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**STERILIZATION OF SUGARCANE JUICE BY OZONE TREATMENT AND
FURTHER FERMENTATION OF JUICE FOR PRODUCTION OF ETHANOL**

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ABSTRACT

Sugarcane juice has a typical shelf life of about 6 hours without treatment because its contents being simple sugars which spoil the juice faster converting it to acid and alcohol. Its shelf life was experimentally increased by pasteurization at 62°C for 30mins. Pasteurization increased shelf life to 60days when stored at room temperature and to 90 days when stored at low temperature after treatment. The major drawback of pasteurization is that it degrades colour, flavour, the sucrose content, °Brix value as well as quality parameters because heat is involved in the process. Due to the heat involved, the sugars get charred and convert into simpler ones which is another drawback. UV disinfection is another sterilization method but UV rays are harmful for human being as well as the UV will be a costlier method. Ozone is a non-thermal treatment. Ozone can be used to inactivate microbial load to extend the shelf life of the juice. Ozone destroys microorganisms by progressive oxidation of vital cellular components and residual ozone decomposes to nontoxic products (i.e. oxygen) making it an eco-friendly anti-microbial agent for use in the jaggery production. Furthermore fermentation of that ozone treated juice can be done for the production of ethanol. Ozone treatment will reduce the microbial load that will interfere and cause contamination during fermentation. Ozone treatment will alsoslightly reduce the nutritional and sensory properties of the juice, but losses will be much less as compared to pasteurization and UV sterilization.

**Keywords: Ozone treatment, Sugarcane juice, pasteurization, UV sterilization,
fermentation, ethanol**

INTRODUCTION

Sugarcane juice is the liquid extracted from sugarcane. It is consumed as a beverage in many places, especially where sugarcane is commercially grown. Sugarcane is one of the main crops used in the production of sugar: 70% of the world's sugar is made from sugarcane. However, this isn't the case for the sugarcane grown in India which happens to be the second largest producer of sugarcane. A lot of the sugarcane grown in India is first used to make jaggery followed by unrefined or brown sugar, and then sugar which is processed using chemicals and sulphur. The remaining fibrous mass can be used as fuel, or to make paper or sound insulating boards and in some countries it's used even to make alcohol. .Nutritional value of Sugarcane: The juice Sugarcane per serving (28.35 grams) contain Energy-111.13 kJ (26.56 kcal), Carbohydrates-27.51 g, Protein-0.27 g, Calcium11.23 mg (1%), Iron 0.37 mg (3%), Potassium41.96 mg (1%), Sodium17.01 mg (1%)

Sterilization refers to any process that eliminates, removes, kills, or deactivates all forms of life (in particular referring to microorganisms such as fungi, bacteria, viruses, spores, unicellular eukaryotic organisms such as Plasmodium, etc.) and other biological agents like prions present in a specific surface, object or fluid, for

example food or biological culture media. Sterilization can be achieved through various means, including heat, chemicals, irradiation, high pressure, and filtration.

Some other important parameters of sugarcane juice: The analysis of the quality indexes of sugarcane juice plays a vital role in the process for its further use. 1. Pol Content: Studies have recorded that the change trend of polarization is consistent with the change trend of sucrose content in juice. This shows a strong correlation between them. 2. Reducing Sugars: It has been proved from many research works that reducing sugar is low when the sugarcane is ripe and reducing sugar is high when the sugarcane is immature or overripe; especially when the quality of sugarcane juice tends to be worse, reducing sugar increases significantly. 3. Brix value : A brix value which is expressed as degrees brix(°Bx), is the number of grams of sucrose present per 100 grams of liquid, which means %sucrose is equivalent to the brix value of sugarcane juice. It is usually near to 23% when the juice is fresh.

After checking the above parameters only, the sugarcane juice was lead to fermentation. Studies showed that fermentation of sugarcane juice with pasteurization or UV lead to the less concentration of ethanol than by Ozone

sterilization. The fermentation is done by the yeast *Saccharomyces cerevisiae* which helped in a better production of ethanol. The objective of our study was to sterilize the sugarcane juice with the help of ozone treatment and to increase the ethanol yield by fermentation [1].

MATERIAL AND METHODS

1 Sample Collection:

The sample of sugarcane juice was collected from Amaltaas landscaping, Pune Maharashtra. The sugarcane juice was grated, filtered and further used for treatment.

2. Analysis of Physicochemical Parameters of raw Sugarcane juice [1]

- pH
- Odour
- Colour
- Sucrose content
- % Brix
- ° Pol

3. Sterilization of the grated sugarcane juice [Tiwari B.K et al, 2008] [2]

$$\text{Formula: cfu/ml} = (\text{no. of colonies} \times \text{dilution factor}) / \text{volume of sample}$$

3.b By Ozone treatment [Tiwari B.K et al, 2008]

The grated 1000 ml filtered sugar cane juice was taken in a cylindrical vessel. Pure oxygen was supplied via an oxygen cylinder and flow rate was controlled using an oxygen flow regulator which was fixed at 0.5 lpm.

3. a. By UV sterilization [Priyadarsini. P et al, 2018]:

The grated 1000 ml filtered sugar cane juice was taken in a conical flask. The conical flask was covered with aluminium foil from top. The juice was allowed to be treated with UV rays at 260nm, for 20mins. The 10ml aliquot of sugarcane juice at every 10min exposure of UV rays was drawn from which 10µl was poured on plates containing Sabouraud dextrose agar for molds, Yeast Extract Mannitol Agar with congo red for yeasts and Luria Bertani Agar for bacteria. The microbial count was taken by plate count method and checked for bacterial, mold and yeast.

This oxygen was introduced into an OTEEN-4E ozonator by silicone tube. Oxygen gas was generated using an OTEEN-4E ozonator. Oxygen gas was directly pumped into the vessel containing 1000 ml filtered sugar cane juice. Pressure was applied at 0 psi, 10 psi and 15 psi respectively. The treatment of all sugar

cane juice samples was carried out upto 60 min with sampling interval of 10 min (i.e 10 min, 20 min, 30 min, 40 min, 50 min and 60 min). The 10ml aliquot of sugarcane juice at every 10min exposure of ozone was drawn from which 10 μ l was poured on plates containing Sabouraud dextrose agar for molds, Yeast Extract Mannitol Agar with congo red for yeasts and Luria Bertani Agar for bacteria. The microbial count was taken and checked for bacterial, mold and yeast count for every time interval by plate count method.

4.a Analysis of Total viable count to determine the better technique and optimisation of Ozone sterilization parameters. [3]

Note: After both the sterilization processes, physicochemical parameters like sucrose content, pol content and %Brix was determined, if favourable parameters of sucrose content, pol content and %Brix were seen only then the juice was proceeded for fermentation. Sucrose content was determined by Phenol sulphuric Method and by Lane Eynon method.

5. Culture preparation for fermentation of sugarcane juice

Saccharomyces cerevisiae was taken from NCIM (National Chemical Industrial Microorganisms). *Saccharomyces cerevisiae* was inoculated in Glucose Peptone Yeast Broth at 28°C for 24hrs.

6. Fermentation Of Sterile Sugarcane Juice[Cardona et al 2010]

2L sterile sugarcane juice either by UV or by Ozone was fermented in the fermenter with 3L capacity. 2L sterile sugarcane juice either by UV or by Ozone was fermented in the fermenter with 3L capacity. The bioreactor was set at 100 rpm agitation, 30°C temperature for 18hrs.

7. Recovery for extraction of Ethanol

The distillation assembly was made manually using glasswares, gas burners and boiling water bath but temperature control was not possible. A distillation assembly setup was used for the second run which had heating mantle and temperature could be controlled. Temperature of the assembly was set to 78°C because the boiling point of ethanol is 78°C. Thus the ethanol was recovered from the mixture of water and ethanol.

8. Qualitative analysis of ethanol by Specific gravity Method

Specific Gravity bottle was taken, cleaned and dry bottle weight was measured. Specific Gravity bottle was filled with water to the rim and weighed. Dry bottle weight was subtracted to know the volume of water. Water was discarded and extracted ethanol was added to the bottle and weighed. Dry bottle weight was subtracted to know the sample weight.

Apparent Specific Gravity was obtained by the formula:

$$\text{Apparent Specific gravity} = \frac{\text{Weight of Ethanol}}{\text{Weight of water}}$$

9. Qualitative analysis of ethanol by Potassium Dichromate Method [Borji et al 20015]

1ml of ethanol was taken. To this 5ml Potassium Dichromate was added. This mixture was kept in a water bath at 60 °C for 20 mins. After cooling Absorbance was recorded at 600nm. The Potassium Dichromate Method was used to plot the standard dose curve of ethanol and for the sample. Thus from the standard dose curve the concentration of ethanol was determined.

RESULTS AND DISCUSSION

In the present investigation, an attempt has been made to explore the possibility of using ozone for the sterilization of sugarcane juice.

The above aliquots were also checked for sucrose content, pH and reducing sugars.

The 2L sterilized juice was proceeded further for fermentation in the fermentation vessel having capacity of 3L. 100ml *Saccharomyces cerevisiae* grown in Glucose Peptone Yeast Broth was used for fermentation with fermentation parameters being 100rpm agitation and 30°C temperature for 18hrs fermentation time.

After fermentation, recovery of ethanol from fermentation broth was done by distillation method keeping the constant temperature of 78°C.

The extracted ethanol was qualitatively analysed by Specific Gravity method and Potassium Dichromate method.

RESULTS

1. Optimization of Ozone parameters:

As a first step, 1000ml grated sugarcane juice was exposed to ozone for 60mins and an aliquot of every 10mins was taken. The bacterial, mold and yeast count was taken at every interval by plate count method. With this, the ozone parameters like pressure and time of exposure were optimised.

Colony count after Plate Count Method (Table 1):

2.c. Sucrose content by Phenol Sulphuric Method (Figure 1):

The sugarcane juice sample when checked for sucrose content by Phenol Sulphuric method, showed absorbance of 0.130 at 490 nm. Hence extrapolating the graph, the sucrose concentration is 1.91mg/10ml which means 19.1%. This is in the desirable range of sucrose content (14-22%) for fermentation by *Saccharomyces*.

3. Sterilization with UV rays:

The sugarcane juice was exposed to UV rays at 260nm for 20mins. The aliquot was taken and checked for bacterial, yeast and mold count. The aliquot was also checked for sucrose content, pH, colour, taste and odour.

Colony count after plate count method (Table 2):

Calculations For Specific Gravity:

Weight of Dry bottle(W_1) = 31.383g

Weight of Bottle + Water(W_2)= 79.298g

Weight of Bottle + Extracted Sample(W_3)=
70.751g

$$\begin{aligned} \therefore \text{Weight of Water} &= W_2 - W_1 \\ &= 79.298 - 31.383 \\ &= 48.015\text{g} \end{aligned}$$

$$\begin{aligned} \therefore \text{Weight of Sample} &= W_3 - W_1 \\ &= 70.751 - 31.283 \\ &= 39.468\text{g} \end{aligned}$$

Specific Gravity = Weight of sample / Weight of Water

$$\begin{aligned} &= 39.468 / 48.015 \\ &= 0.822 \end{aligned}$$

Hence, Specific Gravity was found to be 0.822 which is near to the specific gravity of Ethanol (0.79), therefore, the presence of Ethanol was observed.

5.b. Standard dose curve for determination of recovered product concentration of Ethanol (Figure 2):

From the Potassium Dichromate method of ethanol estimation, the Sample absorbance at 600nm was observed to be 0.159 hence the concentration was 6.783ml ethanol in 10ml water. Therefore 67.8% concentrated ethanol was extracted from ozone sterilised sugarcane juice.

Table 1: Colony Forming Units after Sterilization by Ozone Treatment

Molds- Sabouraud's Dextrose Agar							
Time (mins)	0	10	20	30	40	50	60
Colony Count at 0 psi (cfu/ml)	125400	124900	88500	59600	48700	45200	38900
Colony Count at 10 psi (cfu/ml)	120300	80000	29600	18500	7800	800	400
Colony Count at 15 psi (cfu/ml)	23400	10800	9800	3200	600	300	300
Bacteria- Luria Bertani Agar							
Time (mins)	0	10	20	30	40	50	60
Colony Count at 0 psi (cfu/ml)	128700	127100	120700	98000	78600	69900	65700
Colony Count at 10 psi (cfu/ml)	119000	81200	32200	19100	8000	1600	500
Colony Count at 15 psi (cfu/ml)	78350	64300	50300	25700	8500	3500	1900
Yeast-Yeast Extract Mannitol Agar							
Time (mins)	0	10	20	30	40	50	60
Colony Count at 0 psi (cfu/ml)	78200	68700	59800	49800	41100	32800	28900
Colony Count at 10 psi (cfu/ml)	96300	56300	18900	18500	1800	600	400
Colony Count at 15 psi (cfu/ml)	34500	18100	7700	3600	2800	1900	800

Table 2: Colony Forming Units after UV Sterilization

Media	Time (mins)	Colony Count
Sabouraud's Dextrose Agar	20	9925
Luria Bertani Agar	20	60800
Yeast Extract Mannitol Agar	20	8500

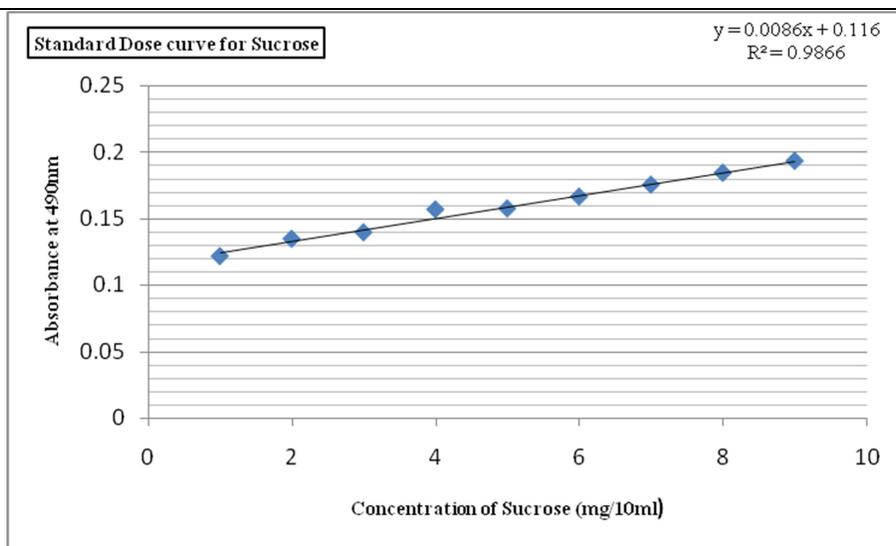


Figure 1: Determination of Sucrose Content after Phenol Sulphuric Method

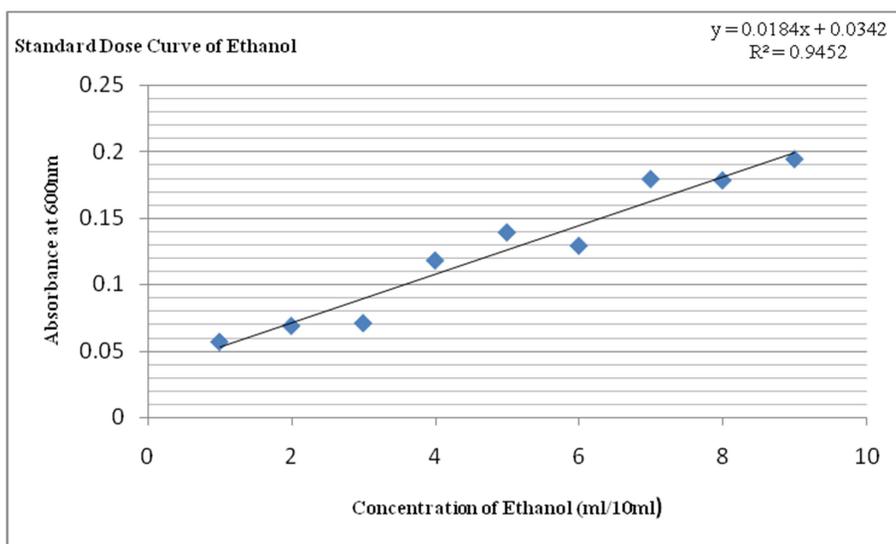


Figure 2: Standard Curve for ethanol estimation by Potassium Dichromate Method

DISCUSSION

One of the main factors that affect the production of ethanol from raw sugarcane juice is it is microbially unstable and the possibility is that particular strains of bacteria, yeast and mold may cause infection. Hence, sterilization to remove unwanted microbes was necessary [13].

The previous studies recorded the pasteurisation method for sterilization which had adverse effects that it converted sugars in the sugarcane juice and also changed the physicochemical parameter like pH, taste, odour and sucrose content of juice due to heating [16]. In the present study, to overcome the problem ozone treatment was done for sterilization of

Sugarcane juice. Ozone sterilization is non-thermal treatment and the most important advantage is that the oxygen is freely available [5].

Economic evaluation of fuel bioethanol production explores that more energy is consumed in recovery steps conducted by distillation due to low ethanol concentration in fermented broth [15].

Therefore, increasing the content of ethanol in the broth can considerably reduce energy consumption in distillation. This was achieved by ozone treatment on juice which eliminated the unwanted components like inhibitors and helped the *Saccharomyces* culture to produce improvised quality of Ethanol [3].

The present study showed that a remarkable quality of ethanol was obtained when sterilization was done by ozone treatment. This process will reduce the cost of fuel by producing the better quality of ethanol.

CONCLUSION

Studies concluded that the application of ozone in clarification of sugarcane juice is a viable alternative, since values of reducing sugars and sucrose were not changed significantly, while the decrease of colour was significantly changed. However, it is important to determine the better way to obtain this technology in the clarification process because the heat treatment applied during pasteurization has great influence in

juice colour, Brix, reducing sugars, %sucrose and pol content of sugarcane juice. This behaviour can affect the best use of ozone as a clarifying agent as seen in many other treatment methods which include preheating sugarcane juice during pasteurization for the clarification process. Ozone is a safe disinfectant when used under controlled conditions. Ozone quickly decomposes to oxygen with little residual effect and hence its effects are short-lived and it is suited for use in preservation of most food materials. Some combination application of ozone treatment with pasteurization, UV, high-pressure processing, membrane processing and freezing may be very effective in microbial inhibition and shelf life extension and of food products. Ozone treatment showed to be an effective method of stabilizing fermentation media based on raw sugar beet juice, allowing for sterilization without inhibiting yeast cell growth. In samples which were sterilized using ozone, it was noted a significant increase in efficiency of process in comparison to fermentations conducted in media that were not sterilized, especially in worts with higher microbial contamination. An additional advantage may be observed if ozone treatment and fermentation in the same fermenter is performed or of treating fermentation media with ozone in a system of flow, as

the fermentation tank is being filled. Use of ozone treatment for the stabilization of fermentation media based on sugar beet juices has been described in some studies.

Saccharomyces cerevisiae had the ability to produce a better quality of Ethanol using Sugarcane juice as the substrate because the fermentation inhibitors present in the juice were killed with the help of ozone treatment.

Overall study reveals that the ozone treatment helped in the better production of ethanol from fermentation without changing the properties of sugarcane juice.

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