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Production of bioethanol from tomato processing residues using *Saccharomyces cerevisiae* in the Adrar region (Southwestern Algeria)

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ABSTRACT

The objective of this study is to obtain second-generation ethyl alcohol from tomato processing residues. To convert this lignocellulosic biomass into ethanol; tomato residues were treated by a thermo-mechanical and chemical process, the acid hydrolysis was carried out. Fermentation was realized using *Saccharomyces cerevisiae* yeast in different dilutions with an incubation time of 72 hours, a pH of 4.7, and a temperature of 30°C. The evolution of pH, sugar concentration, and alcoholic degree were monitored. After 72 hours of fermentation, distillation was carried out, the characterization by GPC and IR confirmed the presence and nature of the compound obtained as ethyl alcohol. The yield of bioethanol obtained was 0,29 g/g of substrate. The results of the study demonstrated the feasibility of bioconverting tomato processing residues into 2nd generation ethanol.

1. INTRODUCTION

A major challenge currently facing the world, particularly in the environmental field, lies in the need to find methods and strategies to effectively manage the waste resulting from the food production chain, such as tomato processing residues. This chain encompasses the stages of production, processing, consumption, and waste generation and contributes around 19–25% of greenhouse gas emissions [1]. The lack of adequate control over the decomposition processes of these wastes has a significant impact on the ecosystem [2], [3]. It is worth noting that processing industries generate considerable quantities of waste, placing those second only to domestic waste [4]. Algeria produces a considerable quantity of tomatoes, with the south-western region of Adrar alone contributing around 59,546.15 metric tons. Of this quantity, some 13,368.8 metric tons are destined for the manufacture of tomato paste. This processing activity generates a large quantity of under-exploited waste, consisting of seeds, skins, and pulp, and thus has a harmful impact on the environment. This lignocellulosic biomass, which is not in competition with human food, is an excellent source of ingredients such as polysaccharides [5], lycopene, β-carotene, phenolic compounds, flavonoids, levulinic acid, phytosterols, cutin [6], vegetable oil [7], antimicrobial compounds, and natural pigments [8]. In the context of reducing the effects of climate change, such as gas emissions and environmental pollution, the energy transition and the search for alternative, renewable, and sustainable energy sources are crucial steps towards diversifying resources and achieving a balance between human needs and ecosystem protection. The first bioethanol generation uses materials rich in sucrose and starch, while the second generation is based on lignocellulosic materials and the third on algal biomass. [9], According to Saini et al. [10]; first-generation feedstocks are insufficient to meet the growing demand for energy. Using residual feedstocks for ethanol will avoid mobilising more arable land, contributing to food security [11].

Lignocellulosic biomass can be used as a bio-resource for the development of new high-value-added products such as biofuels [12], thanks to its availability, abundance, and relatively low cost [13]. This second-generation bioenergy can compete with fuels derived from fossil resources and reduce energy bills [14]. Among approaches exploring the potential of tomato by-products; Using Saccharomyces cerevisiae yeast, Osei et al. [15] produced bioethanol from rotten tomatoes while varying incubation times and temperatures. On the other hand, Aramrueang et al. [16] fermented poor-quality fresh tomato fruit rejected by growers. Meanwhile, Patle et al. [17] used two different bacterial strains to ferment a hydrolysate from tomato waste. Similarly, Kasavi et al. [18] fermented untreated tomato peels using bacteria and yeast. However, tomato pomace was subjected to enzymatic hydrolysis by Lenucci et al. [19]. The importance of eliminating bioconversion inhibitors present in residue components or resulting from pre-treatment processes has not been clearly addressed in research into the recovery of this type of tomato waste.

In this study, tomato industrial processing residues were used as feedstock for the production of second-generation bioethanol by alcoholic fermentation. Processes required to convert sugars from lignocellulosic biomass into bioethanol are essentially pretreatment, hydrolysis, fermentation, and distillation. To improve the efficiency of these processes, we adopted other steps, which are the extraction of inhibitory compounds and detoxification.

2. METHODOLOGIE

2. 1. Raw material preparation

From the concentrate production line at the 'Fouggara' plant in Reggane, southwest of Adrar-Algeria, we retrieved a significant amount of tomato processing residues (figure 1). These residues were ground to increase specific surface area and decrease cellulose's degree of polymerization (DP) and crystallinity after being dried in a well-ventilated area away from sunlight [20]. They were then kept in an airtight container in a dry location. Physico-chemical tests were carried out on our cellulosic biomass to determine moisture and ash content and to quantify cellulose and hemicellulose content. A substrate with 97% dry matter content was used for the experimental pre-treatment work.

Figure 1. Tomato processing residues

The figure 2 shows de various stages of preparation, processing and transformation of the residues from tomato processing to the production ethylique alcaool.

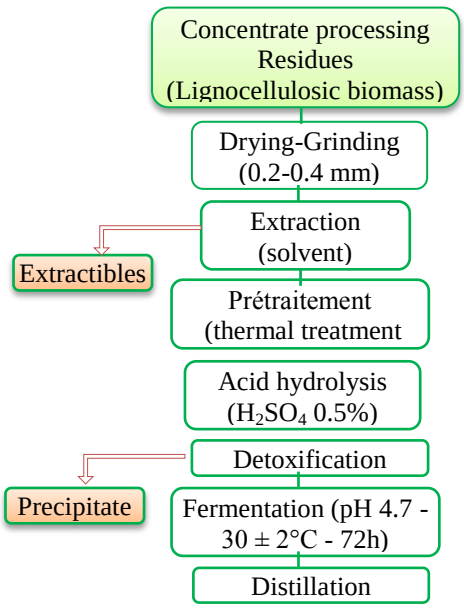

Figure 2. Bioethanol production processes

2.2.1 Soxhlet extraction

Extraction recovers the tannins, oils, fats, resins, and waxes that represent extractible (TAPPI Standard T204 cm-97, 1997). They are extracted under heat using a reflux extraction set-up consisting of a 300-mL flask into which 160 mL of the extracting solvent is introduced and a Soxhlet apparatus topped by a refrigerant. The residues were distributed in filter paper cartridges and introduced into the four station Soxhlet apparatus (behrotest). The residues were left in the solvent for 2 hours at room temperature, and then the temperature was raised to the minimum boiling point of the solvent for 6 hours. A waiting period before extraction was used to reduce thermal contact. Rotating evaporation was used to recover solvent. The extracted residue-containing cartridges were then oven-dried at 110 °C.

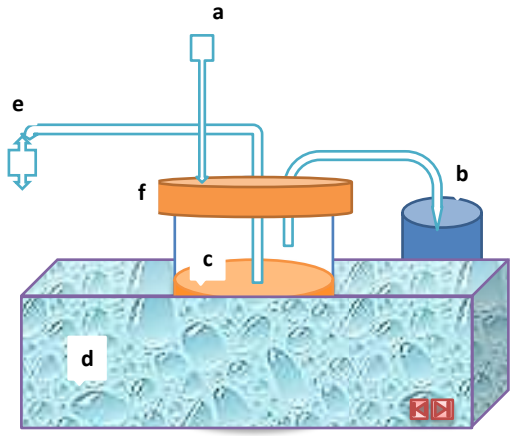

Figure 3 Experimental device for fermentation

a thermometer **b** Co_2 release **c** Reaction medium **d** Bain-marie **e** Sampling syringe **f** bioreactor

2.2 Thermal pre-treatment

The residues were pre-treated with pressurized steam to separate their components, i.e., cellulose, hemicellulose, and lignin, and eventually to remove the lignin while reducing the crystallinity of the cellulose. This process recovers almost all the sugars and requires fewer chemicals and operating conditions [21]. The substrate is pretreated under steam pressure in flasks with a solids-to-solvent ratio of 15% (w/v) in a 'Micro 8' type autoclave for 40 minutes at 134 °C. We chose to extend the residence time and lower the temperature to avoid the formation of inhibitors for subsequent fermentation [22], Depressurization was carried out every 5 minutes for 1 to 2 seconds.

2.3 Acid hydrolysis

The steam-pretreated residues were treated with 0.5% sulfuric acid for 50 minutes. Following the pre-treatment stage, acid hydrolysis promotes the recovery of hemicellulose [23] and the depolymerization of the cellulose component into glucose [24].The dilute acid hydrolysis process consists of breaking osidic bonds and releasing fermentable sugars [25].

2.4 Hydrolysate detoxification

The alkaline process, also known as overliming, is a method used to reduce the presence of inhibitory compounds in alcoholic fermentation.

This method involves raising the pH of the hydrolysate by adding calcium dihydroxide $(CaOH)_2$ until a precipitate forms [26]. This precipitate is then removed by filtration.

2.5 Fermentation

Hydrolysate acidity was corrected with NaOH solution to pH 4.7 using a pH meter 'Ohaus ST3100-F'. Inoculum was prepared by pre-cultivating Saccharomyces cerevisiae dry yeast (1 g/l) [27], with a 12% (v/v) sucrose solution. Continuous agitation was maintained for 90 minutes at a temperature of 30 ± 2 °C [28]. The bioreactor (figure 3) was immersed in a water bath where the temperature was maintained at 30 ± 2 °C [29], and fermentation was carried out anaerobically for 72 hours [30]. Agitation was provided by the movement of bubbles from the CO2 released. During fermentation, samples were taken every 24 hours to monitor the acidity of the reaction medium and density, changes in the color and odor of the medium, and the sugar content, which was determined by Dubois methods [31].

2.6 Alcoholic distillation and bioethanol characterization

After fermentation, the tomato residues wine was distilled to extract the ethanol using a conventional assembly comprising a heating mantle, a cooling column, and a recovery ampoule at a temperature of 78 °C. This process purifies ethanol by separating it from water and impurities [32]. Parameters for qualitative and quantitative characterization of bioethanol after fermentation include density measurement to assess the purity and alcohol content of our distillate using a pycnometer with a calibration curve plotted by different concentrations of absolute ethanol; flammability testing; IR spectroscopy using an Agilent Technologies model: CARY660 FTIR' to identify the specific functional groups present in bioethanol and to identify any impurities or contaminants; gas chromatography (GC) using a 'Clarus 500-650S10041402' to separate and quantify the various components.

3. RESULTS AND DISCUSSION

The fraction of hemicellulose in tomato processing residues is 14%, 27% cellulose and 3.4% lignin. Biopolymers such as hemicellulose and cellulose are valuable components for bioethanol production. However, due to its complexity and resistance to degradation, the presence of lignin requires more advanced degradation methods to maximize the use of these residues in bioconversion.The residues were pre-treated with pressurized steam to separate their components, i.e., cellulose, hemicellulose, and lignin, and eventually to remove the lignin while reducing the crystallinity of the cellulose. This process recovers almost all the sugars and requires fewer chemicals and operating conditions [21], Following the pre-treatment stage, acid hydrolysis promotes the recovery of hemicellulose [23] and the depolymerization of the cellulose component into glucose [24], with dilute acid H2SO4 being widely used. During alcoholic fermentation, the fermentable sugars obtained are converted into ethanol, when the sugar supply is almost entirely converted to ethanol, fermentation is considered complete [33].

3.1 pH evolution

Figure 4 of pH variation provides information on the metabolic activity of yeast during the conversion of sugars into alcohol. The pH curve shows a gradual drop in the pH of the fermentation medium, from 4.7 to 4.08 after 72 hours.

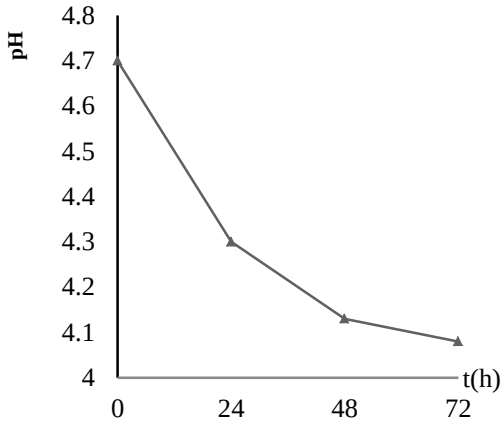

Figure 4. pH evolution during fermentation

The determination of the pH is essential for controlling the fermentative process, both before and during fermentation. Its variation provides information on the metabolic activity of the yeast, the acidification of the medium is indicative of the intensification of fermentation activity over time, is due to the consumption of carbon and nitrogen

substrates, accompanied by the production of acid metabolites and the formation of CO2 and ethanol by *Saccharomyces cerevisiae* [34]. This variation gives information about the metabolic activity because the lower pH facilitates the yeast's ability to convert the sugar in the medium into ethanol.

3.2 Evolution of sugars

Examination of the data presented in Figure 5 reveals that as fermentation time progressively increases, the total sugar concentration in the fermentation medium decreases over the 72-hour incubation period. Total sugar levels start at around 0.008 g/L at the beginning of fermentation, but decrease steadily to approximately 0.006 g/L at the end of the 72-hour period.

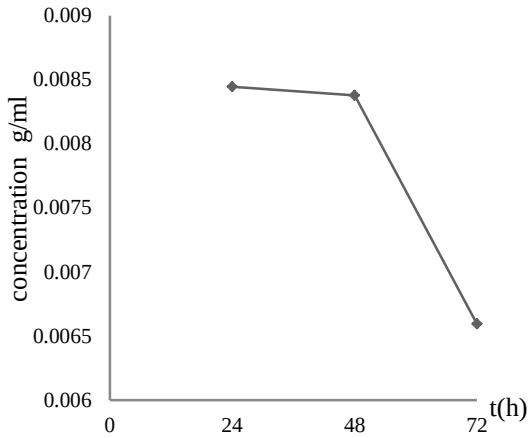

Figure. 5 Evolution of total sugars during fermentation

The decrease in total sugars over extended fermentation time is an expected trend, as reported by Schügerl et al. [35]. The decrease in sugar levels over time can be attributed to Saccharomyces cerevisiae yeasts actively consuming and utilizing available carbohydrates as the fermentation process progresses, consistent with their role as a carbon source for growth, metabolism and bioethanol production during the 72 hour incubation [36].

3.3 Bioethanol distillation and characterization

Analysis of the distillate density and yield curve (figure 6), measured at a 72-hour fermentation interval reveals a significant decrease in density, from 1.0661 to 1.042, reaching its maximum after 72 hours of fermentation. The ethanol produced in this study is volatile, flammable, clear, and has a characteristic pungent alcohol odor. 18.5 (v/v); is the distillate's volume fraction after the first distillation.

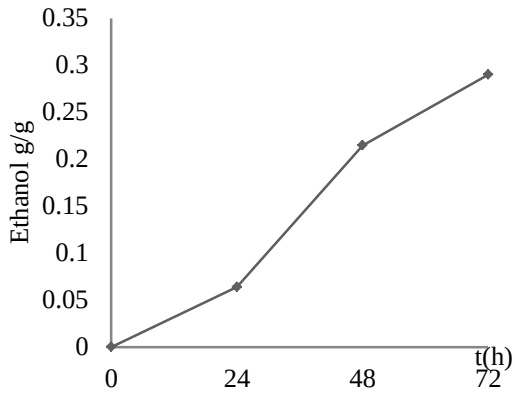

Figure 6 Evolution of ethanol production during fermentation

The decrease in distillate density during fermentation reflects a steady increase in ethanol concentration over time according to Hadri et al. [37], with the amount of ethanol extracted from tomato residues due to the pre-treatment effect of pressurized steam and the elimination of compounds inhibiting alcoholic fermentation by calcium dihydroxide (CaOH) [24].

3.4 Analysis using gas chromatography

After a second distillation of the ethanol obtained, qualitative and quantitative analysis was carried out using the chromatogram (GPC) (figure 7).

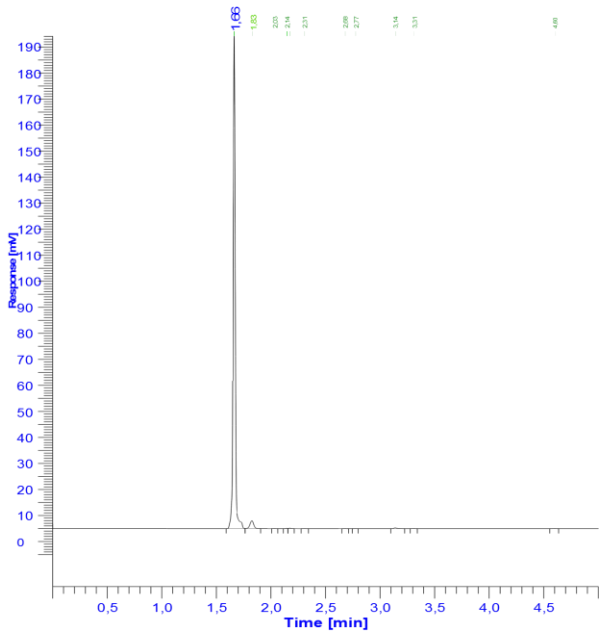

Figure 7. GPC chromatogram spectrum of the bioethanol obtained

A comparison of the retention times of reference ethanol (1.59) with those of bioethanol produced from tomato lignocellulosic biomass processing residues (1.66) shows that they are very close. The close correspondence of retention times is a significant key to the identification of specific chemical compounds in chromatographic analysis. In additional, the proportion of the total peak area corresponding to this component further validates its presence and relative abundance in the sample.

The similar retention times and the percentage of total chromatographic area confirm the presence and identity of the compound obtained as ethyl alcohol (C2H5OH). This compound has a purity of approximately 96.39%, slightly higher than the content measured according to Tadmourt et al. [38].

3.5. Infrared IR spectroscopy analysis

In order to verify the purity of the bioethanol obtained, analysis of the infrared spectrum of the bioethanol produced (figure 8), enables us to highlight the specific molecular vibrations associated with the different chemical bonds present in the sample. We note the presence of a wave at 3367 cm-1 representing the hydroxyl (OH) functional group characteristic of alcohols. In addition, we observe the presence of a wave at 1045 cm-1 resulting from the stretching of the alcohol's C-O bond [38], confirms this presence and nature [32].

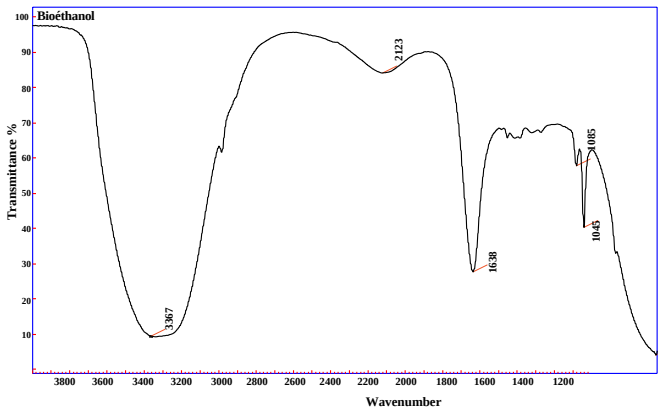

Figure 8. Infrared IR spectrum of the bioethanol obtained

The results showed that alcoholic fermentation of these residues led to a decrease in sugar content as the alcohol content increased. Bioethanol production was achieved, with a yield of 0,29 g/g of substrate. In addition to our experimental results with other types of substrate, Patle et al. [17] have obtained 14 g/L of ethanol using tomato waste and a co-culture of *Z. mobilis* and *Candida tropicalis* , while Kasavi et al. [18] achieved 0.16-0.27 g/L by fermenting tomato peels. This shows the effectiveness of pre-treatment by pressurized steam, because Lignocellulosic feedstock cannot be effectively saccharified by enzymes to achieve substantial yields without a prior pretreatment, primarily because the lignin contained within plant cell walls creates a barrier against enzymatic degradation, and the process of eliminating inhibiting compounds in alcoholic fermentation using calcium dihydroxide (Ca(OH)2), with the yeast *Saccharomyces cerevisiae* being the most preferred as it offers advantages in terms of production and has the best potential [39]. The valorization of tomato processing residues into bioethanol appears to be a promising sector with proven economic, environmental, health and energy benefits, and its development deserves to be supported.

4. CONCLUSIONS

In this work we have presented the main results relating to the study of the bioconversion of tomato processing residues without extractives, to produce second-generation bioethanol. We monitored the evolution of various fermentation parameters: pH, density of the reaction medium, total sugar content, alcoholic strength of the bioethanol produced, and so on. The results show that the alcoholic fermentation of these residues led to a decrease in the total sugar content as the alcohol content increased. The concentration of bioethanol obtained by the fermentation process of tomato residues is influenced by the effect of pre-treatment by steam explosion and the process of elimination of inhibiting compounds in alcoholic fermentation by calcium dihydroxide (Ca(OH)2). The effective extraction and removal of inhibitors generated during the pre-treatment stages is essential for creating a favorable environment and optimizing the performance of fermentation processes using micro-organisms. This study has enabled us to diversify energy production resources by exploiting the lignocellulosic biomass produced by the agri-food industries.

5. ACKNOWLEDGEMENTS

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