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Synthesis and Mass Balance of Citric Acid from Sugarcane Molasses Using Baker's Yeast and Submerged Batch Fermentation Technique

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Abstract— **Citric acid is one of the most important organic acids manufactured in bulk quantity due to its widespread usage in food and pharmaceutical industries as acidulant and preservative. Though, it is very common to produce citric acid using fungi (Aspergillus niger) from different sources of carbohydrates, such as molasses and starch based media, in this present research work an attempt has been made to isolate citric acid from sugarcane molasses from Wonji sugar factory, Ethiopia using baker's yeast (*Saccharomyces cerevisiae*) by submerged fermentation. During the isolation process various nutrients required have been calculated carefully and added. Also maintained a temperature of 26 ±1 ^{o}C, Oxygen in excess of 25% than the saturation level with a constant stirring at a pH of 5. The fermentation process was carried out for 6 days. At the end, citric acid crystals were isolated, purified and characterized by using appropriate techniques mentioned in the literature. Finally, mass balance was also carried out for the entire process and values were reported.**

Keywords: *Citric acid; Yeast; Saccharomyces cerevisiae; Molasses; Mass balance*

I INTRODUCTION

Citric acid (2-Hydroxypropane-1,2,3-tricarboxylic acid), a tribasic acid with pKa values of 3.1, 4.7 and 6.4 respectively was first isolated by Carl Wilhelm Scheele, a Swedish chemist in 1784. Although its main source of production is fruits of citrus family, it can also be found in fruits like pineapple, pear, peach etc. First successful commercial production of citric acid by Aspergillus niger was carried out by J.N. Curie [1]. Due to high solubility, palatability and low toxicity citric acid can be used in food, bio chemical, beverages, cosmetics and pharmaceutical industries [2]. There are also chemical methods to synthesize citric acid but better results can be obtained by microbial fermentation methods [3]. Selection of raw materials and optimization of process parameters are essential in the manufacturing process. Numerous investigations have been reported with cheap raw materials such as sugarcane, beet and starch molasses [4].

Various microorganisms such as fungi (*Aspergillus wentii, A. carbonarius, A. aculeatus, A. awamori* and *Penicillium janthinellum*); bacteria (*Bacillus licheniformis, Arthrobacter paraffinens,* and *Corynebacterium* sp.) and Yeasts (*Saccharomycopsis lipolytica, Candida tropicalis,* C. *oleophila* C. *guilliermondii,* C. *citroformans* and *Hansenula anamola*) [5 – 10]. Since citric acid is a metabolite of energy metabolism, commercially less product yield is obtained unless drastic imbalance conditions are maintained. The product yield depends not only on the strain but also on the fermentation technique. One microbial strain which gives good yield by one particular fermentation technique may not give good yield with another technique [10].

Most preferred substrates for the citric acid fermentation process are molasses as they are cheaply available and contain high sugar content (40 – 55%) [5].

In available fermentation techniques, about 80% of commercial citric acid production is carried out by submerged fermentation alone [9, 11, 12]. Beet molasses are more suitable and give greater yields of citric acid than cane molasses as they contain less amounts of trace elements. Therefore, a suitable treatment of cane molasses to reduce/eliminate trace elements is required [13 – 21]. The fermentation process is also depends on the conditions such as aeration [22], temperature [23, 24, 25] and pH [26, 27]. However, as no attempt has been done on the production of citric acid from Ethiopian grown sugarcane molasses and Baker's yeast (*Saccharomyces cerevisiae*) as microorganism lead us to select this investigation.

II MATERIALS AND METHODS

Microorganism

Saccharomyces cerevisiae type-II strain from Merck obtained from local supplier. Fermenter is cleaned and sterilized in an autoclave after adding appropriate nutrients for yeast to grow colonies. Yeast strains were added to the fermenter (Streak Plating Method) and allowed to grow for 6 days at 26 ^{o}C.

Apparatus

A 500 ml glass fermenter at 600 rpm was used. Experimental set up consisting of a working volume of 300 ml substrate at 26 ±1^{o}C temperature, pH 5 and 25% excess oxygen [28] was supplied using a compressor with air filter.

Media

Yeast culture was prepared by taking 5 g yeast (*Saccharomyces cerevisiae*), 1g urea, 10 g glucose, and 100 ml distilled water. 150 ml of sugar cane molasses collected from Wonji sugar factory, Ethiopia which contained 30% sugars was diluted by adding 150 ml distilled water to contain 15% sugars. The resulting solution was boiled for 20 minutes to reduce sugars followed by cooling and filtering. 1 N sulfuric acid was added to the solution, the same boiled for 10 min, cooled, neutralized with lime-water, $Ca(OH)_2$ and was left to stand overnight for clarification. H_2SO_4 was added to break down complex sugars to simpler sugars so that microorganisms can utilize them. Filtered the solution again using filter paper to

remove the impurities of the first phase. The following nutrients were added in the flask containing the solution.

- Potassium biphosphate (KH_2PO_4) - 0.5g
- Ammonium nitrate (NH_4NO_3) - 0.5g
- Magnesium sulfate ($MgSO_4$) - 0.5g
- Ferric chloride ($FeCl_3$) - 0.01g
- Zinc sulfate ($ZnSO_4$) - 0.0025g

The pH of solution was adjusted using H_2SO_4 since no further break down was required. The pH was finally adjusted to 5. 150 ml of molasses medium (sugar 15%, pH 5) in 1000 ml open beaker is now sterilized. Mixed the yeast growth in the medium prepared. The flask incubated at 26 °C in the rotary shaker at 600rpm for 6 days with 25% excess oxygen pumped continuously using a compressor through an air filter and humidifier [22]. No Fe^{2+} salt was added in to the media. Polypropylene glycol was used as antifoaming agent.

III PRODUCT RECOVERY

Taken out the flask from the shaker incubator. Filtered the same using muslin cloth and again filtered using filter paper. Added $Ca(OH)_2$ to the filtrate until the solution gets neutralized. Filtered again and collected the precipitate this time. Washed the same with distilled water and again filtered through Whattman 42 filter paper. Kept the flasks in shaker again for continued washing for 24 Hrs. Filtered again with filter paper. The precipitate was filtered and washed with water several times. It was then treated with H_2SO_4. The solution was again filtered to remove $CaSO_4$. Added activated charcoal to the filtered citric acid and filtered the mother liquor to remove color and odor. Crystallized the product and dried it.

The experiment was repeated several times until to get reproducible values and the parameters were fixed.

IV RESULTS AND DISCUSSION

The isolated citric acid was characterized by verifying various physical and chemical properties using appropriate techniques and equipment and found them good agreement with the literature values.

From this experiment we have obtained the required results by utilizing the above mentioned ingredients. The strain yeast was found to enhance citric acid synthesis.

1. **Volume fermenter 1L**
2. **Working volume 300 ml**
3. **pH value=5**
4. **Incubation temperature=26 °C**
5. **Raw molasses sugar mainly sucrose (substrate)= 150 ml**
6. **Fermentation hours =144 hrs**
7. **Distilled water 150 ml**
8. **Product of citric acid obtained was 12 gl^{-1}**

$$\textbf{Concentration} = \frac{\textbf{Number of Moles}}{\textbf{Volume of Water}}$$

$$\mathbf{C = \frac{m}{VM}}$$

Where C = concentration of citric acid
m = mass of citric acid obtained
M = molecular weight of citric acid
V = volume of water

$$C = \frac{1.01\ g}{192\ g\ mol^{-1} x\ 150\ ml} = \mathbf{3.51 \times 10^{-5}\ mol\ ml^{-1} = 0.0351\ mol\ l^{-1}}$$

By unit conversion,

$$C = \frac{0.0351 mol\ l^{-1} \times 1g}{0.00292144\ mol} = \mathbf{12.00\ g\ l^{-1}}$$

$$\text{Yield of product from substrate} = Y_{P/S} = \frac{\mathbf{12.00\ g\ l^{-1}}}{\mathbf{150\ g\ l^{-1}}} = \mathbf{0.08}$$

Dry cell mass, yeast (biomass) = 109.88 gl^{-1}

$$C = \frac{\text{mass of biomass obtained}}{\text{molecular weight of sacchromyces x volume of water}}$$

$$= \frac{1.22\ g}{25.34\ g\ mol^{-1} x 150\ ml} = \mathbf{0.321\ mol\ l^{-1}}$$

By unit conversion,

$$C = \frac{0.321\ mol\ l^{-1} \times 1\ g}{0.00292144\ mol} = \mathbf{109.88\ g\ l^{-1}}$$

$$\text{Yield of biomass from substrate} = Y_{X/S} = \frac{109.88\ g\ l^{-1}}{150\ g\ l^{-1}} = \mathbf{0.7325}$$

Mass Balance

1. Develop the product stoichiometric equation of citric acid

$$C_wH_xO_yN_z + aO_2 + bH_gO_hN_i \longrightarrow cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O + fC_jH_kO_lN_m$$

Stoichiometric coefficient balance:

C balance: w = c + d + fj
H balance: x + bg = cα + 2e + fk
O balance: y +2a + bh = cβ + 2d + e + fl
N balance: z + bi = cδ + fm

$$C_{12}H_{22}O_{11} + aO_2 + bNH_4NO_3 \longrightarrow cCH_{1.82}O_{0.58}N_{0.16} + dCO_2 + eH_2O + fC_6H_8O_7$$

2. Calculate the stoichiometric coefficient balance

C balance: 12 = c + d + 6f
H balance: 22 + 4b = 1.82c + 2e + 8f

O balance: 11 + 2a + 3b = 0.58c + 2d + e + 7f
N balance: 2b = 0.16c

$$Y_{X/S} = c\left(\frac{\text{MW Cell}}{\text{MW Substrate}}\right)$$

where $Y_{X/S} = 0.7325$
MW Cells = 25.34 + ash (7.3%)

$$= \frac{25.34}{(1 - 0.073)} = 27.34 \text{ g mol}^{-1}$$

MW substrate (sucrose) = 342 g mol^{-1}

$$0.7325 = \left(c\frac{27.34}{342}\right)$$

$$c = 9.16$$

N balance: 2b = 0.16c

$$b = 0.733$$

$$\mathbf{Y_{P/S} = 0.08}$$

$$Y_{P/S} = f\left(\frac{\text{MW Product}}{\text{MW Substrate}}\right)$$

MW Product (citric acid) = 192 g mol^{-1}

MW Substrate (sucrose) = 342g mol^{-1}

f = 0.1425

C balance: 12 = c + d + 6f
d = (12 - 9.16) - (6 x 0.1425) = **1.985**
H balance: 22 + 4 x 0.733 = 1.82 x 9.16 + 2e + 8 x 0.1425

$$e = \frac{22 + 4 \times 0.733 - (1.82 \times 9.16 + 8 \times 0.1425)}{2} = \mathbf{3.5604}$$

O balance: 11 + 2a + 3b = 0.58c + 2d + e + 7f

$$a = \frac{11 + 3 \times 0.733 - (0.58 \times 9.16 + 2 \times 1.985 + 3.5604 + 7 \times 0.1425)}{2} = \mathbf{0.32085}$$

$$C_{12}H_{22}O_{11} + 0.32085\,O_2 + 0.733\,NH_4NO_3 \longrightarrow 9.16\,CH_{1.82}O_{0.58}N_{0.16} + 1.985\,CO_2 + 3.5604\,H_2O + 0.1425\,C_6H_8O_7$$

1 mole $C_{12}H_{22}O_{11}$ produced 0.1425 mole $C_6H_8O_7$
1 mole $C_{12}H_{22}O_{11}$ produced 9.16 mole $CH_{1.82}O_{0.58}N_{0.16}$
1 mole $C_{12}H_{22}O_{11}$ produced 1.985 mole CO_2
1 mole $C_{12}H_{22}O_{11}$ produced 3.5604 mole H_2O

Calculation of limiting and excess reactants or reagents

Limiting reactants: is the reactants to run out or consumed
: is the reactant that gives the smallest yield of products.
: is the reactant that determines the final amount of product.
Excess reactant: is the reactant that is left over an excess.

Calculate the moles of each reactants

$$\text{Number of moles of ammonium nitrate} = \frac{1.05\text{g}}{80 \text{ g mol}^{-1}} = \mathbf{0.013125 \text{ mol}}$$

Calculating the number of moles of products

a) Using sucrose

$$\underset{1 \text{ mole}}{C_{12}H_{22}O_{11}} + 0.32085\,O_2 + \underset{0.6245 \text{ mole}}{0.733\,NH_4NO_3} \longrightarrow 9.16\,CH_{1.82}O_{0.58}N_{0.16} + 1.985\,CO_2 + 3.5604\,H_2O + \underset{0.0481 \text{ mole}}{0.1425\,C_6H_8O_7}$$

X mole

$$\text{X mole} = \frac{0.6245 \text{ mol} \times 0.1425 \text{ mol}}{1\text{mol}} = \mathbf{0.08899 \text{ mol}}$$

b) Using ammonium nitrate

$$\underset{0.733 \text{ mole}}{C_{12}H_{22}O_{11}} + 0.32085\,O_2 + \underset{0.013125 \text{ mole}}{0.733\,NH_4NO_3} \longrightarrow 9.16\,CH_{1.82}O_{0.58}N_{0.16} + 1.985\,CO_2 + 3.5604\,H_2O + \underset{0.0481 \text{ mole}}{0.1425\,C_6H_8O_7}$$

X mole

$$\text{X mole} = \frac{0.013125 \text{ mol} \times 0.14251\text{mol}}{0.733 \text{ mol}} = \mathbf{0.002552 \text{ mol}}$$

Therefore,
Limiting reactant is ammonium nitrate and excess reactant is sucrose

Calculation of percentage yield by using limiting reactant

$$\underset{1.05 \text{ g}}{\overset{80 \text{ g mol}^{-1}}{NH_4NO_3}} \longrightarrow \underset{X \text{ g}}{\overset{192 \text{ g mol}^{-1}}{C_6H_8O_7}}$$

$$\text{X g} = \frac{1.05\text{g} \times 192 \text{ g mol}^{-1}}{80 \text{ g mol}^{-1}} = \mathbf{2.52 \text{ g}}$$

Actual yield= **1.01g**

Theoretical yield= **2.52 g**

Now,

$$\% \text{ Yield} = \left(\frac{\text{actual yield}}{\text{theoretical yield}} \right) \text{x } 100$$

$$= \left(\frac{1.01\text{g}}{2.52\text{g}} \right) \text{x } 100 = \mathbf{40.1}$$

The % yield, 40.1 is less by this method as against *Aspergillus niger* method which is about 65% yield [17].

V CONCLUSION

Citric acid has been isolated successfully from sugarcane molasses obtained from Wonji Sugar Factory, Ethiopia by using yeast (saccharomyces cerevisiae – baker's yeast) for which a submerged fermentation technique (batch process) was used. All appropriate nutrients used and suitable conditions (pH, temperature, aeration etc.) were maintained during the fermentation process. Isolated citric acid was purified and characterized. Mass balance of the process was also carried out. Overall, 40.1% yield was obtained and the same has been reported.

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