Review on the Production of Citric Acid through Solid-State Fermentation on Sugarcane Bagasse

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Abstract— For citric acid production four isolates of Aspergillus Niger (viz CA16,14/20,HB3 and 318) were used. Sugarcane bagasse was used as a substrate and sucrose solution used as a moistening agent. The highest citric acid production was obtained when 10 ml of 14% sucrose solution was used as moistening agent. For 11 days fermentation for all isolates of Aspergillus Niger.

Keywords: Citric Acid, Solid-State Fermentation, Aspergillus Niger, Sugarcane Bagasse

I. INTRODUCTION

Citric acid is one of the most commonly used organic acid in the food and pharmaceutical industry, which is produced mainly by submerged or surface fungal fermentation. Aspergillus Niger fermentation is the world's leading source for commercial production of citric acid. Solid-state fermentation is regarded as better option for citric acid production because of lower energy requirement, higher product yield with little risk of bacterial contamination, generation of less waste water and environmental concerns regarding the disposal of solid-waste. Another benefit for solid-state fermentation is that, inhibition of citric acid production due to presence of metal ions is ineffective in solid-state fermentation. Since 1920, all commercial citric acid was produced from lemon and lime juices. In 1923, at first time Aspergillus Niger, a fungus used for production of citric acid. Factors which affect the production of citric acid by fermentation are the nutritional composition of the media, environmental conditions, deficiency of manganese and other metals, pH, and dissolved oxygen concentration. Now a days citric acid is commercially produced by using mutant strains of Aspergillus Niger, and with a significant amount by Saccharomyceslipolytic., large amounts of sugarcane bagasse are produced as a by-product of sugar industries. Sugarcane bagasse is the best source for solid state fermentation for production of citric acid. The study was done to investigate the possibility of using sugarcane bagasse as a substrate fortified with different quantity of sucrose solution as moistening agent and additional carbon source for solid-state fermentation for citric acid production by four isolates of Aspergillus Niger.

II. MATERIAL & METHOD

A. *Area of study*

This study was conducted at the Department of Chemical Engineering All experiments were accomplished aseptically in the laboratory of chemical engineering..

B. *Collection of sample*

Three samples were collected from air, bread, and soil in depth of 15 cm from Tuti islands' farms (Erika et al., 2013). Forty litres of sugarcane molasses sample were obtained from the Distillery Unit of Kennan Sugars (D.U.K.S) Company – White Province – Sudan. The sugarcane molasses were collected in clean, durable plastic container and stored at room temperature for further uses.

C. *Isolation of test microorganism (Aspergillusniger)*

One gram of the soil sample was placed in the test tube containing 10 ml of sterile distilled water to make a soil suspension and tenfold serial dilution was made by transferring one ml of the soil suspension to another test tube containing 9 ml of sterile distilled water. This step was repeated ten times to obtain a dilution of 10-10. An amount of 0.1 ml from each of the first three test tubes (10-1, 10-2, and 10-3) was taken and placed on the plate containing Sabouraud dextrose agar medium supplemented with rose bengal to inhibit the growth of saprophytes fungi other than A. niger. Another plate was opened inside the laboratory of microbiology to isolate A. nigerfrom the air; the third plate was inoculated with the A. nigerfrom infested bread; all plates were incubated aerobically at 25°C for 72 hrs. After the incubation period, the culture characteristics were observed and the growth was examined microscopically to confirm its purity using lactophenol cotton blue stain technique (Cheesbrough, 2008).

D. *Physical characteristics of the sugarcane molasses sample*

The physical characteristics of sugar cane molasses, such as moisture content, ash measurement and pH, were analyzed following standard methods (APHA, 2000).

1) *Moisture content and ash measurement*

The moisture content and ash measurement of molasses was performed by taken 10 grams of molasses sample and oven dried in a crucible at 104°C for 30 minutes (Hubert, 2006). Then the results were calculated using the following equations:

$$\text{Moisture content}(\%) = (A - X \div A) \times 100 \quad (1)$$

$$\text{Ash (unit)} = \text{Weight of molasses before burning (A)} - \text{Weight of molasses after burning (X)} \quad (2)$$

where A is the weight of molasses before burning. While X is the weight of molasses after.

2) *The pH value*

The pH value was measured before and after inoculation of molasses samples using pH meter device (pH 213 Microprocessor-based Bench pH/mV/C Meters. Hanna Instruments).

E. *Production of citric acid from raw sugarcane molasses*

Isolates of A. nigerwere transferred to the 15 flasks containing raw sugarcane molasses media with different concentrations, i.e., each three flasks have the equal amount of molasses (20%, 30%, 40%, 50%, and 60%) by taking 100 ml, 150 ml, 200 ml, 250 ml, and 300 ml of sugarcane molasses and the volume was completed to 500 ml using sterile distilled water. The flasks were autoclaved at 115°C for 10 minutes. An amount of 50 ml of distilled water was

added to the fungal pure culture to make a fungal suspension and then 10 ml from this suspension was transferred to the sugarcane molasses media. All flasks were incubated at 28°C for 144 hrs till 10 days. After incubation, the suspension was distilled to monitor the growth and observe the results (Elholi and Al-Delaimy, 2003).

F. Production of citric acid from sugarcane molasses with determined concentration of sucrose

In another experiment, different concentrations of sucrose (10%, 25%, 35%, and 50%) were measured using a hand refractometer device. After sterilization, an amount of 0.5 grams of urea powder was added to each flask containing sugarcane molasses with known concentration. Then 3 ml from pure A. nigerwere added to the media and the culture was incubated at 28°C for144 hrs till 10 days. After incubation, the suspension was distilled to monitor the growth and observe the results (Dubey, 2003).

G. Detection of citric acid

The detection of citric acid was done chemically by the addition of three drops of bromocrysol green indicator to the 10 ml of distillation yield (Soccol1 et al., 2006).

H. Determination of citric acid concentration

Citric acid was determined by titration using 0.1N NaOH and Phenonphthalein as indicator and calculated as percentage according to the following formula (Soccollet al., 2006):

- Normality of Citric acid = normality of NaOH× NaOH volume ÷ volume of Citric acid
- Concentration of Citric acid = Citric acid normality ×equivalent ×100 ÷ volume of distillation
- (Equivalent = 96, volume of distillation = 10)

III. Results & Discussion

A. Isolation of Aspergillusniger

Three isolates were isolated from three different sources air, bread, and soil using sterile culture media, the isolates were purified, examined microscopically to show its purity and characterized by its culture characteristics.

B. Physical characteristics of the sugarcane molasses sample

The physical characteristics of sugarcane molasses were determined and calculated. The present study shows that the percentage moisture content was 65%. The ash was calculated as 6.50%.While the pH shown 6.0±0.2.

These findings were in disagreement with the findings of Gasmallaet al. (2012) who reported that the pH value of obtained molasses was 5.8±0.35. The ash was 12.69% on wet weight basis. Also these findings were in disagreement with the findings of Osunkoya and Okwudinka (2011) who reported that the pH value of obtained molasses was 5.1. The ash was 8.24%.

C. Production of citric acid from raw sugarcane molasses

As can be seen in Table 1, the best yield by all strains was 37.5 ml, 37.0 ml, 35.0 ml which were obtained at concentration of 20%. At concentration 30% they were 37.0 ml, 37.5 ml, and 33.5 ml, while at concentration 40% the two strains that were isolated from soil and bread gave a similar yield 35.0 ml and the air isolate strains gave 20.0 ml citric acid. The present study was almost in agreement with Sikanderet al. (2002) who reported four A. nigerisolates produced citric acid with the concentration of 18.86±1.8 – 42.56±2.0 g/l on 150g/l molasses sugar.

The lowest yields were obtained at the concentration of 50% as 10.0 ml, 5.5 ml, and 8.0 ml citric acid. At 60% sugarcane molasses concentration the microorganism did not exhibited any growth due to the effect of hypertonic solution. These findings were in disagreement with Sikanderet al. (2002) who stated that three A. nigercultures gave concentrations of citric acid ranged between 58.14±2.7 – 78.18±18 g/l on 150g/l molasses sugar. Also, the present study was in agreement with Peksel and Kubicek (2003) who reported that the concentration and type of sugar influence the yield of citric acid production by A. niger. The present findings also were in agreement with Laboniet al. (2010) who reported that the citric acid production increased with the increase of the fermentation period and the maximum citric acid production was found on day 13.

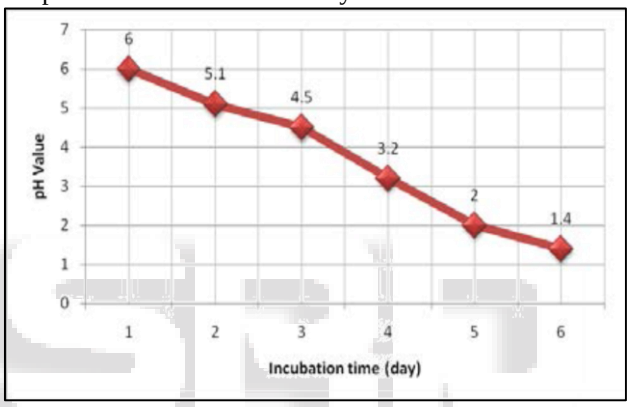

Fig. 1: Citric acid indicated by pH reduction

(2014) who stated that the production of citric acid by A.niger, cultured on Parkiabiglobosafruit pulp, showed that the highest yield (1.15 g/L) of citric acid was obtained at pH 2 and it declined as the pH increased from being acidic to alkaline (pH8) with the yield of (0.86 g/L).

During the fermentation process there was a gradual reduction (Figure 1) of pH noticed in all the experiments and it indicated the production of citric acid.

	Solids/ g	Soil yield (ml)	Bread yield (ml)	Air yield (ml)
20	28.50	37.5	37.0	35.0
30	42.75	37.0	37.5	33.5
40	57.00	35.0	35.0	20.0
50	71.25	10.0	5.5	8.0
60	85.50	No production	No production	No production

Table 1. Production o of citric acid from raw molasses Sugarcane Molasses concentration%

D. Production of citric acid from molasses with determined concentration of sucrose

As can be seen in Table 2, the yield of citric acid was high when using the A. nigerwhich was isolated from soil at all concentrations compared with other isolates (air, bread), followed by bread isolates, then air isolates which was the lowest yield. These findings were in disagreement with Kareem et al. (2010) who stated that the inoculation of A.

nigeron medium supplemented with sucrose (15% w/v) gave the highest citric acid value (36.6 g/kg). Also these findings were in agreement with the same author who stated that A. niger, when inoculated on medium containing pineapple peels, gave 17.23 g/kg at 5 days fermentation period. The increase in citric acid production and biomass values was accompanied with a steady decrease in sugar along the incubation time.

The addition of urea as a nitrogen source did not affect the production of citric acid; this is in agreement with Sadiaet al. (2011) who reported that all concentrations (0.1 to 0.6%) of ammonium sulphate, peptone and yeast extract, used as a nitrogen source, were found to be inhibitory to fungal growth, sugar utilization and citric acid production. Also, the findings of this study were in agreement with Laboniet al. (2010) who stated that in the presence of prescott salt, citric acid production was found lower than it is with the absence of prescott salt and mixed substrate prepared with molasses and pumpkin media proved to be the best and potential for citric acid production. The natural oils with high unsaturated fatty acids content when added at concentrations of 2% and 4% (v/v) to Beet Molasses (BM) medium caused a considerable increase in citric acid yield from A. niger. The maximum citric acid yield was achieved in surface culture in the presence of 4% olive oil after 12 days incubation.

E. Production of citric acid from molasses with determined concentration of sucrose

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	Solids/g	Urea /g	Soil yield (ml)	Bread yield (ml)	Air yield(ml)
10	14.25	0.5	10.6	10.0	9.0
25	35.63	18.0	15.0	13.0	
35	49.88	19.5	17.0	10.5	
50	71.25	9.0	7.5	8.5	

Table 2: Production of citric acid from molasses (sucrose + urea) Sucrose%

IV. Conclusion

We found in this study that using sugarcane bagasse as a natural fermentation medium fortified with 10 of 15% sucrose as additional carbon source and moistening agent for citric acid production was superior when supplemented with Prescott salt. Maximum citric acid was produced by A. niger 318 on sugarcane bagasse in solid-state fermentation.

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