Research Paper Biotechnology

Effect of Various Parameters on Citric Acid Production using Different Substrates

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ABSTRACT

Citric acid is a weak organic acid having many applications in food and pharmaceutical industries. Mostly *Aspergillus niger* is used for production of Citric acid due to its ease of handling and its ability to utilize variety of substrates. In the present study, Citric acid was produced using different substrates such as Molasses, Pumpkin and mixed substrate prepared from both. Highest level of Citric acid was noticed on 15th day of fermentation by all the substrates. Among the three substrates used, mixed substrate produced higher level of citric acid i.e. 49.89 mg/ml in pumpkin, 51.87 mg/ml in molasses and 56.44 mg/ml in mixed substrate. With the extension in fermentation period up to 15 days, there was increase in biomass and citric acid, and decrease in residual sugar as well as pH. Estimation of citric acid was done by titration method and Pyridine Acetic Anhydride method.

KEYWORDS Citric acid, *Aspergillus niger*, substrates-pumpkin, molasses, mixed substrate, factors- pH, Temperature, production of citric acid.

Introduction:

Organic acids such as citric, acetic, tartaric, malic, lactic and gluconic acid have being utilized in food industries for preventing food deterioration and extending shelf life of food ingredients. Most commonly used organic acid is citric acid (Sukesh K et al.,2013). Citric acid (2-hydroxy-propane-1,2,3-tricarboxylic acid) is a weak organic acid found in citrus fruits. About 70% of total Citric acid produced is consumed by food industry, 12% by pharmaceutical industries and rest of 18% by other industries (Nadeem et al., 2010). It is being used in food industry to add an acidic (sour) taste to foods, soft drinks and other food products as jams, jellies, candies, water ice and wines (Khalil et al., 2010; Nadeem et al., 2010). It has applications such as acidulation, antioxidation, flavor enhancement, preservation, plasticizer and synergistic agent (Nadeem et al., 2010). These application demands production of Citric acid on large scale.

Citric acid can be produced by many micro-organisms such as *Artrobacter paraffinens, Bacillus licheniformis, Corynebacterium sp., Lacytobacillus casei, L. helveticus, L. paracasei, Streptococcus thermophilus,* Aspergillus niger*, A. aculeatus, A. carbonarius, A. awamori, Yarrowia lipolytica* and related yeast species (Sukesh K et al., 2013, Kubicek et al., 1985, Gardner et al., 1956). Mostly *Aspergillus niger* is used for Citric acid production due to its ease of handling (Nadeem et al., 2010), its ability to utilize various substrates such as apple, grape pomace, orange peel, kiwi fruit peel, cotton waste (Hildegard Kiel et al., 1981), okara soy-residue, cane molasses (Sikandar Ali et al., 2002), bagasse, potato residue, wheat bran, coffee husk (Kareem et al 2010; Sukesh K et al., 2013) because of well developed enzymatic system and also produces more citric acid per time unit. Citric acid production can be done by solid state fermentation or by submerged fermentation. Various factors such as nutritional composition of the media, environmental conditions; deficiency of manganese and other metals, pH & dissolved oxygen tension affects production of citric acid by fermentation.

At present, there is need of commercial production of citric acid using cheap raw materials due to its demand in industries. The present study deals with production of citric acid using different substrates such as molasses, pumpkin and its mixture with the effect of incubation time on pH, cell biomass, residual sugar and citric acid production.

Material & Method:

1. Selection of Micro-organism:

Citric Acid producing micro-organism *Aspergillus niger* was selected from PVP College, Loni microbial culture collection and maintained on Potato Dextrose Agar slants (Himedia, India).

2. Selection of Substrates and media preparation :

Three substrates namely Cane molasses, Pumpkin and mixture of both were selected for production of Citric acid. Preparation of Molasses, Pumpkin and mixed substrate medium was done as per Khalil et al., 2010. Along with different substrates regular fermentation media containing Sucrose 140gm, NH_4NO_3 2.23 gm, $MgSO_4$ 0.23gm, KH_2PO_4 1gm and Distilled water 1000ml was used as control for comparison.

3. Inoculum preparation and fermentation:

Aspergillus niger was inoculated on PDA medium and incubated at 28^{0}C for 3-5 days. Spores were collected and used as inoculums. Each flask containing different media (pH.6) were inoculated with 1 ml spore suspension and incubated at 28^0 C for up to 15 days. After fermentation, the medium was filtered and the filtrate was used for further analysis.

4. Determination of Optimum incubation time for citric acid production, cell biomass, residual sugar and pH:

Citric Acid, cell biomass, residual sugar and pH was measured at interval of 5 days i.e. 5th, 10th and 15th day to determine optimum incubation time based on production.

5. Effect of different substrates on citric acid production, cell biomass, residual sugar and pH:

Citric Acid, cell biomass, residual sugar and pH was measured at interval of 5 days i.e. 5th, 10th and 15th day of each substrate to determine cheap and best medium for citric acid production.

6. Determination of Citric acid:

6.1. Determination of citric acid titrimetrically (El-Holi and Al-Delaimy, 2003)

Citric acid was determined using 0.1 N NaOH and phenolphthalein indicator titrimetrically (AOAC, 1995). % Citric acid was determined using following formula:

% Citric acid = Normality x Volume of 0.1 N NaOH x Equiv. wt of citric acid / Weight of sample (g) x 10

6.2. Determination of citric acid by pyridine –acetic anhydride method (Marrier and Boulet , 1958)
Filtrate was taken in clean test tubes and final volume made to 1 ml with distilled water. 8 ml acetic anhydride was added and placed at 60^{0}C for 10 min, which was followed by addition of 1 ml pyridine and placed at 60^{0}C for 40 min. Color was changed to yellow and later absorbance, was taken at 420 nm spectrophotometrically.

7. Determination of Residual Sugar:
Estimation of residual sugar was done by 3, 5-dinitrosalicylic acid (DNS) method (Miller 1959).

8. Determination of Biomass:
Biomass was determined according to the method reported by Shah et al., 2002.

9. Determination of pH:
pH was measured using Anolog pH meter daily (Kareem et al.,2010). Experiment regarding each substrate was done in triplicates.

Results:

Table 1: Effect of incubation time and different substrates on Citric acid production

Substrates	5th day (mg/ml)	10th day (mg/ml)	15th day (mg/ml)
Control (fermentation medium)	26.92	42.53	54.74
Molasses	23.94	40.88	51.87
Pumpkin	12.90	32.24	49.89
Mixed substrate	21.50	42.56	56.44

Table 2: Effect of incubation time and different substrates on Cell Biomass

Substrates	5th day(g)	10th day(g)	15th day(g)
Control	2.138	4.729	5.287
Molasses	1.959	2.392	2.725
Pumpkin	0.863	3.027	3.545
Mixed substrate	1.52	2.931	3.437

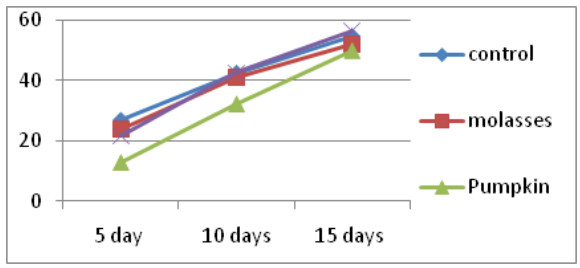
Table 3: pH observed on different days with varying substrates

Substrates	5th day	10th day	15th day
Control	3.05	1.09	0.8
Molasses	3.8	2.0	0.7
Pumpkin	3.75	2.1	1.0
Mixed substrate	3.4	2.0	0.9

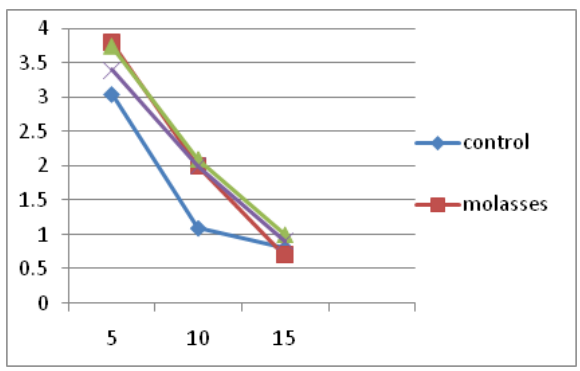
Table 4: Effect of incubation time and different substrates on Residual sugar consumption

Substrates	5th day (mg/100ml)	10th day(mg/100ml)	15th day (mg/100ml)
Control	13	10	4.5
Molasses	15.75	13	7
Pumpkin	15.5	12.5	5.75
Mixed substrate	17	13.75	8.25

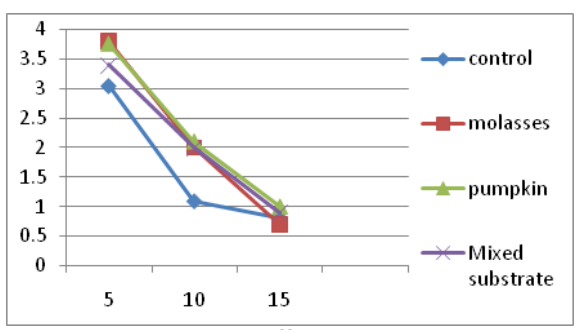
Results (Graphs):



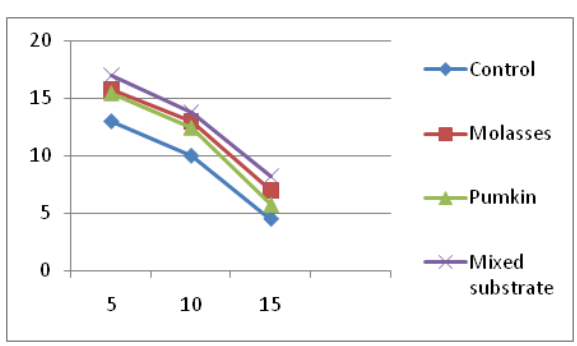
Graph 1: Effect of incubation time and different substrates on Citric acid production



Graph 2: Effect of incubation time and different substrates on Cell Biomass



Graph 3: pH observed on different days with varying substrates



Graph 4: Effect of incubation time and different substrates on Residual sugar consumption

Discussion:
Citric acid production was estimated at an interval of 5 days i.e. 5th, 10th and 15th day of fermentation using different substrates. Highest level of Citric acid production was noticed on 15th day of fermentation in each substrate. Among the three substrates used, mixed substrate showed higher level of citric acid production (56.44 mg / ml) as compared to molasses (51.87 mg / ml) and pumpkin (49.89 mg / ml).Similar results were observed in the report of Khalil et al., 2010. With the

increase in citric acid production, there was increase in cellular growth (cells/ml) and cell biomass (dry cell weight) in each substrate. Also there was gradual decrease in residual sugar and pH with the increase in incubation time & citric acid production. A steady increase in biomass for mixed substrate from 1.52 to 3.437 g/Kg, for pumpkin 0.863 to 3.545 g/Kg and for molasses from 1.959 to 2.725 g/Kg was observed from 5^{th} to 15^{th} day while decrease in pH from 3.4 to 0.9 for mixed substrate, 3.75 to 1.0 for pumpkin and 3.8 to 0.7 for molasses was noted after 15 days of fermentation. Also constant decrease in residual sugar content was observed .Thus the decrease in pH indicates production and accumulation of citric acid with an extension in incubation time.

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