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RESEARCH ARTICLE

OBTAINING COMBUSTIBLE BIOETHANOL-GEL FROM *EICHHORNIA CRASSIPES* PLANTS

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Abstract

Eichhornia crassipes is a plant that alters the functioning of aquatic ecosystems by threatening fisheries, river transport, tourism and biodiversity conservation. The aim of this work is to enhance the value of *Eichhornia crassipes* plants, through the study of the kinetics of its conversion into second-generation bioethanol by the enzymatic process and by fermentation to obtain a combustible bioethanol-gel. During the study, the fermentation performance of *Angel super alcohol active dry yeast* in the production of bioethanol from the wort of *Eichhornia crassipes* was tested. The monitoring of fermentation kinetic parameters such as pH, acidity, density and Brix, showed a high variability of these parameters during the fermentation process. The distillation of the musts at the end of fermentation allowed to obtain ethanol extraction rates (% v/v at 20°C) of between 4.42 and 12.36 after a first distillation and 12.78 to 73.18 after a second distillation. The combustible bioethanol-gel was produced after the total dehydration of the ethanol obtained. In conclusion, this study made it possible to set up a process for upgrading water hyacinth into combustible bioethanol-gel with a view to large-scale production.

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Introduction:-

In 1912 Rudolf Diesel stated that: "the use of vegetable fuel as a liquid fuel in engines may seem insignificant today. But, these oils will soon become as important as oil and coal tar" (Rudolf 1912). Similarly, Henry Ford stated in 1906 that: "There is gasoline in any vegetable matter that can be fermented" (Bret 2014). Thus, the decrease in non-renewable energy resources (Foteinis *et al.*, 2011) such as crude oil, limited refining capacity and growing concern

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about environmental degradation offer excellent prospects for bioethanol (Gbohaida *et al.*, 2015). This renewable energy is a credible alternative to fossil fuels (Vinson 2016). Its development is a huge challenge in terms of diversification and energy security (Leclerc *et al.*, 2014). Indeed, *Eichhornia crassipes* is a perennial freshwater aquatic plant that floats on the surface of the water thanks to its petioles which have a rounded bulge at their base and which acts as floats (Kouwanou *et al.*, 2018). It has become a real scourge in waters around the world. The consequences of this biological invasion on the aquatic ecosystem and human activities are not trivial. Water hyacinth alters the functioning of aquatic ecosystems by threatening fisheries, river transport, tourism, etc. (Adjahatode *et al.*, 2016). One of the inconveniences generally mentioned when addressing the issue of the proliferation of this plant is its propensity to completely cover the surface of the water it colonizes, hence its negative impact on navigation, irrigation, fishing and on the conservation of biodiversity, as it causes the disappearance of many species of flora and fauna (Kouwanou *et al.*, 2018). We are thus witnessing the asphyxiation of aquatic animals living in environments where the Water Hyacinth is proliferated. This state of affairs alters the physico-chemical and organoleptic quality of the water and then reduces the fishing stocks. It is therefore urgent to find techniques for the recovery of water hyacinth that respect the environment in a sustainable way in all its forms. It is in this context that the present study aims to valorize the plants of *Eichhornia crassipes* through its bioconversion into ethanol for the production of combustible bioethanol-gel

Materials and Methods:-

Hardware :-

The plant (leaves and stems) of *Eichhornia crassipes* subject of our study was collected in three lake areas of the political capital of Benin (Porto-Novo). These are Adjohoun, Bonou and Dangbo. Once brought back to the laboratory, the samples were washed, dried at a temperature of 25°C and then mechanically crushed using the crusher.

Methodology:-

After grinding, the raw material underwent a series of pre-treatments before being transformed into bioethanol. These include pre-treatment by steam explosion, enzymatic hydrolysis, fermentation, distillation and finally gelling.

Steam pretreatment / Steam explosion:-

Steam pre-treatment is a very promising technique for lignocellulosic biomass before bioconversion. 1kg of cleaned *Eichhornia crassipes* leaves and stems, washed with pure water to remove impurities, cut crushed and deposited in an Erlenmeyer flask containing 0.5 liters of distilled water, was pre-treated with steam in an autoclave for 1 hour at a temperature of 270 C°.

Enzymatic hydrolysis:-

After the pre-treatment stage of the raw material, a hydrolysis phase is necessary to obtain the fermentable sugars. In the present study, this is the enzymatic hydrolysis which is catalyzed by enzymes generically called cellulases (composed of endo- and exo-glucanases and β -glucosidases). The continuous and combined action of these two enzymes (endo- and exoglucanases) leads to the complete conversion of cellulose into cellobiose and small oligosides. Finally, B-glucosidase or cellobiase acts on cellobiose and oligosides to give glucose. Thus, the enzymatic hydrolysis of the pre-treated and water-soaked *Eichhornia crassipes* musts was carried out by adding *Aspergillus Niger* cellulase at respective concentrations of 6g of enzyme per 1kg of pretreated substrate in order to optimize the process. The incubation temperature is 50°C for 144 hours

The different stages of alcoholic fermentation:-

Preparation of the inoculum:-

The ferment in this work is *Angel super alcohol dry yeast* of *saccharomyces cerevisiae* mainly industrial and marketed by the Chinese company "Angel Yeast Co., Ltd". The inoculum was prepared by introducing 2g and 4.0g of this dry yeast strain (freeze-dried) at a temperature of $37 \pm 1^\circ\text{C}$ in 9mL of buffered peptone water.

Preparation of fermentation musts:-

At the end of the enzymatic hydrolysis, we proceeded to press and filter the different samples while adjusting the volume of each hydrolysate to 2L with distilled water. After dividing each hydrolysate by two, a pre-fermentation was carried out by incorporating the inoculum into 1/10 of the total volume of each hydrolysate and leaving it under agitation for 24 hours in a closed chamber and in a controlled environment in order to acclimatize the yeast strains with the substrate to be fermented. Then the mixture was added to 9/10 of the volume of the remaining must.

Alcoholic fermentation:-

The musts prepared in this way are kept at 25°C under intermittent agitation. Fermentation is batch or discontinuous. Each fermenter is kept hermetically closed. The alcoholic fermentation of the musts took place over 4 days. Periodic sampling followed by analysis made it possible to monitor the evolution of physico-chemical parameters such as pH, titratable acidity (expressed for the must as % acetic acid), density and Brix degree.

Distillation:-

The extraction of bioethanol from the fermented broth was carried out by distillation from a still, The temperature is maintained at 79 ° C at the top of the column until the alcohol is completely exhausted in the fermented broth.

Gelling of the bioethanol obtained:-

The gelling method used is gelling by soap. Bars of soap were cut into strips. Using the hot plate, a volume of water was boiled in a reactor (beaker) at about 80°C. After the pouring of the cut soap into the boiling water, the mixture was stirred until the soaps were completely dissolved in the water (maintain the temperature at 80°C). Then sodium carbonate (NaHCO₃) and glycerin were added to the mixture followed by the reduction of the temperature to 60°C and the gradual addition of ethanol. Finally, the whole thing was cooled. Remember that glycerin plays the role of emollient.

Analytical methods:-

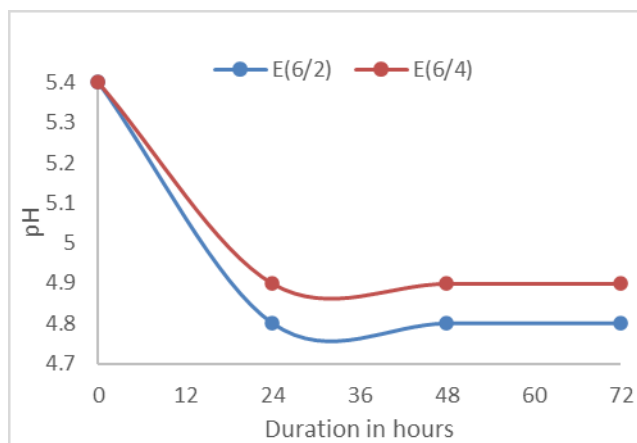
The pH of the musts was measured with an OHAUS ST10 pH meter, and the total soluble dry matter (Brix) was determined using an Abbe 201 MISCO digital refractometer. The alcoholic content (% v/v) of the musts at the end of fermentation is determined according to the pycnometric method recommended by AOAC (1984). The kinetic parameters determined are essentially the Limit Attenuation (Al), the average fermentation times which represent the time between the start of fermentation and the cessation of sugar consumption (Novidzro *et al* 2013), and the ethanol production yield (Rp) which is the ratio of the volumes of ethanol obtained per volume of fermented wort.

Statistical analysis**Statistical analysis:-**

The tests were repeated three times and the data were processed using Microsoft Excel 2010 software. The statistics, data analysis and comparison of independent data averages were established at the 5% threshold by SPSS 16.0.

Results and Discussion:-**pH:-**

Figure 1 presents the results of the evolution of the pH of the different musts during fermentation. From the analysis of this figure, we note a decrease in pH (5.4 to 4.8) after 24 hours of fermentation with *Angel super alcohol active dry yeast*). This phase of pH decrease could therefore correspond to the phase of exponential growth of yeasts, which often results in the production of secondary metabolites. We then note a stabilization of the pH at the level of the musts. This could correspond to the depletion of the medium of fermentable sugars or to the saturation of the medium by secondary metabolites likely to inhibit the growth of yeasts or slow down their fermentation activity (Garcia-Gonzalez *et al* 2007). The evolution of the pH observed is in line with that observed during the fermentation of grape musts by Akin in 2008. According to the author, the drop in the pH value is linked to the assimilation of the nitrogenous source by the yeasts, and therefore to the metabolism of the yeast, whereas the rise is due to a physico-chemical phenomenon because the production of alcohol from sugars leads to a modification of the dissociations of the constituents of the must and mainly of the organic acids initially present in the must. Thus, in the presence of ethanol, dissociation is less important and therefore results in a lower proton concentration and thus a high pH.



6/2 and 6/4 indicate samples hydrolyzed with 6 grams of enzyme and fermented with 2 and 4 grams respectively
Angel super alcohol active dry yeast

Figure 1: Evolution of pH during fermentation

Brix Degree:-

Figure 2 shows the results of the evolution of the Brix degree during fermentation. The analysis of this figure shows a two-phase evolution of the Brix of the different fermenting musts, characterized by a slight decrease in Brix during the first 48 hours of fermentation at the level of the two musts. As far as the second phase is concerned, there is a stabilization of the Brix of the different musts at values depending on each type of yeast used. The lowest Brix value (1.5°Bx) is obtained with the must fermented with 4g of strain. This stabilization then signals the end of fermentation

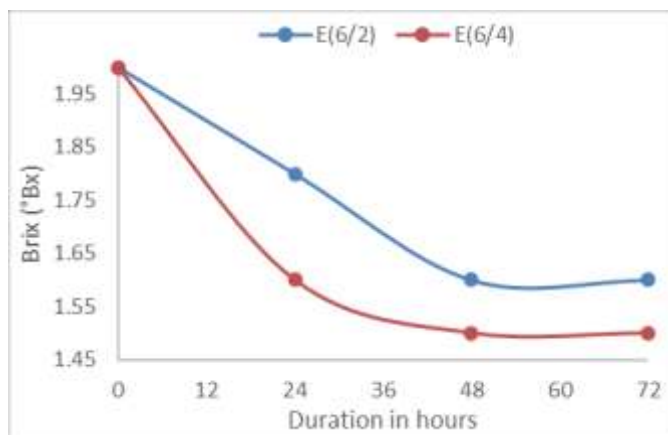


Figure 2: Evolution of density during fermentation

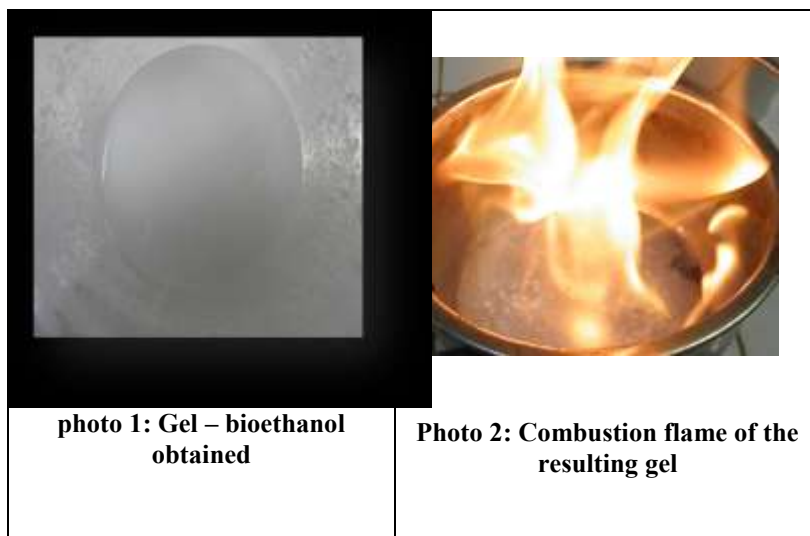
Some parameters of the efficiency of the fermentation process and the alcoholic content of the distillates:-

Table 1 below presents respectively the results on the fermentation time, the limit attenuation, the ethanol production yield and the alcoholic content of the bioethanol produced. From the analysis of this table, it can be seen that the fermentation time of the two musts is 48 ± 2 hours. This shows that this duration does not depend on the concentration of the strain used. These results are better compared to the results of adjahatodé et al (2016) showing a fermentation time of 96 hours and that of fragoso (2011) with a fermentation time of 121 hours. As far as the attenuation limit is concerned, it should be noted that the concentrations of the strains used do not have the same tolerances to alcohol, which is reflected in the different attenuation values (rate of consumption of fermentable sugars) recorded. The rate of consumption varies between $20 \pm 0.05\%$ and $25 \pm 0.06\%$, which is also reflected in low values of the alcohol content obtained. These attenuation values are respectively lower than the values (53.1% to 74.4%) obtained by Mossi et al; in 2018 during the fermentation of *S. saccharatum* juices (Mossi 2018) and the values (56.06% to 56.60%) obtained by Gbohaida et al in (2018) on the pressing residues of cashew apples. As for the ethanolic yield, it should be noted that it depends on the concentration of the strain used. It varies between values 65 ± 3 and 79 ± 2 g/kg. The best ethanolic yield was obtained for must fermented with 2 g of strain.

As regards the alcoholic degrees of the distillates obtained after the first and second distillations, they range from $4.22 \pm 0.23\%$ to $12.36 \pm 1.08\%$ after the first distillation and then from $12.78 \pm 0.67\%$ to $73.18 \pm 0.88\%$ at the end of the second distillation. The best alcoholic content was obtained at the level of the must fermented with 4g of strain while the lowest alcohol content was obtained with the must fermented with 2g of strain. This work made it possible to obtain an alcohol content much higher than that obtained by Mishima et al. (2008) who found 0.14g of pure bioethanol per gram of *Eichhornia crassipes* and 0.955% V/V by Adjahatodé et al. (2016). This difference observed in the alcohol content would be linked to the different processes used during the release of fermentable sugars and the way in which the distillations were made. Finally, the bioethanol produced underwent dehydration and then gelling to give the gel-bioethanol that will be used in the stove manufactured for the occasion.

Table 1: Some parameters of fermentation process efficiency and the alcohol content of distillates

	Fermentation time (h)	Limiting Attenuation (%)	Production Yield	Alcohol content (%V/V)	
				1st distillation	2nd distillation
2g strain	48 ± 2	20 ± 0.05	79 ± 3	4.22 ± 0.23	12.78 ± 0.67
4g strain	48 ± 2	25 ± 0.06	65 ± 2	12.36 ± 1.08	73.18 ± 0.88



Conclusion:-

In general, these results have shown that *Eichhornia crassipes* plants are a potential source of production of second-generation bioethanol. It has also made it possible to formulate a combustible bioethanol-gel that will be used in households for cooking food. All this will contribute to the liberation of the surface of waterways, the preservation of the environment and the ecosystem. In perspective, we plan to determine the fuel characteristics of the bioethanol obtained for its use in internal combustion engines and also the fuel characteristics of the combustible bioethanol-gel obtained.

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