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Production of Ethanol from Sudanese Sugar Cane Molasses and Evaluation of Its Quality

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Abstract

The objective of the present study was to produce ethanol from final sugar cane molasses and to evaluate its quality. Urea was used as nitrogen source and added at different concentrations 0.15%, 0.5%, and 0.25% (w/v) to the molasses mash. Experiments were conducted using four treatments depending upon molasses sugar concentration which was calculated as percentages 10, 15, 20 and 25(w/v). The pH of the mash was adjusted to 4.8 using concentrated sulphuric acid. 5% (w/v) baker's yeast was added. The fermentation was conducted for 72 hours at 33°C. The microbiological analysis revealed absence of bacteria, yeasts and moulds in dilutions 10-3, 10-4, 10-5 of molasses samples. The yield of ethanol obtained was 20 ml per 100 g of molasses, and ethanol with 96% purity could be obtained when the main medium of production (molasses) includes 0.25% (w/v) urea and 20% (w/v) sugar concentration.

Keywords: Sugar cane; Molasses; Ethanol; Chemical composition; Fermented mash

Introduction

Ethanol known as ethyl alcohol or grain alcohol is a flammable, colorless, mildly toxic chemical compound with a distinctive perfume –like odor, and the ethanol is found in alcoholic beverages. In common usage, it is often referred to simply as alcohol [1]. Natural energy resources such as petroleum and coal have been consumed at high rates over the last decades. The heavy reliance of the modern economy on these fuels is bound to end, due to their environmental impact (and the corresponding pressure of society) and to the fact that they might eventually run out. Therefore, alternative resources such as ethanol are becoming more important. Bio-ethanol is one of the most important renewable fuels contributing to the reduction of negative environmental impacts generated by the worldwide utilization of the fossil fuels [2]. Hoefnagels et al. [3] also reviewed and examined methodological choices and premises in the estimation of the life cycle greenhouse emissions of biofuels. The properties of ethanol stem primarily from the presence of its hydroxyl group and the shortness of its carbon chain. Ethanol's hydroxyl group is able to participate in hydrogen bonding, rendering it more viscous and less volatile than less polar organic compounds of similar molecular weight. Ethanol has slightly more refractive than water with a refractive index of 1.36242 (at λ=589.3 nm and 18.35°C) [4].

Molasses, a by-product of sugar processing, is produced in large amount in Sudan. Sucrose is lost in sugarcane molasses which affect factory profit; therefore transformation of molasses to ethanol is possible alternative to maximize the use of molasses. Ethanol is extensively used as a motor fuel additive [5]. The United States became the world's largest producer of ethanol which produced 49.2 billion liters of ethanol fuel in 2010 [6]. Yeasts are the most commonly used microorganisms for ethanol fermentation. Anaerobic fermentation of *Saccharomyces cerevisiae* generates, besides ethanol, carbon dioxide, glycerol and cell biomass as the most significant byproducts. Carbon dioxide is an inevitable fermentation product, but the off-gas can be sold as a high-quality raw material. Glycerol can be produced as a compatible solute during osmotic stress [7]. The fermentative yeast *Saccharomyces cerevisiae* is largely employed in ethanol production using renewable biomass such as sugar cane, sugar beet and molasses as the main carbon source [8] because this strain exhibits typical values for fermentation parameters, such as fermentation ability in both low sugar (5% of sugar) and high sugar (30% of sugar) [9]. Among them, sugar-cane blackstrap molasses is a very useful raw material for that purpose, because it is cheap and plentiful in the sugar industry. The ethanol fermentation can be carried out in batch, fed-batch or continuous mode [10]. Ethanol for use in alcoholic beverages, and the vast majority of ethanol for use as fuel, is produced by fermentation. When certain species of yeast, most importantly, *Saccharomyces cerevisiae*, metabolize sugar in the absence of oxygen, they produce ethanol and carbon dioxide. The chemical equation below summarizes the conversion: $C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$ [11]. The objective of this study was to determine the effect of urea and sugar concentrations on ethanol production yield.

Materials and Methods

Materials

Molasses samples were obtained from a local sugar factory (Elguneid, Al Jazirah State, Sudan), A strain of baker's yeast (*Saccharomyces cerevisia*), urea, sulphuric acid, sodium hydroxide, hydrochloric acid, fehling A, fehling B, methylene blue and EDTA were purchased from Elwataneia Co. (Khartoum, Sudan).

Chemical composition of black strap molasses

The pH of the molasses was measured using pH-meter (PHS-3C Digital) at ambient temperature according to ICUMSA [12]. The total soluble solids, the total sugar content and reducing sugars content were determined according to ICUMSA [12]. The sucrose content was

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determined according to ICUMSA [13]. Ash content was determined according to Chen and Chou [14].

Mash preparation and fermentation

Sample (100 g) was weighed into a beaker of one-liter volume and 500 ml of water was added to the molasses. The weight of yeast was taken to be as a percentage 5% (w/v) of mash weight. The required nutrients (urea) of different concentrations 0.15%, 0.5%, and 0.25% (w/v) were then added. The pH of the mixture was adjusted to 4.8 using concentrated sulphuric acid. The mash was transferred into clean reinforced plastic container of about 900 ml volume. Fermentation was conducted for 72 h under controlled temperature. For final production, the fermented mash was distilled and the ethanol amount was recorded.

Ethanol measurement

The yield of ethanol (%) in the fermented mash was measured using an Ebulliometer (Model 170-1652, Kessler Co., and Washington 98272, USA). The density, viscosity and purity values of ethanol were determined according to (AOAC) [15].

Microbiological analysis

Preparation of media and samples, total viable count, yeast and mould count of molasses were determined according to APHA [16].

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan multiple range test using SPSS 16. All data were expressed as mean ± SD. The significance of results was at 5%.

Results and Discussion

Chemical composition of black strap molasses

The chemical composition of black strap molasses is presented in Table 1. The molasses contained 84° brix, 17% reducing sugars, 32% sucrose, 49% total sugars, 12.69% ash (w/v) on wet weight basis. The, pH value of obtained molasses was 5.8. The brix value determined in this study was lower than the value (85.4°) reported by Paturau [17]. Results indicated that chemical composition of Sudanese sugar cane molasses were in close agreement to those reported by Chen and Chou [14], who found that molasses contained 52% total sugars, 16% reducing sugars, 34% sucrose, 12% ash and pH 5.0.

Parameter	Mean (w/v)
Brix	84 ± 2.51°
Reducing sugars	17 ± 2.0%
Sucrose	32 ± 3.51%
Total sugars	49 ± 5.50%
Ash	12.69 ± 0.26%
pH	5.8 ± 0.35

Table 1: Chemical composition of black strap molasses (on wet weight basis).

Dilutions	Total viable counts (c.f.u./ml)	Yeast and mould counts(c.f.u./ml)
10^{-1}	$3×10^2$	$2×10^2$
10^{-2}	$0.7×10^2$	$0.9×10^2$
10^{-3}	ND	ND
10^{-4}	ND	ND
10^{-5}	ND	ND

Table 2: Microbiological analysis of molasses.

Molasses weight (g)	Sugar concentration (%)	Urea%	pH	Temperature (°C)	Yield% (w/v)
100	8.3	0.15	4.8	33	4.8 ± 0.10
100	8.3	0.50	4.8	33	5.5 ± 0.15
100	8.3	0.25	4.8	33	5.9 ± 0.21

Table 3: Ethanol yield in fermented mash using different urea concentrations.

Molasses weight (g)	Water mash added (ml)	Sugar concentration %(w/v)	Urea concentration % (w/v))	pH	Temperature (°C)	Yield% (w/v)
100	300.20	10	0.25	4.8	33	5.5 ± 0. 20
100	200.20	15	0.25	4.8	33	7.8 ± 0.10
100	100.20	20	0.25	4.8	33	11 ± 0.40
100	70	25	0.25	4.8	33	10.3 ± 0.35

Table 4: Effect of sugar concentration on ethanol yield.

Properties	Mean value
Purity (%)	96 ± 1.45
Density (g/ml)	0.807 ± 0.03
Viscosity (cP)	0.83 ± 0.04

Table 5: Physicochemical characteristics of the obtained ethanol in this study.

Microbiological analysis of molasses

The microbiological analysis of molasses samples is shown in Table 2. The results revealed the presence of 3×10^2 and 0.7×10^2 (c.f.u/ml) of total microbial counts in 10^{-1} and 10^{-2} molasses residual dilution, respectively. While other dilutions (10^{-3}, 10^{-4} and 10^{-5}) were devoid of microorganisms, it seems that the high sugar concentration reduced the total number of microorganisms as a result of reduction in water activity. On the other hand, the yeast and mould counts at dilutions of molasses 10^{-1} and 10^{-2} were found to be 2×10^2 and 0.9×10^2 (c.f.u/ml), respectively while other dilutions of molasses (10^{-3}, 10^{-4} and 10^{-5}) were free from yeast and moulds. This could be attributed to the good hygienic conditions during sampling.

Effects of nutrient concentration on the yield of ethanol in fermented mash

The effect of different urea concentrations (0.15%, 0.50%, and 0.25%) on ethanol yield from fermented molasses mash is shown in Table 3. The highest nutrient concentration which gave the highest ethanol yield in fermented mash after period of fermentation (72 hours) was 0.25% (w/v). Calm [18] reported that the use of $(NH_4)2\ SO_4$ as a nitrogen source in molasses medium is greatly recommended for ethanol production.

Effects of sugar concentration on the yield of ethanol in fermented mash

Sugar concentration plays an important role in ethanol fermentation by yeast. For economic reasons the residual sugar for maximum ethanol formation should be negligible at the end of fermentation. Therefore, the optimum level of sugar was determined by using 20% (w/v) sugar in molasses medium (Table 4). Maximum amount of ethanol 11% (w/v) was produced when the sugar concentration was 20% (w/v). Further increase in the sugar concentration, however, resulted in the decrease of its conversion to ethanol. The decrease in fermentation efficiency by increasing the sugar level above 20% may be due to the substrate inhibition or due to the increased accumulation of residual sugar [19]. Monot et al. [20] studied the effect of sugar in synthetic medium. The workers found the yield of ethanol was maximum when sugar level ranged from 4.0 to 6.0% (w/v). The volume of ethanol production was 20 ml per 100 g of molasses.

Physicochemical characteristics of ethanol

In Table 5 physicochemical characteristics of ethanol was shown. Ethanol had 96% purity, 0.80 g/ml density and 0.83 cP viscosity. The purity value determined in this study was slightly greater than the most popular method of purification in which the purity reached 95.6% [21]. The bio-ethanol produced by Ghosh and Ghose [22] was in the form of hydrous ethanol (95% v/v). The density value was higher than the standard density value (0.78097 g/ml) in the same temperature [23]. The viscosity value was greater than the value 0.37 cP reported by Perry's [5].

Conclusion

Experimental results of producing ethanol from molasses showed high alcohol yield, especially when urea (as a nutrient source) and sugars were used at 0.25% and 20% (w/v) concentrations, respectively. That formulation gave 11% (w/v) ethanol in fermented mash. After distillation, the volume of ethanol produced was 20 ml per 100 g of molasses, these conditions were considered suitable for yeast activity and high yield of alcohol.

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