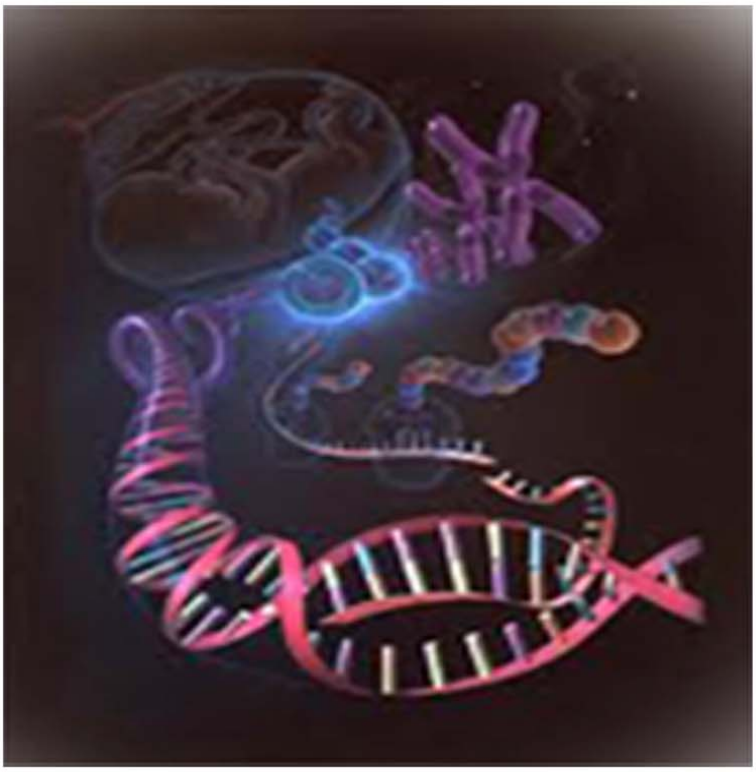
IJLBPR

ISSN 2250 - 3137
Vo1. 3, No. 4, October 2014

International Journal of Life Sciences Biotechnology and Pharma Research



www.ijlbpr.com

International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA

Email : editorijlbpr@gmail.com or editor@ijlbpr.com

International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com
Vol. 3, No. 4, October 2014

Research Paper

CITRIC ACID PRODUCTION USING *ARTOCARPUS HETEROPHYLLUS* AND ITS WASTE WITH ALCOHOL AS A STIMULANT

Sashi Prabha M[1*] and Subba Rangaiah G[2]

Corresponding Author: **Sashi Prabha M ✉ shashi23581@rediffmail.com*

Citric acid is a 6-Carbon containing tricarboxylic acid which was first isolated from lemon juice and was crystallized by Scheele in 1784. It is responsible for the tart taste of various fruits, e.g., lemons, limes, oranges, pineapples, pears and gooseberries. In the present study more focus was made on the economical production of citric acid from Artocarpus heterophyllus and its peel, which was in turn compared with a citric acid production rate from sucrose as a substrate. *Aspergillus niger* MTCC 281 is the choice of the organism for the present study. Jack fruit (*Artocarpus heterophyllus*) is a seasonal fruit and its peel will be dumped indiscriminately after using the edible portion. So, this waste is considered for the present study as a substrate for citric acid production. In the second part of the work alcohols were also used at the time of inoculums to check the role of alcohol in the production rate. Three different alcohols were used, i.e., methanol, ethanol and butanol at different concentrations.

Keywords: Citric acid, *Artocarpus heterophyllus*, Peel, Stimulants, Substrate, Methanol, Ethanol and Butanol

INTRODUCTION

Citric acid obtained through the microbial fermentation is considered synthetic while that of present in fruits is referred to as natural (Ranya *et al.*, 1999; Karklins *et al.*, 2001). Citric acid can be produced by the fermentation of glucose with aid of *Aspergillus niger* or synthetically from an acetone or glycerol (Fernando *et al.*, 2000; Adham 2002; Adachi *et al.,* 2003; Ikram-Ul *et al.*, 2004). Approximately, 75.0% commercial use of this acid is for food and 12.0% for pharmaceutical industries (Haq *et al.*, 2001a; Johnson 2003). The worldwide demand for citric acid is about 6.0×10^5 tons per year (Karaffa and Kubicek, 2003). Many microorganisms have been evaluated for the citric acid production including bacteria, fungi and yeasts. However, *Aspergillus niger*, a filamentous fungus remained the organism of choice for citric acid production (Maddox and Brooks, 1998; Arzumanov *et al.*, 2000; Schuster *et al.*, 2002).

[1] Department of Microbiology, Dr. Lankapalli Bullayya PG College and Research Center, Visakhapatnam, Andhra Pradesh, India.
[2] Department of Microbiology, Andhra University, Visakhapatnam, Andhra Pradesh, India.

Citric acid is a primary metabolic produced by *A.niger* during idiophase stages when growth drops and acid production becomes the main cellular activity. A submerged process appears to be highly desirable and many articles and patents have appeared in the literature (Rajoka *et al.,* 1998; Vandenberghe *et al.*, 1999; Haq *et al.*, 2002a, b).

The main aim of the present study is to study the citric acid production on the economical grounds using fruit and its waste as a substrate which are considered as a municipal waste, because of environmental concern regarding the disposal of solid wastes. In addition, this process may give the encouraging results in terms of citric acid production. A variety of solids have been reported as substrate for the citric acid bioproduction, including kiwifruit peel (Hang *et al.,* 1987), apple pomace, grape pomace (Hang and Woodams, 1985), Wheat bran (Shankaranand and Lonsane, 1994), sugar cane baggage, concentrated liquor of pineapple waste (Wang and Liu, 1996). Sweet potato (Maddox *et al.*, 1995; Yuguo *et al.*, 1999) and carrot (Takita *et al.,* 1999; Roukas, 2000).

The specific fruit that was selected is *Artocarpus heterophyllus* (Jack fruit) and its waste. *Aspergillus niger* (MTCC281) was selected for the production of citric acid. Comparative study was done with the sucrose as a substrate to that of the fruit and its waste as a substrates.

The present study also deals with effect of alcohols as stimulants on citric acid production using fruit and its waste, so that we can get maximum amount of citric acid even from fruit waste which is considered as municipal waste. Three different alcohols were selected, i.e., Methanol, ethanol and butanol at different concentrations to check at which concentration we are getting more rate of yield.

MATERIALS AND METHODS

Materials Used

Organism used: *Aspergillus niger* MTCC281. The growth medium for the organism is Czapek Yeast Extract Agar medium (CYA).

Instruments: pH meter, Autoclave, Orbital shaking Incubator, Colorimeter, Water bath, Electronic weighing balance.

Substrates: *Artocarpus heterophyllus* (Jack fruit) and its waste.

Methods

The initial sugar concentration has been found to determine the amount of citric acid and other organic acids produced in the culture broth (Honeeker *et al.*, 1989; Iwaki *et al.,* 1990; Navaratnam *et al.,* 1998). Normally strains of *A. niger* need a fairly higher initial sugar concentration (15-18%, w/v) in the medium (Klasson *et al.*, 1989; Ali *et al.*, 2002). The higher sugar concentrations lead to greater amounts of residual sugars making the process uneconomical (Kubicek, 1988). So, presence of sugar concentration in the production media plays a crucial role in the rate of citric acid production. So, in order to determine the amount of initial sugar present in the substrate Anthrone's method was used.

The Anthrone Method for the Determination of Carbohydrates

There are different techniques for the estimation of carbohydrates. However, these determinations are associated with numerous difficulties. In order to overcome the difficulties Morse (1947) and

Morris (1948), have described the use of anthrone for the quantitative estimation of carbohydrates which is both quicker and more accurate and suites well for the determination of carbohydrates. To obtain this degree of accuracy, it was found necessary to heat the mixture of the carbohydrate sample and the anthrone reagent at 100°C. for 5 to 10 min after mixing.

Anthrone Reagent

Anthrone reagent is prepared by dissolving 2 g. Anthrone in 1 L of 95% sulphuric acid. This reagent has to be prepared fresh daily and was between 4 to 8 h old. After this time gradual increase in color occurred. After which it should not be used and has to be discarded (Ludwig and Goldberg, 1956).

Using the above stated method the amount of carbohydrate present in the *Artocarpus heterophyllus* (Jack fruit) and its waste was determined, in order to do so, for the sample preparation the Jackfruit and its waste was collected separately and macerated, together with the expressed juice dried in a hot air oven at less than 60°C. They were then pulverized and stored in dark bottles (Williams *et al.*, 1940; Roukas, 1998, 1999). Aliquots of ½ to 2 g. Pulverized material were used for analysis (William, 1940) and followed the Morris anthrone method. The amount of carbohydrate in the test sample was estimated from a standard curve.

Citric Acid Production

Microorganism Used

The microorganism used was the *Aspergillus niger* MTCC 281, received from Microbial Type Culture Collection and Gene Bank.

Shake Flask Studies

The *Aspergillus niger* cultures were used for citric acid production by submerged fermentation in 250 mL Erlenmeyer flasks.

Preparation of Conidial Inoculum

Conidial inoculum was used in the present study. The spores from 4-6 days old slant cultures of PDA medium were used for the inoculation. Inoculation is carried out using spores of *A. niger.*

Preparation of Vegetative Inoculum

To 100 mL of sterile fermentation media in a 1 L conical flask, 1 mL of the *A.niger* conidial suspension (1.2×10^6 culture per mL) was used for inoculation. The flask was incubated at 30°C in a rotary shaking incubator at 200 rpm for 24 h.

Fermentation Technique

Vegetative inoculums were transferred into the sterile fermentation medium at a level of 4.0% (v/v). The incubation temperature was kept at 30°C throughout the fermentation period of 144 h. The shaking speed of the orbital shaker was adjusted to 160 rpm. The pH of fermentation medium was adjusted to 3.5 by 0.1 N NaOH/ HCl before autoclaving.

After the incubation period the ingredients of the flasks were filtered and the filtrate was used for the estimation of citric acid produced and residual sugar content. The dry cell mass was also calculated.

Recovery

Partial citric acid recovery was accomplished by the precipitation method (Kristiansen *et al.*, 1999). After fermentation was completed fermentation broth was filtered completely. The filtrate was boiled with equivalent amount of lime and tri-calcium citrate, this involves precipitation method. The calcium citrate was filtered off and then treated with sulphuric acid (60-70%, v/v) to obtain citric acid and precipitate of calcium sulphate.

Effect of Different Alcohols at Various Concentrations

The effect of different alcohols such as methanol, ethanol and butanol at varying concentrations on citric acid fermentation by the strain *Aspergillu niger* MTCC*281*, using *Artocarpus heterophyllus* fruits and its peels as a carbohydrate substrate in shake flasks, was carried out. The concentration of alcohols varied from 0.5 to 2.5 % (v/v) in each case, i.e., with fruit and its waste, the same was performed with the standard production medium and was compared.

RESULTS

The critical parameters for citric acid production by *Aspergillus niger* were defined empirically, include high carbohydrate concentration but should not be more that 15 to 20%. So, in order to fulfill the requirement the concentration of carbohydrates in Jack fruit and its peel was estimated and calculated (Table 1). So, 15 g/100 mL concentration of each fruit and its peel were calculated and were used for the present study of citric acid production using Jack fruit and its waste.

Table 2 has shown the data regarding the production of citric acid with *Aspergillus niger* MTCC 281 using Jack fruit and its wastes in shake flask method. The amount of sugar consumed, dry cell mass and citric acid produced was estimated (Table 2). According to Table 2, the amount of citric acid obtained with control is 52.96±0.56 g/L, using sucrose as a substrate, where as with Jack fruit and its waste the yield obtained is 16.47±0.73 g/L (Table 2) and 10.51±0.49 g/L (Table 2) respectively. The rate of yield from Jack fruit and its waste were compared with that of the control yield.

The effect of alcohols as stimulants at various concentrations were also tested, alcohols used were Methanol (Table 3), Ethanol (Table 4) and

Table 1: Estimation of Carbohydrates in *Artocarpus heterophyllus* and Its Peel

S.No.	Name of the Sample	Vol. of Sample[1] (ml)	Conc. of Sample for 0.1 mg (μg)[2]	Conc. of Sample for 100 gm (gm)	Vol. of Anthrone (ml)	O.D. at 620 nm
1	Jack fruit	1	26.01	26.01	4	0.25
2	Jack fruit peel	1	12.48	12.48	4	0.12

Note: 1. 1ml of volume of the sample = 0.1 mg of dried powder of the fruit/sample.
2. Concentration of sample was determined from the standard graph.

Table 2: Citric Acid Production in Shake Flask Using *A.niger* MTCC281*

S.No.	Sample	Dry cell mass (g/l)	Sugar consumed (g/l)	Citric acid (g/l)
1	Sucrose (Control)	15.97±0.49	97.99±0.56	**52.96±0.56**
2	Jackfruit	7.49±0.04	112.15±0.70	**16.47±0.73**
3	Jack fruit peel	8.23±0.12	89.44±0.25	**10.51±0.49**

Note: * Fermentation period 168 h, Sugar concentration 150 g/l, Initial pH 2.5, incubation temperature 30 ^{0}C; ± Indicate standard error mean (SEM) of the mean.

Table 3: Effect of Alcohols at Various Concentration on Citric Acid Fermentation by the *Aspergillus niger* MTCC281 Using Sucrose Salt Medium in Shake Flasks*

S. No.	Sample	Alcohol	Concentration%	Dry Cell Mass(g/l)	Sugar Consumed(g/l)	Citric Acid(g/l)
1	Sucrose- Control	-	-	15.97±0.49	97.99±0.56	52.96±0.56
2	Sucrose	Methanol	0.5	16.02±0.42	95.31±0.29	56.60±1.29
			1.0	**15.69±0.50**	**96.74±0.07**	**61.98±0.03**
			1.5	15.33±0.06	95.87±0.29	61.66±0.38
			2.0	14.92±0.53	94.92±0.38	57.79±0.39
			2.5	16.43±0.73	95.24±0.33	53.45±0.18
3	Sucrose	Ethanol	0.5	16.51±0.37	100.40±0.35	49.60±1.29
			1.0	**16.93±0.26**	**101.44±0.74**	**53.98±0.03**
			1.5	16.96±0.03	101.92±0.88	53.66±0.38
			2.0	16.48±0.51	102.70±1.31	50.79±0.39
			2.5	16.75±0.38	101.26±0.59	46.45±0.18
3	Sucrose	Butanol	0.5	13.98±0.39	101.29±0.25	38.93±0.57
			1.0	**13.68±0.49**	**102.76±0.06**	**42.31±0.87**
			1.5	13.35±0.06	101.86±0.28	39.66±0.38
			2.0	12.90±0.50	100.93±0.38	36.46±0.28
			2.5	14.42±0.70	101.26±0.33	32.79±0.31

Note: * Initial sugar concentration 150g/l, Fermentation period of 168 h, incubation, 30°C, initial pH 2.5. Each value is an average of three parallel replicates. ± Indicates standard error mean among the replicates.

Butanol (Table 5). After using different concentrations of different alcohols as stimulants on all the three substrates, i.e., sucrose, Jack fruit and its waste we got highest of 61.98±0.03 g/L (Table 3) of citric acid with sucrose as a substrate at 1.0% Methanol as a stimulant, for Jack fruit and its waste, the highest amount of citric acid obtained is 27.19±1.47 g/L and 17.02±0.45 g/L, respectively (Tables 4 and 5). In all the three cases 1.0% Methanol is acting as a good stimulants in compared to that of Ethanol and Butanol and other concentrations of methanol.

Even though the amount of citric acid obtained with Jack fruit 16.47±0.73 g/L (Table 4) and its peel 10.51±0.49 g/L (Table 5) is less than the citric acid obtained from sucrose 52.96±0.56 g/L as a substrate, but the amount produced from fruit and its peel were not negligible, which has enhanced after the addition of stimulants to 27.19±1.47 g/L and 17.02±0.45 g/L, for Jack fruit and its waste respectively. The point to be noted here is that the Ethanol and Butanol were not acting as a stimulant, in turn it is decreasing and inhibiting the rate of production in both the cases, i.e., with fruit and its peel.

Table 4: Effect of Methanol, Ethanol & Butanol at Various Concentration on Citric Acid Fermentation by the *Aspergillus niger* 281 Using Jack Fruit as a Substrate in Shake Flasks*

S. No.	Sample	Alcohol	Concentration%	Dry Cell Mass (g/l)	Sugar Consumed (g/l)	Citric Acid (g/l)
1	Jack fruit Control	-	-	7.49±0.04	112.15±0.70	16.47±0.73
2	Jack fruit	Methanol	0.5	6.35±0.72	107.58±0.64	20.71±0.32
			1.0	**6.85±0.43**	**107.72±0.68**	**27.19±1.47**
			1.5	6.07±0.46	107.79±0.69	24.04±0.32
			2.0	6.95±0.33	107.73±0.53	16.62±0.50
			2.5	7.26±0.30	108.08±0.63	16.64±0.16
3	Jack fruit	Ethanol	0.5	7.43±0.21	118.11±0.33	13.04±0.26
			1.0	**7.83±0.31**	**116.63±0.37**	**16.00±0.48**
			1.5	8.16±0.14	116.52±0.47	14.81±0.30
			2.0	8.19±0.21	117.30±0.43	11.40±0.35
			2.5	7.89±0.38	117.52±0.28	10.43±0.38
3	Jack fruit	Butanol	0.5	5.01±0.50	114.91±0.32	6.66±0.32
			1.0	**5.54±0.37**	**114.72±0.66**	**11.18±0.62**
			1.5	5.43±0.55	115.11±0.41	8.36±1.08
			2.0	5.60±0.54	114.68±0.54	4.32±0.98
			2.5	5.97±0.68	115.37±0.87	1.64±0.13

Note: * Initial sugar concentration 150g/l, Fermentation period of 168 h, incubation, 30°C, initial pH 2.5. Each value is an average of three parallel replicates. ± Indicates standard error mean among the replicates.

DISCUSSION

Citric acid produced from Jack fruit and its waste were compared with sucrose as a substrate for citric acid production (Table 2). In order to increase the yield, alcohols as a stimulants were added, as expected the addition of methanol has increased the yield (Tables 3, 4 and 5). The explanation for how the methanol is acting as a stimulants is, addition of low molecular weight alcohols to the medium increases fungal tolerance to trace metals during fermentation (Zakowska and Joloka, 1984) (Sanjay and Sharma, 1994). In addition, methanol markedly depressed the synthesis of cell proteins in the early stage of cultivation (Moyer, 1953) and also increased the metabolic activity of enzyme citrate synthase. When methanol concentration was further increased, it resulted in the decreased citric acid production (Tables 3, 4 and 5) because of the disturbance in fungal metabolism. Methanol has also some role in conditioning the mycelia without impairing their metabolism. Similar, type of work has also been carried out by Hang and woodams (1986) and Navaratnam *et al.* (1998). Zulay *et al.* (1995), proved the use of methanol as a stimulant and butanol had adverse affect on the rate of citric acid fermentation.

Table 5: Effect of Methanol, Ethanol & Butanol at Various Concentration on Citric Acid Fermentation by the *Aspergillus niger* 281 Using Jack Fruit Peel as a Substrate in Shake Flasks*

S. No.	Sample	Alcohol	Concentration%	Dry Cell Mass (g/l)	Sugar Consumed (g/l)	Citric Acid (g/l)
1	Jack fruit peel- Control	-	-	8.23±0.12	89.44±0.25	10.51±0.49
2	Jack fruit peel	Methanol	0.5	6.45±0.35	86.06±0.33	15.02±0.25
			1.0	**6.81±0.28**	**84.65±0.41**	**17.02±0.45**
			1.5	7.14±0.16	84.51±0.46	15.83±0.29
			2.0	7.14±0.21	85.62±0.73	12.41±0.36
			2.5	6.84±0.38	85.87±0.43	9.41±0.36
3	Jack fruit peel	Ethanol	0.5	8.67±0.36	95.68±0.30	6.57±0.23
			1.0	**8.73±0.43**	**94.20±0.64**	**9.67±0.22**
			1.5	9.06±0.44	94.42±0.71	7.48±0.25
			2.0	9.09±0.51	94.90±0.16	4.40±0.35
			2.5	9.12±0.41	95.78±0.28	3.43±0.38
3	Jack fruit peel	Butanol	0.5	5.54±0.17	94.78±0.45	0.00
			1.0	**5.60±0.31**	**94.59±0.70**	**4.38±0.13**
			1.5	5.83±0.16	94.65±0.33	1.56±0.10
			2.0	6.00±0.21	94.54±0.81	0.00
			2.5	5.77±0.38	94.91±0.95	0.00

Note: * Initial sugar concentration 150g/l, Fermentation period of 168 h, incubation, 30°C, initial pH 2.5. Each value is an average of three parallel replicates. ± Indicates standard error mean among the replicates.

Thus, yield of citric acid can be enhanced more by considering all other physical and chemical parameters. By doing so we can produce one of the important bulk producing organic acid, i.e., citric acid economically using a municipal waste, Jack fruit waste.

CONCLUSION

Citric acid which is one of the important organic bulk product is produced by *Aspergillus niger* MTCC 281 using Jack fruit and its waste separately. And the rate of production was compared with the rich carbohydrate source, i.e., sucrose as a substrate for the citric acid production. In order to increase the rate of production alcohols addition at the time of inoculation was done to check whether the three alcohols used were acting as a stimulant or the inhibitor for the rate of citric acid produced. The three alcohols used are methanol, ethanol and butanol, at different concentrations ranging from 0.5 to 2.5% v/v.

Finally, it is proved that even with the municipal waste, i.e., jackfruit waste we can produce one of the important organic acid, i.e., citric acid. Of the three alcohols used methanol at 1% v/v is proved to act as a stimulant and the ethanol and butanol are showing adverse effect. In the present work we have considered only the stimulant but other than this we have many parameters for the production of citric acid so, considering the other parameters we can increase the rate of yield even with jackfruit waste.

REFERENCES

1. Adachi D M, Toyama H, Yamada M, Shinagawa E and Matsushita K (2003), "New developments in oxidative fermentation", *Appl. Microbiol. & Biotechnol.,* Vol. 60, pp. 643-653.
2. Adham N Z (2002), " Attempts at improving citric acid fermentation by *Aspergillus niger* in beet molasses medium", *Bioresource Technol*., Vol. 84(1), 97-100.
3. Ali S, Haq I and Iqbal J (2002), "Exploitation of raw sugar cane molasses for citric acid production by *Aspergillus niger*", *Online J. Biol. Scis.*, Vol. 32, No. 3, pp. 31-35.
4. Arzumanov T E, Shishkanova N V and Fingoinava T V (2000), "Biosynthesis of citric acid by *Yarrowia lipolytica* by repeated batch culture on ethanol", *Appl. Microbiol. & Biotechnol.*, Vol. 53, No. 5, pp. 525-529.
5. Fernando A V, Carlos G A and Torres N V (2000), "Metabolism of citric acid production by Aspergillus niger", *Biotechnol. & Bioengin.,* Vol. 70, No. 1, pp. 82-108.
6. Hang Y D and Woodams E E (1985), "Grape pomace, a novel substrate to microbial production of citric acid", *Biotechnol. Lett.*, Vol. 7, p. 763.
7. Hang Y D, Luh D S and Woodams E E(1987), "Microbial production of citric acid by solid-state fermentation of kiwifruit peel", *J.Food Sci.,* Vol. 52, pp. 226-227.
8. Haq I, Ali S and Iqbal J (2002b), "The role of Mn^{++} ions for high and consistent yield of citric acid in recycling fed-batch bioreactor system and its novelty on kinetic basis", *Electronic J. Biotechnol.,* Vol. 5, No. 2, pp. 110-117.
9. Haq I, Ali S and Qadeer M A (2001a), "Fed-batch culture studies during citric acid fermentation by *Aspergillus niger* GCMC-7", *Biologia,* Vol. 45, No. 2, pp. 32-37.
10. Honecker S, Bisping B, Yang Z and Rehm H J (1989), "Influence of sucrose concentration and phosphate limitation on citric acid production by immobilized cells of *Aspergillus niger*," *Appl. Microbiol. & Biotechnol.,* Vol. 31, pp. 17-24.
11. Ikram-Ul H, Ali S, Qadeet M A and Iqbal J (2004), "Citric acid production by mutants of *Aspergillus niger* from cane molasses", *Bioresource Technol.*, Vol. 93, No. 2, pp. 125-130.
12. Iwaki Y, Kohashi K, Eya H, Honda K and Arai Y (1990), "Measurements and correlation of oxygen solubilities in aqueous solutions containing salts and sugars", *Kagaku Kogaku Ronbunsha*., Vol. 16, pp. 1247-1251.
13. Johnson S (2003), "Do Mitochondria regulate cellular iron homeostasis through citric acid and haem production? Implication for cancer and other diseases", *Med. Hypoth.*, Vol. 60, No. 1, pp. 106-111.
14. Karaffa L and Kubicek C P (2003), *"Aspergillus niger* citric acid accumulation: Do we understand this well working black box ?", *Appl. Microbiol. & Biotecnol.,* Vol. 61, pp. 189-196.

15. Karaklins R, Lemba J and Ciesturs U (2001), " Biotechnology for organic acid production", *J. Biotechnol*., Vol. 5, No. 6, pp. 281-283.

16. Klasson T K, Clausen E C and Gaddy J L (1989), "Continuous fermentation for the production of citric acid from the glucose", *Appl. Biochem. & Biotechnol*., Vol. 20, pp. 491-509.

17. Kubicek C P (1988), "The role of the citric acid cycle in fungal organic acid fermentations", *Biochemical Sym*., Vol. 54, pp. 113-126.

18. Maddox, I.S. & Brooks, J.D. (1998), " Application of multilayer packed bed reactor to citric acid production in solid-state fermentation using *Aspergillus niger*", Process Biochem., 33(2), 117-123.

19. Maddox I S, Lu M Y and Brooks J D (1995), "Citric acid production by *Aspergillus niger* in solid substrate fermentation", *Bioresource Technol.,* Vol. 54, pp. 235-239.

20. Morris D L (1948), "Determination of carbohydrate in Biological Fluids", *Science,* Vol. 107, p. 254.

21. Morse E E (1947), "Anthrone for Estimation of Sucrose in Low Concentrations", *Indust. and Engin. Chem.*, (Anal. Ed.) Vol. 19, p. 1012.

22. Navaratnam P, Arasaratnm V and Balasubramaniam K (1998), "Channelling of glucose by methanol from citric acid production from *Aspergillus niger*", *Worl J. Microbiol. & Biotechnol*., Vol. 14, No. 4, pp. 559-563.

23. Rajoka M I, Ahmad M N, Shahid R, Latif F and Parvez S (1998), "Citric acid production from sugarcane molasses by cultures of *Aspergillus niger", Biologia*, Vol. 44, No. 1, pp. 241-253.

24. Ranya A A, Osama M S and Mustafa A B (1999), "Production and purification of citric acid by *A.niger*", *Alex. J. Pharm, Sci.*, Vol. 13, No. 1, pp. 57-60.

25. Roukas T (2000), "Citric acid gluconic acid production from fig by *Aspergillus niger* using solid-state fermentation", *J. Ind. Microbiol. & Biotechnol*., Vol. 25, No. 6, pp. 298-304.

26. Schuster E, Dunn-Coleman N, Frisvad J C and Van Dijck P W (2002), "On the safety of *Aspergillus niger:* A review*", Appl. Microbiol. & Biotechnol.*, Vol. 59(4-5), pp. 426-435.

27. Shankaranand V S and Lonsare B K (1994), "Ability of *Aspergillus niger* to tolerate metal ions and minerals in a solid-state fermentation system for the production of citric acid", *Biotechnol.*, Vol. 29, No. 1, pp. 29-37.

28. Takita F, Koyama H and Hara H (1999), "Organic acid metabolism in aluminium phosphate utilizing cells", *Cell Physiol.,* Vol. 40, No. 5, pp. 489-495.

29. Vandenberghe L P S, Carlos R S, Pandey A and Lebault J M (1999), " Microbial production of citric acid"*, Braz. Arch. Biol. Technol.,* Vol. 42, No. 3, pp. 263-276.

30. Wang J L and Liu P (1996), "Comparison of citric acid production by *Aspergillus niger* immobilized in gels and crysogels of polyacrylamide", *J. Ind. Microbiol.*, Vol. 16, pp. 351-353.

31. Yuguo Z, Zhao W and Xiaolong C (1999), "Citric acid production from the mash of dried sweet potato with its drugs by *Aspergillus niger* in an external-loop airlift bioreactor", *Process Biochem*., Vol. 35, Nos. 3-4, pp. 237-242.

International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA. Ph: +91-09441351700, 09059645577
E-mail: editorijlbpr@gmail.com or editor@ijlbpr.com
Website: www.ijlbpr.com

ISSN 2250-3137