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Production, Comparative and Quantitative Analysis of Citric acid by *Aspergillus niger* using Food Waste as a Substrate

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Abstract

In this study the main emphasis is given on the techniques by which citric acid can be produced at low cost. The potential of agricultural waste (Apple pomace, carrot waste and pineapple peel) as a substrate was examined for citric acid production by *Aspergillus niger* using Solid State Fermentation technique. The citric acid concentration and biomass was determined during fermentation period. The amount of citric acid was determined by titration using 0.1 N NaOH and biomass was determined by oven drying method. The optimization of three parameters (temperature, Low molecular weight alcohol and nitrogen source) was carried out. The study revealed that these parameters effect citric acid production extremely. The maximum yield was obtained in case of apple pomace followed by pineapple peel and then the carrot waste. In case of alcohol, 4% methanol gives the maximum yield as compared to isopropyl alcohol. In case of carbon and nitrogen source, sucrose 5% and NH_4NO_3 0.25% give more citric acid yield as compared with the glucose 5% and NH_4NO_3 1% respectively. When fermentation media was kept at different temperature the maximum yield was obtained at temperature 30°C as compared with the 4°C. The study has revealed that food waste material can be used for citric acid production by SSF using *Aspergillus niger*. The use of these wastes might represent an efficient method of reducing the environmental problem due to their disposal and also help in the reduction of the substrate cost.

Keywords: Citric acid; *Aspergillus niger*; Apple pomace; Pineapple peel; Carrot waste; substrates; Solid state fermentation

Introduction

Citric acid also called as tricarboxylic acid which is found in animal and plants. Citrus acid is first isolated from lemon [1]. CA is an intervene product of metabolism [2]. CA is solid at room temperature and melt at 153°C [3]. Citric acid is one of the most important organic acids produced by various fermentation techniques and also the most utilized biotechnological product [2]. The yearly growth of its demand/utilization is 3.5-4.0%. The food industry devour around 70% of aggregate citric acid produced and pharmaceutical industries devour around 12%, and the remaining 18% are devour by other industries. Citric acid sold in the market as anhydrous and monohydrate form only [4].

CA is recognized worldwide as GRAS which was authorized by the Joint FAO/WHO Expert Committee on Food Additives [5]. Therefore, CA is not only used in the food industry and pharmaceutical industry but also used in biopolymers, for drug delivery and in vast area of biomedical application [5].

Commercial production of Citric acid can be done by either solid-state fermentation or with SmF [4]. Now days, the attention has been shifted from the production of Citric acid by Solid State Fermentation rather than Submerged Fermentation. Solid State fermentation means cultivation of micro-organism in a low-water activity condition on a non-dissolvable material acting both as a supplement source and a physical support acid. The advantage of using SSF techniques include SSF process utilize low energy, give high product yield, little risk of contamination and less waste generation [6].

Many Microorganism including bacteria such as *Bacillus licheniformis*, *B. subtilis*, *Corynebacterium spp.*, fungi such as *A. niger*, *A. awamori*, *A. foetidus*, and yeasts such as such as *Candida lipolytica*, *C. intermedia* and *Saccharomyces cerevisiae* can be used for the production of citric acid [7]. Out of these fungi *A. niger* is mildly used for CA production as it grow on wide range of substrate, secret CA from the mitochondria and the cytosol has led to the massive accumulation of CA which was not possible in case of other microorganism [5].

To minimize the cost of citric acid production Fruit waste was used. Fruit waste produced in India in immense amount which is not only used as an animal feed but also disposed in the soil causing environmental problem. These fruit waste are rich in carbohydrate, starch and other nutrients so, it can be used as a substrate for citric acid production [6].

The main purpose of this study is to determine the amount of citric acid produced by *Aspergillus niger* form three different substrate i.e. apple pomace, pineapple peel and carrot waste and also examine the effect of the various parameters such as Nitrogen source, Temperature and low molecular weight alcohol on citric acid production.

Materials and Methodology

Sample and substrate collection

Soil sample for serial dilution was collected from D-Bolck sector 10 Noida and substrate was collected from juice shop in New Ashok Nagar, Noida.

Preparation of *conidia* suspension

Serial dilution: Soil sample collected was homogenised and 1 gram of soil sample was weighed. In six sterile test tubes, 9 ml of distilled water was poured and weighed soil sample was poured in 1 test tube and about 1 ml of soil sample was transferred in the next test tube. The step repeated for the all the test tube.

Isolation of Microorganism: 100 ml of PDA media was prepared and pour into 6 sterile petri plates and was allowed to solidify. On the solidified media spread about 50-100 µl of the serial dilution sample one by one and incubate it at 30°C for 3 days in an incubator. Yellow colony was selected and identification of *A. niger* is done by microscopic examination using 10% KOH.

Suspension: PDB media of about 150 ml was prepared and in that the colony was inoculated and then incubated for three days at 30°C. After three days microscopic examination of the broth is done by taking the culture on a slide in a Laminar Air Flow.

Pre-treatment of substrates

The substrate which was collected form the juice shop was kept in an incubator at 60°C for 1 hour and then grounded with the help of the pestle and mortal.

Fermentation media

The fermentation media was prepared by introducing apple pomace 1.5 g, pineapple peel 1.5 g and carrot waste 1.5 g in 15 ml of fermentation media. The composition of fermentation media for 1L: Sucrose (150-200 g), NH_4NO_3 (2.23 g), KH_2PO_4 (1.0 g), $MgSO_4$ (0.23 g). The effect of the low molecular weight alcohol was studied by adding 4% of methanol and 4% of isopropanol alcohol to the fermentation media before autoclaving it. Also the effect of Nitrogen source was studied by varying the concentration of ammonium nitrate i.e. 0.25% w/v and 1% w/v in the media composition. The effect of the different temperature was studied by keeping the fermentation media at 30°C and 4°C.

Biomass estimation:

Biomass was determined by filtering the fermentation media with whatman filter paper and washing the residue left on the filter paper with the distilled water three times. Take the initial weight of the fermentation media and then was kept in an oven at 105°C to constant mass and the residue was weighed again to calculate the biomass [8].

Citric acid determination:

CA was determined by a titration method. Pipette 4 ml of the filtrate in the conical flask and to that add 20 ml distilled water followed by 3-4 drops of phenolphthalein indicator and titrate with 0.1 N NaOH. The amount of citric acid was determined by material balance equation [9].

Results and Discussion

Out of the three substrates, the maximum production in all cases was obtained in case of Apple pomace followed by Pineapple peel and carrot waste. The high production rate was obtained in case of apple pomace because the initial amount of carbon and energy source present in the sample was higher as compared with the pineapple peel and carrot waste according to USDA National Nutrient Database.

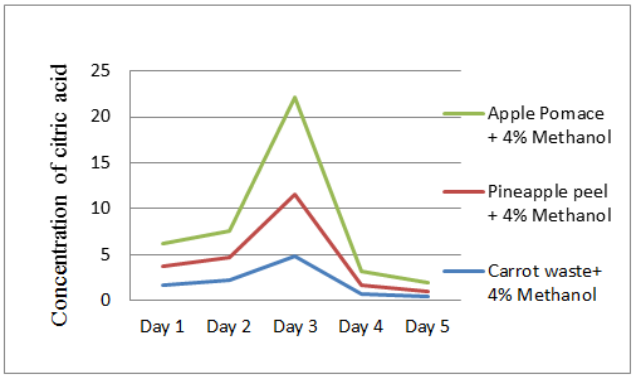

Figure 1. Effect of 4% Methanol on citric acid production.

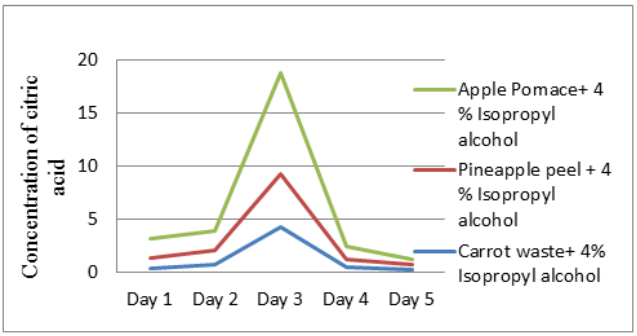

Figure 2. Effect of 4% Isopropyl alcohol on citric acid production.

When low molecular weight alcohol was added in the fermentation media, the maximum production of 10.56 g/15ml citric acid was obtained in case of apple pomace with 4% methanol on 3rd day. After third day with the increase in the incubation period the citric acid production decreases in all the cases (Figure 1). The reason for the decrease in the citric acid production is the inhibitory effect of high concentration of citric acid and decay of the enzyme needed for citric acid production [4].

As compared to the effect of the methanol and isopropyl alcohol the production rate was higher in case of methanol in case of all the substrate used. The use of the methanol in the fermentation increased the permeability of the cells to citrate [6]. Out of all variation done in the parameters for citric acid production the maximum production was obtained in case of alcohol due to the fact that the use of alcohol in the citric acid fermentation media shift the pH of the media to an acidic level does inhibit the production of gluconic acid and increase the production yield [10]. The low production of citric acid (Figure 2) in case of isopropyl alcohol was due to use of high concentration of isopropyl alcohol as it was effective only at low concentration [11].

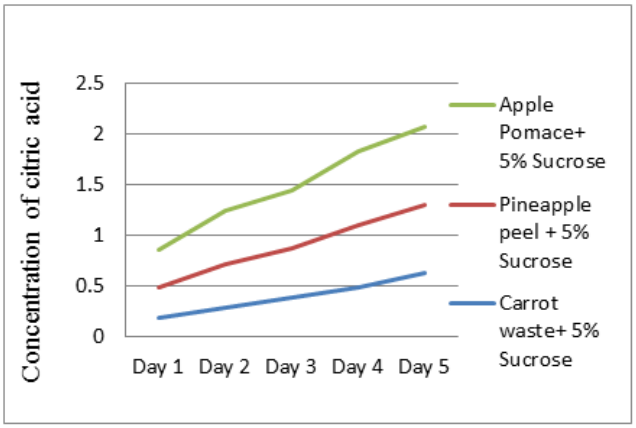

Figure 3. Effect of 5% sucrose on citric acid production.

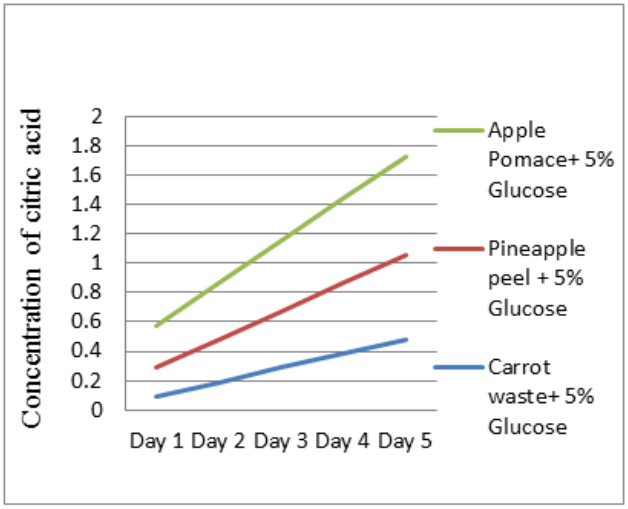

Figure 4. Effect of 5% glucose on citric acid production.

When the carbon source was varied in the fermentation media the maximum production was obtained in case of 5% sucrose as compared with the 5% glucose. The maximum production was obtained in case of Apple pomace (Figure 3) with 5% Sucrose on 5th day of 0.7684 g/15ml and least production was obtained in case of carrot waste (Figure 4) with 5% glucose of 0.48025 g/15 ml. The rate of production in case of sucrose was higher because it easily transferred into the cell for hydrolysis by intracellular enzyme [12].

The effect of different temperature on citric acid production was monitored by keeping it at 30°C and 4°C. The optimum temperature for production was obtained when fermentation media was kept at 30°C (Figure 5). The maximum production was obtained in case of Apple pomace at 30°C of 0.079 g/15 ml on 3rd day and least was obtained in case of carrot (Figure 6) at 4°C of 0.432 g/15 ml. The citric acid production was low at 4°C because at low temperature the activity of the enzyme responsible for production was low [13].

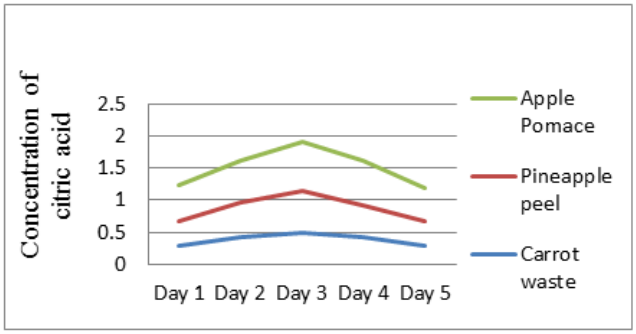

Figure 5. Effect of 30°C temperature on citric acid production.

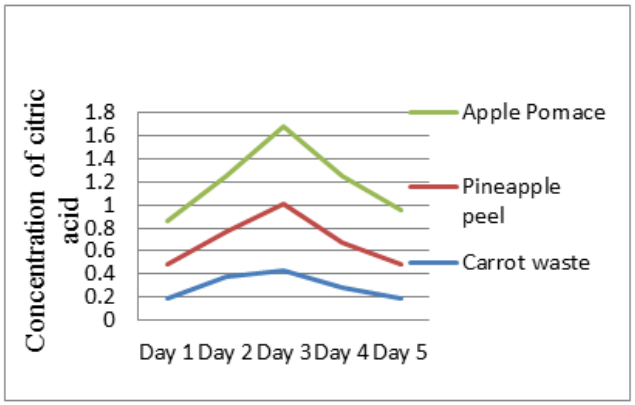

Figure 6. Effect of 4°C temperature on citric acid production.

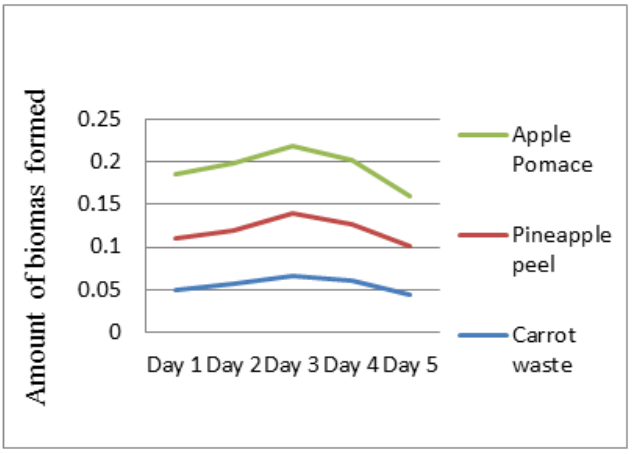

Figure 7. Effect of 30°C temperature on biomass.

The biomass at temperature 30°C (Figure 7) and 4°C was increased up to 3rd day and then decreased (Figure 8), this is due to drastic degradation of some enzymes which play an important role in the accumulation of carbon [8].

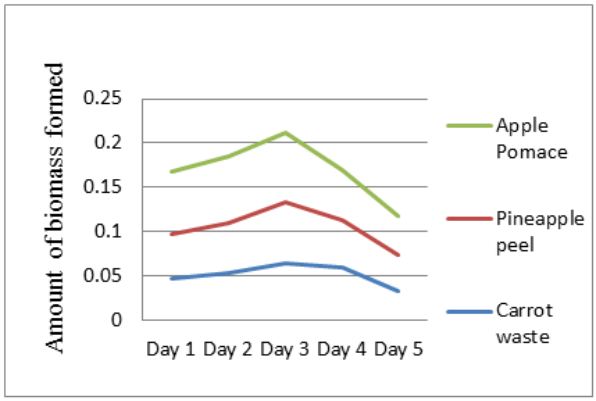

Figure 8. Effect of 4°C temperature on biomass.

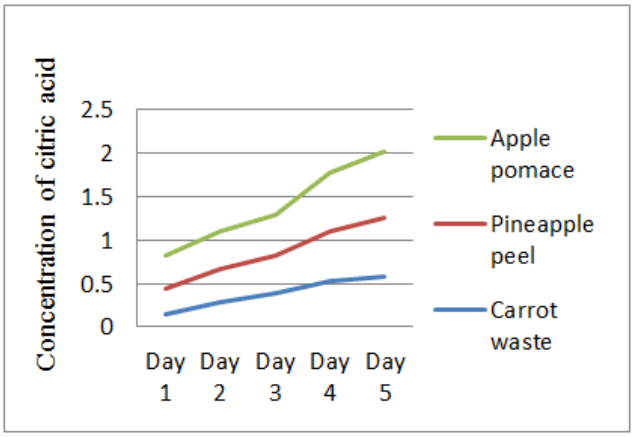

Figure 9. Effect of 0.25% w/v ammonium nitrate on citric acid production.

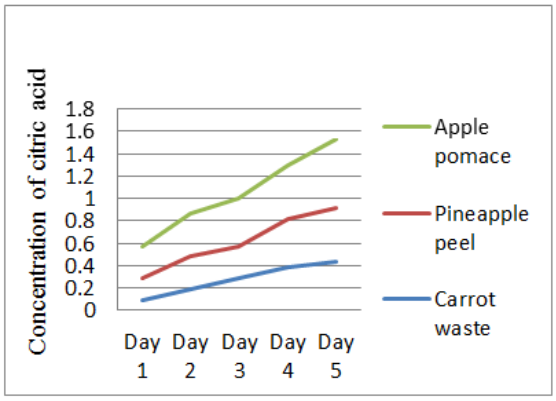

Figure 10. Effect of 1% w/v Ammonium nitrate on citric acid production.

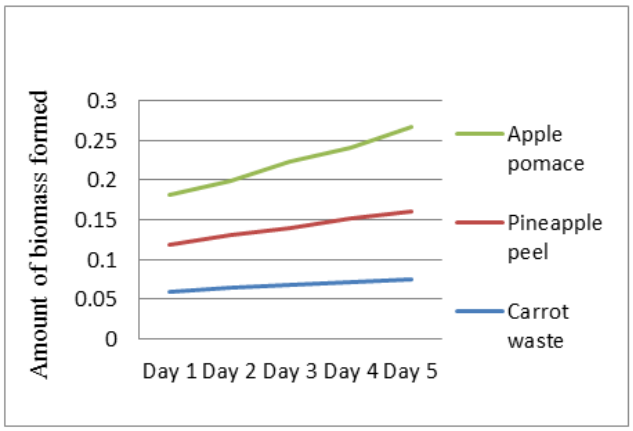

Figure 11. Effect of 0.25% w/v ammonium nitrate on biomass.

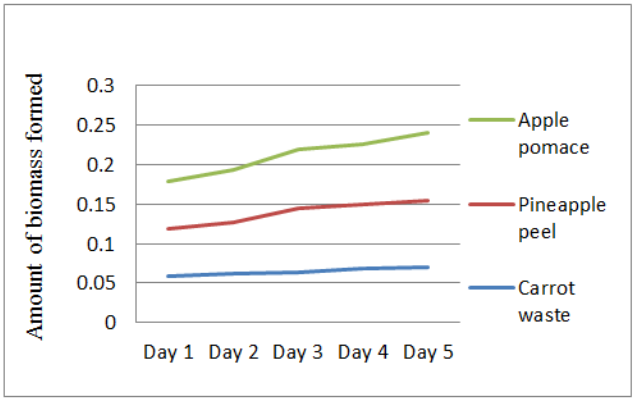

Figure 12. Effect of 1% w/v ammonium nitrate on biomass.

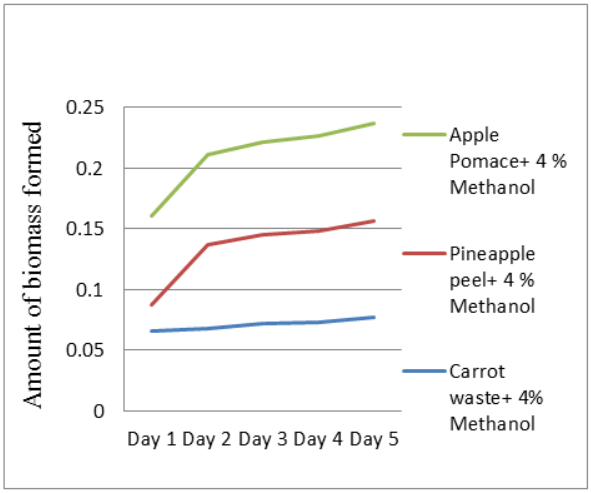

Figure 13. Effect of 4% methanol on biomass.

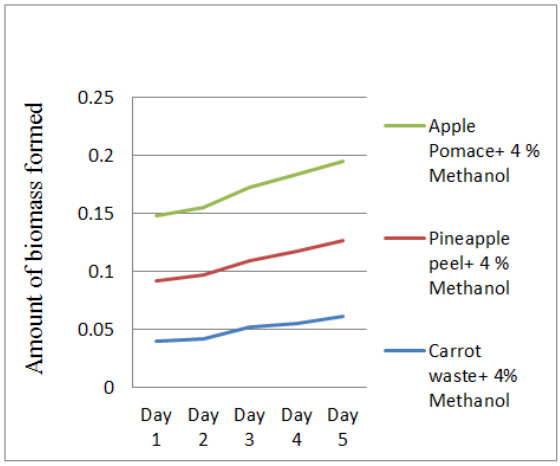

Figure 14. Effect of 4% isopropyl alcohol on biomass.

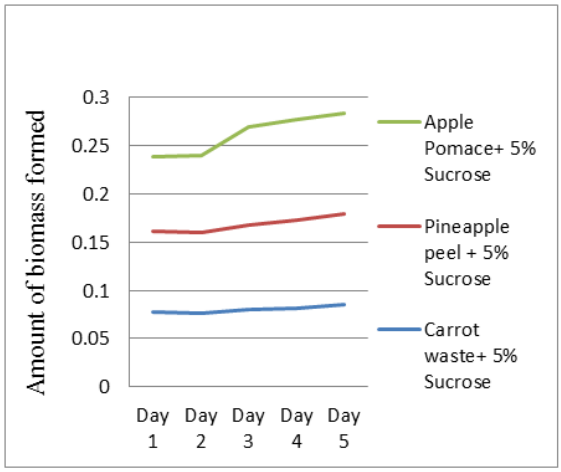

Figure 15. Effect of 5% Sucrose on biomass.

When nitrogen source was varied, maximum production was obtained in case of 0.25% NH_4NO_3 as compared with 1% NH_4NO_3.

The maximum yield 0.768 g/15 ml of citric acid was obtained on 5th day in case of apple pomace (Figure 9) with 0.25% NH_4NO_3.

The low production rate in case of 1% NH_4NO_3 (Figure 10) was due to increase in fungal growth and sugar concentration [2].

In case of Nitrogen source (Figures 11 and 12), low molecular weight alcohol (Figures 13 and 14) and carbon source (Figures 15 and 16), the biomass increased with the increase of incubation period due to the accumulation of the carbon by the cell [8].

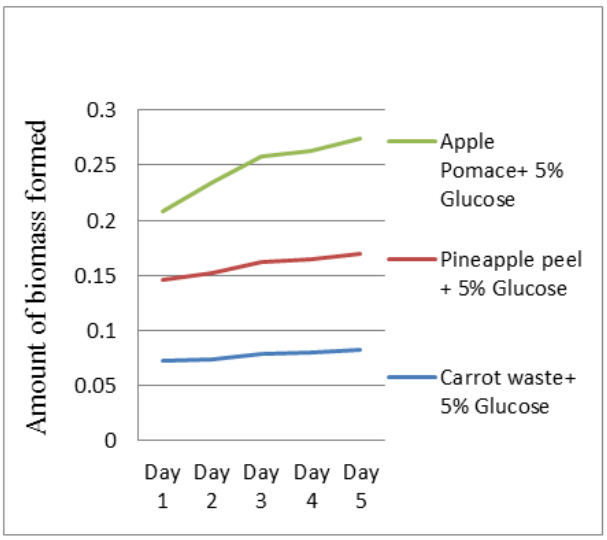

Figure 16. Effect of 5% glucose on biomass.

Conclusion

The study has revealed that food waste material can be used for citric acid production by SSF using *Aspergillus niger*. The maximum production at various parameters was obtained in case of Apple pomace followed by pineapple peel and carrot waste. The use of this waste might represent an efficient method of reducing the environmental problem and also help in the reduction of the substrate cost. The observation indicates that it might be possible to manipulate the morphology parameters in order to improve process yields.

Acknowledgement

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