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RESEARCH PAPER

Standardization of Process Parameters for Production of Citric Acid from *Mahua* Flowers (*Madhuca indica*) by Surface Fermentation using A*spergillus niger* NCIM-545 and NCIM-595

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Abstract

Madhuca indica commonly known as *mahua*, is a large tree which produces edible flowers rich in reducing sugars. In the present investigation suitability of *mahua* flowers as a substrate for production of citric acid was evaluated. The environmental parameters such as pH, temperature, incubation period, inoculum and nutritional parameters viz. initial sugar concentration, nitrogen source, and methanol contration affected the production of ciric acid using *Aspergillus niger* NCIM-545 and NCIM-595. The production of citric acid was optimum when fermentation was carried out at initial pH of 5.0, temperature of 30°C, incubation period of 168 h and inoculum level of 2.0 per cent for both the strains. Further it was observed that at sugar level of 14 per cent, yeast extract (0.07 per cent nitrogen level) and methanol (4 per cent), the citric acid production was optimum. At the optimised conditions the citric acid production was comparatively higher for *Aspergillus niger* NCIM-595 than NCIM-545.

Keywords: Mahua flowers, surface fermentation, *Aspergillus niger,* fermentation, citric acid

Madhuca indica (syn. *Bassia latifolia*) commonly known as *mahua,* is a large tree. The production potential of *mahua* flowers in India is 4.9 million tones, out of which only 0.85 million tones are collected. In India, in various parts of Andhra Pradesh, Maharashtra, Chhat isgarh, some tribal communities are cultivating and harvesting *mahua* flowers for preparation of alcoholic beverages using traditional methods (Yadhav *et al.* 2009). Beside this, these can be used for various value added products as detailed earlier (Patel and Naik, 2010). The *mahua* produces edible flowers which on dry weight basis contains reducing sugars in the range of 48-57%, non-reducing sugars 3-18%, and is also a good source of proteins and minerals like Ca, P, Fe and K, and vitamins like thiamine, riboflavin, niacin, folic acid and ascorbic acid (Awasthi *et al.* 1975; Church, 1986; Jaysree *et al.,* 1998). The *mahua* flowers have been found to contain 70 per cent reducing sugar as glucose without hydrolysis of the flowers (Mande *et al.* 1949). The syrup of extract of *mahua* flowers can be a good substitute for honey (Shrivastava *et al*. 1970).

The extraction of sugars has also been carried by autoclaving *mahua* flowers with water (Yadhav *et al.* 2009) and the flowers have been empreyed

for production of vinegar and alcohol. Since alcohol is intended for use as potable spirits (Anon, 1964). Not only this, dried *mahua* flowers have successfully been empreyed for production of alcohol, brandy, acetone lactic acid (Tiwari *et al*. 1980; Patel *et al.* 1982; Malavade and Jadhav, 2000; Swain *et al*. 2007 and Behera *et al*. 2010, Rupesh Singh *et al*. 2013). In another approach, bacterial synthesis of polyhydroxybutyrate-co-hydroxyvalerate using *mahua* flowers has been reported by Anil Kumar *et al*. 2007 while excellent bakery and confectionary goods, jam, jelly, sauce etc. using *mahua* flowers can also be prepared (Patel 2008).

Citric acid is one of the most commonly used organic acids in food and pharmaceutical industries. The food industry is the largest consumer of citric acid, using almost 70% of the total production, followed by about 12% for the pharmaceutical industry and 18% for other applications (Briffaud and Engasser, 1979a, b, Roehr *et al*. 1981, Berovic and Cimerman, 1982, Meers and Milsom, 1987, Milsom, 1987, Haq *et al*. 2001).

Its worldwide demand citric acid is about 6.0×10^5 tons per year (Karaffa and Kubicek, 2003) while its world annual production stood at approximately 16 lakh tones. Despite its wide application in food and pharmaceutical industries, citric acid is receiving little attention in the tropics. Commercial production of citric acid is generally carried out by submerged fermentation of sucrose or molasses using the filamentous fungus *A. niger* or synthetically from acetone or glycerol (Torres *et al*. 1998; Fernando *et al*. 2000; Adachi *et al*. 2003; Haq *et al*. 2004). Surface fermentation was the first fermentation system used for the production of citric acid on an industrial scale (Blain *et al*. 1979; Anderson *et al*. 1980; Jernejc *et al*. 1982 and Allen and Robinson, 1989).

Today, almost all the citric acid is produced by fermentation of strains (*A. niger*) in submerged and surface culture and depends strongly on an appropriate strain and on operational conditions such as aeration, type and concentration of the carbon source, nitrogen and phosphate limitation, pH, concentration of trace elements and the morphology of the producer organism (Khan and Shaukat, 1990; Qazi *et al*. 1990; Sakurai and Imai, 1992; Papagianni, 2007).

Many microorganisms have been evaluated for the production of citric acid including bacteria such as *Bacillus licheniformis, B. subtilis*, *Corynebacterium* spp. (Kapoor *et al*. 1983), fungi such as *A. niger*, *A. awamori*, *A. foetidus*, *Penicillium restrictum* (Mattey and Allan, 1990; Kubicek, 1998) and yeast such as *Candida lipolytica*, *C. intermedia* and *Saccharomyces cerevisiae* (Archer *et al*. 2001; Crolla and Kennedy, 2001 and Kamzolova *et al*. 2003). However, *A. niger* remained the organism of choice for citric acid production due to ease of its handling, ability to ferment a variety of cheap raw materials, and high yields (Schuster *et al*. 2002). Mutants of *Aspergillus niger* are being used for commercial production of citric acid (Jianlong *et al*. 2000).

A cost reduction in citric acid production can be achieved by using cheap agricultural wastes such as apple and grape pomace, orange peel, kiwi fruit peel, cotton waste, okara, soy-residue and cane molasses (Kiel *et al*. 1981; Hang and Woodams, 1986; 1987 & Khare *et al*. 1995). The basic substrates for citric acid fermentation using submerged technique of fermentation are beet or cane-molasses (Pazouki *et al.* 2000) and the *mahua* flowers could offer a potential substrate for the production of citric acid. They are the second most source of raw materials after sugarcane.

Looking in to the potential, the present investigation was undertaken to standardize the fermentation parameters and medium for the production of citric acid using *mahua* flowers by *Aspergillus niger* (NCIM-545) and (NCIM-595).

Materials and Methods

Materials

Dried *mahua flowers* (*Bassia latifolia*) were obtained from the farmer of Bhokar Dist. Nanded (Maharashtra). The flowers were dried and powdered and stored in a refrigerator for further investigation.

Two strains of *Aspergillus niger* NCIM-545 and NCIM-595 were procured from the Biochemical Science

Division, NCL, Pune (India). The cultures were grown for about 7 days at 30°C on PDA slants and preserved at 4°C, till use. The cultures were maintained by sub-culturing ones in every month to maintain good viability and stability. All the chemicals used were of analytical grade and were obtained from M/S, SD Fine Chemicals, Mumbai, India.

Analysis of Mahua flowers

The *Mahua* flowers were analysed for per cent moisture, crude protein, titratable acidity and total ash as per the standard procedures described (A.O.A.C., 1995). The total sugar content was determined by using the procedure described by Duboise (1956) while reducing sugars were estimated by using dinitrosalicylic acid (DNS) reagent method as described by Miller (1972). Total soluble solids of the extract were determined in terms of degree Brix by using hand refractometer. The pH of the medium was measured by using pH – meter and Citric acid as per the method described by Drysdale and McKay (1995). The Coefficient of conversion was calculated on the basis of reducing sugars utilized during fermentation by employing following formula.

$$\text{Coefficient of conversion (\%)} = \frac{\text{Citric acid (g/100ml)}}{\text{Sugar utilized (g)}} \times 100$$

Preparation of mother culture

The citric acid producing strains of *Aspergillus niger* NCIM-545 and NCIM-595 were grown on a potato dextrose agar slant at 30 °C for 7 days. A spore inoculum was prepared by adding 3 ml of sterilized distilled water to each slant and shaking for one minute. It was used for the preparation of mother culture as described by Hang and Woodams (1987).

Basal medium

The *mahua* flowers were ground and after adding ten times water heated at 80 °C for 10 min to obtain the extract with 18% total sugar. This extract was diluted to have 12% sugar and used as a basal medium for production of citric acid.

Production of citric acid

The citric acid was produced by surface fermentation as described by Singh *et al.* (1998).

Standardization of cultural and nutritional parameters

The basal medium for citric acid production was standardized for initial pH (4.0, 4.5, 5.0, 5.5, and 6.0), temperature (25, 30, 35 and 40°C), fermentation period (120, 144, 168, 192 and 216 h) and inoculum level (1.5, 2.0, 2.5, 3.0 and 3.5 per cent). The nutritional parameters were also optimized with respect to initial sugar concentration (12,14,16 and 18 per cent), different nitrogen sources such as yeast extract, urea and ammonium sulphate, keeping the nitrogen level, same (0.07 per cent), and also for different methanol levels (1.0, 2.0, 3.0 and 5.0 per cent).

Results and Discussion

Chemical composition of dry mahua flowers

The results showed that the dry *mahua* flowers contained 11.8 % moisture and 88.2 % dry matter. The dry matter consisted of appreciable quantities of both total sugar (58.2%) and reducing sugars (52.0%). The protein and ash content of 3.8 and 3.45%, were respectively. Earlier it was found that *mahua* produces edible flowers which on dry weight basis contain reducing sugars in the range of 48-57%, non-reducing sugars 3-18 %, and also a good source of proteins and minerals like Ca, P, Fe and K and vitamins like thiamine, riboflavin, niacin, folic acid and ascorbic acid as observed earlier (Awasthi *et al*. 1975 & Jayasree *et al.* 1998). Dried *mahua* flowers contained sucrose 2 per cent, invert sugar 52.6 per cent, ash 4.8 per cent, moisture 15 per cent and undetermined matter 12.6 per cent (Church, 1986).

Effect of cultural parameters on production of citric acid

pH

The initial pH level has been considered as a key factor in influencing the production of citric acid. The maximum citric acid concentration (6.40 and 6.80 g/100ml), conversion coefficient (66.67 and 72.03

per cent) and per cent yield (53.33 and 56.67) were obtained at pH 5.0 after 192 h of fermentation with *Aspergillus niger* NCIM-545 and NCIM-595, respectively (Table 1). The production of citric acid declined on increase as well as decrease of pH from 5.0. Which might be attributed to accumulation of oxalic acid at higher pH while lower pH could be inhibitory for growth of *Aspergillus niger* (Sikander and Haq, 2005 & Shadafza *et al.* 1976). The optimum production of citric acid was found in pH range of 4.5-7.0 from cane molasses by solid state fermentation and was obtained using *Aspergillus niger* as documentd earlier (Sikander and Haq, 2005).

Table 1: Effect of initial pH on citric acid production[a] by *Aspergillus niger* (NCIM-545) and (NCIM-595) from *mahua* flowers

pH	Sugar utilized (g/100ml)	Citric acid (g/100ml)	Coefficient of conversion (%)	Yield (%)
Aspergillus niger NCIM-545 and *Aspergillus niger* NCIM-595 (values in parenthesis)				
4.0	10.00 (10.08)	3.28 (3.20)	32.80 (31.75)	27.33 (26.67)
4.5	9.76 (10.00)	4.80 (4.96)	49.18 (49.60)	40.00 (41.33)
5.0	9.60 (9.44)	6.40 (6.80)	66.67 (72.03)	53.33 (56.67)
5.5	9.20 (8.80)	5.44 (5.60)	59.13 (63.64)	45.33 (46.67)
6.0	10.24 (10.24)	3.84 (4.00)	37.50 (39.06)	32.00 (33.33)

Values in parenthesis are for *Aspergillus niger* NCIM-595
[a] Conditions: Initial sugar concentration 12 %, Fermentation period 192 h

Temperature

The citric acid production increased significantly with the increase in fermentation temperature and was the highest at 30°C and thereafter it decreased (Table 2). The sugar utilization also increased slightly with the increase in fermentation temperature from 25 to 40°C.

The maximum citric acid concentration (6.64 and 6.88 g/100ml), conversion coefficient (68.03 and 71.67 per cent) and per cent yield (55.33 and 57.33) was obtained at a temperature of 30°C after 192 h of fermentation for *Aspergillus niger* NCIM-545 and NCIM-595, respectively. The biosynthesis of citric acid decreased at lower temperature which might be due to lower enzymatic activity, thus, impacting the of citric acid production.

Table 2: Effect of temperature on citric acid production[a] by *Aspergillus niger* (NCIM 545) and NCIM-595 from *mahua* flowers

Temperature (°C)	Sugar utilized (g/100ml)	Citric acid (g/100ml)	Coefficient of conversion (%)	Yield (%)
Aspergillus niger NCIM-545 and *Aspergillus niger* NCIM-595 (values in parenthesis)				
25	9.20 (9.76)	4.40 (4.64)	47.83 (47.54)	36.67 (38.67)
30	9.76 (9.60)	6.64 (6.88)	68.03 (71.67)	55.33 (57.33)
35	10.40 (9.76)	5.44 (5.76)	52.31 (59.02)	45.33 (48.00)
40	10.80 (11.12)	3.60 (3.84)	33.33 (34.53)	30.00 (32.00)

Values in parenthesis are for *Aspergillus niger* NCIM-595
[a] Conditions: Initial sugar concentration 12 %, initial pH 5.0 and fermentation period 192 h

The decreased biosynthesis of citric acid was also observed when temperature of medium was increased above 30°C which might be due to denaturation of enzyme citrate synthase of micro-organisms at higher temperatures, and may also be due to accumulation of other by products such as oxalic acid and enzyme catabolite repression (Haq *et al.* 2004). Similar findings for citric acid production in submerged fermentation using cane molasses by *Aspergillus niger* GCMC-7 have also been reported. The maximum citric acid concentration at 30°C temperature was obtained from carob pod by solid state fermentation using *Aspergillus niger* ATCC-9142 (Roukas, 1999)

Fermentation period

The maximum citric acid concentration (6.80 and

7.04 g/100ml), coefficient of conversion (70.83 and 72.13 per cent) and per cent yield (56.67 and 58.67) were observed at 168 h for *Aspergillus niger* NCIM-545 and NCIM-595, respectively. The concentration of citric acid increased with increase in the fermentation time upto 168 h and thereafter, there was a decline in critic acid production by *Aspergillus niger* NCIM-545 and NCIM-595 (Table 3).

Table 3: Effect of fermentation period on citric acid production[a] by *Aspergillus niger* NCIM-545 and NCIM-595 from *mahua* flowers

Time period (h)	Sugar utilized (g/100ml)	Citric acid (g/100ml)	Coefficient of conversion (%)	Yield (%)
Aspergillus niger NCIM-545 and *Aspergillus niger* NCIM-595 (values in parenthesis)				
120	6.80 (6.88)	3.20 (3.36)	47.06 (48.84)	26.67 (28.00)
144	7.60 (7.92)	4.08 (4.40)	53.68 (55.56)	34.00 (36.67)
168	9.60 (9.76)	6.80 (7.04)	70.83 (72.13)	56.67 (58.67)
192	10.00 (10.08)	6.48 (6.40)	64.80 (63.49)	54.00 (53.33)
216	10.40 (10.24)	5.84 (4.08)	56.15 (39.84)	48.67 (34.00)

Values in parenthesis are for *Aspergillus niger* NCIM-595

[a] Conditions: Initial sugar concentration 12%, initial pH 5.0 and fermentation at 30°C

Table 4: Effect of inoculum level on citric acid production[a] by *Aspergillus niger* NCIM-545 and NCIM-595 from *mahua* flowers

Inoculum level (%)	Sugar utilized (g/100ml)	Citric acid (g/100ml)	Coefficient of conversion (%)	Yield (%)
Aspergillus niger NCIM-545 and *Aspergillus niger* NCIM-595 (values in parenthesis)				
1.5	9.76 (9.44)	6.08 (6.24)	62.30 (66.10)	50.67 (52.00)
2.0	9.60 (9.76)	6.88 (7.12)	71.67 (72.95)	57.33 (59.33)
2.5	9.84 (9.92)	6.24 (6.40)	63.41 (64.52)	52.00 (53.33)
3.0	10.00 (10.08)	5.60 (5.44)	56.00 (53.97)	46.67 (45.33)
3.5	10.24 (10.40)	4.80 (4.88)	46.88 (46.92)	40.00 (40.67)

Values in parenthesis are for *Aspergillus niger* NCIM-595

[a] Conditions: Initial sugar concentration 12 %, initial pH 5.0, fermentation at 30°C and fermentation time 168 h.

In the batch fermentation of citric acid, the production starts after a lag phase and reaches maximum at the onset of stationary phase or late exponential phase. Further increase in incubation period did not however enhance citric acid production which might be due to the aged of fungus, decreased availability of nitrogen in the fermentation medium and depletion of sugar contents in the culture broth (Arzumanov *et al*. 2000).

The optimum production of citric acid has been reported in 144 h fermentation period (Sikander and Haq 2005). Slightly higher optimum fermentation period in this experiment may be due to use of different substrate and the mold strain

Inoculum level

The maximum citric acid concentration (6.88 and 7.12 g/100ml) was obtained with 2.0 per cent inoculum level for both the strains of *Aspergillus niger* (Table 4). The increased inoculum size above 2.0 per cent showed a significant reduction in citric acid production and also lowered coefficient of conversion and per cent yield. The inoculum size of 1.0 per cent has been optimum for citric acid production from cane molasses by using *Aspergillus niger* GCBT-7 (Sikandar and Haq, 2005).

Effect of nutritional parameters on production of citric acid

Concentration of Sugar

The effect of initial sugar concentration was that the maximum amount of citric acid (7.28 and 7.47 g/100ml) was obtained in the medium containing 14 per cent sugar, while the consumption of sugar was 10.08 and 9.99 g/100ml and per cent yield was 60.67 and 62.22 for *Aspergillus niger* NCIM-545 and NCIM-595, respectively (Table 5).

Table 5: Effect of sugar concentration on citric acid production[a] by *Aspergillus niger* NCIM-545 and NCIM-595 from *mahua* flowers

Sugar concentration (%)	Sugar utilized (g/100ml)	Citric acid (g/100ml)	Coefficient of conversion (%)	Yield (%)
Aspergillus niger NCIM-545 and *Aspergillus niger* NCIM-595 (values in parenthesis)				
10	8.67 (9.00)	5.73 (6.33)	66.15 (70.37)	47.78 (52.78)
12	9.60 (9.76)	6.88 (7.12)	71.67 (72.95)	57.33 (59.33)
14	10.08 (9.99)	7.28 (7.47)	72.22 (74.77)	60.67 (62.22)
16	12.80 (13.01)	6.40 (6.61)	50.00 (50.82)	53.33 (55.11)
18	15.60 (15.84)	4.92 (5.16)	31.54 (32.58)	41.00 (43.00)

Values in parenthesis are for *Aspergillus niger* NCIM-595

[a] Conditions: Initial pH 5.0, fermentation at 30°C, inoculum level 2.0 % and fermentation time 168 h.

However, reduction in citric acid production was observed when the initial sugar concentration of fermentation medium was increased above 14% which may be due to the higher growth of mycellium (Mat ey and Allan, 1990), resulting in increased viscosity of the medium (Haq *et al*. 2004). It is reported earlier that out that a sugar concentration higher than 16-18 % leads to a greater amount of residual sugars and lower yields of citric acid due to accumulation of oxalic acid in the culture broth (Pazouki *et al.* 2000). Citric acid accumulation is strongly influenced by the type and concentration of carbon source (Roukas and Kotzekidou, 1987; Soccol and Vandenberghe, 2003). The presence of carbohydrates which are rapidly taken up by microorganisms has been found essential for a good production of citric acid (Yokoya, 1992). Among the easily metabolized carbohydrates, sucrose is the most favourable carbon source followed by glucose, fructose and galactose (Dasgupta *et al*. 1994; Vandenberghe *et al*. 1999). Hossain *et al.* (1984) explained that the nature of sugar source has a marked effect on citric acid production by *A. niger*. Moreover, it is the traditional commercial substrate for citric production although glucose, fructose and maltose have also been used as substrates for citric acid production (Xu *et al*. 1989). Further, sucrose is of relatively low molecular weight and is readily transported into microbial cells for hydrolysis by intracellular enzymes (Drysdale and McKay, 1995). Kubicek (1998) reported that the final yield of citric acid in fermentation by *A. niger* was strongly dependent on the type and concentration of carbon source.

Nitrogen source

The maximum citric acid concentration (8.96 and 9.33 g/100ml), coefficient of conversion (76.80 and 78.13 per cent) and per cent yield (74.67 and 77.78) was obtained by using yeast extract as a nitrogen source for *Aspergillus niger* NCIM-545 and NCIM-595, respectively (Table 6). Kristicsen and Sinclair (1979) using continuous culture concluded that nitrogen limitation was necessary for citric acid production. Nitrogen sources have profound effect on citric acid production because nitrogen is not only important for metabolic rates in the cells but it is also basic part of cell proteins.

Its production is directly influenced by the concentration and nature of the nitrogen source. Physiologically, ammonium salts, such as urea, ammonium nitrate and sulphate, peptone, malt extract, *etc*. are preferred (Grewal and Karla, 1995 and Vandenberghe *et al*. 1999) as their consumption leads to pH decrease, which is essential for the citric fermentation. The concentration of nitrogen source required for citric acid fermentation range from 0.1 to 0.4 g/L. (Yokoya, 1992). High nitrogen concentrations increased fungal growth and sugar consumption but decreased the amount of citric acid produced (Grewal and Karla, 1995).

Nitrogen is considered as an important factor in fermentation processes due to an increase in C/N ratio (Pande, 2003). The fermentation medium for citric acid biosynthesis should consist of substrates necessary for the growth of microorganism, primarily the carbon,nitrogen and phosphorus sources (Kubicek and Rohr, 1986).

Methanol level

The maximum concentration of citric acid (9.80 and

Table 6: Effect of nitrogen sources on citric acid production[a] by *Aspergillus niger* NCIM-545 and NCIM-595 from *mahua* flowers

Nitrogen source	Nitrogen level (%)	Sugar utilized (g/100ml)	Citric acid (g/100ml)	Coefficient of conversion (%)	Yield (%)
Control	—	10.08 (9.99)	7.28 (7.47)	72.22 (74.77)	60.67 (62.22)
Yeast Extract	0.07	11.67 (11.95)	8.96 (9.33)	76.80 (78.13)	74.67 (77.78)
Urea	0.07	11.39 (11.48)	8.40 (8.68)	73.77 (75.61)	70.00 (72.33)
Ammonium Sulphate	0.07	10.73 (10.92)	7.93 (8.21)	73.91 (75.21)	66.11 (68.44)

Values in parenthesis are for *Aspergillus niger* NCIM-595

[a] Conditions: Initial sugar concentration: 14 %, initial pH 5.0, fermentation at 30°C, inoculum level 2.0 % and fermentation time 168 h.

10.27 g/100ml), coefficient of conversion (83.33 and 87.30 per cent) and per cent yield (81.67 and 85.56) were observed at methanol concentration of 4 per cent (v/w) for *Aspergillus niger* NCIM-545 and NCIM-595, respectively (Table 7). The citric acid production is found to be increased with increase in methanol concentration in the medium up to 4 per cent and thereafter, the citric acid production was reduced for both strains of *Aspergillus niger.*

Table 7: Effect of methanol level on citric acid production[a] by *Aspergillus niger* NCIM-545 and NCIM-595 from *mahua* flowers[b]

Methanol level (%)	Sugar utilized (g/100ml)	Citric acid (g/100ml)	Coefficient of conversion (%)	Yield (%)
Control	10.08 (9.99)	7.28 (7.47)	72.22 (74.77)	60.67 (62.22)
1	10.17 (10.27)	7.37 (7.75)	72.48 (75.45)	61.44 (64.56)
2	10.27 (10.73)	7.65 (8.12)	74.55 (75.65)	63.78 (67.67)
3	11.20 (11.67)	8.59 (8.87)	76.67 (76.00)	71.56 (73.89)
4	11.76 (11.76)	9.80 (10.27)	83.33 (87.30)	81.67 (85.56)
5	11.57 (11.67)	8.77 (8.87)	75.81 (76.00)	73.11 (73.89)

Values in parenthesis are for *Aspergillus niger NCIM*-595

[a] Conditions: Initial sugar concentration: 14%, initial pH 5.0, fermentation at 30°C, inoculum level 2.0%, Yeast extract 0.6% and fermentation time 168 h.

The addition of methanol in medium has been found to retard growth, delay sporulation and increase citric acid yields is an earlier study (Haq *et al.* 2003). It has a stimulatory effect on the biosynthesis of citric acid in fermented broth. The addition of low molecular weight alcohol to the medium increases the tolerance level of trace metals during the fermentation. Methanol markedly depressed the synthesis of cell protein in the early stages of the cultivation and also increased the metabolic activity of the enzyme citrate synthase (Hang and Woodams, 1984).

The *Aspergillus niger* NRRL-567 has alos produced the greatest amount of citric acid from apple pomace in the presence of 4 per cent methanol (Kumar *et al*. 2003) while its optimum production by solid state fermentation was obtained using sugar cane bagasse by *Aspergillus niger* at 6 per cent methanol level (Moyer, 1953). The presence of methanol (2%) in fermentation media may increase citric acid production by *A. nige*r (Hossain *et al.* 1984). The inductive effect of methanol for citric acid production may be due to reduction of the inhibitory effects of metal ions (Kiel *et al*. 1981). The use of low molecular weight alcohols i.e. methanol, isopropanol as adjuncts to the culture medium are help found the greatly to increase citric acid production in both surface and submerged cultures (Moyer, 1953).

Conclusion

The production of citric acid from *mahua* flowers was found to be affected by cultural and nutritional

parameters such as pH, temperature, time, inoculum level and nutritional parameters such as initial sugar concentration, nitrogen source, and methanol level by *Aspergillus niger* NCIM-545 and NCIM-595. It was found that production of citric acid was optimum when fermentation was carried out at an initial pH of 5.0, temperature of 30°C, time period of 168 h, inoculum level of 2.0 per cent and sugar level of 14 per cent, yeast extract (0.07 per cent nitrogen level), and methanol (4 per cent) were used as nutrients in media for both the strains. Further, the citric acid production was slightly higher for *Aspergillus niger* NCIM-595 than NCIM-545.

It could be concluded that the *mahua* flowers can be utilized and converted into citric acid which otherwise may remain unutilized and go waste due. It however needs further studies for standardization and pilot scale production of citric acid also economical feasibility for making this as a viable alternative for utilization of *mahua* flowers.

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