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Research Article

ECO-FRIENDLY UTILIZATION OF CITRUS PEELS FOR CITRIC ACID PRODUCTION BY *ASPERGILLUS NIGER*

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Cost effective and eco-friendly solid state fermentation method has been developed for citric acid production from citrus fruits waste by *Aspergillus niger* by shake culture method. The solid substrate supplemented with different concentration of Carbon, nitrogen, methanol, temperature and shaker speed were optimized. With 10 % nitrogen supplement, the maximum yields of citric acid obtained were 38.16 %, 33.60 %, 27.84 % and 27.60 % from four different citrus peels waste. With 15% carbon supplements, the yields were 32 %, 29 %, 22% and 24 %. With the minimum concentration of 3 % methanol, the yields of citric acid were 27.12 %, 22.56 %, 26.88 % and 38.88 % and this is due to the high effect of methanol as an enhancer. These results indicate the use of citrus peels as inexpensively available medium for the production of commercially valuable organic acid by using *Aspergillus niger*.

INTRODUCTION

Citric acid is an important organic acid and it was first extracted from citrus fruits. Nowadays it is largely produced by fermentation. Citric acid is commercially used in foods, soft drinks, pharmaceuticals, leather tanning, electroplating etc. Citric acid is most commonly produced by *Aspergillus niger* (*A. niger*). *A. niger* is one of the most important fungi used in industrial microbiology and has been used for the commercial production of citric acid for many years (Schuster *et al.,* 2002). Citric acid is commercially produced by the fermentation of hydrated materials and by-products of sugar production by *A. niger* (Wang and Liu, 1996; Lesniak *et al.,* 2002). The worldwide demand for citric acid is increasing faster than its production and more economical processes are required for its production (Tran *et al.,* 1998 Alvarez-Vazquez *et al.,* 2000). Microbial growth and production of *A. niger* are strongly affected by the medium composition, fermentation parameters and stimulators. Thus, the scale up of citric acid production using *A. niger* can be improved by optimizing the fermentation conditions (Hang, and Woodams, 1987).

The important physico-chemical fermentation strategies influencing the growth of *A. niger* on a solid state and its production of citric acid are the solid substrate composition, moisture content, incubation temperature, power of hydrogen ions, fermentation time and inoculum density [Xu *et al.,* 1989]. Most fungi are known for better citric acid production under acidic pH ranging between 3 and 6, but some fungi are able to grow at pH below 2 to compete with bacteria (Fawole and Odunfa, 2003). Citric acid production is also known to be affected by inoculums and incubation time (Lee and Yun, 1999). Up to a specific limit, acid production generally increases with inoculums density (Kota and Sridhar, 1999).

The present study is based on the use of fruit peels as substrate for the production of citric acid. Citric acid is an intermediate in the TCA cycle and its accumulation is strongly influenced by the balance of nutrients (Kareem *et al.,* 2010). In general, the final concentration of citric acid increases as the initial concentration of the carbon source is increased. Also, citric acid production by *A. niger* also depends on presence of other nutrients such as nitrogen, phosphorous, potassium and other salts (Jianlong and Ping, 1998). The limitation or absence of nitrogen, phosphorus or other trace elements during the fermentation results in the limited growth of *A. niger* and to the enhancement of citric acid production (Mirminachi *et al.,* 2002). In addition to the basal nutrients to improve citric acid production, stimulators such as organic solvents and lipids can

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be applied in production of citric acid (Nampoothiri *et al.*, 2003).

MATERIALS AND METHODS

Microorganisms and inoculum preparation

Aspergillus niger was used for this study. Fungal cultures were maintained on Sabroud dextrose agar (Himedia, India) slants at 4 °C and sub-cultured. The sporulated culture on SDA slant was poured with 5.0 mL of sterile Tween80. Spore suspensions for seeding were obtained from Sabroud dextrose agar (SDA) slant culture incubated at 30 °C for 5 days.

Fermentation media

The basal medium was prepared using different fruit peels such as sweet lime, lemon, orange and pineapple peels weighing 10g into 250 ml Erlenmeyer flask. The production medium was supplemented with carbon, nitrogen at 5, 10, 15% w/v and 2, 4, 6% volume fraction of methanol. The production media were sterilized at 121 °C for 15 min. After cooling, each flask was inoculated with 0.1% of *A. niger* spore suspension. All flasks were placed into a shaker incubator at different temperature ranges such as 24, 26 and 28 °C for 5 days. After incubation, 10g of fermented substrate was diluted with 100 ml of distilled water and filtered using Whatman No. 1 filter paper. The filtrates were analyzed for citric acid estimation.

Optimization of fermentation parameters

The parameters for lemon, orange pineapple and sweet lime peels fermentation using *A. niger* were optimized. The effect of nitrogen sources, carbon sources, methanol concentration, incubation temperature, shaking time and inoculums supplement was noted.

Effect of nitrogen source

Ammonium sulphate was used as nitrogen sources at a concentration of 5 %, 10 % and 15 % for sweet lime, lemon, orange and pineapple and the experiment was carried out at 24, 26 and 28 °C.

Effect of shaker culture

The effect of shaker incubation was studied at 100, 150, 200 rpm for carrying out the fermentation of sweet lime, lemon, orange and pineapple peels at 24, 26 and 28 °C.

Effect of incubation temperature

Temperatures at 24, 26 and 28 °C were tested to find out the optimum temperature for citric acid production using sweet lime, lemon, orange and pineapple peels. For sweet orange peel, the fermentation media were prepared with a composition of substrate 10 % and varying concentration of carbon sources 5 %, 10 %, 15 % and KH_2PO_4 0.1 %. The production medium was inoculated with 2 % inoculum.

Recovery of citric acid

Citric acid was determined by titration using 0.1M NaOH and phenolphthalein as indicator and calculated as % according to the formula:

$$\%\ \text{Citric acid} = \frac{\text{Normality} \times \text{Volume of 0.1M NaOH} \times \text{Equivalent weight of citric acid} \times \text{Dilution factor}}{\text{Weight of sample (g)} \times 10}$$

RESULTS AND DISSCUSSION

Citric acid is an important organic acid and it was initially extracted from citrus fruits. Nowadays it is largely produced by microbial fermentation. Citric acid is commercially used in foods, soft drinks, pharmaceuticals, leather tanning, electroplating etc. *Aspergillus niger* is the most commonly used species for the production of citric acid. Tables 1-4 present the production of citric acid by *A. niger* on lemon, orange, pineapple, sweet lemon waste with various concentration of nitrogen (5, 10, 15 %). A minimum citric acid yield of 16.80 %, 17.28 %, 22.08 % and 20.40 % was obtained respectively. Maximum yields of citric acid 38.16 %, 33.60 %, 27.84 % and 27.60 % was obtained in 10 % nitrogen waste. Nitrogen had been reported to be an important factor in fermentation processes due to an increase in C/N ratio (Pandey, 2003). Javed *et al*. (2011) reported that all concentrations (0.1 to 0.6%) of ammonium sulphate, peptone and yeast extract used as a nitrogen source were found to increase fungal growth by sugar utilization and resulted in citric acid production. Ammonium sulphate is required for the growth of fungi and hence proved to be a good nitrogen source (Alben and Erkmen, 2004).

Effect of nitrogen concentrations on citric acid production

Table 1 Effect of nitrogen concentrations on citric acid production in lemon peel

Lemon	N (%)	Temperature	rpm	Yield (%)
SM-7	0.5	24 °C	100	38.16
SM-15	0.10	26 °C	150	16.80
SM-23	0.15	28 °C	200	30.72

Table 2 Effect of different nitrogen concentrations on citric acid production in orange peels

Orange	N (%)	Temperature	rpm	Yield (%)
SM-8	0. 5	24 °C	100	28.80
SM-16	0. 10	26 °C	150	33.60
SM-24	0.15	28 °C	200	17.28

Table 3 Effect of different nitrogen concentrations on citric acid production in pine apple peels

Pine apple	N (%)	Temperature	rpm	Yield (%)
SM-9	0. 5	24 °C	100	22.08
SM-17	0. 10	26 °C	150	27.84
SM-25	0.15	28 °C	200	27.36

Table 4 Effect of different nitrogen concentrations on citric acid production in sweet lemon peels

Sweet Lemon	N (%)	Temperature	rpm	Yield (%)
SM-10	0. 5	24 °C	100	23.52
SM-18	0. 10	26 °C	150	27.60
SM-26	0.15	28 °C	200	20.40

Effect of carbon concentration on citric acid production

Citric acid production by *A. niger* from different fruits peels as a fermentation media with the different concentrations of carbon sources was shown in Table 5.

Table 5 Effect of different carbon concentrations on citric acid production from lemon peels by *A. niger*

Lemon	Carbon	Temperature	rpm	Yield
SM-11	5%	24 °C	100	30.72
SM-19	10%	26 °C	150	24.00
SM-27	15%	28 °C	200	32.56

In this study, addition of carbon to fruits waste enhanced citric acid production than glucose.

Table 6 Effect of different carbon concentrations on citric acid production from orange peels by *A. niger*

Orange	Carbon	Temperature	rpm	yield
SM-12	5%	24 °C	100	20.72
SM-20	10%	26 °C	150	24.68
SM-28	15%	28 °C	200	29.84

Table 7 Effect of different carbon concentrations on citric acid production from pine apple peels by *A. niger*

Pine apple	Carbon	Temperature	rpm	Yield
SM-13	5%	24 °C	100	19.04
SM-21	10%	26 °C	150	21.91
SM-29	15%	28 °C	200	22.76

Table 8 Effect of different Carbon concentrations on citric acid production from sweet lemon peels by *A. niger*

Sweet lemon	Carbon	Temperature	rpm	Yield
SM-14	5%	24 °C	100	17.96
SM-22	10%	26 °C	150	15.60
SM-30	15%	28 °C	200	24.72

As can be seen in Table 5-8, the best yield by all fruits peel waste were 32 %, 29 %, 22%, 24 % which were obtained with 15% carbon supplements. Four isolates produced citric acid with the concentration of 18 to 42 g/L on 150g/L molasses sugar (Sikander *et al.,* 2002). The medium supplemented with sucrose (15 % w/v) gave the highest citric acid value (36.6 g/kg) (Kareem *et al.,* 2010). The minimum yields obtained at the concentration of 5 % as 24 %, 20.72 %, 19.04 % and 15.60 % of citric acid. In our results, 5% carbon source showed low % of citric acid production while 15 % showed better results. The citric acid production was directly proportionate to the increase of the carbon source and the maximum citric acid was obtained with 15% carbon supplement (Laboni *et al.,* 2010).

Effect of methanol concentration on citric acid production

Effect of methanol on citric acid production was shown in Table-9. Maximum citric acid production (38.88 g/kg) was obtained at 3 % concentration.

Table 9 Effect of different Methanol concentrations on citric acid production from lemon peels by *A. niger*

Lemon	Methanol	Temperature	rpm	Suspension	Yield
SM-2	2 %	30 °C	200	1 mL	13.60
SM-5	3 %	30 °C	200	1 mL	27.12
SM-31	4 %	30 °C	200	1 mL	16.08

Table 10 Effect of different Methanol concentrations on citric acid production from orange peels by *A. niger*

Orange	methanol	Temperature	rpm	Suspension	Yield
SM-4	2 %	30 °C	200	1 mL	18.96
SM-32	3 %	30 °C	200	1 mL	22.56
SM-36	4 %	30 °C	200	1 mL	15.6

Table 11 Effect of different Methanol concentrations on citric acid production from pine apple peels by *A. niger*

Pine apple	Methanol	Temperature	rpm	Suspension	Yield
SM-3	2 %	30 °C	200	1 mL	13.92
SM-33	3%	30 °C	200	1 mL	26.88
SM-37	4 %	30 °C	200	1 mL	18.24

Table 12 Effect of different Methanol concentrations on citric acid production from sweet lemon peels by *A. niger*

Sweet Lemon	Methanol	Temperature	rpm	Suspension	Yield
SM-1	2 %	30 °C	200	1 mL	6.50
SM-6	3 %	30 °C	200	1 mL	38.88
SM-38	4 %	30 °C	200	1 mL	22.56

The effects of methanol as an enhancer at various concentrations were also tested on all the four samples. Various concentrations of methanol were used 2, 3 and 4 % (v/v) and was added into the fermentation medium at the time of inoculation. Our results showed methanol to a good enhancer, the high yield of citric acid obtained was 27.12 %, 22.56 %, 26.88 %, and 38.88 % in 3 % methanol concentration. The entire three cases 3.0 % methanol gave better result than any other concentration.

CONCLUSION

In the present studies, the production of citric acid using citrus peels by *Aspergillus niger* has been reported. It is easy, economic, rapid, and eco-friendly way to produce citric acid and provides better results under optimum growth conditions. This method produced fairly high yield of citric acid and also it is an effective waste management method for the production of citric acid in the food and pharmaceutical industries.

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Conflict of Interest

None of the authors has any conflict of interest in submitting this manuscript.

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