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Optimization Of Sugar Molasses Fermentation Using Saccharomyces Cerevisiae For Bioethanol Production

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ABSTRACT: This work gives a contribution to improve the production of bioethanol using molasses from sugar refinery industry as substrate.In this study, Saccharomyces cerevisiae strain has been used under two forms i.e. the lyophilized form and the precultured form.Both strains have been used for the fermentation of the substrate with different molasses concentration. The values of the different parameters, pH, quantity of inoculum and sucrose concentration were varied in order to determine the optimal values to obtain the highest production of bioethanol. The highest bioethanol production was obtained with the lyophilized cells for a value of pH equal to 5, a quantity of inoculum equal to 0.12 g and a substrate containing 10% of molasses.

This study revealed Saccharomyces cerevisiae under lyophilized form can be used effectively in the perspective of an intensive production of bioethanol from molasses produced by a sugar refinery industry. Nevertheless, more studies in this field will be necessary to enhance the production of bioethanol.

KEYWORDS: molasses, bioethanol, lyophilized, precultured, saccharomyces cerevisiae

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I. INTRODUCTION

Today, due to a pollution steadily growing and oil reserves which are shrinking, a question arises: how to limit or even replace the use of oil while reducing emissions of pollutants and greenhouse gases? The available resourcesare limited, and the researches for sustainable alternatives are therefore needed. Thus, the renewable energies constitute a set of solutions since they reduce the dependency on oil and the pollution of our environment [1]. The advantage of biomass is their ability to produce fuels with the properties similar to those of oil [2]. In this context, the conversion of biomass into biofuels seems to be a choice solution to alleviate these problems [3]. While in the middle of the years 2000, the International Energy Agency estimates that approximately 1% of the cultivated area are devoted to the production of biofuels (liquid) and that they substitute in a similar proportion our global consumption of fossil fuels [4].The Biofuel the most consumed in the world is the bioethanol. Its production is the result of the fermentation of a material rich in sugar [5]. During many years, sugarcane molasses has been used as substrate for the production of ethanol since it is rich in carbon source such as glucose and sucrose [6]. In the optimal conditions for the production of bioethanol, we use Saccharomyces cerevisiae[7]. Whereas Saccharomyces cerevisiae is the strain the least expensive available for the conversion of the substrate of biomass [8].

In our study, we are interested in the enhancement of bioethanol production from the Molasses produced by a sugar refinery. The molasses is subject to an alcoholic fermentation by Saccharomyces cerevisiae (yeast of the beer). Three fermentation parameters including the concentration of sucrose in the medium, the microbial load and the pH were investigated in this study.

II. MATERIALSAND METHODS

2.1 Materials

2.1.1 Molasses characterization and contents

The molasse wereobtained from a sugar refinery and were used as a fermentation medium. The physico-chemical compositions of the molasses are given in Table 1.

Table 1. Composition of the molasse used for experiment

Parameters	Total nitrogen (%MS)	Total Sugar (%MS)	mineral substances (%MS)	Brix (%)	Purity (%)	pH
Value	5.7	63.7	13.8	80.7	71.85	4.9

2.1.2 Yeast strain

The Strain of Saccharomyces cerevisiae used in this study is a beer yeast provided under lyophilized by a brewery.

2.2. Yeast culture conditions

The fermentation was carried out in batch mode using two forms of strain of yeast (precultured and lyophilized), in order to compare the consumption of sucrose in molasses and the production of bioethanol.

2.2.1 Culture using precultured yeast strains

Preculture of yeast strains

1g of lyophilized yeast is dissolved in 10 ml of sterile distilled water. After having well homogenized, the mixture is pouring into boxes Petri dishes containing a Sabouraud medium composed of 10g of peptone, 20g of glucose, 15g of agar-agar, 1000ml of distilled water, vitamins and factor of growth with a pH adjusted to 5 and then incubated at 30° C during 60 h.

Inoculation of precultured strains in the fermentation medium

The colony obtained on the Sabouraud medium is transferred into a Carlsberg medium composed of 20g of yeast extract, 100g of sucrose, 5ml of magnesium sulphate at 20%, 5ml of ammonium phosphate at 20%, 100ml of distilled water with a pH adjusted 4.5. Subsequently, 7.5 ml of strains corresponding to OD of 0.3 is inoculated in a bottle of 500 ml containing 150 ml of fermentation medium and incubated without shaking during 60 h.

2.2.2 Direct culture using the lyophilized yeast

A quantity of lyophilized yeast is poured progressively into bottles of 500ml containing 150 ml of fermentation medium, by ensuring that the temperature of the environment is greater than 20° C and then incubated at 20° C for 60 h.

2.3 Fermentation Process

The fermentations are carried out in bottles of 500 ml containing 150ml of the fermentation medium at a temperature of 20° C during 60 hours under anaerobic condition. In order to investigate the effect of various parameters on the fermentation, the process is repeated several times with different initial quantities of sucrose in the substrate, different values of pH and different microbial loads keeping the temperature and fermentation duration constant.

2.4 Analytical Methods ofSucroseandbioethanol

The quantity of sucrose in the fermentation broth is determined using the refractometer. 2 ml of the sample to be analyzed is collected using a syringe and centrifuged at 6000 rpm during 10 min. The supernatant is recovered and a drop is spread on the blade of the refractometer. It follows the reading of the value of the scale.

To detect the production of bioethanol, a small amount of potassium dichromate and some drop of H2SO4 are added to 5ml of fermentation broth (the change of the colour to green indicates the production of the bioethanol). After filtration of each fermentation broth, the determination of the quantity of bioethanol product is carried out with the aid of a fermentoFlash in a brewery and measured per unit % v/v or per unit of mass.

III. RESULTSAND DISCUSSION

3.1 Effects of yeast culture conditions on the rate of sucrose consumption

The different percentage of molasses and sucrose content in the substrate used for the fermentation are given in the Table 2.

Table 2. Percentage of molasses and sucrose concentration in the substrate

Molasse percentage (%)	Sucrose concentration (%)
2	2.5
4	4.5
10	12
15	14
25	19

Figures 1 and 2 shows respectively, the percentage of sucrose in the samples taken during the molasse fermentation using the culture strains precultured and lyophilized yeast strains. The results show that the more molasses solution is diluted the less the sucrose consumption by both yeast is considerable. Besides, the decrease in the concentration of sucrose begin from the second day for the precultured cells and after 6 h for the lyophilized cells. This can be explained by the slow adaptation of the precultured cells from the Carlsberg medium to the fermentation (molasses) medium, while the rapid consumption of sucrose by the lyophilized cells responds to their needs for their activation. This consumption lasts until the third day. The growth of yeast Saccharomyces cerevisiae on a medium of molasses starts always by a latency phase, whether for the cell multiplication or substrate concentration, or the one in bioethanol.

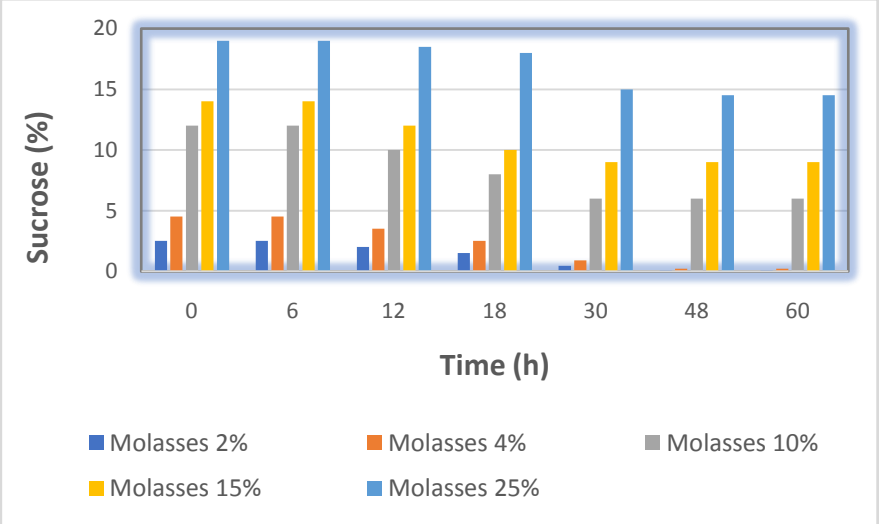

Figure1:Sucrose consumption during molasses fermentation by the precultured yeast strains

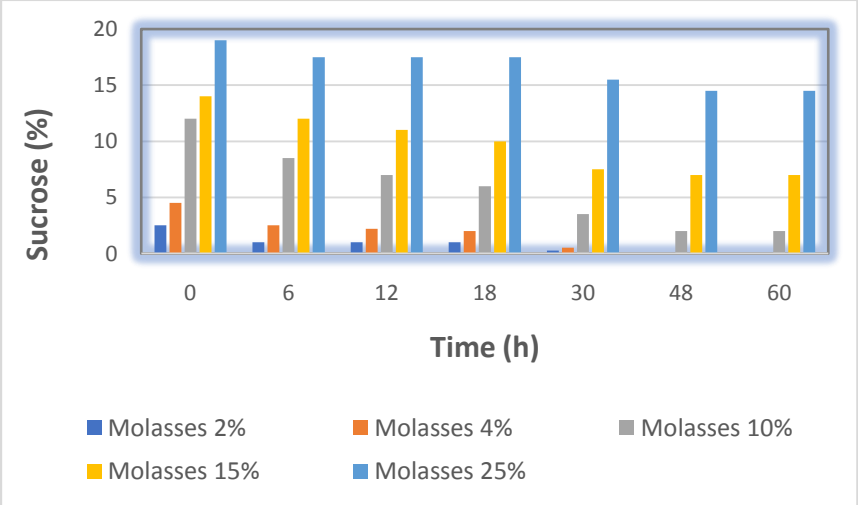

Figure 2: Sucrose consumption during molasses fermentation by the lyophilized yeast strains

The existence of this phase is related to the adaptation of cells in the medium, reflecting the inhibition by the substrate and by the non-sugars [9]. The concentrations of sucrose stabilize at the end of the third day of the fermentation.

3.2 Effects of yeast culture conditions on the production of bioethanol

The analysis of the samples using a fermentoFlash at the Laboratory of a brewery has given the results in figures 3 and 4.

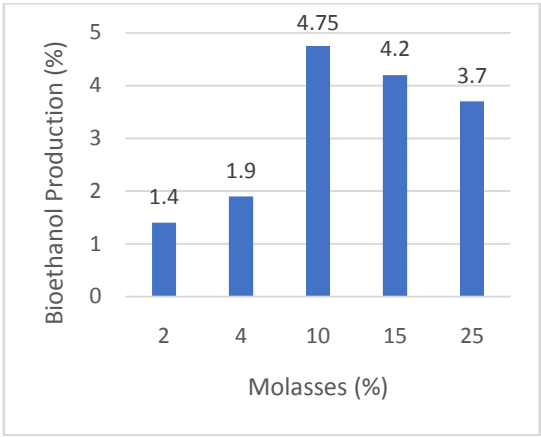

Figure 3: Bioethanol production (% v/v) by lyophilized cells

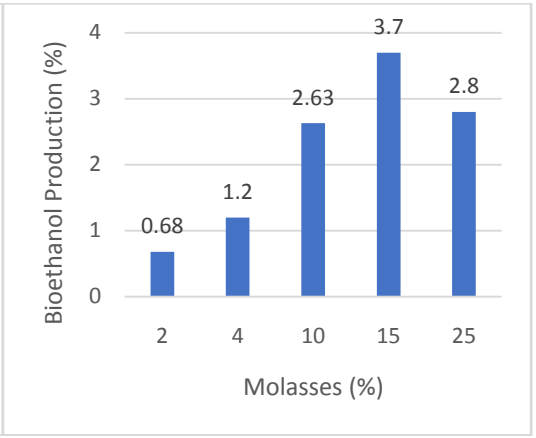

Figure4: Bioethanol production (% v/v) by precultured cells

As shown by the results obtained in the Figures 3 and 4, for each molasses concentration in the fermentation medium, the production of ethanol obtained with the lyophilized cells is always higher compared to that of precultured cells. This may be due to the phase of lyophilization of the cells in the culture. They are lyophilized to the stationary phase, which allows them to enter directly into the fermentation, whereas the cultures precultured have had losses of production of bioethanol in the pre-fermentation medium. The best production is detected for a fermentation medium containing 10% of molasses with the lyophilized yeast strains and 15% of molasses with the precultured yeast strains. That highlights the influence of the osmotic pressure on the cultures [10]. However, the precultured cells are more adapted to high concentrations in sucrose.

According to the results obtained for the consumption of sucrose and the production of bioethanol by the two types of condition of cells, we can affirm that the lyophilized cells of Saccharomyces cerevisiae showed a good performance regarding the production of ethanol. For this reason, only the lyophilized strains and the molasses medium containing 10% of molasses will be used to determine the optimal pH and the quantity of inoculum optimal for the highest production of bioethanol.

3.3 Effects of pH values on the production of bioethanol

The results obtained in the Figure 5 below indicate the different bioethanol production with different pH values between 3.0 and 7.0. The production of bioethanol gradually increases along with the increase in pH and reaches a maximum production for a pH equal to 5. The production decreased slightly for the pH values higher than 5. For the pH of basic medium, the value of the production of bioethanol remains relatively stable (3,67-3,89 %V). The lowest ethanol production is relative to the pH of fermentation medium more and more acid.

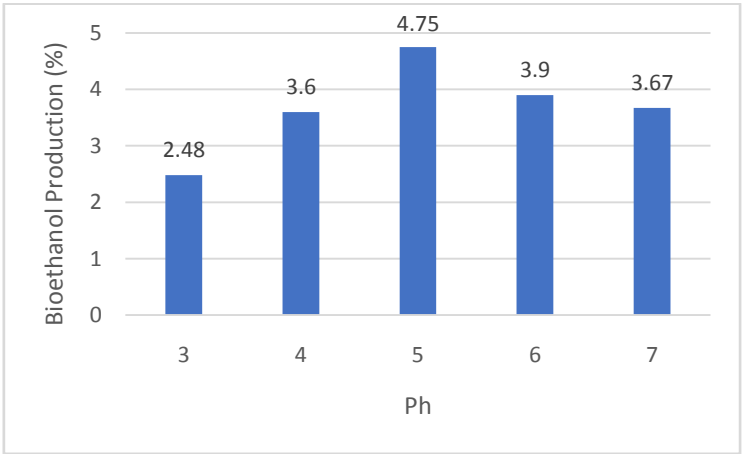

Figure 5: Effects of pH values on the production of bioethanol (% v/v)

These results are similar to those described by the study of (Thenmozhi and Victoria., 2013) [11]. The experiments were conducted with the strain Saccharomyces Cerevisiae MTCC 178 at pH equal to 5. However, the authors reported that the strain was more tolerant to acid pH.

3.4 Effects of yeast load on the production of bioethanol

As mentioned above, the best production of ethanol was obtained with a pH equal to 5. This value of pH was maintained to determine the value optimal quantity of inoculum to achieve a best production of bioethanol. Figure 6 reveals that the increase in the quantity of yeast does not enhance the production initially but with the continuous increase of yeast quantity, the production of bioethanol increases considerably. The best production of bioethanol is obtained with a quantity of inoculum equal to 0.12g (this quantity is equivalent to 4,8.106 cells/ml). Some authors have obtained in their work similar results using 6.106 cells/ml [12]. The studies have been conducted on strain of Saccharomyces cerevisiae ITV-01. In addition, the authors showed in the same study that the increase of the inoculum quantity reduces the lag phase. Another study conducted by Osunkoya and Okwudinka (2011) has allowed to obtain the maximum production of ethanol with a quantity of 5g [13]. the work of Acourene et al. [14], with respect to them have allowed to obtain the best production with an inoculum of 4%.

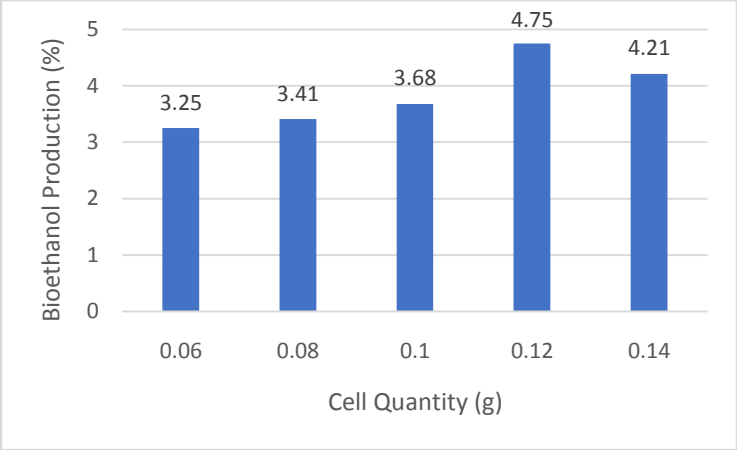

Figure 6:Effects of yeast load on the production of bioethanol (% v/v)

The values of the production of ethanol obtained are low compared with those of the literature cited above. This can be explained by the deficiency of the of fermentation medium in nitrogen material which is an indispensable substance in the nutrition of the yeast. The quantity of nitrogen necessary for the nutrition of the yeast varies between 6.5 and 9.3% M.S (Acourene. S 2013) [14], while the molasses medium for our study does not even contain the minimum.

IV. CONCLUSION

According to the results of the production of ethanol obtained by fermentation of the molasses, it is deduced that this molasses is a source quite rich in carbon and calcium but presents a poor environment in nitrogen. That has led to the production more or less weak of bioethanol. However, it must emphasize that the cultures of lyophilized yeast have given higher yields compared to the precultured yeast. According to the results obtained, the best production of bioethanol has been obtained in the fermentation medium that contains 10% of molasses, equivalent to 12 % of sucrose with pH equal to 5 and a yeast load of 0 .12g /L after incubation for 60 hours at a temperature of 20° C. However, this production of ethanol remains relatively low (4.75 % v/v). Indeed, the molasses alone does not present a complete environment for the production of bioethanol. This value of rate of ethanol even if it is low did not exclude any evidence of enhancing of bioethanol production by optimizing the parameters conditions Consequently, more studies in this field will be necessary to enhance the production of bioethanol using molasses from sugar refinery industry.

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