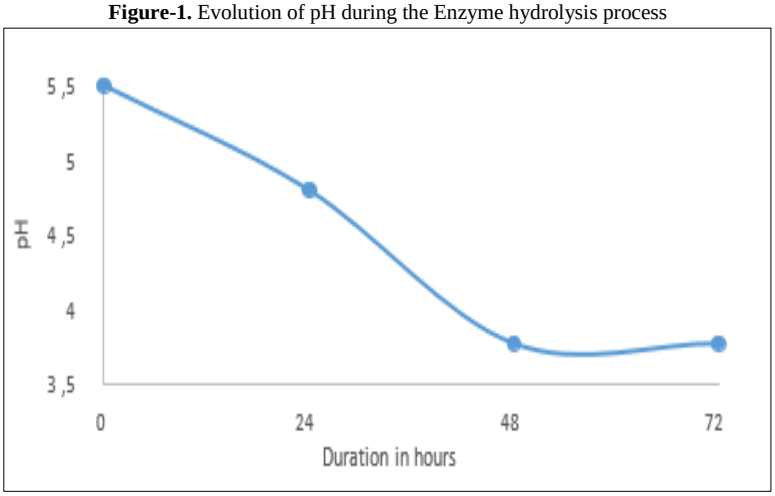
2.5. Statistical Analyses

The data generated from these studies were analyzed using Statistical Analysis Software (SAS) and SYSTAT 5.05. The statistical analyses carried out were mean and standard deviation and analysis of variance (ANOVA).

3. Results and Discussion

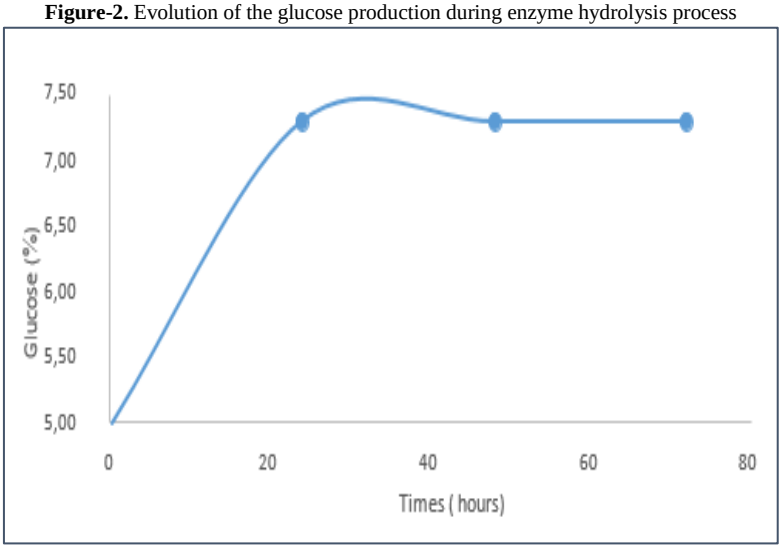
Figure 1 indicated the evolution of the pH during enzyme hydrolysis process of biomass from Phragmite karka. Results revealed a decrease of pH during the first 48 hours of enzyme hydrolysis process, followed by a stabilization period during the last 24 hours of enzyme hydrolysis process. The decrease of pH could be due to could be due to the release of glucuronic acid molecules from plant macromolecules, under the action of the enzymatic complex. These same observations were reported by Agbo and Simard [9], and Adjou, *et al.* [10] during the enzymatic hydrolysis of biomass from rônier fruits (Borassus aethiopum Mart.).

Figure-1. Evolution of pH during the Enzyme hydrolysis process



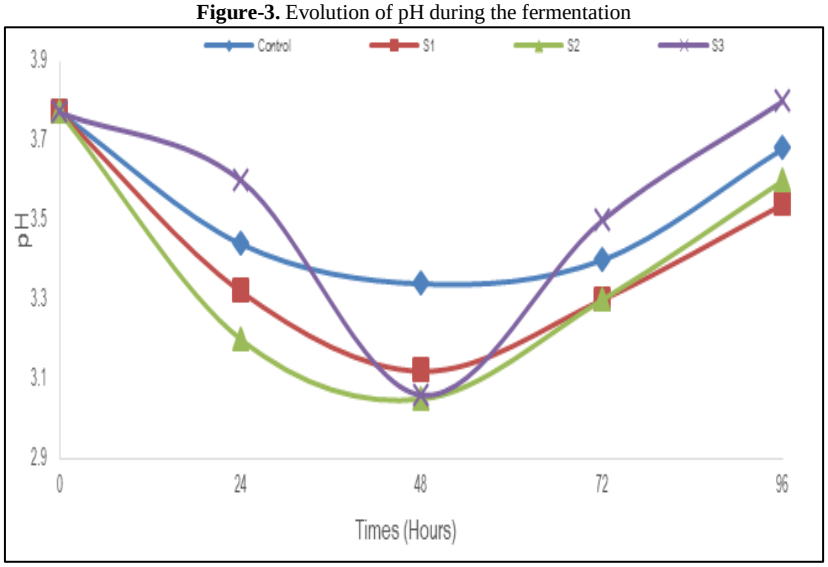
These observations are confirmed by the results obtained on the evaluation of glucose production during the enzyme hydrolysis process (Figure 2).

Figure-2. Evolution of the glucose production during enzyme hydrolysis process



In general, an increase in glucose production was observed during enzymatic hydrolysis. This principle is fundamental, because the complex sugar molecules (Celluloses) are broken down into simple sugar molecules by the synergistic action of the enzymatic complex, in particular cellulases endo 1,4 ?-glucanases and exo 1,4 ?-glucanases, which hydrolyse the celluloses into cellobiose, and ?-glucosidases which hydrolyze cellobioses into glucose available for yeasts during the fermentation process [11]. Figure 3 presented the results of pH during the fermentation process. Results indicated a decrease in the pH after 48 hours of fermentation, followed by pH increase during the last 48 hours of fermentation times. The highest pH value (3.8) was recorded in the must fermented with the Angel super alcohol active dry yeast, while the lowest pH value (3.54) was recorded in the must fermented with Angel Brand Thermal-tolerant alcohol active dry yeast. Analyses of results revealed that the evolution of pH in different musts during fermentation is two-phase, including a phase of pH decrease, followed by a phase of pH increase.

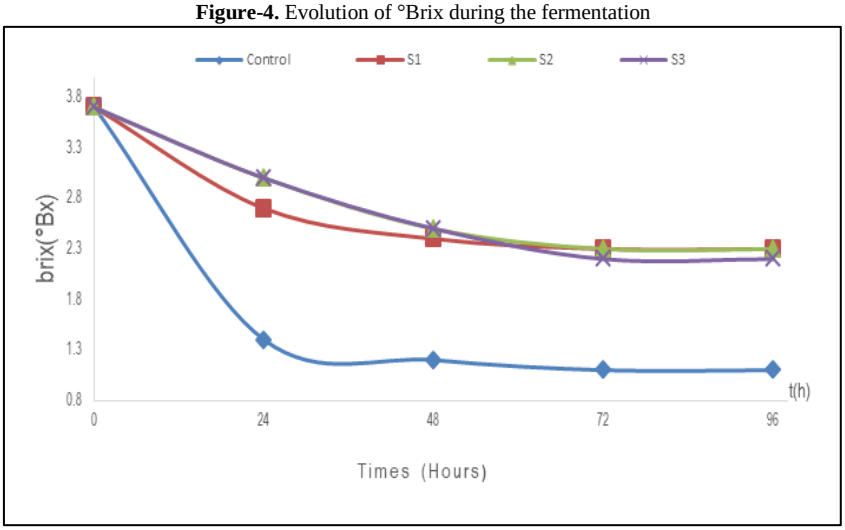
Figure-3. Evolution of pH during the fermentation



The evolution of pH observed is consistent with that reported during the fermentation of grape musts in 2008 by Akin [12]. According to this authors, the decrease of pH coud be linked to the metabolism of yeasts, whereas the rise of pH could be due to the production of alcohol, which could leads to a change in the dissociation of the constituents of the must and mainly of the organic acids initially present in the must. Thus, in the presence of ethanol, the dissociation is less important and this could therefore results in a lower proton concentration and therefore a high pH [12].

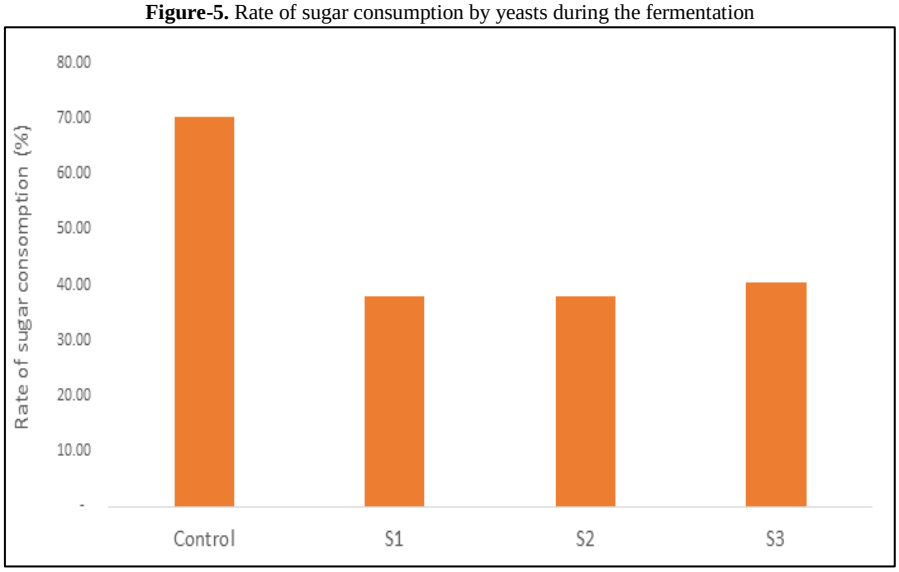
Results obtained on the monitoring of °Brix during the fermentation of musts (Figure 4), indicated a decrease of °Brix value from 3.7°Bx to 1.1°Bx during the first 72 hours of fermentation, followed by stabilization during the last 24 hours of fermentation. This results underlined the efficacy of enzyme hydrolysis process which leads to production of reducer sugars from the lignocellulosic materials. This carbohydrates were quickly used by the yeasts during the fermentation activity, revealed by the strong decrease in Brix observed during the fermentation process.

Figure-4. Evolution of °Brix during the fermentation



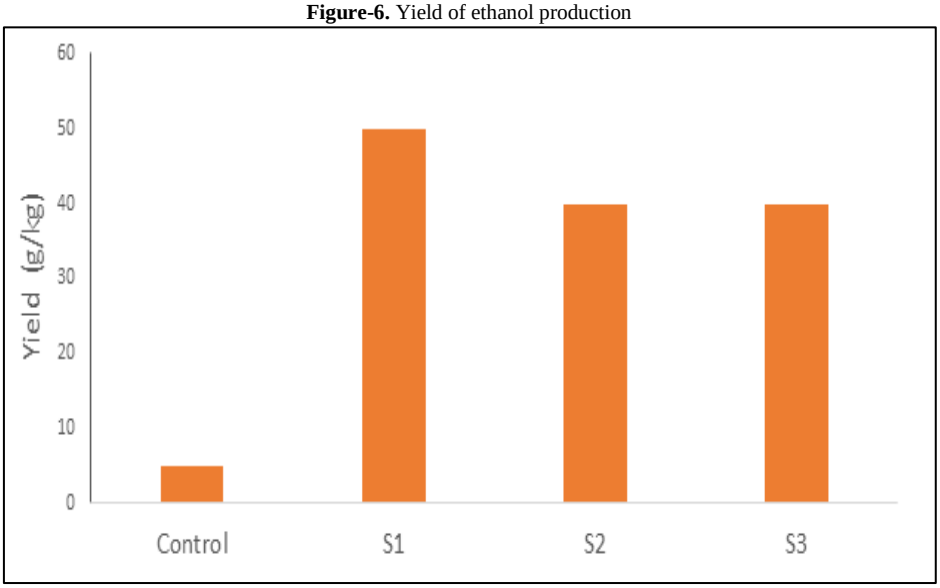
These results are similar to those reported by reported by Novidzro, *et al.* [13] during the fermentation of sucrose, and by Gbohaïda, *et al.* [14], during the fermentation of must from cashew apple residues. Indeed, fermentation process is a metabolic activity during which yeast cells obtained the energy necessary for their enzymatic activity and their multiplication in the culture medium. However, the stationary phase (arrest of the fermentation process), characterized by the stabilization of °Brix, indicated the cessation of the fermentation reaction, due either to the lack of simple sugars in the medium, or either a response to the various stresses undergone by the microorganisms involved in the fermentation. The cessation of sucrose consumption by yeasts depends on the performance of each strain. It could be caused by inhibition phenomena linked to the concentration of the ethanol excreted by the yeast or by a high osmotic pressure in the fermentation medium. Moreover, ethanol is not the only product obtained during an alcoholic fermentation, because other compounds such as carbon dioxide and co-metabolites (acetic acid, lactic acid, formic acid, succinic acid, glycerol) are also produced and could inhibited the fermentation metabolism of Saccharomyces cerevisiae strains used. This performance of yeasts could be evaluate in the dermination of the Attenuation Limits which indicated the rate of sugar consumption by yeasts during the fermentation (Figure 5).

Figure-5. Rate of sugar consumption by yeasts during the fermentation



From the analysis of the results obtained in the present study, the rate of sugar consumption by yeasts, is between 32.43 and 70.27%. According to results reported by Gbohaïda, *et al.* [14], the Attenuation Limits is between 69.5 and 75% during the fermentation of must from cashew apple juice. Results obtained during the evaluation of yield of ethanol production of yeasts (Figure 6) indicated that Angel Brand Thermal-tolerant alcohol active dry yeast was found to be more effective with the highest yield of ethanol production of 50g/kg. These values are low than those reported in Brazil by Rocha, *et al.* [15].

Figure-6. Yield of ethanol production



4. Conclusion

The present study underlined the importance of enzymatic hydrolysis of lignocellulosic biomass in the bioconversion of aquatic plant Phragmite karka for second-generation bioethanol production. These findings could be used in the perspective of intensive production of second-generation bioethanol with lignocellulosic plant materials. The results obtained from this work make it possible to consider the deepening of research in the development of a method of valorization of lignocellulosic materials. The development of this process is important, because it will greatly reduces the quantity of solid waste by recovering it through the production of simple substances which could be used for bioconversion in good form of renewable energy.

References

[1] Novidzro, K. M., 2013. *Bioethanol production by alcoholic fermentation of fruit juice: desert date*. Curcubitapepo: Dialium guineense and Opilia amentacea. p. 202.

[2] Ward, O. P. and Singh, A., 2002. "Bioethanol technology: Development and prospects." *Adv. Applied Microbiol*, vol. 51, pp. 53-80.

[3] Kouwanou, C. S., Agbangnan, D. C. P., Adjou, E. S., Mossi, I., Wotto, V. D., and Sohounhloué, D. C. K., 2019. "Valorization of Typha Australis stems in bioethanol production using enzymatic hydrolysis and biofermentation." *Academic Journal of Chemistry*, vol. 4, pp. 90-95.

[4] Bernard, B., 2007. "Le programme Proalcool au Brésil." In *Conférence Prononcée À Saint Dié-Des-Vosges Festival De La Géographie*.

[5] Diesel, R., 1912. *Die enstehung of dieselmotors (the appearance of the diesel engine): The diesel engine*. Berlin: Shipbuilding Company in Berlin. Trad. Fr. L and H Mendez (France).

[6] Kaïdi, F. and Touzi, A., 2001. "Production de bioalcool à partir des déchets de dattes: production et valorisation-biomass." *Rev. Energ. Ren.*, pp. 75-78.

[7] Cleveland, C. J., Costanza, R., Hall, C. A. S., and Kaufmann, R., 1983. "Energy and the united states economy. A biophysical perspective." *Science*, vol. 225, pp. 890-897.

[8] Kouwanou, C. S., Agbagnan, D. C. P., Adjou, E. S., Tchobo, F. P., Bonou, C., Soumanou, M. M., and Sohounhloué, D. C. K., 2018. "Physicochemical and enzymatic hydrolysis of Eichhornia crassipes for the production of second-generation bioethanol." *American Journal of Chemistry*, vol. 8, pp. 41-44.

[9] Agbo, G. N. Z. and Simard, R. E., 1992. "Characteristics of juice from palmyrah palm (Borassus) fruit." *Plant Foods for Human Nutrition (Dordrecht, Netherlands)*, vol. 42, pp. 55-70.

[10] Adjou, E. S., Amamion, H., Tchobo, F. P., Aissi, V. M., and Soumanou, M. M., 2013. "Extraction assistée par enzyme du jus de la pulpe fraîche du rônier (Borassus aethiopum Mart.) acclimaté au Benin: caractérisation physico-chimique et microbiologique." *International Journal of Biological and Chemical Sciences*, vol. 7, pp. 1135-1146.

[11] Gbohaïda, V., Kouwanou, S. C., Bothon, F. T. D., Olayé, T., Agbangnan, D. C. P., Avlessi, F., and Sohounhloué, K. C. D., 2020. "Performance of Saccharomyces carlsbergensis and Saccharomyces cerevisiae yeasts in the formulation of bioethanol from cashew apple residues." *Int. J. Curr. Res. Biosci. Plant Biol.*, vol. 7, pp. 15-25.

[12] Akin, H., 2008. "Evolution du pH pendant la fermentation alcoolique de moûts de raisins : modélisation et interprétation métabolique." *Thèse de doctorat de l'Institut National Polytechnique de Toulouse,* p. 136.

[13] Novidzro, K. M., Agbodan, K. A., and Koumaglo, K. H., 2013. "Study of the performance of four strains of saccharomyces cerevisiae during ethanol production from enriched sucrose musts." *Journal of the West African Chemical Society*, vol. 35, pp. 1-7.

[14] Gbohaïda, V., Kouwanou, C. S., Agbangnan, P. C. D., Adjou, H. E., Avlessi, F., and Sohounhloué, K. C. D., 2018. "Biofuel power of bioethanol Obtained by enzymatic biocatalysis from cashew apple bagasse with Saccharomyces carlsbergensis and Saccharomyces cerevisiae." *I. J. G. H. C. Sec.*, vol. 7, pp. 541-550.

[15] Rocha, M. V. P., Rodrigues, T. H. S., Macedo, G. R., and Gonçalves, L. R. B., 2009. "Enzymatic hydrolysis and fermentation of pretreated cashew apple bagasse with alkali and diluted sulfuric acid for bioethanol production." *Appl. Biochem. Biotechnol.*, vol. 155, pp. 407-417.