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Effect of Operating Conditions on Molasses fermentation for Bioethanol production

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Abstract

The aim of the presented study was to evaluate the potential of molasses as bioethanol feedstock by studying the effect of different operating conditions on fermentation yield including initial sugar concentration (25-150 g/L), pH (4.5-9.5), and temperature (30-50°C). Molasses composition analyses indicated its richness with sucrose and fermentable sugars which qualify it as a promising feedstock for bioethanol production. The highest ethanol production (49 g/L) was achieved with an initial sugar = 150 g/L, pH = 4.5 but the maximum ethanol yield has been noted for: initial sugar concentration = 50 g/L, pH=4.5 and 30 °C of temperature which represent the optimum conditions for the fermentation. The kinetics study of fermentation experiment carried out under optimal conditions revealed that the fermentation reaction occurs in 3 phases: lag phase, acceleration phase and final phase.

Keywords: Bioethanol; fermentation; feedstock; molasses; *Saccharomyces cerevisiae.*

INTRODUTION

In the last decade, a huge increase in the demand for energy derived from conventional fossil fuel resources such as coal, oil, and natural gas has been remarkable. Meanwhile, conventional fuels should be replaced partially by new energy sources to fulfil the energy needs of humanity where the industrial products derived from biomass is considered as alternative to substitute fossil resources for renewable resources (Davis et al., 2009; Kikuchi et al.,2013). This is mainly due to growing concerns over the consequences of climate change. A difficult choice between energy needs and environment could precipitate a major economic crisis, an environmental crisis, or both (Hester et al., 2010). Bioethanol is the most widely used liquid biofuel with the markets expected to reach 100 x 10^9 litres in 2015 (Licht et al., 2005). Bioethanol production using agro-industrial residues can be an alternative, given the need to replace fossil fuels. The bioethanol industry aims to promote the use of biofuels, with a dual economic and ecological interest. The United States is the world's largest producer of ethanol, producing over 14 billion gallons in 2014. Together, the U.S. and Brazil produce 83% of the world's ethanol. The majority of U.S. ethanol is produced from corn while Brazil primarily uses sugar cane (Licht et al., 2015).

The production of liquid fuels from biomass currently occurs in four major steps: pretreatment to make the feedstock more amenable to enzymatic degradation, hydrolysis of cellulose and hemicellulose to monomer and oligomer fermentable sugar, fermentation of monomeric sugars to fuels, and the recovery of the fuel from the reactor bulk (Hanley et al., 2013**)**.In several countries, research is currently working on the development of advanced biofuels, which are produced from non-food, cellulosic biomass, such as woody and straw residues from agriculture and forestry, fast-rotation plants, non-food crops (possibly grown on marginal, non-arable land), organic fraction of urban waste and algae-based feedstock. These types of feedstock require advanced, capital-intensive processing to produce biofuels, but they can be more sustainable, offering higher emissions reductions and less sensitivity to fluctuations in feedstock costs (IEA.,2013). Molasses seems to be a promising feedstock for bioethanol production with high yield and low cost. In this regard, research has highlighted production of bioethanol from molasses using *Saccharomyces cerevisiae* (Periyasamy et al.,2009; GABRIELA et al.,2009; Abdalbasit et al.,2012). In this study, we have focused on bioethanol production from molasses residues from sugar refinery to evaluate its potential as a feedstock besides and to determine the effect of different factors such as temperature, initial sugar concentration and pH on fermentation yield followed by fermentation kinetics study to comprehend the mechanism of the fermentation process.

MATERIALS AND METHODS

Molasses characterization and contents:

Molasses of « Rassila Sucre factory-Algeria» were analysed to determine its composition and to investigate its potential as a feedstock for bioethanol production. Different tests including: sucrose concentration, reducing sugar concentration, sulphated ash, water, and pH were determined. The water percentage was determined using Refractometer (Atago Digital Brix Refractometer, PAL-1) water % = 1- Brix degree. The concentration of sucrose was measured using 30123.01 Disc polarimeter.Reducing sugars (glucose and fructose) were determined using Fehling's test .The sulphated ash was determined using the method B reported by WHO (WHO.,2013).

Microorganism and culture media preparation :

2-3 loops of active dry *Saccharomyces cerevisiae* yeast were dissolved in 50 mL of distilled water with then were added directly into 200 mL of culture media containing diluted molasses, ammonium sulfate (0.7g/L) ammonium phosphate (0.4 g/L) and incubated at 35°C and shaking with 250 rpm for 6 hours.

Fermentation yield and Kinetics :

Aneorobic fermentation was carried out in batch mode using bioractor 1 L volume containing 250 mL of culture media with different intial sugar concentrations, different temperatures and different pH values.

To investigate the effect of different operating conditions on the fermentation, the fermentation process described above was carried with 3 duplicates during 72 hours , pH value was adjusted to 4.5, 7 and 9.5 with sulphuric acid (0.1M)and sodium hydroxide (0.1M); Inoculum for fermentation assays was prepared with different dilution rates for (25 , 5 ,100,150 g/L) of intial sugar.The fermentation temperature was kept at 20 ,30,50 °C .

After studying the effect of different parameters on the fermentation yield and determining the optimal condition for bioethanol production, we have conducted the fermentation under the optimal conditions for 5 days to follow the fermentation kinetics regarding the sugars consumption and ethanol production.

Sucrose and reducing sugars concentration were determined using the methods mentioned above for molasses characterization, Ethanol concentration was determined using densitometry method after distillation of the broth at 78°C (Bettin et Spieweck.,1990).

RESULTS AND DISCUSSION

Determination of the composition of sugar molasses:

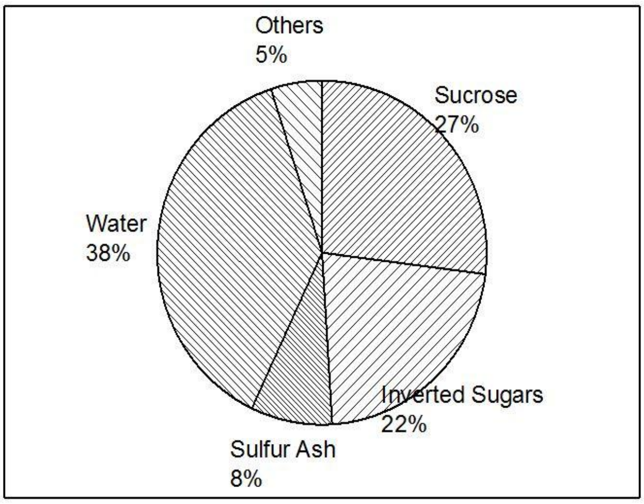

Figure 1: The chemical composition of molasses

Regarding to the results shown in the figure (Fig.1), it is clear that molasses of the sugar refinery 'Rassila sugar is characterized by very high sucrose content. Moreover, the important amount of reducing sugars was remarkable and higher than the reducing sugar percentage reported in previous studies (Olbrich.,1963; Hashizume al.,1966). Molasses analysis have also shown its richness of molasses of sulfur ash as nitrogen source represented 8% of the total mass.

Regarding to its composition with its slightly acidic nature (pH=4.8) and its richness of carbon and nitrogen sources, the molasses can be considered as a suitable medium for *Saccharomyces cerevisiae* yeast growth ;Therfore, it is a promising feedstock for bioethanol production at large scale. Considering the theoretical ethanol yield ~0.51g EtOH/g sugar and the total sugar content ~49%, it is expected that 1 kg of molasses may produce 249.9 g of ethanol which represents a high yield.

Effect of different operating conditions on fermentation yield:

Theoretically regarding the sugar fermentation equation, it is expected that any increase in sugar amount leads to an increase in ethanol production and yield.

Glucose + 2 ADP + 2 Pi ⟶ 2 Ethanol +2 CO2 + 2 AT

The produced ethanol amount increased as initial sugar concentration increased and achieved 47 g/L for 150 g/L of initial sugar with 0.31 g Eth /g sugar yield **(Fig 2)**. Although the produced ethanol in the cases of 50 and 100 g/l initial sugar was less than the produced ethanol produced by 150 g/l, the ethanol yield was higher where it reached 0.48 g ethanol/g sugar which represents 94% of theoretical yield.

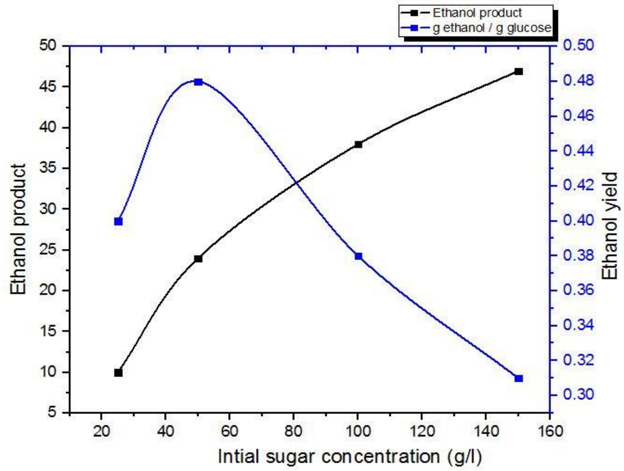

Figure 2: Effect of initial sugar concentration on fermentation yield

The optimal sugar concentration depends primarily on the physiological properties of the yeast; a high sugar concentration can create an extracellular osmotic pressure greater than that of the intracellular environment; the water in the cell tends to diffuse through a membrane of a hypotonic solution to a hypertonic solution **(**Klis et al.,2006). In the very dilute sugar solution; there is a passage of water from the external medium to the intracellular environment which creates internal pressure leads to swelling and bursting of the cells. Moreover, an increase in initial sugar concentration increased the concentration of product (ethanol) presence of which has been shown to have an inhibition effect on yeast growth, fermentation activity which totally stops in high ethanol concentration (Zhang et al.,2015).

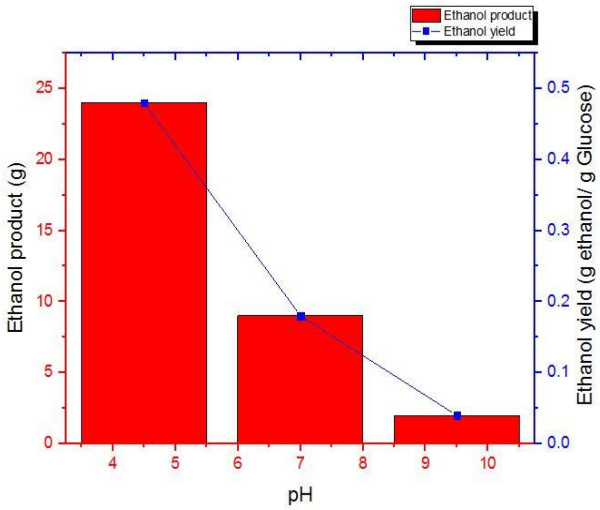

Figure 3: Effect of pH on fermentation yield

Determining the effect of f pH on fermentation yield **(Fig.3)** revealed that pH = 4.5 produced the highest ethanol concentration and had the highest ethanol yield (0.48 g ethanol/g sugar). It is clear that the acidic medium is favorable for the activity, therefore, we could say that *S.cerevisiae* yeast is an acidophilus microorganism, Moreover, the acidic pH is favorable for the degradation of sucrose to fermentable sugars by the invertase enzyme. Meanwhile, the fermentation was too slow in neutral medium (pH =7), and the produced ethanol decreased to 0.8 which means 3 times less than the ethanol produced in acid medium (0.18 g eth / g sugar yield). In basic medium, the fermentation there was no conversion of sugars into ethanol.

The effect temperature was determined **(Fig.4)** revealing that 30 ° C was the optimal temperature for alcoholic fermentation which accelerate and stimulate the fermentation process to reach the maximum yield (0.48 g eth / g sugar). At 20 ° C, the fermentation reaction was slower, however, the produced ethanol reached 16 g with 0.31 g eth / g sugar as yield. When the temperature was increased up to 50 ° C; a small amount of ethanol was produced (11 g) with low yield (0.23) but after few hours most of the yeast cells died and decanted at the bottom of the bioreactor, and the fermentation reaction stopped.

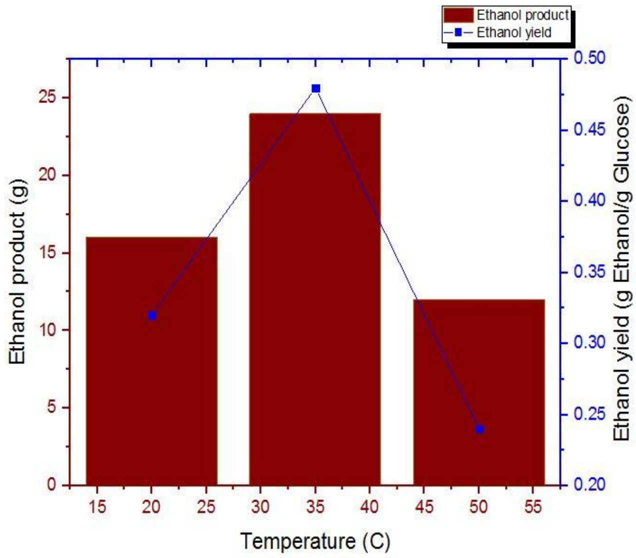

Figure 4: Effect of temperature on fermentation yield

Fermentation reaction kinetic:

The results of kinetic study of fermentation process carried out under optimal conditions (pH=4.5, T=30 °C, initial sugar concentration =50 g/L) are represented in Fig.5 where the variation in produced ethanol, Sucrose and inverted sugar were followed to investigate the fermentation mechanism and progress.

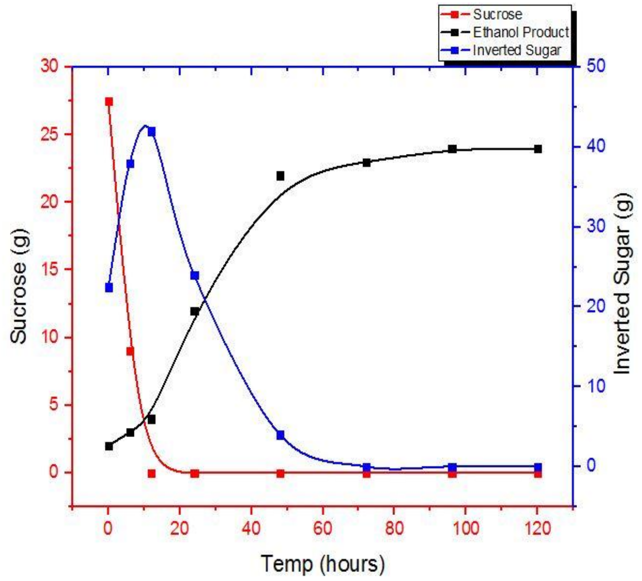

Figure 5: Fermentation Kinetics

The produced ethanol during the first 10 hours was 4g, it was noted that this period was characterized by a remarkable increase in inverted sugar (equimolar Glucose-Fructose mixture), two reaction mechanisms have occurred in this stage: The first one produces invert sugar: the degradation of the sucrose to inverted sugar in acidic medium **(Fig 6)**.

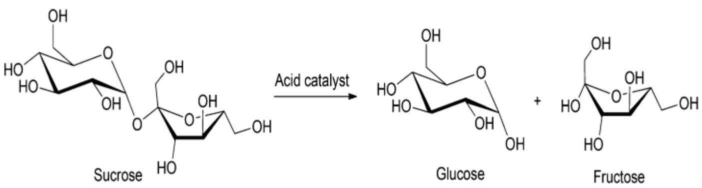

Figure 6: Conversion of sucrose to glucose and fructose (Chatterjee et al.,2015)

The second reaction have consumed the inverted sugar: the fermentation reaction. It is also noted from the curve shape of the graph in this stage that the degradation was faster than reaction fermentation. After the complete degradation of the sucrose, the fermentation reaction that consumed the inverted sugar present. The fermentation accelerated and the ethanol amount has exponentially increased and produced 22 g at 72 hours of fermentation. After 3 days, the sugars were totally consumed and fermentation stopped.

As it is shown in Fig.5, three phases have been noticed during the fermentation: the first stage is the lag phase (0-10h) where the yeast adapted to its new environment producing some metabolites need for the fermentation reaction in this period with limited ethanol production. After 10 hours, the yeast started rapidly consuming the sugar and produced ethanol, the produced ethanol in this stage was 19 g (82% of the total ethanol), we call this stage “Acceleration phase”. After 72 hours, we have noticed the depletion of sugar which ends up the fermentation reaction, it is the final phase.

CONCLUSION

The study of the influence of different operating conditions on fermentation yield have shown that the optimal conditions for molasses fermentation were: pH = 4.5, T=30 ° C, S=50 g/L.

By running a fermentation using molasses as a feedstock under the optimal conditions, yield of (0.48 g eth / g sugar) has been successfully achieved, too close to the theoretical yield (0.48 g eth / g sugar), which means the production of 235 g of ethanol using 1 kg of molasses.

The kinetic study has revealed that fermentation process occurs in three stages: lag, acceleration and final phase.

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