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RESEARCH ARTICLE

Reducing heavy metals content in sugarcane molasses and its effect on ethanol fermentation efficiency

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Abstract

The world energy scene is undergoing a period of transition. As the inevitability of exhaustion of fossil fuels is becoming increasingly intensive, efforts are exerted to find and use substitutes for energy. Bioethanol is one of the most substitute renewable fuels contributing to the reduction of the global warming effect and negative environmental impact. Bioethanol production generally utilizes derivatives from food crops such as corn grain and sugarcane. In Egypt, sugarcane molasses is mainly used as feedstock for bioethanol production. However, molasses contains a concentration of heavy metals. Heavy metals are presented in high concentrations in the fermentation medium causing a critical problem during fermentation. This study focuses on reducing heavy metals content in molasses to improve bioethanol fermentation using heating, centrifugation, sulfuric acid, and phosphoric acid. Heating and centrifugation were sufficient to decrease Ca with less effect on other metals. Sulfuric acid reduced heavy metals content and the reduction addition of phosphoric acid had less effect on lowering the levels of heavy metals in molasses. Pretreatment of molasses with 0.3% H2SO4 decreased the contents of various inhibitory metals: Ca, Cd, Cu, Fe, Ni, Pb, and Zn making molasses healthier for fermentation by yeast strains and increasing subsequent ethanol yield as well as high fermentation efficiency.

Keywords: Molasses; Heavy metals; Inhibitory effect; Ethanol fermentation.

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Introduction

Biofuels have great importance as they are directly related to energy consumption in the near future. Many research organizations and other energy-related multi-national companies are now trying to focus their attention on the production of biofuels with an increase in its efficiency and reduction of harmful release products if any (Azenha et al. 2000; Bai et al. 2008).

The energy crisis necessitates studying and discovering new processes involved in the production of utilizable compounds as alternative energy sources. As a result of this fermentation of ethanol, there is a significant strategy; especially as ethanol has shown promise to partially substitute for gasoline as a fuel (Ueda et al.1981). Bioethanol source of particular interest whose production by microbial fermentation is increasing to replace gasoline (Ogbonna et al. 2001; Limtong et al. 2007; Dhaliwal et al. 2011). This situation has led many countries to yield to a policy of blending ethanol at the rate of 5 – 10 % with petrol. This has received worldwide interest to improve and optimize the process for a quicker and cheaper ethanol product (Rattanapan et al.2011). Among the widely used substrates for ethanol production, there are molasses, the byproduct of sugar industries from sugarcane and sugar beet (Zohri et al. 2022; Kaman and Amor 2009). This is because they are cheap raw materials; readily available and ready for conversion with limited pretreatments as compared with starchy or cellulosic materials. Also, most sugars in molasses are presented in a readily fermentable form (Razmovski and Vučurdvić 2011). In Egypt, mainly sugarcane molasses is used in ethanol production. Curtin (1983) reported that the composition of molasses is influenced by many factors such as soil type, ambient temperature, moisture, and season of production. Also, the variety and technology of sugar mills can control the amount of sucrose extracted. Therefore, the sugar content of molasses produced in different countries varies according to the production techniques employed. Also, Damtew (2008) reported that changes in the design of centrifuges used to separate sugar. and syrup constitute one of the major advancements in the cane sugar industry.

Moreover, the sugar manufacturing process of cane molasses will generate some hazardous substances such as excessive metallic ions, which are toxic to microbial cells (Zohri et al. 2019; Xu and Xu 2014). The commonly used in ethanol production in the industry is Sacharomyces cervisiae (zaldovar et al.2001).

Roukas (1998) and Goksungur et al. (2004) reported that molasses contains harmful compounds, which may inhibit yeast activity. Heavy metals are presented in high concentrations in the fermentation medium causing a critical problem during fermentation and causing various kinds of technological difficulties. Therefore, its fermentation processing requires manipulations without which the fermentation and yield of the ethanol become lower (Andersen et al.2002; Jauert et al.2002; Soares et al.2003) reported that heavy metal accumulation in soils was influenced by many factors such as the type and amount of clay, soil organic matter content, phosphatic fertilizers, crop residues, and soil pH. This study focuses on the improvement of the bioethanol production process from sugarcane molasses using various methods for managing and decreasing heavy metals by using heating, centrifugation, sulfuric acid, and phosphoric acid.

Materials and Methods

Sugarcane molasses

Different samples of sugarcane molasses were obtained from different sugar factories (Deshna, Kous, Nag Hammady, and Edfu), Egyptian integrated industries companies (ESIIC's) at the beginning of mills season 2020/2021, at the Hawamdia distillation factory. Separately samples of molasses were taken directly from receiving station (hawamedia's transportation Affairs) by using Nile units at receiving it before mixing all molasses comes from ESIIC's factories from Upper Egypt before discharging into one tank as it runs nowadays. Different four samples (5 Kg each) of molasses from of each the four factories were collected, mixed well, and stored in a jar in a refrigerator until physic-chemical analysis and used for different experiments in this study.

Yeast strains.

Saccharomyces cerevisiae F-514 which is already applied for ethanol production in Egyptian distillation factories supplied by Microbial Chemistry Lab. National Research Centre, Dokki, Giza, Egypt used in this study. This yeast strain was maintained at 4˚C on agar slants. The composition of the agar medium was (g/L): yeast extract 3, malt extract 3, peptone 5, glucose 10, and agar 20. The cultures were maintained by sub-culturing every 20-days and the test tubes were then incubated at 30˚C for 36 h.

Inoculum preparation.

Sterilized 500 ml capacity conical flasks each contained 200 ml of medium containing (g/L) malt extract, 3, yeast extract, 3, peptone, 5, and sucrose, 30 was steam-sterilized at 121°C for 20 minutes, cooled to room temperature, then inoculated with a loop of yeast strain S. cerevisiae F-514 and incubated at 34°C for 24 h. This inoculum was used to inoculate the prepared fermentation vessels at 2% v/v (Fadel et al.2013).

Fermentation of molasses medium

The sugarcane molasses was diluted with water to give the demand sugar amount in fermentation containers, 18%. This diluted molasses was supplemented with 2 g/L diammonium phosphates as a source of nitrogen and phosphorus and 0.5 g/L magnesium sulfate as a source of magnesium and sulfur. Molasses medium was dispensed into 500 ml Erlenmeyer flasks containing 200 ml. The molasses medium was inoculated by 2 % of the prepared inoculum and incubated statically to complete fermentation at 32° C under anaerobic conditions.

Analytical determination

Measuring of pH.

The pH was measured by using a digital bench pH-meter, model pH-526/sentix – 20/ASDIN/. SIN/ STH/ 650.

Determination of specific gravity.

Specific gravity was measured according to ICUMSA (International Commission for Uniform Measurement of Sugar Analysis 2003).

Determination of total solids (Brix solids)

Mean the total applicable solids content of sugarcane molasses. The Brix value was determined using a Brix hydrometer corrected to 20°C.

Determination of total sugar

The total sugars of molasses solution are determined by the volumetric method (Lane and Eynon 1923). The Lane and Eynon method is also described in ICUMSA (2003), which measures reducing sugars by titration. Reducing sugars are those sugars that reduce Fehling reagents. Glucose and fructose reduce Fehling reagents, sucrose does not, so the sucrose was inverted using hydrochloric acid (1N) and the titration was repeated and the total reducing sugars can be calculated.

Determination of non-fermentable sugars

Total non-fermentable sugars are reducing matters that have a reducing effect on Fehling's solution and cannot ferment by S. cerevisiae and are determined by the volumetric method (Lane and Eynon 1923). incubated at 32 °C for 24 hours. Transferred quantitatively into 250 ml ml of 10 % neutral lead acetate solution diluted to the mark, mixed well, and filtrated. Transferred 150 ml of filtrate into 200 volumetric flasks then added 5 ml of 4% potassium oxalate, cooled to 20 °C, and diluted to the mark with water. Mixed well then filtrated and titrated the filtrate with 10 ml of Fehling's solution its factor is known and determines the volume consumed in titration (V1) Total non-fermented sugars% g molasses =

Lane - lynon's factor x Fehling's factor x250 x200 x100 /12 x 150 x (V1) x 1000.

Determination of ash

Total ash in the molasses sample was determined by the methods described by AOAC (2000).

Determination of ethanol yield

Ethanol percent was determined by Ebulliometer apparatus approved in distillation factory which consists of condenser, thermometer, burner and Calibrate scale. It depends on a constant boiling temperature for two liquids mixture having different boiling temperatures ethanol and water according to Jacobson (2006).

The proper statistical analyses of variance according to Gomez and Gomez (1984). Mean comparison between treatments and their interactions was determined using Duncan's multiple range tests at a 0.05 probability significance level.

Determination of heavy metals

Different heavy metals: cadmium (Cd), nickel (Ni), lead (Pb), copper (Cu), iron (Fe), and zinc (Zn) were determined in crude samples of molasses of the different four sugar factories according to the method described by AACC (2000). Each sample (2 g) was dried in an oven (105°C). The dried material was ashes in a muffle furnace at 450 - 500°C until the sample was completely combusted (ash turned white/gray or slightly colored). The obtained ash was dissolved using 1 ml conc. HCl. at crucible walls. Dissolved samples were transferred to a 50 ml volumetric flask and de-ionized water was added to the complete volume. The solution was filtered through ashless filter paper Whatman No. 42 and stored in a refrigerator until a determination by Atomic Absorption Spectrophotometer (PG-990). The maximal absorbance was obtained by adjusting the specific hollow cathode lamps for each element at a specific wavelength for the element Cd, Ni, Pb and Cu, Zn, and Fe, respectively. The concentration (K) of metal in the sample was calculated according to the following formula:

$$K = \frac{(a-b) x V}{m}$$

Where K = concentration of metal in sample (mg/kg); a = concentration in sample solutions (mg/l); b = mean concentration in blank solutions (mg/l); V = volume of sample solution (ml); m = weight of sample (g).

Treatment of molasses

Centrifugation

A mixed sample (MS) of collected molasses was diluted to desired concentration (18 %) of fermentable sugar. Then centrifuged at 8000 rpm for 15 minutes. The centrifuged molasses was fermented by S. cerevisiae F-514 as previously mentioned in this study.

Heating

Centrifuged molasses was heated to 90 °C for 2 hours then cooled and fermented by S. cerevisiae F-514 as previously mentioned in this study.

Acid treatments

Using sulfuric acid

Centrifuged and heated molasses were treated by 0.1, 0.2, 0.3, 0.4 and 0.5% of 98% conc. sulfuric acid is then fermented by S. cerevisiae F-514 as previously mentioned in this study.

Using phosphoric acid

Also, centrifuged and heated molasses were treated by 0.1, 0.2, and 0.3 % conc. phosphoric acid and then fermented by S. cerevisiae F-514 as previously mentioned in this study.

Results and Discussion

Sugar content and ethanol yield

Data presented in Table 1 shows that chemical composition included total sugar, total-fermentable, and non-fermentable sugars involved in sugarcane molasses samples collected from the four factories in addition to the mixed samples of the four factories. The result appeared that the sugar contents varied between samples collected according to the location source. This fact was reported previously by many investigators (Mohamed 1999; Curtin 1983) reported that the composition of molasses is influenced by many factors such as soil type, ambient temperature, moisture, and season of production. Also, the variety and technology of sugar mills can control the amount of sucrose extracted. Therefore, the sugar content of molasses produced in different countries varies according to the production technology employed (Barzega et al.2005). Also, Damtew (2008) reported that changes in the design of centrifuges used to separate sugar and syrup constitute one of the major advancements in the cane sugar industry.

The ethanol yield and fermentation efficiency were different despite the fermentation medium whic contained the same concentration of fermentable sugars (18 %) as recorded in Table 1. This may be attributed to the presence of inhibitors and deleterious substances in Diluted 12 g of molasses by boiled distilled water in 500 mL conical flask, cooled, added 0.2g urea, 0.2g di-ammonium phosphate, 25g of fresh yeast, and drops of antifoam, mixed and closed by cotton, and incubated at 32 °C for 24 hours. Transferred molasses such as heavy metals (Yadav et al. 1998; Abd El-Rahman 2010), microbial contamination (Fadel et al. 2018), and other yeast inhibitors such as furfural, hydroxyl-methyl furfural and volatile acids (Bronn 1985; Goble 2002) involved in sugarcane molasses. Moreover, the sugar manufacturing process of cane molasses will inevitably generate some hazardous substances such as excessive metallic ions, which are toxic to cells (Xu and Xu 2014).

Table 1. Sugar content in samples of sugarcane molasses from different sugar factories mixed sample also the ethanol yield as well as FE % by fermentation of these samples with 18% invert sugar.

Parameters	pH.	TS%	TFS%	NFS%	Ash	Ethanol V/V	EF%
NH	5.3	52.60	48.30	4.3	10.63	9.25	84.10
Kous	5.6	46.91	42.11	4.8	12.39	8.84	80.37
Edfu	5.4	51.90	47.50	4.4	11.03	8.90	80.90
Deshna	5.5	52.12	47.82	4.3	10.53	9.20	83.65
MS	5.2	51.40	47.20	4.2	10.90	8.98	81.65

NH; Hagh Hamadi, MS; Mixed sample, TS; Total sugar. TFS; Total fermentable sugar, NFS; Non fermentable sugar, FE; Fermentation efficiency.

Some heavy metals and calcium involved in sugarcane molasses.

Table 2 shows different heavy metals: Cu, Ni, Cd, Zn, Pb, and Fe concentrations in addition to Ca percent in sugarcane molasses produced by different four sugar factories and mixed samples. The results showed that the concentrations of these elements varied between samples collected from different factories. This variation may be attributed essentially to soil composition. Also, the sugar manufacturing process of cane will generate some hazardous substances such as excessive metallic ions, which are toxic to cells (Xu and Xu 2014).

The variations in heavy metals concentrations in the soil of different areas as well as sugarcane plant, juice, and sugar cause variation in heavy metals concentrations in cane molasses of different regions. Mohamed et al. (1989) stated that the presence of heavy metals in molasses maybe return to the transportation of metals from soil to sugarcane plant, which can rely on the preferential absorbability of plant, fertilizers, and irrigation. Furthermore, Mohamed et al. (1989) concluded that variations of heavy metals in molasses may be related to different compositions of sugarcane plants, the season of production, ecologic changes in soil, the additives of fertilizers to sugarcane plant, the irrigation water, the additives to sugarcane juice during sugarcane production stages and the corrosion effects on containers due to passage of CO_2 and SO_2.

Table 2. Some heavy metals and calcium concentrations involved in sugarcane molasses.

Element	Edfu	Nag Hamady	Dshna	Kous	Mixed sample
Calcium (%)	6.8	8.20	4.40	6.30	6.60
Cupper (ppm)	18.7	18.40	21.70	14.90	18.70
Nikel (ppm)	1.22	1.63	1.82	1.85	1.60
Iron (ppm)	91.00	82.00	206.00	63.00	115.00
Cadmium (ppm)	0.78	0.64	0.80	0.82	0.74
Zinc (ppm)	22.00	22.00	22.00	23.00	22.00
Lead (ppm)	6.64	7.36	6.64	9.46	6.40

Besides, Mohamed (1999) reported that the variation between heavy metals concentrations in molasses of different locations maybe return to the variation of their concentrations in the soil of the different areas. Andersen et al. (2002) and (Jauert et al. (2002) reported that heavy metal accumulation in soils was influenced by many factors such as type and amount of clay, soil organic matter content, phosphatic fertilizers, crop residues, and soil pH. The variation in heavy metals and calcium concentrations can affect the ethanolic fermentation and activity of S. cerevisiae employed in the fermentation process as it can inhibit the yeast enzyme and reduce the growth rate of a yeast cell (Goksungur et al.2004).

Effect of some pretreatments on heavy metals content of the mixed sample (MS) of sugarcane molasses.

Data presented in Tables 3, 4 and 5 shows the effect of some economic treatments on decreasing the load of some heavy metals as well as calcium contents in the mixed sample of sugarcane molasses. Centrifugation and heat treatment affect principally on calcium reduction. Sulfuric acid reduced heavy metals content and the reduction effect was increased by increasing the percentage of its addition. The addition of phosphoric acid had less effect on lowering the levels of heavy metals in sugarcane molasses.

The obtained data agreed with that reported by many investigators. Roukas (1998) studied the effect of pretreatment of beet molasses with cation exchange resin, sulfuric acid, tricalcium phosphate, potassium ferrocyanide, and EDTA. He reported that sulfuric acid gave better results as the technique was used for the removal of heavy metals from molasses, compared with other techniques. Moreover, Roukas (1998) reported that molasses contains great amounts of some metals (iron, zinc, copper, manganese, magnesium, calcium, etc.) caused a critical problem during fermentation. They reported that molasses can be treated with sulfuric acid to reduce heavy metals.

Table 3. Effect of some pretreatments (centrifugation and heating) on some heavy metals and calcium contents of the mixed molasses sample (MS).

Treatment	Ca (%)	Ni (ppm)	Fe (ppm)	Zn (ppm)	pb (ppm)	Cu (ppm)	Cd (ppm)
Control	6.6A	1.60A	115A	22A	6. 40A	18.70A	0.74A
Centrifugation	6.3B	1.49A	110B	20A	6.36A	17.30B	0.72A
Heat	6.2B	1.38B	107B	19AB	6.31A	16.13C	0.71A
Heat + Centrifugation	5.9C	1.32B	102C	18AB	6.26A	15.80C	0.70A
P value	0.04*	0.06ns	0.03*	0.04*	0.08ns	0.04*	0.09ns

*, **, ns; significant at 5%, 1% level, and not significant, respectively.

The numbers followed by the same capital letters in each column are not significant at 5% probability.

Table 4. Effect of some pretreatments (centrifugation and heating with sulfuric acid) on some heavy metals and calcium contents of the mixed molasses sample (MS).

Treatment	Ca (%)	Ni (ppm)	Fe (ppm)	Zn(p (ppm)	pb (ppm)	Cu (ppm)	Cd (ppm)
Control	6.6A	1.60A	115A	22A	6. 40A	18.70A	0.74A
Heat + Centrifugation + Sulfuric acid 0.1%	3.40B	1.12B	84B	15B	5.11B	12.10B	0.56B
Heat + Centrifugation + Sulfuric acid 0.2%	2.80BC	0.80BC	68C	12C	4.26C	11.00BC	0.48BC
Heat + Centrifugation + Sulfuric acid 0.3%	1.60C	0.60C	52D	9D	3.18D	9.40C	0.41C
Heat + Centrifugation + Sulfuric acid 0.4%	0.90CD	0.40CD	46DE	6E	2.34E	7.10D	0.36CD
Heat + Centrifugation + Sulfuric acid 0.5%	0.60D	0.20D	38E	4EF	2.08F	6.80D	0.31D
P value	0.01**	0.01**	0.01**	0.01**	0.01**	0.01**	0.01**

*, **, ns; significant at 5%, 1% level, and not significant, respectively.

Goksungur et al. (2004) studied the effect of pretreatment of beet molasses by three methods (sulfuric acid, potassium ferrocyanide, and sulfuric acid with activated carbon). They reported that the highest polysaccharide concentration was obtained in molasses treated with sulfuric acid with activated carbon. Meanwhile, Liu et al. (2008) and Yadav et al. (1998) used cane molasses treated with sulfuric acid and heated at 90° C for 2 h., then centrifugation at 8000g for 15 min for acetone, butanol, and ethanol production. Rose (1976) says the critical concentration of the metal such as Mn, Fe, Zn, Cu, Ni, Co, and others are toxic to yeast growth at 100 µ mol but calcium is more than 2.16 W/V.

Table 5. Effect of some pretreatments (centrifugation and heating with phosphoric acid) on some heavy metals and calcium contents of the mixed molasses sample (MS).

Treatment	Ca (%)	Ni (ppm)	Fe (ppm)	Zn (ppm)	pb (ppm)	Cu (ppm)	Cd (ppm)
Control	6.6A	1.60A	115A	22A	6. 40A	18.70A	0.74A
Heat + Centrifugation + Phosphoric acid 0.1%	5.4B	1.23B	83B	15B	5.17B	14.21B	0.64B
Heat + Centrifugation + Phosphoric acid 0.2%	5.2B	1.04C	77C	12BC	5.04B	10.88C	0.54C
Heat + Centrifugation + Phosphoric acid 0.3%	4.8C	0.90D	64D	8C	3.39C	8.70D	0.45D
P value	0.02*	0.01**	0.01**	0.01**	0.01**	0.01**	0.01**

*, **, ns; significant at 5%, 1% level, and not significant, respectively.

The presentation of different, removable, and heavy metals concentrations through the previous processing:

Tables 6, 7 and 8 show the concentrations which were removed from the heavy metals (Ca, Cd, Cu, Fe, Ni, Pb, and Zn) of the mixed symbols (MS) of sugarcane molasses according to the processing which is done as the results are shown in the table.
The fate of heavy metals and calcium is removed. These metals react with acid. Some of Them are precepted and get rid of them in muds by centrifugation and filtration the others go in vinasses after distillation Yadav et al. (1998) and Liu et al. (2008).

Table 6. Concentration of heavy metals and calcium removed by centrifugation and heating.

Treatment	Ca (%)	Ni (ppm)	Fe (ppm)	Zn (ppm)	pb (ppm)	Cu (ppm)	Cd (ppm)
Control	00C	00D	00D	00D	00D	00D	00C
Centrifugation	0.30B	0.11C	5C	2C	0.04C	1.4C	0.02BC
Heat	0.40B	0.22B	8B	3B	0.09B	2.57B	0.03AB
Heat + Centrifugation	0.70A	0.28A	13A	4A	0.14A	2.90A	0.04A
P value	0.03*	0.01**	0.01**	0.02*	0.01**	0.01**	0.2*

*, **, ns; significant at 5%, 1% level, and not significant, respectively.

Table 7. Concentration of heavy metals and calcium removed by Sulfuric acid addition.

Treatment	Ca (%)	Ni (ppm)	Fe (ppm)	Zn (ppm)	pb (ppm)	Cu (ppm)	Cd (ppm)
Control	00C	00F	00E	00C	00E	00E	00F
Heat + Centrifugation + Sulfuric acid 0.1%	3.2B	0.48E	0.31D	7BC	1.29D	6.6D	0.18E
Heat + Centrifugation + Sulfuric acid 0.2%	3.8B	0.80D	0.47C	10B	2.14C	7.7C	0.26D
Heat + Centrifugation + Sulfuric acid 0.3%	5.0AB	1.00C	0.63B	13B	3.22B	9.3B	0.33C
Heat + Centrifugation + Sulfuric acid 0.4%	5.7A	1.20B	0.69B	16AB	4.06A	11.6A	0.38B
Heat + Centrifugation + Sulfuric acid 0.5%	6.0A	1.40A	0.77A	18A	4.33A	11.9A	0.43A
P value	0.01**	0.01**	0.01**	0.01**	0.01**	0.01**	0.01**

*, **, ns; significant at 5%, 1% level, and not significant, respectively.

Table 8. Concentration of heavy metals and calcium removed by phosphoric acid addition.

Treatment	Ca (%)	Ni (ppm)	Fe (ppm)	Zn (ppm)	pb (ppm)	Cu (ppm)	Cd (ppm)
Control	00C	00C	00C	00D	00C	00D	00C
Heat + Centrifugation + Phosphoric acid 0.1%	1.2B	0.37B	0.32B	7.00C	1.24B	4.49C	0.10B
Heat + Centrifugation + Phosphoric acid 0.2%	1.4B	0.46A	0.48A	10.0B	1.40B	7.82B	0.20AB
Heat + Centrifugation + Phosphoric acid 0.3%	1.8A	0.50A	0.51A	14.0A	3.01A	10.00A	0.29A
P value	0.01**	0.03*	0.02*	0.01**	0.01**	0.01**	0.02*

*, **, ns; significant at 5%, 1% level, and not significant, respectively.

Effect of pretreatment of sugarcane molasses on ethanol production

Tables 9, 10 and 11 show the effect of pretreatment molasses to get rid of some load of heavy metals as a deleterious substance in molasses which hinders yeast activity, leading to a decrease in ethanol yield and fermentation efficiency Pretreatment with centrifugation and heating may be sufficient to decrease Ca with less effect on heavy metals. So, the increase in fermentation efficiency is not significant (Table 6).

Pretreatment with sulfuric acid is the most one and the best pretreatment with 0.3% sulfuric acid is more suitable than other pretreatments. pretreatment with low or high than 0.3% sulfuric acid is not suitable. The obtained results in Table 7 can be discussed in the light of 1- at a low concentration the removal of heavy metals is not enough for yeast to make good fermentation for fermentable sugars; 2 - at a high concentration than 0.3% sulfuric acid a deficiency of heavy metals to the level demand to yeast to make healthy fermentation for fermentable sugars; 3- changes in pH of molasses medium make some essential nutrient not metabolized by yeast; in addition, pretreatment with sulfuric acid release sulfur in fermentation medium as bacterial contamination management (Fadel et al. 2018).

Table 9. Effect of the pretreatments for decreasing the heavy metals and calcium contents in the mixed sample (MS) of molasses from the four factories on ethanol production by S. cerevisiae F-514 for 48 h at 32C (centrifugation and heating).

Treatment	pH	Et OH% (v/v)	Fermentation efficiency	Alcohol increasing (%)
Control	5.4	8.98	81.65	00
Centrifugation	5.4	9.02	82.00	0.44
Heat	5.1	9.04	82.10	0.66
Heat+ Centrifugation	4.9	9.06	82.30	0.88

Table 10. Effect of the pretreatments for decreasing the heavy metals and calcium contents in the mixed sample (MS) of molasses from the four factories on ethanol production by S. cerevisiae F-514 for 48 h at 32C (centrifugation and heating with sulfuric acid).

treatment	pH	Et OH% (v/v)	Fermentation efficiency	Alcohol increasing (%)
Control	5.4A	8.98B	81.65C	00D
Heat + Centrifugation + Sulfuric acid 0.1%	4.7B	9.10AB	82.70B	1.33C
Heat + Centrifugation + Sulfuric acid 0.2%	4.5B	9.18A	83.50AB	2.22B
Heat + Centrifugation + Sulfuric acid 0.3%	4.4B	9.25A	84.10A	3.00A
Heat + Centrifugation + Sulfuric acid 0.4%	4.2B	8.93B	81.10CD	- 0.5E
Heat + Centrifugation + Sulfuric acid 0.5%	4.1B	8.58C	78.00D	-4.4F
P value	0.04*	0.04*	0.03*	0.02*

*, **, ns; significant at 5%, 1% level, and not significant, respectively.

Table 11. Effect of the pretreatments for decreasing the heavy metals and calcium contents in the mixed sample (MS) of molasses from the four factories on ethanol production by *S. cerevisiae* F-514 for 48 h at 32C (centrifugation and heating with phosphoric acid),

Treatment	pH	Et OH% (v/v)	Fermentation efficiency	Alcohol increasing (%)
Control	5.4A	8.98A	81.65B	00B
Heat + Centrifugation + Phosphoric acid 0.1%	4.6B	9.08A	82.6A	1.1A
Heat + Centrifugation + Phosphoric acid 0.2%	4.4B	9.10A	82.74A	1.33A
Heat + Centrifugation + Phosphoric acid 0.3%	3.9C	9.12A	82.9A	1.55A
P value	0.02*	0.06 ns	ns	ns

*, **, ns; significant at 5%, 1% level, and not significant, respectively.

Pretreatment with sulfuric acid considers a more economic method for sugar molasses treatment in distillation factories (Fadel 2014) also Pretreatment with phosphoric acid is good in reducing the heavy metal but less effective than sulfuric acid (Table 8).

Conclusion

Sugarcane molasses contains heavy metals which hinder the activity of yeast to utilize the fermentable sugars for ethanol production, as it inhibits the yeast's enzymes and reduces the growth rate for yeast cells. Pretreatment of molasses with 0.3% sulfuric acid decreased the contents of inhibitory substances and increased subsequent ethanol yield from the molasses unit.

References

AACC (2000) American Association of Cereal Chemists. Approved Method of the AACC. 10h ed.American Association of Cereal Chemists, St. Paul, Minnesota, USA, 2570 p.

AOAC (2000) Official Association of Official Analytical Chemistry. Washing ton, DC, USA.

Abd El-Rahman, GN (2010) Effect of some heavy metals in molasses medium on the produced baker's yeast properties. M.Sc. Thesis, Food Technology Dep. Fac. Agric., Cairo Univ. Egypt.171 p.

Andersen M, Refsgaard A, Rasmussen K, Strobel B, Hansen H (2002) Content, distribution, and solubility of cadmium in arable and forest soils. Soil Sci. Soc. Am. J. 66:1829–1835.

Azenha M, Vasconcelos T, Ferreira M (2000) The influence of Cu concentration on ethanolic fermentation by Saccharomyces cerevisiae. J. Biosci. Bioeng. 90:163-167.

Bai FW, Anderson WA, Moo-Young M (2008) Ethanol fermentation technologies from sugar and starch feedstocks. Biotechnology advances. 26(1):89-105.

Barzegari A, Koochekzadeh A, Xing B, Herbert S (2005) Concentration changes of Cd, Ni and Zn in sugarcane cultivated soils. Water, Air, and Soil Pollution. 161: 97 - 112.

Bronn WK (1985) The technology of yeast production, Baker yeast. Pp:1-30.

Curtin VL (1983) Types of molasses "Molasses–General Consideration, National Feed Ingredient Association, pp:3 – 11.

Damtew W (2008) Studies on the development of Baker's yeast using cane molasses. M.Sc. Thesis, Fac. Technol. Addis Ababa Univ. Addis Ababa. 189 p.

Dhaliwal SS, Oberoi HS, Sandhu SK, Nanda D, Kumar D, Uppal SK (2011) Enhanced ethanol production from sugarcane juice by galactose adaptation of a newly isolated thermotolerant strain of Pichia kudriavzevii. Bioresour. Technol. 102 (10): 5968 - 5975.

Fadel M, Zohri AA, El-Heeny MH, Abdel Aziz AM (2018) Managing of bacterial contamination in alcoholic fermentation of sugarcane molasses. Asian Journal of Science and Technology. 9(1): 3783 - 3791.

Fadel M (2014) Production of fuel ethanol from cane molasses. In: N. Garg and A. Aaeron (eds.) Microbes In Process. Nova Science pub. Inc New pp:93-117.

Fadel M, Keera AA, Mouafi FE, Kahil T (2013) High level ethanol from sugarcane molasses by a new Thermotolerant Saccharomyces cerevisiae strain in industrial scale. Biotechnology Research International vol. 2013: 24-29.

Godbole J (2002) Ethanol from molasses praj industries Itd. India, Hawaii ethanol workshop.

Goksungur Y, Ucan A, Guvenc U (2004) Production of Pullulan from beet molasses and synthetic medium by Aureobasidium pullulans. Turk J. Biol. 28: 23 - 30.

Gomez KA, Gomez AA (1984) Statistical procedure for agricultural research, 2nd ed. John Wiley and Sons Inc. Croda.

ICUMSA (2003) International commission for Uniform Measurement of sugar Analysis 477 (2 & 3): 187 - 200.

Jacobson JL (2006) Introduction to wine laboratory practices and procedures. Springer Science & Business Media.

Jauert P, Schumacher T, Boe A, Reese R (2002) Rhizosphere acidification and cadmium uptake by strawberry clover. J. Environ. Qual. 31: 627 - 633.

Limtong S, Sringiew C, Yongmanitchai W (2007) Production of fuel ethanol at high temperature from sugarcane juice by a newly isolated Kluyveromyces marxianus. Bioresour Technol. Bazilian journal of microbiology 4 8: 461 – 475.

Liu YP, Zheng P, Sun ZH, Ni Y, Dong JJ, Zhu LL (2008) Economical succinic acid production from cane molasses by Actinobacillus succinogenes. Bioresource technology, 99(6):1736-1742.

Mohamed AE (1999) Environmental variations of trace element concentrations in Egyptian cane sugar and soil samples (Edfu factories). Food Chem. 65: 503-507.

Mohamed A, Awadallah R, Hassan A (1989) Determination of trace elements in Egyptian molasses by instrumental neutron activation analysis. J. Radioanal. Nucl. Chem. 129 (2): 453 - 457.

Ogbonna JC, Mashima H, Tanaka H (2001) Scale up of fuel ethanol production from sugar beet juice using loofa sponge immobilized bioreactor. Bioresour Technol. 76 (1): 1 - 8.

Rattanapan A, Limtong S, Phisalaphong M (2011) Ethanol production by repeated batch and continuous fermentations of blackstrap molasses using immobilized yeast cells on thin-shell silk cocoons. Applied Energy 88 (12): 4400 - 4404.

Razmovski R, Vučurović V (2011) Ethanol production from sugar beet molasses by S. cerevisiae entrapped in an alginate-maize stem ground tissue matrix. Enzyme and Microbial Technology 48 (4-5): 378 - 385.

Rose AH (1976) In survival of vegetative Microbes society for General Microbiology Symposium 26:155 – 182.

Roukas T (1998) Pretretmemt of beet molasses to increase pollution production. Process Biochem. 33: 805 – 810.

Kaman S, Amor D (2009) Commercial production of fullerenes from waste molasses, a major commercial break through for sugar industry, New Orleans, USA sugar industry Technologists Inc. University of Lucknow, India, P. 1.

Soares E, Hebbelink K, Soares H (2003) Toxic effect caused by heavy metals and the yeast Sacharomyces cervisiae. A comparative study. Can. J. Microbial. 49: 336 – 343.

Ueda S, Zemn CT, Monteiro DA, Parker YK (1981) Production of ethanol from row cassava starch by a nonconventional fermentation method. Biotechnal. Bioeng. 23:291 - 299.

Xu K, Xu P (2014) Efficient production of L-lactic acid using co, feeding strategy based on cane molasses/glucose carbon sources. Bioresource Technology 153: 23 – 29.

Yadav A, Bajaj B, Sharma S (1998) Environment and Ecology. 16 (3): 579 - 583.

Zalidovar J, Nielsen J, Olsson L (2001) Fuel ethanol production from lignocellulose, is a challenge for metabolic engineering and process integration. Apple Microbial Bioethanol 56: 17 – 34.

Zohri A, Soliman M, Ibrahim O, Abdel Aziz A (2022) Deterioration of sugarcane molasses during storage and its effect on ethanol fermentation efficiency in distillery factories. Egyptian Sugar Journal, 18: 32-39. doi: 10.21608/esugj.2022.128090.1006

Zohri A, Kamal El-Dean A, Elbanna K, Abdalrahim S (2019) Oligosaccharides Production by Leuconostoc holzaapfelii Strain S7 Using Sugarcane Molasses as Fermentation Medium. Egyptian Sugar Journal 13: 137-160. doi: 10.21608/esugj.2019.219364