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RESEARCH PAPER

Citric acid production from pre-treated sugarcane bagasse by *Aspergillus niger* under solid state fermentation

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Citric acid is one of the important commercially produced organic acid. The aim of the present work was to study the utilization of pre-treated sugar cane bagasse for citric acid production using *Aspergillus niger* under solid state fermentation. The maximum value of citric acid was observed in acid treated substrate followed by urea and heat respectively. Fermentation conditions were also optimized and maximum production of citric acid occurred when the pH, Initial moisture content and temperature of the fermentation medium were 5, 65 per cent and 30^{0}C, respectively. As a globally required organic acid for various industrial applications, citric acid can be produced at large scale by utilizing pre-treated agro residues such as sugarcane bagasse. Application of agro residues in the production of value added product can be a positive step towards agricultural waste management.

Key words : Citric acid, *Aspergillus niger*, Pre- treatment, Solid state fermentation

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INTRODUCTION

Citric acid is considered as having great commercialization potential because of its multiple uses, particularly in food and beverage industries as a flavour enhancer and antioxidant agent. It also has other industrial uses, such as in pharmaceutical, cosmetic, and various chemical industries (Heinzle *et al.,* 2007). Industrial production of citric acid is mainly carried out by microbial fermentation. A large number of microorganisms have been employed for citric acid production, but only a few of them can produce citric acid in industrial scale (Soccol *et al.,* 2006). *Aspergillus niger* is reported as almost exclusively used micro-organism for industrial scale production of citric acid (Lofty *et al.,* 2007). Solid-state fermentation is considered as better option for citric acid production because of lower energy requirement, higher product yield, low risk of bacterial contamination and environmental concerns regarding the generation of less waste water and disposal of solid-waste (Lu *et al.,* 1997).

Large amounts of sugarcane bagasse are produced world wide as a by-product of sugar industries. Many investigators have successfully utilized Sugarcane bagasse as a substrate for solid-state fermentation for citric acid production (Kumar *et al.*, 2003 and Vandenberghe *et al.,* 2000). In a country like India bagasse is mainly used as a fire fuel, so this agricultural waste can be easily used for citric acid production. The objective of the present work is to utilise pre- treated sugar cane bagasse as carbon source for citric acid production using *A. niger* under SSF.

Research Methodology

Collection of culture and substrate :

Pure culture of *Aspergillus niger* (MCCB0201) was collected from Department of Microbiology and Fermentation Technology, SHIATS, Allahabad. Culture was maintained on PDA media. Required quantity of Sugarcane bagasse was collected from local market of Allahabad. Bagasse was washed under running tap water and sun dried. Size of the substrate was reduced to 2-5 mm particle size by grinding in a grinder. Substrate was divided into four equal parts for pre-treatment.

Pre-treatment of substrate :

Substrate was subjected to acid, urea and heat pre-treatments. One part of the substrate was treated with dilute 1N HCl using a solid liquid ratio of 10 per cent (w/v), and pretreated in a water bath at 100°C for 1 hour, after cooling down, the substrate was washed with distilled water and dried in an oven at 100°C. For urea, pre-treatment substrate was soaked with 2 per cent (w/v) urea solution, using a solid-liquid ratio of 5 per cent (w/v), pre-treated in a water bath at 60°C for 1 hour, after cooling down, the substrate was washed with distilled water and dried in an oven at 100°C. For heat treatment, Substrate was mixed in distilled water using a solid liquid ratio of 10 per cent (w/v) and boiled at 100°C for 1 hour, after cooling down; the substrate was washed with distilled water and dried in an oven at 100°C.

Solid state fermentation :

The fermentation was carried out in 250 ml conical flask containing 5 grams of pre treated substrates and 10 ml of Prescott salt (NH_4NO_3, 2.23 g/l; K_2HPO_4, 1.00 g/l and $MgSO_4.7H_2O$, 0.23 g/l). The contents of flask were mixed thoroughly and autoclaved at 121 °C for 20 minutes. After cooling the flasks containing production media were inoculated with inoculum (10^6spores/ml) and incubated under static condition at 30°C for five days.

Extraction of citric acid :

After fermentation, crud extract containing citric acid was obtained. Citric acid was extracted from fermented substrate by adding 50 ml distilled water and shaking for 1 hour at 150 rpm at room temperature (28±1 °C). The supernatant was collected by filtering with Whatman number 1 filter paper. The concentration of citric acid in supernatant was estimated titrimetrically using 0.1 M NaOH and phenolphthalein indicator (AOAC, 1995).

Process optimization :

Fermentation process was optimized at different parameters to obtained maximum citric acid production from solid state fermentation.

Optimization of initial moisture content :

To evaluate the effect of initial moisture of substrate on citric acid production, fermentation was carried out at different moisture levels such as 50 per cent, 55 per cent, 60 per cent, 65 per cent, and 70 per cent. Moisture level was maintained by using Distilled water.

Optimization of initial pH :

Fermentation was carried out at different initial pH (3-8) for 120 hours and optimum pH for maximum citric acid yield was determined. pH was maintained with the help of HCl and NaOH solutions.

Optimization of fermentation temperature :

Temperature plays very critical role in microbial growth and metabolism. The influence of temperature on citric acid production was studied by carrying the fermentation at different temperatures ranging from 20°C to 60°C for 120 hours. Initial pH of the medium was near to neutral pH.

Optimization of fermentation period :

To determine the effect of fermentation period, fermentation was carried out for various time periods *i.e.* 24, 48, 72, 96, 120, 144, 168 hours at 30º C having initial pH of the medium neutral. All the flasks were incubated in a single BOD incubator and after every 24 hours of fermentation, amount of citric acid was determined.

Optimization of nitrogen sources :

Citric acid production was analyzed by supplementing fermentation medium with different nitrogen sources (0.03 g/g dry substrate) such as Ammonium sulphate, Yeast extract, Peptone and Urea. Each flask was supplemented with one individual supplement and after autoclave the fermentation was carried out and amount of citric acid was determined under the influence of different nitrogen sources.

Statistical analysis of results :

The results obtained were subjected to statistical analysis as Mean and Standard Deviation (SD) (Zar, 1984).

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Screening of pre treated sample for maximum production of citric acid :

After collection of substrate, pre treatments of substrate were performed using three methods of pre treatments *viz*., Acid, heat and urea along with control. After pre treatment fermentation was carried out for required period of time and the amount of citric acid was determination in crude supernatant. The maximum value of citric acid was observed in acid (2.109667±0.004 mg/g) treated substrate followed by urea (1.439±0.092 mg/g) and heat (1.067±0.087 mg/g), respectively. From Fig. 1 it is clear that acid treatment with dilute HCl almost doubled the production of citric acid. Maximum citric acid production was reported when dilute HCl treated wheat straw was fermented by *A. niger* under SSF (Khosravi *et al.,* 2008). Lee *et al.* (1999) also reported that acid treatment has become state of the art technology for pretreating any lignocellulosic biomass.

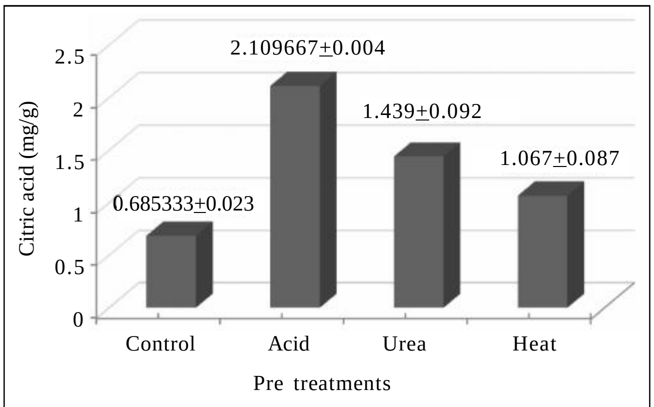

Fig. 1 :Production of citric acid using pre-treated sugarcane bagasse

Process optimization :

Fermentation process was optimized at different parameters to obtained maximum citric acid production from substrate. As acid treated substrate showed maximum citric acid production, it was further optimized at different process parameters to obtain optimum conditions for maximum production of citric acid.

Effect of initial pH :

Successful production of citric acid depends on maintenance of initial pH of the production medium. Fermentation was carried out at different pH (3, 4, 5, 6, 7, 8) and amount of citric acid was determined. Maximum amount of citric acid was observed at pH 5 (3.521± 0.265 mg/g) followed by at pH 7 (3.003667±0.121). Optimum pH range was found to be between 5- 7. Fig. 2 represents the effect of pH on citric acid production. Highest citric acid yield was reported the at pH 5 using Areca husk as solid substrate (Narayanamurthy *et al.,* 2008).

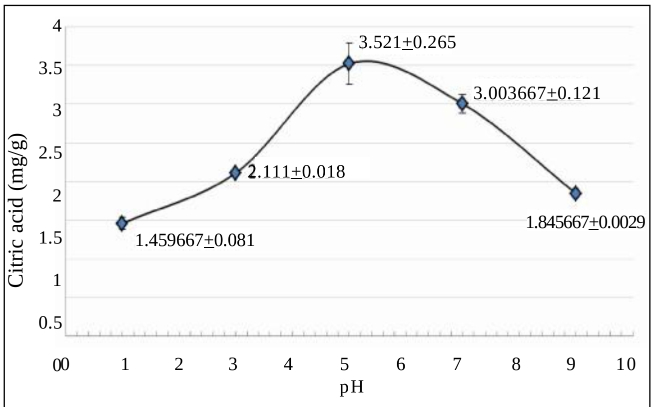

Fig. 2 :Effect of pH on citric acid production

Effect of temperature :

Temperature is a critical factor for citric acid production. Highest level of citric acid was achieved when fermentation was performed at 30°C. From Fig. 3, it was observed that 25°C to 35°C temperature is most suitable range for maximum cell growth and increased production of citric acid. Other researchers also reported 30°C as best temperature for citric acid production (Ali *et al.,* 2002; Kareem *et al.,* 2010 and Kim *et al.,* 2002). It was also reported that 30°C temperature is optimum temperature for citric acid production under submerged condition using *Aspergillus* species (Nwoba *et al.,* 2012).

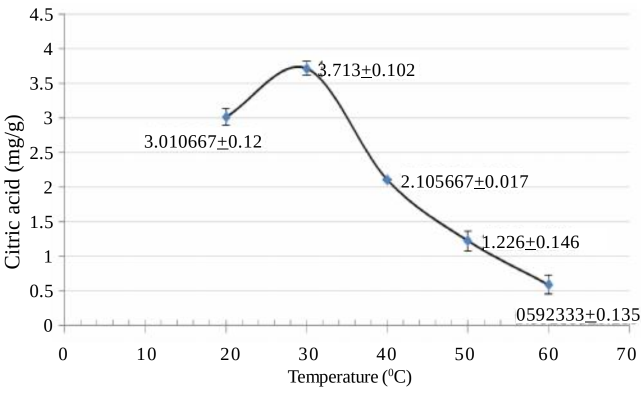

Fig. 3 :Effect of temperature on citric acid production

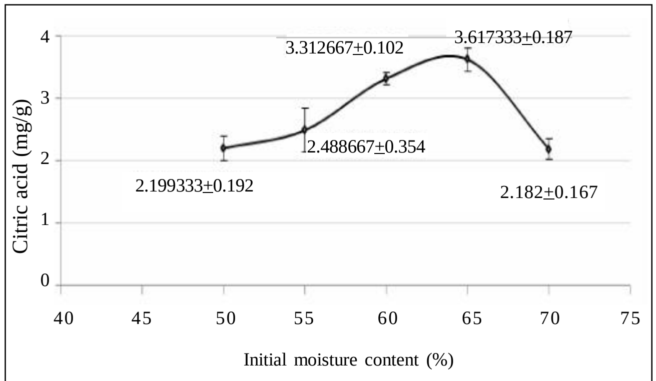

Fig. 5 : Effect of moisture on citric acid production

Effect of fermentation period :

Fermentation was carried out for different course of time to determine the optimum incubation time at which maximum citric acid is synthesized. From the results obtained it was obvious that 120 hours of fermentation time period was most favourable condition for maximum production of citric acid *i.e.* 3.572± 0.232 mg/g followed by 3.046 ±0.078 mg/g at 144 hours (Fig. 4). Other researchers also reported the 5 days of incubation period suitable for maximum citric acid yield using acid pre-treated wheat straw and pineapple waste, respectively under SSF (Khosravi *et al.*, 2008 and Kareem *et al.*, 2010).

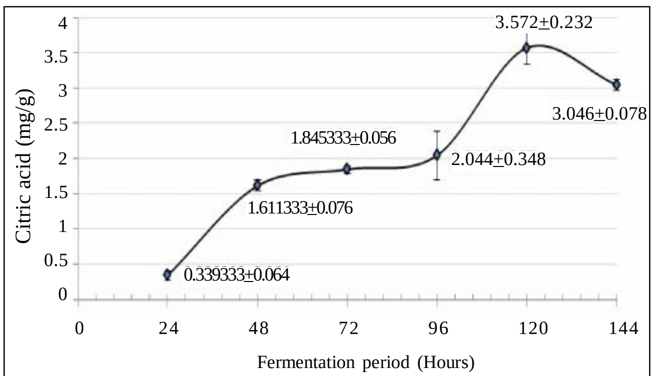

Fig. 4 :Effect of fermentation period on citric acid production

Effect of moisture content :

Varying moisture contents (50%, 55%, 60%, 65%, 70%) were examined for citric acid production (Fig. 5). Fermentation medium with 65 per cent moisture content was found most suitable for maximum product yield. 65 per cent initial moisture content has been reported suitable for fungal production of citric acid by *A. niger* using pineapple waste (Kareem *et al.*, 2010).

Effect of nitrogen source :

The effect of nitrogen sources on growth and citric acid yield was optimized using four different nitrogen sources like peptone, yeast extract, urea and Ammonium Sulphate. Maximum yield was observed with Ammonium Sulphate (5.543667±0.459 mg/g) followed by Yeast extract (3.512667±0.137), Peptone (3.045667±0.459) and Urea (2.618667±0.051). Graphical presentation of effects of nitrogen sources on total yield of citric acid has been expressed in Fig. 6. Ammonium sulphate has been reported as suitable nitrogen source for maximum citric acid production (Boominathan *et al.*, 2012).

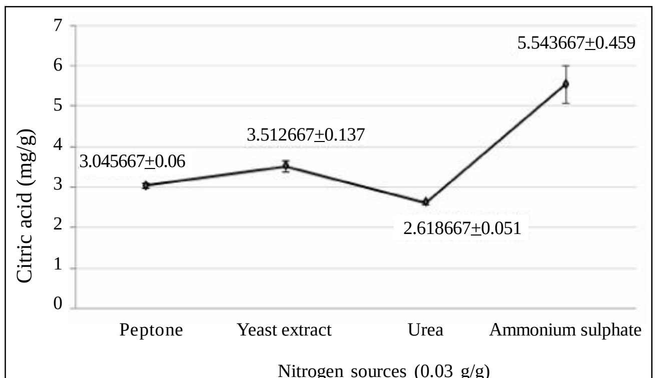

Fig. 6 : Effect of various nitrogen sources on citric acid yield

Conclusion:

The work was undertaken to investigate the efficiency of different pre treatments on sugarcane bagasse for production of citric acid under solid state fermentation by *A. niger.* On the basis of results obtained it can be concluded that as citric acid is a globally required organic acid with various industrial applications, it can be synthesized and produced at large scale by utilizing locally available agro residues and wastes such as sugarcane bagasse. SSF is a very efficient technique to be applied for such type of industrial setup. The problem of agro waste disposal and its impact on environment can also be reduced by using such type of wastes for the production of value added products.

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