ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 11 (1): 44-49, 2012
ISSN 1682-296X / DOI: 10.3923/biotech.2012.44.49

Improved Citric Acid Production by Radiation Mutant *Aspergillus niger* Using Sugarcane Bagasse Extract

[1]Abdullah-Al-Mahin, [2]A.B.M. Sharifuzzaman, [1]M.O. Faruk, [3]M.A. Kader,
[2]J. Alam, [4]R. Begum and [1]Harun-Or-Rashid
[1]Institute of Food and Radiation Biology, Atomic Energy Research Establishment,
P.O. Box 3787, Dhaka 1000, Bangladesh
[2]Department of Genetic Engineering and Biotechnology,
Shahjalal University of Science and Technology, Sylhet, Bangladesh
[3]The Centre for Environmental Risk Assessment and Remediation, Mawson Lakes Campus,
University of South Australia, Mawson Lakes, South Australia-5095, Australia
[4]Bioscience Division, Bangladesh Atomic Energy Commission, Paramanu Bhaban, E-12/A,
Agargaon, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh

Abstract: Due to huge demand of citric acid, attempts are taking to introduce its efficient production either by using low cost substrates or by improving the potency of the fermentation microorganisms. In this study, sugarcane bagasse extract was used for citric acid production using wild type *Aspergillus niger* CA16 and its radiation mutant 79/20 by submerged fermentation. Fermentation was carried out up to 15 days using 5, 10, 15 and 20% of sugarcane bagasse extract which contained 21.06, 32.60, 43.50 and 53.20 g L^{-1} sugar, respectively. The fermentation medium was supplemented with prescott salt. With the increasing concentration of baggase extract, total titratable acidity and citric acid production was increased. Moreover, radiation mutant *A. niger* 79/20 had higher citric acid production than *A. niger* CA16. Maximum amount of citric acid (12.81 g L^{-1}) was produced in the 20% bagasse extract medium by *A. niger* 79/20, whereas, CA16 produced 10.25 g L^{-1} citric acid in the same fermentation medium. Maximum substrate uptake, growth yield co-efficient and productivity were also found higher in case of the strain 79/20. Thus, radiation mutation induced improved citric acid production in *A. niger* 79/20.

Key words: Radiation mutation, *Aspergillus niger*, bagasse extract, citric acid

INTRODUCTION

Citric acid is one of the most commonly used carboxylic acid intermediary of metabolism in most plants and animals. Due to its inoffensive nature, citric acid is extensively used in food preparations, pharmaceuticls and cosmetics. About 70% citric acid is used in food industry and remaining 30% in other industries (Dhillon *et al*., 2010). It also acts as an antioxidant synergist in fatty foods. Nowadays, citric acid is also increasingly utilized as a monomer for the manufacturing of eco-friendly polymers which are extensively used in various medical applications (Yang *et al*., 2004; Namazi and Adeli, 2005; Djordjevic *et al*., 2009; Naeini *et al*., 2010). As this organic acid has received the status of "Generally Recognized as Safe" (GRAS), utilization of citric acid is expanding in biomedicine, biopolymer production and various other applications. Considering high consumption rate and slight increase in price, the market value for this commodity chemical was expected to exceed $2 billion in 2009 (Partos, 2005).

A large number of microorganisms including bacteria, such as, *Arthrobacter paraffinens*, *Bacillus licheniformis, Corynebacterium* ssp., fungi, such as, *Aspergillus niger*, *A. aculeatus*, *A. carbonarius*, *A. awamori*, *A. foetidus*, *A. fonsecaeus*, *A. phoenicis*, *Penicillium janthinellum* and yeasts, such as, *Candida tropicalis*, *C. oleophila*, *C. guilliermondii*, *C. citroformans*, *Hansenula anamola* and *Yarrowia lipolytica* have been employed for citric acid production (Grewal and Karla, 1995; Kubicek and Rohr, 1986; Pandey *et al*., 2001; Vandenberghe *et al*., 1999; Maryam *et al*., 2011a, b; Xie and West, 2007a, b; Afifi, 2011; Lodhi *et al*., 2001; Ali *et al*., 2001; Mazhar *et al*., 2003). However, the fungus *A. niger* has remained the leading candidate for commercial production

Corresponding Author: Abdullah-Al-Mahin, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, P.O. Box 3787, Dhaka 1000, Bangladesh

of citric acid because of its higher productivity. Recently, many (natural or mutant) *Y. lipolytica* strains have also been reported to produce citric acid using various substances. A major disadvantage of producing citric acid by yeasts is the simultaneous formation of isocitric acid. In contrary, application of *A. niger* has many advantages, i.e., easy to handle, can ferment a variety of cheap raw materials, give high yields (Soccol *et al*., 2006).

Citric acid is one of the most widely used organic acid which is produced by using fermentation technology on an industrial scale. Large-scale production of citric acid has been carried out solely by the *A. niger* in submerged fermentation on beet or cane molasses, sucrose or glucose syrup. In recent years, solid state fermentation process has gained global attention as an alternative to submerged fermentation (Soccol *et al*., 2006). Low-cost agro-industrial wastes can be utilized to produce different value added products by solid state fermentation. Thus, the process saves money as well as solves environmental problems. This method can also be adopted to produce citric acid using less expensive raw materials. Large amounts of sugarcane bagasse are produced world wide as a by-product of sugar industries. In our country, bagasse is mainly used as fire fuel. Previously, we reported a successful production of citric acid from sugarcane bagasse in presence of 14% sucrose by solid state fermentation with both wild type and radiation mutant *A. niger* strains (Abdullah-Al-Mahin *et al*., 2008). In this study, we aimed to find out the feasibility of using sugar cane bagasse for citric acid production by submerged fermentation.

MATERIALS AND METHODS

Analysis of the substrate used: In this study, Sugarcane baggase, collected from Rajshahi Sugar Mill, was used as substrates for citric acid production by submerged fermentation. Total Reducing Sugars (TRS) of the substrate were determined as glucose using the Nelson (1944). Fermentable Reducing Sugars (FRS) and Nonfermentable Reducing Sugars (NRS) were determined by the method of Saeman *et al*. (1945). Here, potential FRS and NRS are closely related to the contents of cellulose and hemicellulose, respectively. The lignin was gravimetrically estimated by Mooreand Johnson (1967). Moisture, protein, lipid, ash and fibre contents were determined according to the methods described in AOAC (1980).

Microorganisms used: Two citric acid-producing strains of *A. niger* were used in the present study. The strain CA16 was used as the original parent strain which was a natural isolate from local soil (Abdullah-Al-Mahin *et al*., 2008; Vandenberghe *et al*., 1999; Hannan, 1972; Hannan *et al*., 1973). Another strain of *A. niger*, designated as 79/20, was the second step radiation mutant of *A. niger* CA16 (Abdullah-Al-Mahin *et al*., 2008; Hannan *et al*., 1973). The strains were revived onto potato dextrose broth medium (PDB) for 7-8 days at 30±1°C and 200 rpm. The microorganisms were maintained on Potato Dextrose Agar (PDA) plates for 96 h at 30±1°C and stored at 4±1°C in a refrigerator for future use. The cultures were renewed every four weeks.

Citric acid production medium: Collected sugarcane bagasse was sundried, cut into small pieces, grounded and screened to collect a particle size of about 1.2-1.6 mm. Bagasse powder was then taken in 500 mL Erlenmeyer flasks and mixed with distilled water to make a concentration of 5, 10, 15 and 20% and allowed to soak for 2 h. The suspensions were then filtered through thin cloth and supplemented with Prescott salt (NH_4NO_3, 2.23 g L^{-1}; K_2HPO_4, 1.00 g L^{-1} and $MgSO_4.7H_2O$, 0.23 g L^{-1}). The pH of the medium was adjusted to 5.0, as was suggested by Begum and Chowdhury (2000), for better citric acid production by the studied *A. niger* strains.

Submerged fermentation: One hundred fifty milliliter of liquid substrate was dispensed in a 500 mL Erlenmeyer flask and thoroughly mixed and autoclaved at 121±1°C for 30 min (Fig. 1). The substrates were inoculated with spore suspension of 1×10^7 spores/25 mL. After thorough mixing, Erlenmeyer flasks and their contents were incubated

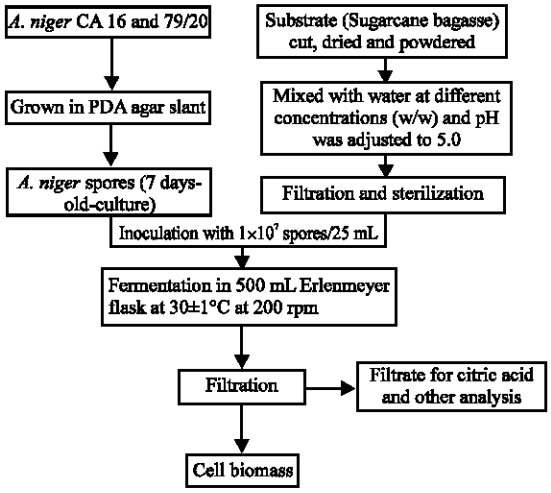

Fig. 1: Flow sheet of fermentation process for the production of citric acid by *A. niger* CA16 and 79/20

in a shaker at 30±1°C at 200 rpm for 15 days (Papagianni, 2007). Unless specified otherwise, these fermentation conditions were maintained throughout the study. All the experiments were conducted in duplicates.

Fermentation analysis: Sugar concentrations at different interval of fermentation were determined according to the method described by Morse (1947) using sucrose as standard. Total Titratable Acid (TTA) values in the media were determined against freshly prepared 0.1N NaOH. Citric acid concentrations were determined spectrophotometrically by the modified method of Marier and Boulet (1958). Appropriately diluted 1 mL sample of fermented filtrate was taken in a test tube and 1.3 mL pyridine was added. The contents were mixed manually by swirling and 5.7 mL acetic anhydride was added to the test tubes. The contents were again mixed by swirling and immediately placed in a constant-temperature (22°C) water bath and incubated for 30 min. The optimal density was recorded at 420 nm with the blank set on 100% transmission. Citric acid concentration was determined by referring to a standard curve for citric acid concentration. Citric acid concentrations were expressed as grams per liter (g L^{-1}).

RESULTS

Analysis of sugarcane bagasse: Total reducing sugar, fermentable reducing sugar, non-fermentable reducing sugar and lignin of sugarcane baggase were 68, 43.5, 20.2 and 24%, respectively. The amounts of moisture, protein, lipid, ash and fibre of the agricultural waste were 45, 1.4, 0.37, 2.3 and 48.5%, respectively (Table 1).

Production of citric acid by *A. niger* CA16: Table 2 shows the production of citric acid by *A. niger* CA16. With the increase of incubation period sugar concentration in the medium was reduced and maximum reduction was found on day 15. Maximum utilization of sugar was detected in case of 20% sugarcane bagasse extract on 15th day. pH values were also decreased with the increase of fermentation period and maximum decrease was recorded in case of 20% bagasse extract medium.

However, TTA value and citric acid production were increased with the increase of time. These values were found just proportional to the utilization of sugar in the fermentation media. Most of the cases, the highest TTA value was obtained on the 12th day. In case of 5% bagasse extract, the highest TTA was obtained on the 7th day. Like TTA values, maximum citric acid production values for 10, 15 and 20% bagasse extract media were detected on the 12th day and for 5% bagasse extract on 7th day of fermentation. After that, these values tended to decrease again. Accumulations of citric acid by the strain CA16 in 5% bagasse extract medium on the 4th, 7th, 12th and 15th day of fermentation were 2.50, 4.65, 3.84 and 2.46 g L^{-1}, respectively (Table 2). In 10% bagasse extract, sugar were estimated periodically. Here, also maximum utilization was detected in case of 20% sugarcane bagasse extract on 15th day (Table 3). However, if we compare the sugar utilization by CA16 and 79/20 (Table 2, 3), we find that 79/20 was more potential in utilizing sugar from all the studied bagasse extract media.

As expected, the decrease in pH during fermentation by the strain *A. niger* 79/20 (Table 3) was higher than that of CA16 (Table 2). Lowest pH was reached after 15 days of fermentation and the values were 4.0, 4.2, 3.25 and 3.3 for the media with 5, 10, 15 and 20% bagasse extract, respectively.

The TTA value during citric acid fermentation by *A. niger* 79/20 in case of 5% bagasse extract was highest on 7th day and the value was highest on 12th day in case each of 10, 15 and 20% bagasse extract (Table 3). Citric acid values were also highest in these days for the respective bagassa extract medium.

Increase of cell biomass during citric acid production: After inoculation of *A. niger* strains in the fermentation

Table 1: Proximate analysis of sugarcane bagasse

Constituent	Dry weight basis (%)
Moisture	45.0
Total reducing sugar	68.0
Fermentable reducing sugar (Celluslose)	43.5
Non-fermentable reducing sugar (Hemicellulose)	20.2
Lignin	24.0
Protein	1.40
Lipid	0.37
Ash	2.30
Fibre	48.5

Table 2: Citric acid production by *A. niger* CA16

	Residual sugar (g L^{-1})				pH				TTA				Citric acid (g L^{-1})			
	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Fermentation period (day)	(%)															
1st	21.06	32.60	43.50	53.20	5.00	5.00	5.00	5.00	0	0	0	0	0	0	0	0
4th	16.65	25.60	35.67	41.05	4.70	4.80	4.50	4.10	0.40	0.40	0.40	1.00	2.50	2.56	3.60	6.40
7th	10.8	19.25	28.84	35.92	4.50	4.45	4.20	3.90	0.80	0.90	0.80	1.20	4.65	4.90	5.09	7.50
12th	8.63	14.59	21.29	25.10	4.30	4.20	3.80	3.60	0.60	1.00	1.20	1.60	3.84	6.40	7.70	10.25
15th	3.85	9.09	15.61	19.28	3.95	3.90	3.50	3.35	0.30	0.60	0.70	1.00	2.46	3.74	5.05	6.75

Table 3: Citric acid production by *A. niger* 79/20

	Residual sugar (g L^{-1})				pH				TTA				Citric acid (g L^{-1})			
Fermentation period (day)	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
	(%)															
1st	21.06	32.6	43.5	53.2	5	5	5	5	0	0	0	0	0	0	0	0
4th	15.64	24.23	34.53	39.95	4.7	4.7	4.1	3.9	0.4	0.4	0.6	0.8	2.55	2.6	2.69	5.12
7th	9.84	17.76	27.76	33.92	4.5	4.5	3.7	3.5	0.6	0.8	1.0	1.4	3.84	5.05	7.68	8.95
12th	5.06	13.84	20.56	23.12	4.2	4.4	3.5	3.5	0.4	1	1.2	2	2.56	6.45	10.15	12.81
15th	1.02	7.84	14.8	17.92	4	4.2	3.25	3.3	0	0.4	1	1.2	0	2.56	6.3	7.65

Table 4: Kinetic parameters and coefficients of citric acid fermentation by *A. niger* CA16 and 79/20

	Strain							
	A. niger CA16				*A. niger* 79/20			
Bagasse extract (%)	5	10	15	20	5	10	15	20
Sugar conc. (g L^{-1})	21.060	32.600	43.500	53.200	21.000	32.500	43.500	53.200
Maximum sugar utilized (g L^{-1}), S	10.260	18.010	22.210	28.100	11.220	18.760	22.940	30.080
Maximum dry cell weight (g L^{-1}), D	2.860	3.430	5.430	7.710	3.430	4.290	6.570	9.710
Maximum substrate uptake rate, Q_s = S/h	0.061	0.063	0.077	0.098	0.067	0.065	0.080	0.104
Maximum citric acid production (g L^{-1}), P	3.650	6.400	7.700	10.250	3.840	6.450	10.15	12.810
Product yield coefficient, $Y_{p/s}$ = P/S	0.356	0.355	0.347	0.365	0.342	0.341	0.445	0.426
Growth yield coefficient, $Y_{x/s}$ = D/S	0.279	0.190	0.244	0.274	0.306	0.229	0.286	0.323
Productivity, Q_p = P/h	0.022	0.022	0.027	0.036	0.023	0.024	0.035	0.044

Qs: g substrate consumed/L/h, $Y_{p/s}$: (gram citric acid produced/gram sugar consumed), $Y_{x/s}$: (gram cell mass/gram sugar consumed), Q_p: g citric acid produced/L/h

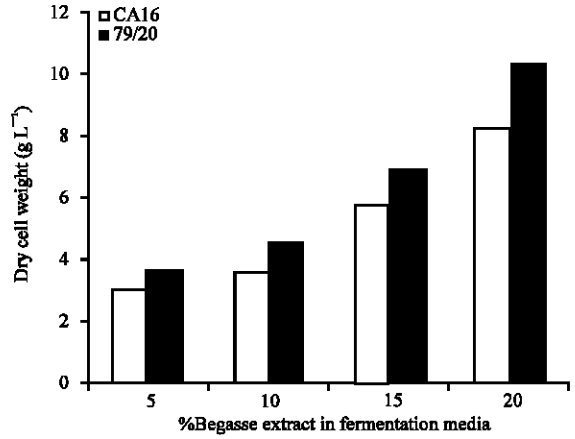

Fig. 2: Cell biomass of *A. niger* CA16 and 79/20 at the end of fermentation

media, the amounts of cell biomass were found to increase with the increase of incubation period. Maximum cell dry weight was detected on 15th day of fermentation (Fig. 2). However, citric acid production was not necessarily increased with the increase of cell biomass for all concentrations of bagasse extracts. Highest cell weight was found in case of 20% bagasse extract in both of the strains. Expectedly, *A. niger* 79/20 had higher biomass than that of CA16 which also produced higher citric acid.

Fermentation kinetics: On the basis of kinetic parameters as shown in Table 4, in case of both of the *A. niger* strains, substrate uptake rate, product yield co-efficient, growth yield coefficient and productivity were found to increase with increase concentration of bagasse extract in fermentation media. A significant improvement in product yield coefficient and productivity were also detected in case of fermentation with *A. niger* 79/80 than that of the wild type strain CA16 in all the media used.

DISCUSSION

In this study, two *A. niger* strains were used for citric acid fermentation using an agricultural wastes which has almost no use in biotechnological purpose in Bangladesh even having a high sugar content (Table 1) for acid or biomass production. Reports of Li *et al.* (2002) also showed similar pattern of constituents in sugarcane bagasse. In the present study, there was a quantitative relationship between sugar utilization and citric acid production. The sugar concentration was decreased throughout the process indicating the utilization of sugar for citric acid production. However, the radiation mutant strain *A. niger* 79/20 utilized sugar to produce citric acid more rapidly than *A. niger* CA16. This finding was in accordance with the findings of Lotfy *et al.* (2007) where they reported improved citric acid production by a radiation induced mutant *A. niger* strain than that of mother strain. Begum and Chowdhury (2000) reported 1.6 fold improved production yield by radiation mutant *A. niger* compared to wild type strain. Our previous study also showed more efficient production of citric acid from sugarcane bagasse (Abdullah-Al-Mahin *et al.*, 2008) than the wild type one by solid-state fermentation.

In this study, pH was adjusted to 5.0 in bagasse extract media for optimum production of citric acid as suggested by Begum and Chowdhury (2000). With the

increase in incubation period the pH of the fermented medium was found to decrease gradually. This indicated the increase of acid production. Expectedly, the decrease of pH in the media inoculated with 79/20 was more than that with CA16. Again, this decrease was also higher with increased concentration of bagasse indicating higher citric acid production as a result of higher sugar utilization. The temperature of fermentation media is one of the critical factors for citric acid fermentation. A temperature of 30°C was reported to be optimum for citric acid fermentation by *A. niger* (Dalmau *et al*., 2000). When the temperature of medium is increased above 30°C, the biosynthesis of citric acid is decreased. It might be due to the accumulation of by-products such as oxalic acid (Kubicek and Rohr, 1986). In the present study, 30°C was maintained for maximum growth of *A. niger* strain CA16 and 79/20.

Total Titratable Acidity (TTA), a quantitative measure of citric acid production, was found just proportional to the utilization of sugar in the fermentation media. However, TTA values were decreased after 12th days of incubation. This might be due to product inhibition. The high level of citric acid in the medium caused an inhibition of product formation (Table 2, 3). For the first four days after incubation the growth rate was very slow (data not shown). From 4th to 12th days there was a sharp increase in citric acid level and from 13th to 15th the citric acid concentration was decreased in the fermentation media. This phenomenon can be explained by the inhibition of fungal enzymes by increased concentration of citric acid (Agrawal *et al*., 1983). Agrawal *et al*. (1983) reported that the activity of citric acid synthase, the terminal enzyme of the pathway responsible for citric acid synthesis, decreased after 8 days when citric acid accumulation was highest.

Table 4 showed that with higher concentration of bagasse extract citric acid production was increased by both of the strains indicating that the production of citric acid is dependent on substrate concentration. Higher values of maximum substrate uptake rate, maximum citric acid production, product yield co-efficient and productivity in case of the strain 79/20 than CA16 clearly indicates improved potential of the strain for citric acid production. In our study, sugar concentrations of different bagasse extracts were relatively lower than the optimum level, therefore some impurities of oxalic acid (Rohr *et al*., 1983) may present in the medium which was not detected in our experiments.

CONCLUSION

Finally, it can be concluded that radiation mutant *A. niger* 79/20 had improved sugar utilization and citric acid production ability. However, it is necessary to unveil the molecular mechanism of improved citric acid production by radiation mutation. The appropriate use of this radiation mutation can help us to generate more robust citric acid producing strains.

REFERENCES

AOAC, 1980. Method of Analysis of the Association of Official Agricultural Chemists. 13th Edn., AOAC, Washington, DC., USA.

Abdullah-Al-Mahin, S.M. Hasan, M.H. Khan and R. Begum, 2008. Citric acid production by *Aspergillus niger* through solid state fermentation in sugarcane bagasse. Bangladesh J. Microbiol., 25: 9-12.

Afifi, M.M., 2011. Naturally occurring microorganisms of industrial waste for citric acid production by solid state fermentation. J. Environ. Sci. Technol., 4: 377-386.

Agrawal, P.K., C.S. Bhatt and L. Viswanathan, 1983. Effect of some metabolic inhibitors on citric acid production by *Aspergillus niger*. Enzyme Microb. Technol., 5: 373-376.

Ali, S., Ikram-ul-Haq, M.A. Qadeer and J. Iqbal, 2001. Biosynthesis of citric acid by locally isolated *Aspergillus niger* using sucrose salt media. J. Biol. Sci., 1: 178-181.

Begum, R. and N. Choudhury, 2000. Citric acid fermentation in different starchy substrates by radiation induced mutants of *Aspergillus niger*. J. Asiatic Soc. Bangladesh Sci., 26: 47-52.

Dalmau, E., J.L. Montesinos, M. Lotti and C. Casas, 2000. Effect of different carbon sources on lipase production by *Candida rugosa*. Enzyme Microb. Technol., 26: 657-663.

Dhillon, G.S., S.K. Brar, M. Verma and R.D. Tyagi, 2010. Recent advances in citric acid bio-production and recovery. Food Bioprocess Technol., 4: 505-529.

Djordjevic, I., N.R. Choudhury, N.K Dutta and S. Kumar, 2009. Synthesis and characterization 27 of novel citric acid- based polyester elastomers. Polymer, 50: 1682-1691.

Grewal, H.S. and A.L. Karla, 1995. Fungal production of citric acid. Biotechnol. Adv., 13: 209-234.

Hannan, M.A., 1972. Variants of *Aspergillus niger* induced by ã rays. Indian J. Exp. Biol., 10: 379-381.

Hannan, M.A., F. Rabbi, A.T.M.F. Rahman and N. Choudhury, 1973. Analysis of some mutants of *Aspergillus niger* for citric acid production. J. Ferment. Technol., 51: 606-608.

Kubicek, C.P. and M. Rohr, 1986. Citric acid fermentation. Crit. Rev. Biotechnol., 3: 331-373.

Li, X., R. Kondo and K. Sakai, 2002. Biodegradation of sugarcane bagasse with marine fungus *Phlebia* sp. MG-60. J. Wood Sci., 48: 159-162.

Lodhi, A.K., M. Asghar, M.A. Zia, S. Ambreen and M.J. Asad, 2001. Production of citric acid from waste bread by *Aspergillus niger*. J. Biol. Sci., 1: 182-183.

Lotfy, W.A., K.M. Ghanem and E.R. El-Helow, 2007. Citric acid production by a novel *Aspergillus niger* isolate: I. Mutagenesis and cost reduction studies. Bioresour. Technol., 98: 3464-3469.

Marier, J.R. and M. Boulet, 1958. Direct determination of citric acid in milk with an improved pyridine acetic anhydride method. J. Dairy Sci., 4: 1683-1692.

Maryam, M., N. Iraj and E. Giti, 2011a. A comparative study on two *Yarrrowia lipolytica* strains for optimum citric acid production. Res. J. Microbiol., 6: 568-574.

Maryam, M., N. Iraj and E. Giti, 2011b. The effect of viscous substances on citric acid production by *Yarrowia lipolytic*. Microbiol. J., 1: 120-125.

Mazhar, R., S. Ali, Ikram-ul-haq and A. Waheed, 2003. Citric acid fermentation by *Aspergillus niger* NG-110 in shake flask. J. Biol. Sci., 3: 360-370.

Moore, W.E. and D.B. Johnson, 1967. Procedures for the chemical Analysis Wood and Wood Products. U.S. Forest Product Laboratory, U.S. Department of Agriculture, Madison, WI., USA.

Morse, E.E., 1947. Anthrone in estimating low concentration of Sucrose. Anal. Chem., 19: 1012-1013.

Naeini, A.T., M. Adeli, M. Vossoughi, 2010. Poly(citric acid)-block-poly(ethylene glycol) copolymers-new biocompatible hybrid materials for nanomedicine. Nanomed.: Nanotechnol. Biol. Med., 6: 556-562.

Namazi, H. and H. Adeli, 2005. Dendrimers of citric acid and poly (ethylene glycol) as the new drug-delivery agents. Biomaterials, 26: 1175-1183.

Nelson, N., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem., 153: 375-380.

Pandey, A., C.R. Soccol, J.A. Rodriguez-Leon and P. Nigam, 2001. Production of Organic Acids by Solid-State Fermentation. In: Solid-State Fermentation in Biotechnology: Fundamentals and Applications, Pandey, A. (Ed.). Asiatech Publishers Inc., New Delhi, India, ISBN-13: 9788187680062, pp: 113-126.

Papagianni, M., 2007. Advances in citric acid fermentation by *Aspergillus niger*: Biochemical aspects, membrane transport and modeling. Biotechnol. Adv., 25: 244-263.

Partos, L., 2005. ADM closes citric acid plant as Chinese competition bites. Food Navigator. http://www.foodnavigator.com/Financial-Industry/ADM-closes-citric-acid-plant-as-Chinese-competition-bites.

Rohr, M., C.P. Kubicek and J. Kominek, 1983. Citric Acid. In: Biotechnology, Rehman, H.J. and G. Reed (Eds.). Vol. 3, Verlag Chemie, Weinheim, pp: 419-454.

Saeman, J.F., E.E. Harris and A.A. Kline, 1945. Analysis of wood sugar. Ind. Eng. Chem., 17: 95-99.

Soccol, C.R., L.P.S. Vandenberghe, C. Rodrigues and A. Pandey, 2006. New perspectives for citric acid production and application. Food Technol. Biotechnol., 44: 141-149.

Vandenberghe, L.P.S., C.R. Soccol, A. Pandey and J.M. Lebeault, 1999. Microbial production of citric acid. Braz. Arch. Biol. Technol., 42: 263-276.

Xie, G. and T.P. West, 2007a. Citric Acid production by *Aspergillus niger* on condensed corn distillers solubles. Res. J. Microbiol., 2: 481-485.

Xie, G. and T.P. West, 2007b. Citric acid production by *Aspergillus niger* on the ethanol dry milling coproduct thin stillage. Res. J. Microbiol., 2: 678-683.

Yang, J., A.R. Webb and G.A. Ameer, 2004. Novel citric acid-based biodegradable elastomers for tissue engineering. Adv. Mater., 16: 511-516.