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Utilization of Mash Treatment Unit for Sterilization and Clarification of Final Molasses in Ethanol Plant

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Abstract: The study aimed to determine the effect of sterilization treatment of molasses in ethanol industry in terms of increased production, reduced operating costs and maintenance, through introducing of a specialized unit to enhance the purification and sterilization of molasses, as well as, taking the advantage of high vinasses temperature (a by-product in the ethanol industry) as a source of heat instead of steam, and lowered temperatures of beer as a source for partial cooling. This study depended on scientific experimentation in lab conditions to measure the value of (brix, pH and temperature) as the main primary data collection. The verification of the results was done through the work of two models for the production of ethanol using treated molasses as first model and non treated molasses as second model. Thereafter, the percentage of ethanol production was determined as well as the number of yeasts in both models. Moreover, the difference between the two models was spotted as well. In addition to that, the two models which have been implemented by controlling the values of brix, pH, and temperature and fermentation period in order to determine the optimum and best values that would give higher and better productivity of ethanol and a greater number of yeast. Results showed that the optimum values were found at 21 brix, pH 5, at 30°C temperature, which gave the highest ethanol production, which was 8.1%, 6.7% and 6.4% respectively, when using molasses without treatment and 8.98%, 7.7%, and 7.3% respectively when using treated molasses with a period of fermentation 48 hours in all cases. Also on the other hand and regarding the yeast growth the results showed that the optimum values were found at 18-19 brix, pH 5, at 30°C temperature, which gave the highest growth of yeast cell count, where it was 13.86×10^7, 9.96×10^7 and 9.16×10^7 respectively, when using molasses without treatment and 14.91×10^7, 10.09×10^7 and 9.21×10^7 when using treated molasses with a period of fermentation 24 hours in all cases. It is recommended to establish a specific treatment unit for the molasses so as to achieve greater productivity of ethanol and to adjust the brix value at 21%, temperature at 30°C, pH at 5 and the period of fermentation should be 48 hours.

Keywords: Molasses, Sterilization, Production of Ethanol

1. Introduction

Production of ethanol is a complicated process. The transformation of such biological resources as energy-rich crops (like sugar cane or corn) or lignocelluloses or starchy biomass requires the conditioning or pretreatment of the feedstock's for fermenting organisms to convert them into ethanol. Then, aqueous solutions of ethanol should be concentrated for obtaining anhydrous ethanol. While ethanol today is largely used as blending component with gasoline to add octane and oxygen, opportunities abound for ethanol to play a role in advanced vehicle technology and alternative fuels markets [8]. For example, as a renewable fuel with an established infrastructure in the US, ethanol is an attractive fuel option for the fuel market in both power generation and transportation. Research is also underway on a mixture of

ethanol, diesel and a blending agent, and there is a largely untapped market for E85, a blend of 85%ethanol and 15% gasoline, to power the growing number of available flexible fuel Vehicles [1].

The process of ethanol production was originally based on the consideration of speed and efficiency of production, where the aim was to obtain maximum conversion of carbohydrates into ethanol. Alcoholic fermentation has become of great interest as an alternative energy source due to the recent increase of petroleum prices, pollution problems and for the shortage of crude oil, compared to the growing population of the world, which has led to extensive research for alternative source [11]. At first sight molasses a perfect feedstock for production of alcohol. All the sugars are present in a readily fermentable form [3]. One-half of the fermented sugar is converted to carbon dioxide, which can be used for industrial application or in green houses for increasing plant growth. The other half of the fermented sugar is converted to ethyl alcohol. Since it contains all the fusel oil, esters and aldehydes, it is not good for drinking but good as burn fuel [6,7]. People have used yeast for fermentation and baking throughout history. The useful physiological properties of yeast have led to their use in the field of biotechnology [13]. Fermentation of sugar by yeasts is the oldest and largest application of this technology [10]. Fermentation can be defined as a process in which chemical changes are brought about in organic substrate through the action of biochemical catalysts called enzymes, collaborated by specific types of living micro-organisms. Fermentation is a metabolic process characterized by: (a) incomplete oxidation, (b) the transformation of large amounts of substances by comparatively small amounts of organisms. The micro-organisms of fermentation include yeast, moulds and bacteria. These organisms, lacking chlorophyll, cannot produce their own food by photosynthesis. And feed upon organic materials; they differ widely in morphology, size, methods of reproduction, etc but they similar in that they all produce enzymes by which they catalyze the reactions ascribed to them. Yeast and bacteria are unicellular. Yeast about 0.005mm in diameter; multiply them by budding while bacteria multiply by binary fission [9].

Many compounds can be produced from molasses, mainly ethanol and yeast. Beside them, butanol, acetone, lactic acid, glycerol, lysine, citric acid, monosodium glutamate, liquid sugar, edible molasses, ammonium sulphate, activated carbon(carbonyl), dextran, hydrogen cyanide, isolation material, betainchlorohydrate, glue, paints, heat resistant bricks, fusel oil, shoe-polish, carbon dioxide, synthetic, resins, potassium salts, soap, carbon block, cement instructions, textile industries, dry batteries(as binder) [8]. The fermented mash, now called “beer” contains about 10% alcohol as well as all the non fermentable solids from the feedstock and the yeast cells. The mash is pumped to a continuous flow, multicolumn distillation system where the alcohol is removed from the solids and the water. The alcohol leaves the top of the final column at about 96% strength and the residue mash called stillage or vinasse is transferred from the base of the column to the co product processing area [4].

Ethanol vinasse is a dark, brown colour liquid, of acid nature, that remains after alcoholic distillation at 107°C, with a smell that goes from astringent to nauseating. It’s also termed distillery wastewater, distillery pot ale, distillery slops, distillery spent mash, dunder, most, and thin stillage. It’s the aqueous by-product from the ethanol distillation processwhose disposal is a major environmental concern mainly due to the great volume generated and its high biological oxygen demand [12, 15]. In general, 12-15 litres of vinasse are produced per litre of alcohol. In Brazil about 170 billion litres of vinasse are produced per year. Considering that every 2 litters of vinasse equivalent to the waste excreted by one person per day, the annual production corresponds to the domestic sewage produced by an estimated population of 232 million people during one year [11].

In this research we have invented a method for sterilization and purification of molasses diluted (mash) by using the vinasses for heating and the beer for cooling in order to lower the energy cost. The unit was designed for the purpose of heating, cooling and purification of molasses diluted (mash) to get a higher production and efficiency, less contamination and less maintenance cost.

2. Materials and Methods

2.1. Materials

Molasses samples were collected from Kenana Sugar Company, Sudan, during the production season 2014-2015.

2.2. Methods

2.2.1. Ethanol Production

250 g of cane molasses with 80 brix were diluted to15 brix value and transferred into two 1000 ml bottles. The first bottle was treated with sulfuric acid to pH 5, heated in 70°C for 30 minutes then poured into cylinder and left to cool for 6 hours. The upper 250 ml supernatant was taken and poured into 400 ml bottle (fermenter no 1). From the second bottle, 250 ml from the same diluted molasses was taken and poured into 400 ml bottle directly without any heat treatment, the pH was adjusted with concentrated sulfuric acid to pH 5 (fermenter no 2). Yeast suspension was prepared by diluting 12.5 g in 100 ml water mixed well, then for each bottle treated and non treated about 20 ml of hydrated yeast was inoculated into each 250 ml of diluted molasses (Brix 15), 0.1 g urea\100 ml mash, 0.2 g DAP (Di ammonium phosphate)\100ml mash and incubated in 30°C for 48 hours with stirring.

2.2.2. Refractometer Brix

The refractometer Brix at 20ºC was measured by dropping about 5 drops from the sample solution in the sample location at the refractometer, then the reading key was pressed, after that the result was appeared in the refractometer screen [14].

2.2.3. PH Measurement

The pH - meter was standardized and adjusted with

standard buffer solution then the electrode was rinsed and receptacle with portion of the molasses sample after that a beaker was filled with the molasses sample to a depth covering the bulb of the glass electrode, then the system was allowed to equilibrium and pH was read [14].

2.2.4. Determination of Reducing Sugars

10g of molasses ± 2mg were weighed into a clean tarred dish, then about 15 ml of water were added to the molasses and mixed thoroughly with a small glass rod and transferred without loss to a 200ml volumetric flask and the volume was made to 200ml and mixed to 5%w/v solution of molasses. After that a 2% w/v solution of the molasses was prepared by pipette 100ml from the molasses solution above into a 250 ml volumetric flak after that 15ml of the EDTA solution was added by means of the graduated cylinder then the solution was mixed and made up to the volume and mixed again at the appropriate temperature. Then the burette rinsed and filled with the 2%w/v solution. After that 20ml of Fehling's solution (A+B) were pipetted into the boiling flask and run in 25ml of the molasses solution. The flask on the heating appliance was placed; allowed the solution to reach boiling point and then 4 drops of the methylene blue indicator were added. The end point was appeared in about 1 minute from the time the solution commences boiling (1 minute after boiling). The end point is denoted by the disappearance of the blue colour and the solution assumes the colour imparted by the precipitated cuprous oxide. The titre was noted.

This formula was followed after the end point of the titration was found, then the value of reducing sugar [2].

$$R.S=(500\backslash \text{Titration reading})\%$$

2.2.5. Ethanol%

50ml of sample were taken and centrifuged with laboratory centrifugal machine to divide the sample in to two layers, down layer and upper layer.20 ml were taken from the upper layer with syringe, the alcolyzer plus instrument flashed with sample, then the sample was injected into the alcolyzer plus. Then the result appeared on the screen.

2.2.6. Viability and Microscopic Yeast Cell Count

In the screw capped test tube 1 cm3 of yeast suspension or beer sample was taken and diluted into 9 ml of Methylene blue solution mixed well and left for one minute. The Neuber chamber slide was prepared in the microscope.Small portion of mixtures was transferred and placed into the chamber slide and covered with cover slip, and active cells number was counted in the reticular central 25 squares [5].

Calculation:

Count of the total number of viable yeast in 5 of the squares, using pattern shown below:

Slurry density= (number of cells counted in 5 squares × 50,000 ×number of dilution factor) million cells/ml

Viability = (No of active cells counted / No of total cells counted) ×100% [5].

3. Results and Discussion

3.1. Effect of Sterilization Treatment of Mash on Ethanol Production at Different Brix

The results in figure 1 have shown that there was a significant difference (P≤ 0.05) between treated and non-treated mash in alcohol production at fermentation time of 24 hours, when brix increased in both treated mash and non-treated mash. However, the production of ethanol in the treated mash was 7.88% and it was higher than that in non-treated mash, which was 6.5% at brix 21.It was obvious that at constant brix, temperature, pH and fermentation time (24 hours), the increase in alcohol production was attributed to mash treatment that improved the alcohol production by 21.23%. Also the data represented by figure 1 revealed that there was a significant difference in alcohol production, whenever brix increased in the treated mash more than in the non-treated mash. In addition, it was clear that at constant brix, temperature, pH and fermentation time (48 hours), alcohol production increased in treated mash by 8.89% which was more than in non-treated mash that was 8.1% at brix 21, and that was attributed to mash treatment that had improved percentage by 10.86%.

3.2. Effect of Sterilization Treatment of Mash on Yeasts Cell Countat Different Brix

The results of the effect treatment of mash on yeasts cell count at different brixare shown in figure 2, the yeasts cell count was increased significantly(P≤ 0.05) with increasingbrix in both treated and non-treated mash after fermentation time of 24 hours, and it was higher in treated one. This increased in yeast cell count in the treated mash more than in the non- treated mash, indicating a double amount of yeast due to the higher consumption of sugar in molasses. At the same time, the production of ethanol increased, when brix value increased in the treated mash from 15 up to 18 more than that in the non-treated mash. However, when the brix value became higher than 18, it was noticed that there was a remarkable drop in the yeast count and that was due to higher sugar concentration which was not appropriate for yeast growth. Moreover, it was apparent that at constant brix, temperature, pH and fermentation time (24 hours) the increase in yeast cell count was found to be associated with mash treatment. On the other hand, it was observed that the yeast cell count decreased at brix 20 and 21 respectively when non treated mash was used. The yeast cell count decreased at brix 19, 20 and 21 respectively when treated mash was used. The decrease in yeast cell count was due to increase of ethanol production which led to inhibition of yeast growth. Also the results in figure 2 revealed a significant difference (P≤ 0.05)in yeast cell count when brix value was increased in the treated mash from 15 up to 18, more than that in the non-treated mash.

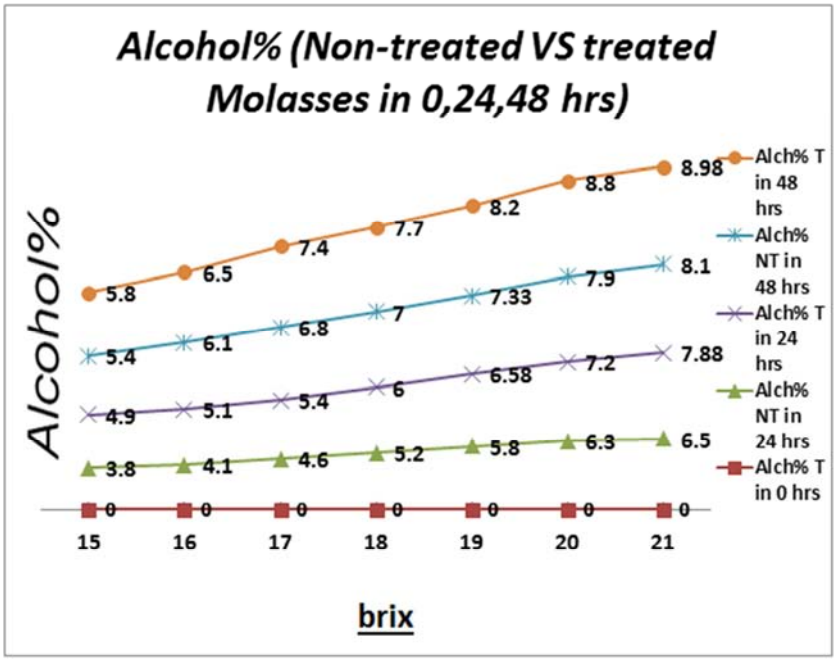

Figure 1. *Effect of treated and non treated mash for ethanol production, at temperature 30°C and pH 5 during zero,24 and 48 hours (at brix 15, 16, 17, 18, 19, 20, 21 respectively).*

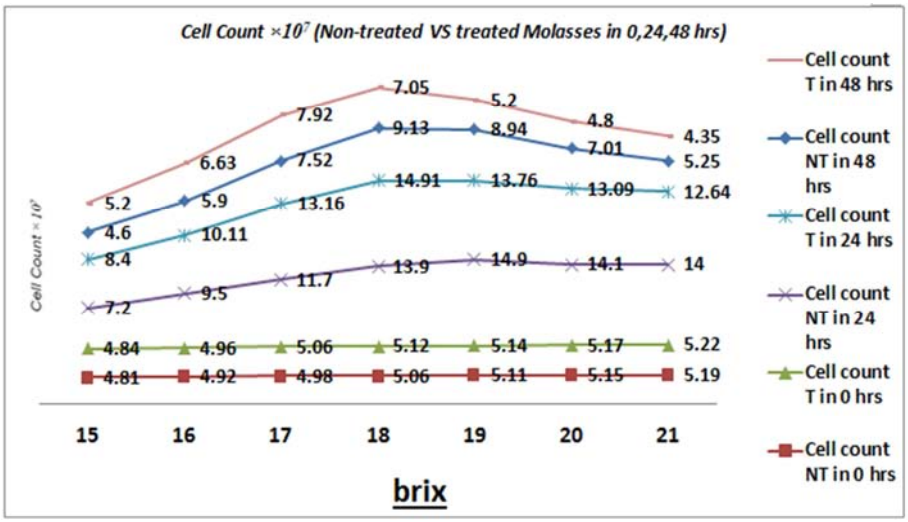

Figure 2. *Effect of treated and non treated mash for yeasts cell count at temperature 30°C and pH 5 (at brix 15, 16, 17, 18, 19, 20, 21 respectively) during zero,24and 48.*

3.3. Effect of Sterilization Treatment of Mash on Ethanol Production at Different PH

The results in figure 3 showed a significant difference ($P \leq 0.05$) between treated and non-treated mash in alcohol production, when the pH value increased from 4 to 6 and that was specifically in the treated mash where alcohol production was more. At constant brix, temperature, pH and fermentation time (24 hours), alcohol production increased in treated mash by 5.4% which was more than in the non-treated mash which was 4.6%. The resultant increase of alcohol output to 17.39% was due to mash treatment. On the other hand, it was observed that the yield of alcohol production decreased when the pH value was more than 5.5. The decrease in alcohol production was attributed to the irrelevant medium growth and production of yeast as well as for the production of ethanol. Also the result of alcohol production in figure 3 revealed that there was a significant difference ($p \leq 0.05$) in treated and non-treated mash. Whenever, the pH value increased from 4 – 6. The increase in alcohol production in the treated mash was higher than that in the non-treated mash. At constant brix, temperature, pH and fermentation time (48 hours), alcohol production increased in treated mash by 7.7% where as in the non-treated it was 6.7%. Mash treatment led to increase of alcohol production to 14.92%. On the other hand, it was observed that the percentage of alcohol production decreased when the pH value was more than 5.5. This decrease in alcohol processing was attributed to irrelevant medium (not acidic) for the growth and generation of yeast as well as for the

production of ethanol.

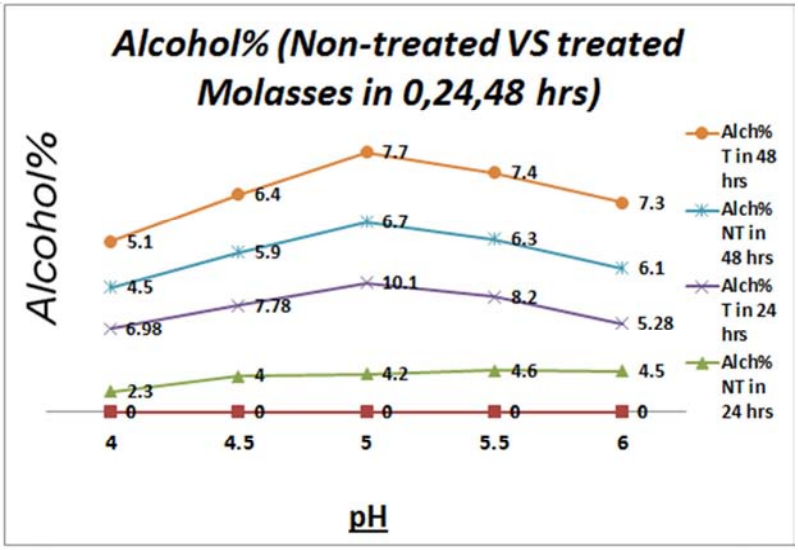

Figure 3. *Effect of treated and non treated mash for ethanol production, at temperature 30°C and brix 18, (at pH 4, 4.5, 5, 5.5, 6 respectively) at zero 24 and 48 hours of fermentation.*

3.4. Effect of Sterilization Treatment of Mash on Yeasts Cell Countat Different PH

The results in figure 4 have shown was a significant difference ($p \leq 0.05$) in yeast cell count when pH increased (from 4 up to 5) in the treated mash more than that in the non-treated mash as in figure 4. When the pH values were 4, 4.5, 5, 5.5 and 6, it was noticed that the increase in yeast cell count in the treated mash was higher than that in the non-treated mash. At constant brix, temperature, pH and fermentation time (24) hours, the increase in yeast cell count was due to mash treatment. On the other hand, it was observed that the yeast cell count decreased when the pH value was more than 5 and that was due to an inappropriate medium (not acidic) for the growth and production of yeast cell. Also it was obvious that there was a significant increase in yeast cell count when pH increased (from 4 to 5) in the treated mash more than that in the non-treated mash as presented in figure 4. Moreover, at pH values 4, 4.5, 5, 5.5 and 6, it was noticed that the increase in yeast cell count in the treated mash was higher than that in the non-treated mash. Additionally, it was apparent that at constant brix, temperature, pH and fermentation time (48) hours, the increase in yeast cell count was due to mash treatment. On the other hand, it was observed that the yeast cell count decreased when the pH value was more than 5 and that was due to the inappropriate medium (not acidic) for the growth and production of yeast cell.

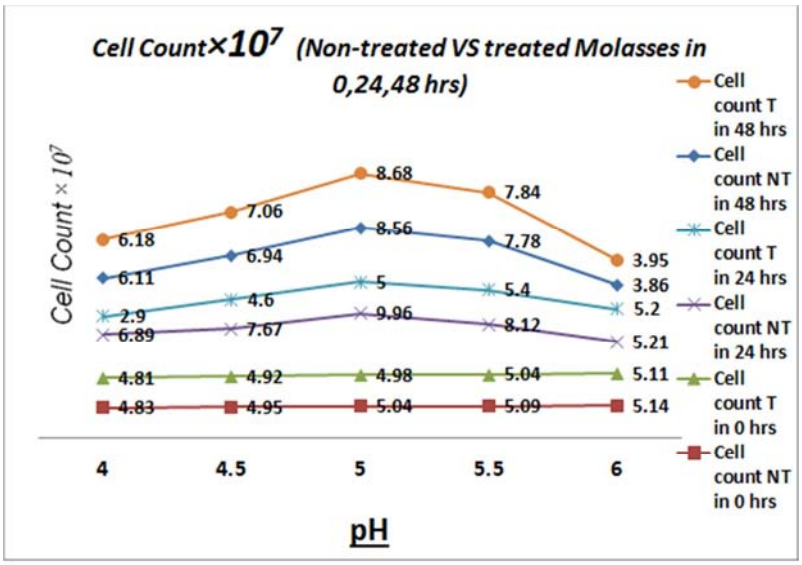

Figure 4. *Effect of treated and non treated mash for yeast cell count at temperature 30°C and brix 18 (at pH 4, 4.5, 5, 5.5, 6 respectively) at zero time,24 and 48 hours.*

3.5. Effect of Sterilization Treatment of Mash on Ethanol Production at Different Temperature

The results in figure 5 illustrated a significant difference (P≤ 0.05) in alcohol production whenever the temperature increased in the treated mash more than that in the non-treated mash. Even so, it was clear that at constant brix, temperature, pH and fermentation time (24 hours) alcohol production increased in treated mash up to 5% more than in the non-treated mash which was only 4.3% at 30°C and alcohol production increased to 16.27% due to mash treatment. Nevertheless, it was observed that the percentage of alcohol production decreased when the temperature became more than 30°C. This decrease in alcohol production was due to the inappropriate medium for the growth and production of yeast as well as for the production of ethanol. Also figure 5 showed a significant difference (p≤0.05) in treated mash and non-treated mash in alcohol production whenever the temperature increased and in the treated mash it was more than in the non-treated mash. Additionally, at constant brix, temperature, pH and fermentation time (48 hours) alcohol production in the treated mash increased by 7.3% more than in the non-treated, which was 6.4% at 30°C and that, was attributed to mash treatment which led to alcohol yield to about 14.06%. Nonetheless, it was observed that the percentage of alcohol production decreased when the temperature became more than 30°C. This decrease in alcohol production was due to the inappropriate medium for the growth and production of yeast as well as for the production of ethanol.

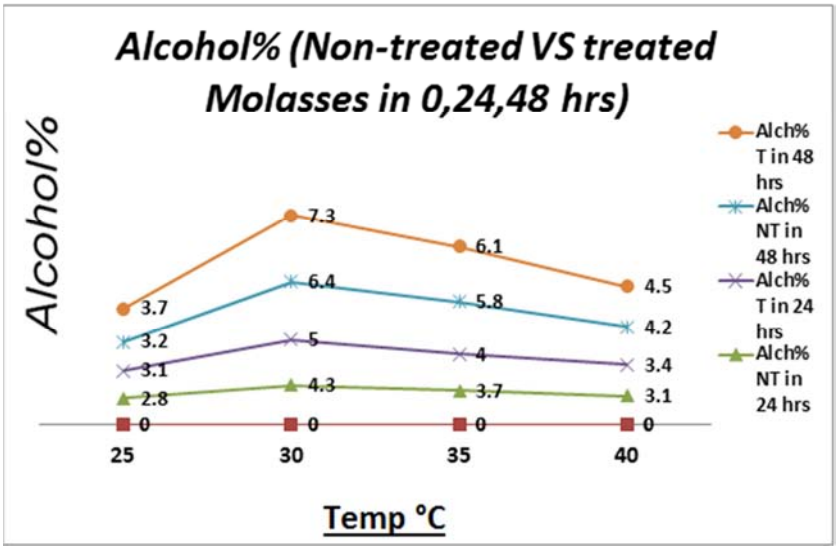

Figure 5. *Effect of treated and non treated mash for ethanol production (at temperature 25°C, 30°C, 35°C, 40°C) at zero,24 and 48 hours of fermentation.*

3.6. Effect of Sterilization Treatment of Mash on Yeasts Cell Count at Different Temperature

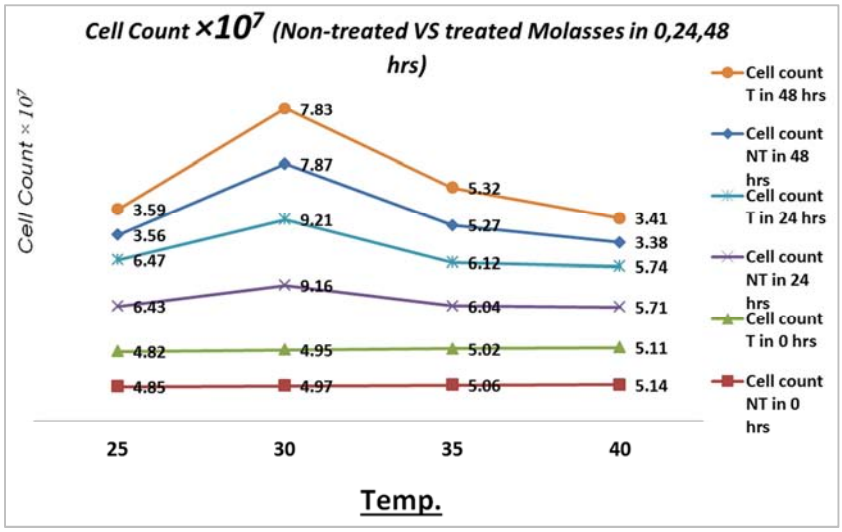

Figure 6. *Effect of treated and non treated mash for yeast cell count at pH 5 and brix 18. (Using Temperature 25°C, 30°C, 35°C, 40°C) at zero, 24 and 48 hours of fermentation.*

From the results presented in figure 6 it was clear that there was a significant increase in yeast cell count when the temperature increased in the treated mash more than in the non- treated mash. While at constant brix, temperature, pH and fermentation time (24 hours), the increase in alcohol production was due to mash treatment. On the other hand, it was observed that the yeast cell count decreased when the temperature was more than 30°C. This decrease in yeasts cell count was found to be due to the inappropriate medium for the growth and production of yeast as well as for the production of ethanol. Also in figure 6, it was obvious that there was a significant increase in yeast cell count when the temperature increased in the treated mash more than in the non- treated mash. Also it was clear that at constant brix, temperature, pH and fermentation time (48 hours), the increase in alcohol production was due to mash treatment. On the other hand, it was observed that the yeast cell count decreased when the temperature was more than 30°C. This decrease in yeast cell count was found to be due to the inappropriate medium for the growth and production of yeast as well as for the production of ethanol.

3.7. Utilization and Method of Work of the Mash Treatment Unit

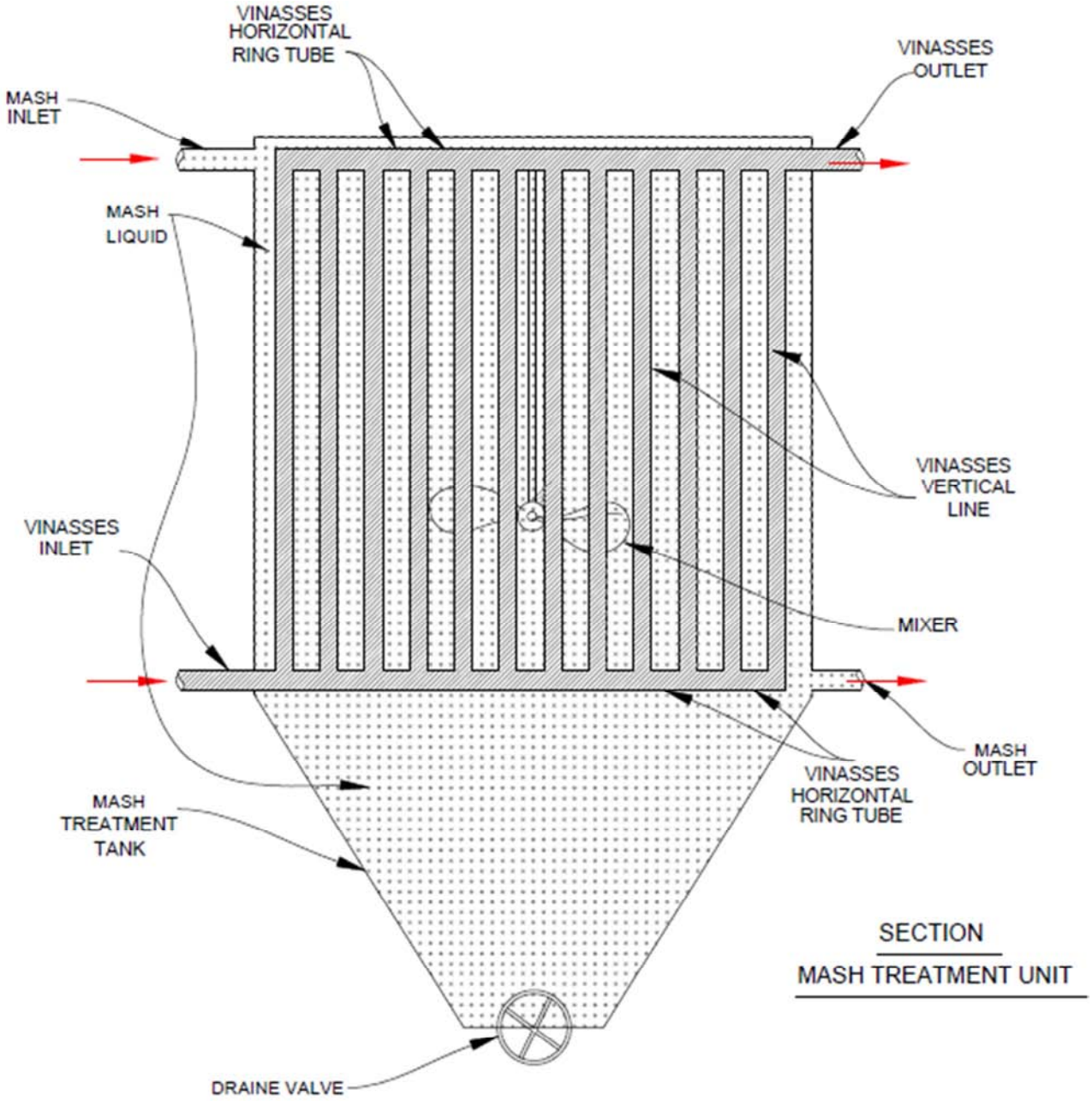

Figure 7. *Section of mash treatment unit.*

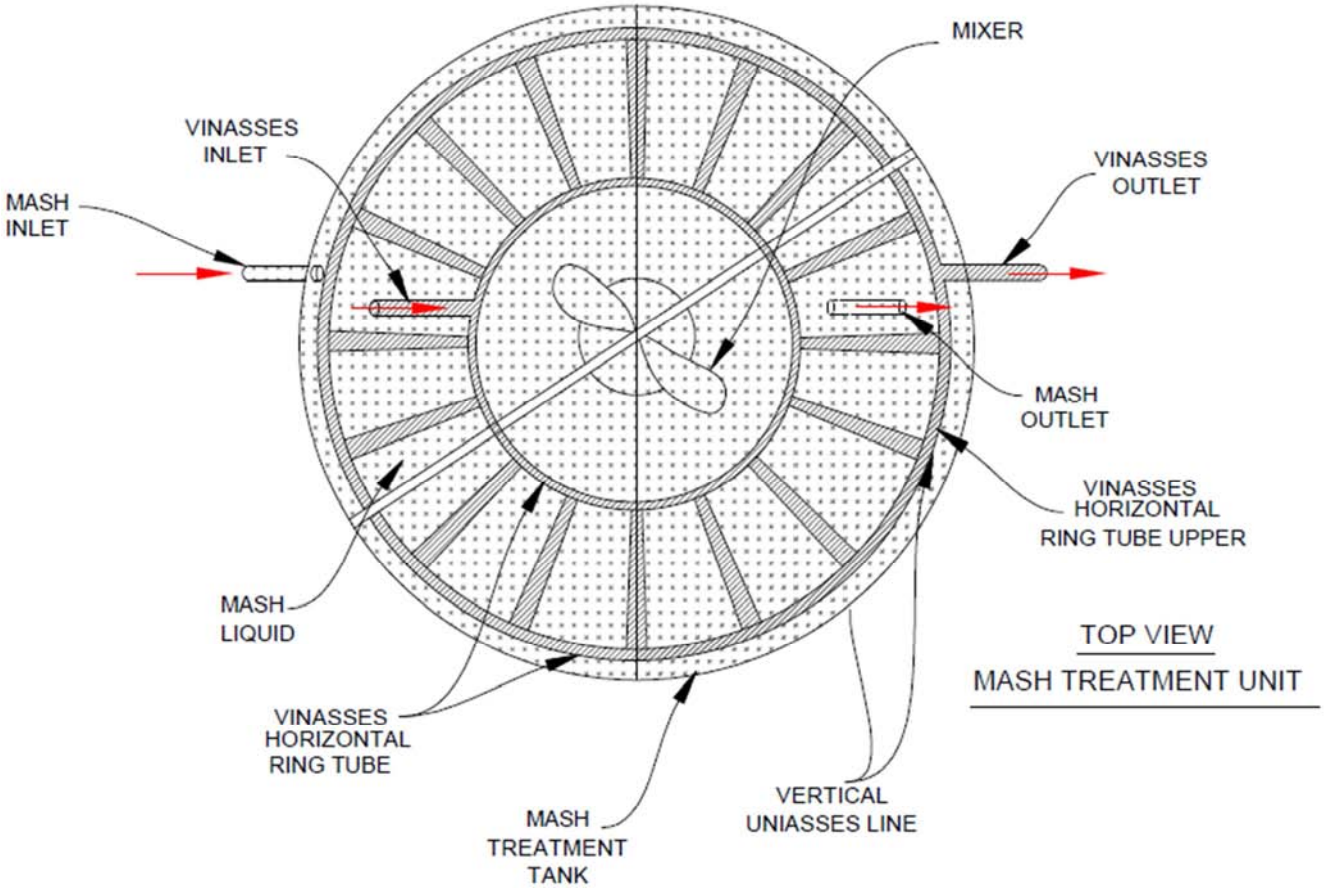

Figure 8. *Top view of mash treatment unit.*

The treatment unit was designed in the form of a cylindrical tank that has a conical base (as in figure 7&8). The tank consisted internally a range of spin-off and longitudinal pipes which were used in the heating process of mash and a mixer for the distribution of heat. The unit tank was filled with the mash from the top and emptied from the bottom after the treatment was completed. Vinasses (the heat source in the treatment unit) was pumped from the bottom of the treatment unit tank and via the spin-off and longitudinal pipes and exist out from the top of treatment unit tank. The vinasses was pumped inside the circular tube that is located in the bottom of the treatment unit tank which is connected to a series of open longitudinal pipes at the sides so that the bottom slot opens in the direction of the circular tube below the upper hole in the other cross section ring tube at the top of the treatment unit tank. The vinasses flow path firstly crosses down the inner circular tube until it is filled, then rises inside the longitudinal tubes also until filled, thirdly it enters the cross-ring tube at the top of the treatment unit tank and finally it exist out of the tank through a side opening in the top of the upper circular tube. The treatment was done by heating through heat exchange between the pipes of the vinasses and the mash stock inside the treatment unit tank, where temperature of mash was raised up to 65°C for 15 minutes in the presence of sulfuric acid which enabled to eliminate the microbes. After that, the mash is transferred to the cooling and sedimentation unit for the purpose of partial cooling and the precipitation of salts, mud and impurities materials. It is worth mentioning that the cooling unit is similar to the heating unit in terms of design, but it only differs in the reverse process where as heating by vinasses in the heating tank and then the operation is changed to partial cooling by beer instead of heating by vinasses. The precipitation of salts, mud and impurities is for 30 minutes, and then the mash is transferred to the fermenters to start fermentation. On the other hand it is worth mentioning that the vinasses used in the mash treatment unit as a source of heat in the heating process instead of steam is a by-product in the ethanol industry and it is disposed off as a waste product in Kenana ethanol industry. We use in the heating vinasses would have made as a source of heating in the treatment process and the use of the free resources available to complete the essential operations without any significant cost, particularly the cost of the generation of steam. In addition, cooling of the mash by beer is considered as an agent for cooling the beer itself, where the hot mash loses its hotness and the beer gains the mash wasted heat and hence the temperature of the beer rises. Thus, we have achieved two goals, the first one was the partial cooling of the mash at a free cost across the beer cooling and secondly, heating up the beer partially as a preparatory step before distillation in the distillation column.

4. Conclusions

The effect of sterilization treatment of molasses in ethanol industry in terms of increased production, reduced operating costs and maintenance, through introducing of a specialized unit to enhance the purification and sterilization of molasses were successfully accomplished. Experimental results demonstrated that the ethanol production from treated molasses was significantly increased and confirmed that the yield of alcohol obtained was remarkably high specifically when brix was 21, pH 5 and the temperature 30°C in the treated molasses. That formulation gave 8.98% (w\w) ethanol in fermented mash. This means an increase of production by 10.86% compared to the use of molasses without treatment. These conditions were considered suitable for yeast activity. The engineering design unit and using vinasses for heating and beer for partial coolingto reduce energy consumption and cost of processing.

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