Mehran University Research Journal of Engineering & Technology
Vol. 38, No. 4, 1021-1032 October 2019
p-ISSN: 0254-7821, e-ISSN: 2413-7219
DOI: 10.22581/muet1982.1904.14

Impact of Storage Time, Rain and Quality of Molasses in the Production of Bioethanol

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RECEIVED ON 25.05.2018 ACCEPTED ON 19.02.2019

ABSTRACT

Due to environmental concerns and the rising prices of petroleum products, usually bio-ethanol is being produced in greater amount from sugar cane molasses in Pakistan. In this work various properties related to molasses are being analyzed by performing experiments at the Distillery Plant, AASML (Al-Abbas Sugar Mills Limited), Mirpurkhas, Sindh, Pakistan. Brix, Sucrose, pH, ethyl alcohol, RS (Reducing Sugar), TSI (Total Sugar as an Invert), TS (Total Sugar), purity and presence of micro-organisms are scrutinized. In addition, the effects of quality parameters of pre-fermentation and fermentation for ethyl alcohol production, time impact on stored molasses and consequences of rain on quality of molasses are studied. It was observed from the study that good quality molasses which is the raw material for alcohol production should be used in fermentation process. Molasses must be stored in steel tanks to prevent it from contamination and loss of fermentable sugars. The prefermenters need to steam sterilized, cleaned and washed before inoculation of yeast cells so that the yeast grows healthy to produce maximum ethyl alcohol in fermenters. The water used for hydrolysis of molasses in fermentation should be clean, filtered and good quality nutrients must be used for the growth of yeasts. Finally, efficiency and yield are calculated and t-test has been performed to depict a direct relationship of the fermentable sugar with efficiency.

Key Words: Molasses, Yeast, Brix, Fermentable Sugar, EthylAlcohol.

1. INTRODUCTION

Molasses, the thick liquid remaining after sucrose has been removed from the mother liquor (of clarified concentrated cane or beet juice), in sugar manufacture. Blackstrap molasses is the syrup from which no more sugar may be removed economically. Therefore, it is called as a by-product of sugar manufacturing process. It has usually been subjected to at least three evaporating and centrifuging cycles to remove the crystalline sucrose. Its analysis varies considerably, depending on many factors, including sugar-mill equipment and operational efficiency, but it may contain approximately 40-45% by weight of

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fermentable sugars and approximately 10% ash (or salts) [1-2]. The composition of black strap molasses varies with the variety of cane and the method of processing. The large amount of inorganic salts in molasses exerting high osmotic pressure and the toxicity to yeast cells are important factors during fermentation. Due to repeated crystallization of sucrose in sugar manufacture, inorganic salts are concentrated which interfere with the enzyme system of the yeast thereby reducing final ethanol yield and typically only 90% of the molasses is utilized [3]. The blackstrap molasses is utilized in numerous industries such as process, polymer, beverages, fuel and farming [4]. Saccharomyces cerevisiae is the species of yeast used for the fermentation of molasses to produce ethyl alcohol with improved efficiency. Nevertheless, molasses is one of the vital components in diverse industrial processes. The biochemical reaction of yeast with diluted molasses produces enzymes called Zymase that works as a catalyst to convert sugar into ethanol, carbon dioxide and heat [5]. The industrial alcohol production capacity of two units of distillery plant, AASML is 170,000 liters per day and the CO_2 is released around 140 tons per day. CO_2 is captured, liquefied for sale to other industries like beverages companies [6].

The advancement in biomass consumption for power sector is the important factor in many parts of the world due to enhanced concerns on the energy sector and environmental pollution. This is true for the developing countries like Pakistan that is an agricultural country and producing huge amount of biomass. Molasses is an indigenous low-cost feedstock producing ethanol which is used to make E 10 fuel. E 10 fuel is a mixture of 90% petrol and 10% ethanol, i.e. a renewable energy source [7]. Sugarcane is an important crop of Pakistan for energy sector because of the higher proportion of the biomass in the shape of fermentable sugar present in it generating biofuels such as ethanol. Generally, the production system of sugarcane is complex, giving various products and by-products such as, sugar, molasses, baggase and filter cake [8-10]. The by-product of sugar milling process baggase is largely used for power generation that supply electricity to the sugar mill, distillery plant, bio-gas plant, due to thepromotion of Independent Power Producers and Small Power Producers [11]. The black strap molasses, a by-product of sugar mill process is currently used as the main feedstock for bioethanol generation. Around 60% of the total bioethanol manufacturing in the year 2013 was from molasses [12-14]. Even though ethanol manufacture in countries like Brazil, Thailand and Pakistan has currently developed into an established industry, there are still concerns on the environmental sustainability [15].

Experimental results proved that the quality of molasses collected from the Punjab and KPK (Khyber Pakhtunkhwa) was better than the Sindh and the results were presented in our previous work [16]. Due to availability of good quality fertilizers, chemicals and pesticides, fertile lands, canal and tube well water, better harvesting practices, well defined process of sugar manufacturing and appliance of up-to-date agricultural studies, the quality of molasses collected from these areas is comparatively better. The three main contributions of this work are to investigate the impact of quality of molasses, storage time and rain for the production of ethyl alcohol.

The rest of the paper is organized as follows. Section 2 presents materials and methods, including procedures for determination of Brix, RS, TSI, TS, purity, efficiency and yield. Section 3 elaborated the results and their discussion. Finally, section 4 states the concluding remarks.

2. MATERIALS AND METHOD

Experimental work was conducted at the laboratory of Distillery plant AASML, Mirwah Gorchani, Mirpurkhas. The laboratory is ISO-9001 certified and the testing techniques are following the standards. Process diagram of Distillery plant AASML [17] is shown in Fig. 1. The two main sections of the plant are distillery and fermentation. Five major steps are involved in the production of ethyl alcohol which are depicted in Fig. 2. The first step is the storage of raw material that is molasses in open pits and steel tanks. After that molasses is diluted with water in the mash preparation section where the sulfuric acid is also added to maintain the pH and kill the micro-organisms which were produced in open pits because of rain and atmospheric effects. The filtered mash is then transferred in the prefermenters where the yeasts are produced in aerobic conditions. The healthy yeasts are moved to the fermenters where the diluted molasses is added for the fermentation reactions. The process of conversion of sugar to ethyl alcohol under the influence of yeast is known as fermentation as demonstrated in the following fermentation reactions.

FIG. 1. DISTILLERY PLANT AL-ABBAS SUGAR MILLS LIMITED [17]

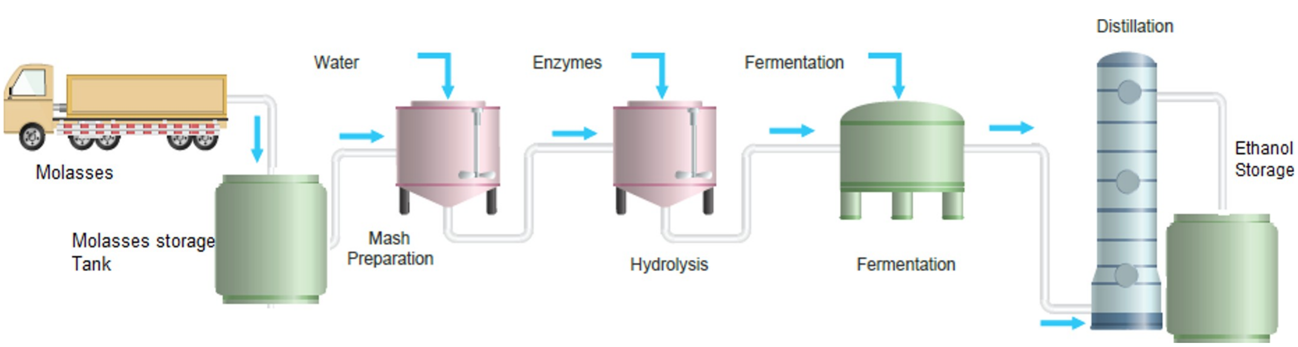

FIG. 2. PROCESS FLOW DIAGRAM OF ETHYL ALCOHOL PRODUCTION

$$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6 \quad (1)$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + Heat \quad (2)$$

The mixture is maintained at about 30-32°C for 40 hours. During this period the enzymes, invertase and zymase present in yeast bring about the conversion of sugar to ethyl alcohol. During this process; the liquor froths owing to the evolution of CO_2 that is collected and liquefied at CO_2 plant for sale to industries including carbonated beverages, urea production, refrigeration, etc. The fermented liquid contains 8-10% of alcohol and then it is introduced to distillation section where ethyl alcohol is produced. Three types of products are obtained which are A-Grade alcohol (96.6%), B-Grade alcohol (94%) and fuel oils. The residue (spent wash) discharged from the distillation column is converted to biogas (methane) that is used as a boiler fuel to convert water into steam. The steam from the boiler is used for two purposes, (a) to run the generators at the power plant to produce electricity for the whole sugar mill and distillery plant along with the residential areas, (b) to supply heat to distillation section. Consequently, distillery plant is known as "Zero fuel" plant.

Molasses samples are collected from open pits and steel tanks from a variety of Sugar Mills of Pakistan as shown in Fig. 3. Molasses is collected during the running period of Sugar Mills in Pakistan i.e. from winter to spring. Molasses is utilized from stored open pits and steel tanks throughout the year as per the capacity of plant i.e. 300±25 tons per day.

2.1 Sampling and Analysis

In this section, analysis procedure for determination of Brix degree, RS, TSI, sucrose, TS, purity and alcohol percentage in pre-fermenters and fermenters are presented [18-19]. The apparatus used during the analysis are: beakers (Pyrex), conical flask (Pyrex), volumetric flask (Pyrex), pipette, burette, funnel, glass rod, glass cylinder, distill water bottle, hydrometer (Cole-Parmer), DJ-Ebulliometer (France), microscope, hot plate stirrer (Wisd Lab Instrument), water bath, pH meter (Bante Instrument China), electrical balance (OHAUS Corporation, USA), test tube, thermometer and stopwatch.

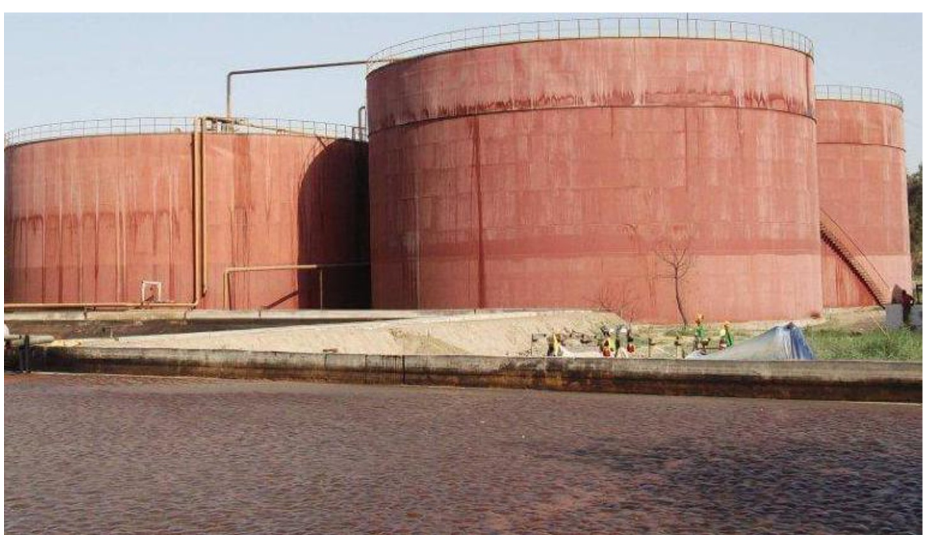

FIG. 3. MOLASSES STORAGE TANKS AND OPEN PIT [17]

2.2 Process to Determine Brix

Brix is a measure of the density or concentration of sugar solution.

Initially, 200 gm of molasses sample was placed in a beaker and 200 ml of distilled water was mixed in it. Then the sample was shifted in 250 ml glass cylinder and was kept in the icebox at 20^0C for 20 minutes. Then hydrometer (40-50 range) was placed in the sample for two minutes and the reading was taken and Brix was calculated as:

$$\text{Brix} = \text{Hydrometer Reading x 2} \quad (3)$$

2.3 Process to Determine Reducing Sugar

The term, reducing sugar denotes the reducing substances in cane or its products calculated as invert sugar. 100 ml of diluted sample was taken in 250 ml volumetric flask. EDTA (Ethylene Diaminetetraacetic Acid) 15 ml solution, 1 drop of Phenolphthale inindicator was added and volume made up to 250 ml by adding distilled water. Then the sample was filled in the burette (50 ml). Fehling solution (A&B) 20 ml each was taken in 50 ml conical flask and the sample was titrated. When green color appeared, readings were noted. After adding five drops of Methylene blue the sample was boiled on hot plate. The sample was titrated and when the color was changed to original color reading was noted. The average of three burette reading was considered during the calculation.

The reducing sugar [18-19] is calculated via,

$$RS = \frac{1000 \times 0.995}{2 \times \text{Burette Reading}} \times 100 \quad (4)$$

2.4 Process to Determine TSI

This test determined the TS percentage which illustrated the quality of molasses. In this experiment, 10 gm sample of molasses was taken in a beaker using weighing balance and mixed with small amount of distilled water. The sample was then shifted in 200 ml volumetric flask and distilled water was added to prepare volume of 200 ml. Then 25 ml of sample was taken in 250 ml volumetric flask and 5ml of 6.34 N HC1 (Hydrochloric Acid) was added in it. 2 N Sodium hydroxide (2N) was added drop wise in the sample till appearance of shiny red color. This color was neutralized with 0.05 N (HCl) till original color appeared. Then 4 ml (EDTA) solution was added and 250 ml volume was prepared by adding distilled water. The sample was filled in burette, 30 ml of sample was taken from the burette and distilled water was added to prepare 75 ml sample and awaited for appearance of green color. Then sample was shifted on hot plate and 5 drops of methyl blue was added to titrate it. Finally, reading was noted when the sample color was matched with the original color.

The values of TSI, sucrose, TS and purity are calculated as follows:

$$TSI = \frac{2000}{\text{Burette Reading}} \times 100 \quad (5)$$

$$\text{Sucrose} = (TSI - RS) \text{ x } 0.95 \quad (6)$$

Sucrose plus reducing substances is expressed as TS.

$$TS = \text{Sucrose} + TSI \quad (7)$$

The percentage ratio of sucrose to the total soluble solids (or Brix) in a sugar product.

$$\text{Purity} = \frac{\text{Sucrose}}{\text{Brix}} \quad (8)$$

2.5 Process to Determine Alcohol Percentage in Fermenters and Prefermenters

The alcohol percentage was checked by Ebulliometer apparatus. Hydrometer was filled with 50 ml sample and then heated. When the rise in temperature was stopped, reading was noted and confirmed into reference temperature disk [18-19].

2.6 Fermentation Efficiency and Actual Yield

The fermentation Efficiency percentage and actual yield percentage were calculated using the following Equations (9-10).

$$\text{Efficiency (\%)} = \frac{\text{Ethanol Produced}}{\text{Suguar Utilized}} \times 100 \quad (9)$$

$$\text{Yield (\%)} = \frac{\text{Ethanol Produced}}{\text{Intital Sugar}} \times 100 \quad (10)$$

3. RESULTS AND DISCUSSION

In this section the effect of prefermentation on fermentation, effect of time on stored molasses, consequences of rain on molasses quality, overall efficiency and yield are discussed.

3.1 Effect of Prefermentation on Fermentation

The quality of molasses plays an important role in the fermentation process. Tables 1-2 are showing the influence of prefermenters on fermentation. The prefermenters are the tanks where the yeast cells grow, which convert the sugar into alcohol. There are some important factors which effect on fermentation such as; the prefermenters must be cleaned and sterilized with steam before inoculation.

TABLE 1. UNIT-1 PREFERMENTER COMPARISON WITH FERMENTERS

Parameters	Unit-1 Prefermenters			Unit-1 Fermenters			
	R-411	R-412	R-413	R-421	R-424	R-426	R-428
Brix	5.3	5.4	5.4	10.9	11	10.9	10.9
pH	4.49	4.51	4.45	4.81	4.86	4.78	4.8
Alcohol%	3.3	3.36	3.3	8.4	8.3	8.2	8.3
Cell 10 million/cm^3	27.3	29	27.8				

TABLE 2. UNIT-2 PREFERMENTER COMPARISON WITH FERMENTERS

Parameters	Unit-2 Prefermenters		Unit-2 Fermenters			
	R-1411	R-1412	R-1421	R-1423	R-1425	R-1427
Brix	5.3	5.2	10.4	10.4	10.5	10.5
pH	4.4	4.47	4.78	4.67	4.38	4.76
Alcohol%	3.1	3.16	7.7	8.1	7.7	8
Cell 10 million/cm^3	23	24.9				

The molasses should be treated before use in prefermenters. The canal water must be clean and filtered. The air used in prefermenters should be filtered. The Brix, pH and temperature values must be controlled as per process requirement. The chemicals and fertilizers used in prefermenter should be of good quality.

In Table 1, R-411, R-412 and R-413 are showing unit-1 preferementers and R-421, R424, R426 and R-428 are referring to unit-1 fermenters. In Table 2, R-1411, R-1412 are showing unit-2 preferementers and R-1421, R1423, R1425 and R-1427 are referring to unit-2 fermenters. From comparison of Tables 1-2, it is obvious that unit-1 is giving higher alcohol percentage than unit-2. Because in unit-1 higher quality molasses was used which demonstrated higher alcohol percent and production than the unit-2 in which low quality molasses was used. As the result the cell value of unit-1 is more than unit-2. However, the Brix and pH values of both units are approximately alike.

3.2 Time Impact on Stored Molasses

The storing of molasses for a long period has a profound effect on molasses, sugar content as it readily dissipates with the passage of time. The lactic and butyric acid bacterias present in molasses cause fermentation, which give rise to conversion of sucrose molecules into long chains of sucrose polymers (dextrans). It results in increase of quantity of non-fermentable sugar, which is objectionable in molasses, as it greatly affects fermentation and reduces alcohol production. The stored molasses will contain great sludge due to sedimentation. The sediment will influence yeast activity as well as fermentation efficiency. Dextran polymers play as an enzyme inhibitor. These inhibitors reduce fermentation efficiency or alcohol recovery. Dextran polymers affect enzyme-catalytic activity by blocking the active sites (pores). At these sites sucrose molecules (key) fit into it (lock) and ultimately producing ethanol, the required product. Now dextran polymers block active sites and do not permit sucrose/glucose molecules for converting into glucose and alcohol. The high amount of mud and fibrous materials reduce the fermentation efficiency. These objectionable impurities limit the yeast activity and dose not give them much space for their growth and survival. Therefore, when molasses is stored for long time; the infection increases and we could not attain the desired results. It is better to avoid use of such molasses to get rid of infection. Liang et. al. [14] have studied successfully the application weak *Saccharomyces cerevisiae* strain on sugarcane molasses for the improvement in the industrial alcohol production.

Table 3 highlights the changing values of Brix, sucrose, purity, TSI, TS and RS with respect to time. The values of these six parameters are observed with the time interval of 50, 20 and 7 days in five tanks. Due to development of dextrans with the passage of time, the percentage of sucrose and purity is decreasing in all tanks. As the result, the percentages of TSI and TS are also depicting declining trend. Consequently, the RS percentage is portraying rising trend as the passage of time is increasing.

In order to observe the minimum, maximum and median values of the Brix degree, sucrose, purity, TSI, TS and

RS with respect to time in all tanks, box chart is depicted in Fig. 4. The minimum, median and maximum brix degree values are 82, 84 and 88.4 respectively. All the maximum values are shown by * sign in the box chart. The minimum, median and maximum sucrose % values are 9.5, 14 and 33.8 respectively. The minimum, median and maximum purity % values are 11, 16 and 39.7 respectively. The 43, 42 and 13 are the minimum TSI, TS and RS % values respectively. It is apparent from the box chart that the maximum TSI, TS and RS % values are 47.7, 46.9 and 36.87 respectively. In addition, the median values of TSI, TS and RS % are 44, 43.5 and 29.9 respectively.

3.3 Consequence of Rain on Quality of Molasses

During the rainy season last year the quality of molasses was badly affected which was stored in open ponds. Fig. 5 and Table 4 represent the analysis of samples tested during the rainy season and shows that after rain the Brix degree, sucrose percentage, TSI percentage, TS percentage, purity percentage were decreased, and the micro-organisms population were increased. This determines that after the heavy rain; the bacteria present in soil and atmosphere diluted the molasses, converted the sugar to different acids and some alcohol percent as pH is increased.

TABLE 3. TIME IMPACT ON MOLASSES STORAGE TANKS

Sources		Brix (Degree)	Sucrose (%)	Purity (%)	TSI (%)	TS (%)	RS (%)
Tank-A	First day	84	15.06	17.93	45.57	44.78	29.72
	After 50 days	84	11.86	14.12	44.81	44.19	32.33
	After 70 days	83.8	10.95	13.07	44.15	43.57	32.62
	After 77 days	83.7	9.53	11.39	43.5	43	33.47
Tank-B	First Day	82	16.42	20.02	45.06	44.2	27.73
	After 70 Days	84.2	10.85	12.88	44.36	43.79	32.94
Tank-C	First Day	85	33.76	39.72	44.2	42.67	13.7
	After 50 Days	84	21.37	25.44	43.66	42.54	21.17
	After 70 Days	83.9	20.83	24.83	43.57	42.47	21.64
	After 77 Days	83.9	19.65	23.42	43.45	42.42	22.77
Tank-D	First Day	88	14.12	16.04	47.73	46.99	32.87
	After 50 Days	88.2	11.46	13	47.36	46.76	35.3
	After 70 Days	88.2	10.77	12.21	47.28	46.71	35.94
	After 77 Days	88.3	9.64	10.91	47.02	46.51	36.87
Tank-E	First Day	83.6	18.97	22.69	44.39	43.39	24.42
	After 50 Days	83.8	13.97	16.67	44.11	43.38	29.41
	After 70 Days	83.8	14.24	16.99	44.04	43.29	29.05
	After 77 Days	84	12.82	15.26	43.057	42.9	30.08

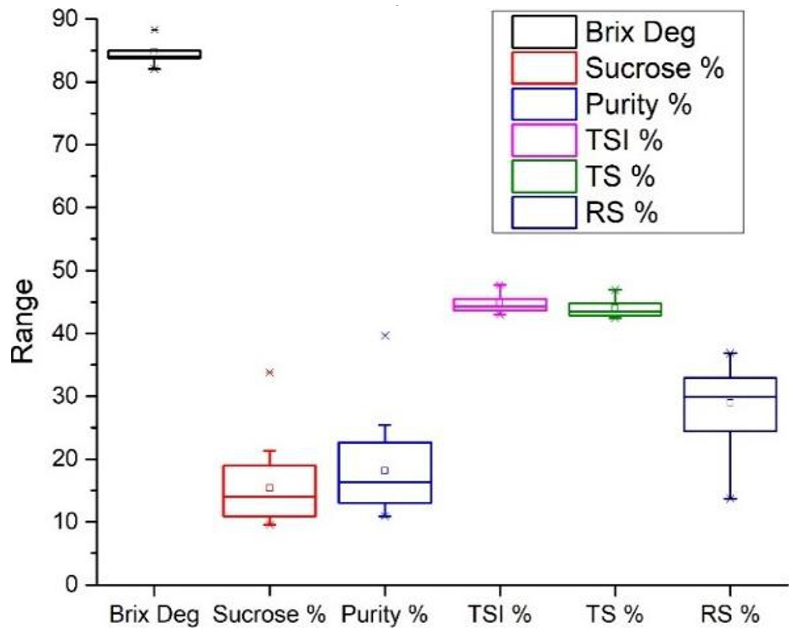

FIG. 4. TIME IMPACT ON MOLASSES STORAGE

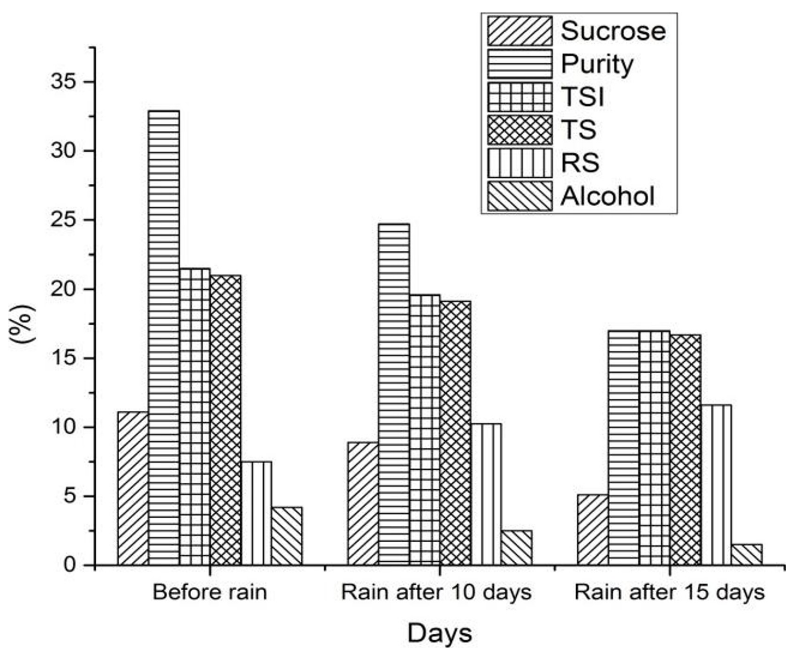

FIG. 5. ANALYSES OF MOLASSES STORAGE PITS DURING RAINY SEASON

TABLE 4. ANALYSES OF MOLASSES WITH BRIX DEGREE AND MICROORGANISM POPULATION DURING RAINY

Date	Brix (Degree)	pH	Wild Yeast (Million/cm^3)	Lactic Bacterias (Million/cm^3)	Butyric Bacterias (Million/cm^3)
Before Rain	38	4.6	15	20	17
Rain After 10 Says	36	4.35	23	28	25
Rain After 15 Days	29	4.3	33	34	37

3.4 Relationship of Fermentable Sugar with Efficiency and Yield

In Fig. 6, fermentation efficiency, actual expected alcohol yield according to fermentable sugar in the molasses are depicted. The values of efficiency and yield show direct relationship with the increase of fermentable sugar percentage. The good quality molasses having higher fermentable sugar gives higher efficiency and alcohol yield during process. Also during the fermenation reaction carbon dioxide (CO_2) is evolved, which is liquified in (CO_2) capture plant for further use in beverages industries. Sheth et. al. [4] discussed the importance of first and second generation ethanol and recovery of by-products based on biorefinery concept. According to Ahmed [10], molasses is a supply of fast energy and source of minerals for farm animals and even human beings. It is also a main ingredient for cost effective management of feeds and pastures.

The t-test has been performed on the sample size of 21 values of fermentable sugar and efficiency percentages. The null hypothesis is defined as that the means of fermentable sugar and efficiency percentages are not showing an increasing trend. The calculated t-value is 73.5. The p-value in the t-table for alpha, 0.05 and degree of freedom, 20 is 1.725. The calculated t-value is greater than the t-table value at an alpha level of 0.05. Thus, the null hypothesis is not being followed and it is supported that the means of fermentable sugar and efficiency are showing increasing trends.

4. CONCLUSION

Due to increasing cost of oil, it is common practice to utilize sugarcane and molasses in the production of bioethanol in Pakistan. In this work it has been analyzed that the molasses with enhanced fermentable sugar levels yields more yeast cells and alcohol as compared to molasses having diminutive fermentable sugar. The rain also affects negatively because bacteria's were producing acids at low Brix as the molasses was diluted with rain water. The experimental results also revealed that better quality raw material (molasses) obtained maximum yield

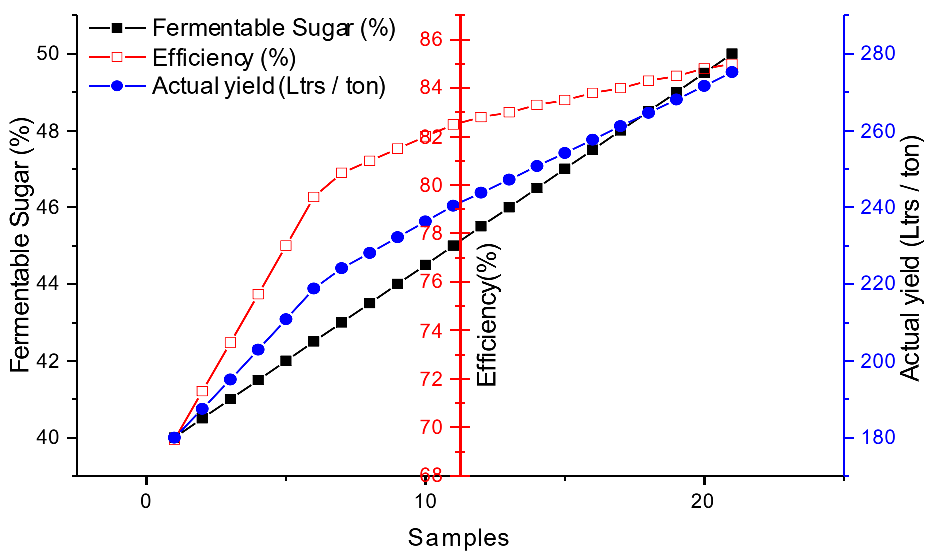

FIG. 6. RELATION OF FERMENTABLE SUGAR WITH EFFICIENCY AND ACTUAL EXPECTED ALCOHOL YIELD

(liters/ton), efficiency (%) and hence the profit. The research showed that yeast activity was unfavorably affected by inhibitors present in the molasses. It is suggested that the good quality molasses must be used in distillery plants to get maximum yield, efficiency, and profit and to minimize the losses. Molasses should be cooled and mixed after sugar manufacturing centrifugal stage in sugar mill to avoid caramelization of sugar. Molasses must be stored for a month before use in distillery as fresh molasses is difficult to ferment. Fresh molasses has high foaming tendency, buffering capacity and suspended sludge. It is also suggested that molasses should not be stored more than 6 months as fermentable sugar could be reduced.

ACKNOWLEDGEMENT

The authors would like to thankEngineers and Laboratory Staff, Distillery Plant, Al-Abbas Sugar Mills Limited, Mirpurkhas, Sindh, Pakistan, for conducting experimental work and technical support.

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