WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 17, 810-818. Research Article ISSN 2277– 7105

OPTIMIZATION OF CITRIC ACID PRODUCTION FROM *ASPERGILLUS NIGER* USING PINEAPPLE WASTE AS FEEDSTOCK IN SUBMERGED FERMENTATION

Diptendu Sarkar[1]* and Kamalesh Das[2]

[1]Assistant Professor, Dept. of Microbiology, Ramakrishna Mission Vidyamandira, Belur Math, Howrah, Pin-711202, West Bengal, India & Visiting Faculty, Dept. of Microbiology, Uluberia College, Uluberia , Howrah, Pin-711315,West Bengal, India.

[2]Assistant Professor, Dept. of Physiology, Uluberia College, Uluberia, Howrah, Pin-711315, West Bengal, India.

Article Received on 01 Nov. 2017,
Revised on 22 Nov. 2017,
Accepted on 13 Dec. 2017
DOI: 10.20959/wjpr201717-10389

***Corresponding Author**
Diptendu Sarkar
Assistant Professor, Dept. of Microbiology, Ramakrishna Mission Vidyamandira, Belur Math, Howrah, Pin-711202, West Bengal, India &Visiting Faculty, Dept. of Microbiology, Uluberia College, Uluberia , Howrah, Pin-711315,West Bengal, India.

ABSTRACT

A solid state fermentation was developed for citric acid production from pineapple waste by *Aspergillus niger*. The medium was supplemented with different concentration of glucose, sucrose, ammonium nitrate and ammonium sulphate. It was found that pineapple waste with 15% (w/v) sucrose and ammonium nitrate (0.25% w/v) gave the optimum citric acid production (44.10 g/kg), in the presence of methanol (4% v/v), when fermented for 7 days at 30 °C, with the initial moisture content of 60 %. Our present investigations show the use of pineapple peel as a cheap medium for the production of commercially valuable organic acid (citric acid) by *A. niger*.

KEYWORDS: *Aspergillus niger*, citric acid, pineapple waste, solid state fermentation.

INTRODUCTION

Citric acid is an organic acid naturally present in all aerobic organisms. It is produced during the process of cellular respiration.[1] This organic acid has found many uses in food, beverage, pharmaceutical, chemical, cosmetic and other industries. It is used to provide a characteristic taste to foods and beverages. This organic acids produced by fermentation and is the most exploited biochemical product in various industries till date. The natural supply of citric acid is very limited but its demand is more. Microbial fermentation is the most common method for large scale production of citric acid. Many microorganisms such as bacteria, fungi and yeasts can produce citric acid.[2] *Aspergillus niger* is the most commonly used strain for commercial production of citric acid, as it is easy to handle, can ferment a variety of raw materials and it produces higher quantity as compared to other microorganisms.[3] Different methods of fermentation have been employed by different workers for producing citric acid such as solid-state fermentation, submerged fermentation and surface fermentation.[4] Cost reduction in citric acid production can be achieved by using several cheap agricultural wastes such as apple and grapepomace, orange peel, kiwi fruit peel, cotton waste, okarasoy-residue and cane molasses (Kiel *et al*., 1981; Hang and Woodams, 1986; 1987; Khare *et al*., 1995; Haq *et al*.,2004).[18,10] So, the objective of this study was to use of pineapple peel as a cheap constituents of medium for the production of citric acid by *A. Niger* in submerged fermentation process.

MATERIALS AND METHODS

Pre-treatment of pineapple peels

Peels from pineapples bought from a local market in Bengaluru, Karnataka, were used in the presentstudy. Pineapple peels were oven-dried at 60 °C after cutting into 2 mm mesh size.[5]

Screening of the fungal cultures

Four fungal species isolated from garden soil sample and morphological characteristics were examined. Czapek-Dox agar medium (10 mL) was poured into individual sterile petri plates and allowed to cool at roomtemperature. Approximately 0.5 mL of the conidial suspension of each culture was transferred to each of the petriplates. The plates were incubated at 30 °C for 3–5 days.The plates were observed after incubation for yellow zones due to citric acid formation. *Aspergillus niger* culture had a zone of clearance above 3cm. On the basis of screening method, the isolate *Aspergillus niger* found to be a potential source of citric acid production. This culture was used for optimization of citric acid production using solid state fermentation process.[6]

Fermentation media

The medium was prepared by introducing pineapple peels (30 g) into 200 mL Erlenmeyer flasks. The medium was supplemented with glucose and sucrose at 5, 10,15% w/v. Effect of nitrogen supplements was studied by adding ammonium nitrate (0.1–0.5%) and ammonium sulphate (0.1–0.5%) to the basal medium and moistened to varying moisture content (50–

70%). The flask was cotton plugged and autoclaved at 121 °C for 15min. After cooling at room temperature, each medium was inoculated with the *A. Niger* (6.0 x 10^6) suspension and incubated at different temperature range (20–50 °C) in arotary shaking incubator for 7 days. Methanol (0–5%) was added to the flasks before fermentation. After fermentation, the medium was diluted with distilled water (1:4 w/v). The medium was then filtered and the filtrate was used for the subsequent analyses.[7]

Citric acid determination

Citric acid % was determined titrimetrically (AOAC, 1995) by using 0.1 NaOH and phenolphthalein as indicator and calculated as per the formula;[1,8]

% of Citric acid = (Normality x Vol. of 0.1 N NaOH x equivalent wt. of Citric acid x Dilution Factor) / (Wt. of sample in gm x 10)

RESULTS AND DISCUSSION

Screening of stock-cultures of *A. Niger* and pineapple waste medium

Citric acid producing strains of *A. Niger* isolated from various sites in Bangaluru, Karnataka were purified and their cultural and morphological characteristics were examined.Out of 4 strains screened for citric acid production, *A.niger* was the best producer of citric acid. The pineapple peel used throughout this study was found to present crude protein 4.89%; crude fat1.87%; crude fiber3.45%; carbohydrate content 52.5%;sugars content 42.6%; ash content 3.8% and moisture9.25%.Our result shows that pineapple waste is asuitable constituent of media for fermentation of citric acid by *A. Niger* due to its nutritional content and achieved the optimum citric acid production at the seventh day.

Table 5.1: Species showing the zones of clearance in cm.

SPECIES	DISTANCE OF ZONE OF CLEARANCE (cm)
Aspergillus flavus	2.1
Penicillium italicum	1.8
Penicillium glaucum	2.7
Aspergillus niger	3.3

Effect of different concentration of sugars

Citric acid production by *A. Niger* from pineapple peels as a basal fermentation media with the different concentrations of sucrose and glucose was shown in Table 1. The medium supplemented with sucrose (15%w/v) gave the highest citric acid value (39.6 g/kg) while the control contain only pineapple peels gave 18.4 g/kg at 7 days fermentation period. As per Adachi *et al.*, (2003),[2,9] citric acid accumulation by *A. niger* in higher concentrations of sucrose or glucose is simultaneously increase in the intracellular concentration of fructose 2,6 phosphate. Hossain *et al.*, (1984)[10,12] suggested that the chemical nature of source has a marked effect on citric acid production by *A. niger*. In our study, supplementation of sucrose to pineapple waste found to increase citric acid production than glucose. It is may be due to the relatively low molecular weight of sucrose and is readily transported into microbial cells for hydrolysis by intracellular enzymes (Drysdale and McKay, 1995).[5,13] Xu *et al*., (1989) suggested that sucrose is the traditional commercial substrate for production of citric acid, although glucose, fructose and maltose have also been extensively used as substrates for citric acid production.[27]

Table 1: Effect of different sugar concentrations on citric acid production from pineapple waste by *A. niger*.

Citric acid amount (g/kg) at different incubation time (Days)								
Fermentationmedia	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Pineapple waste	0	13.4	13.9	14.7	16.9	17.2	17.9	18.4
Pineapple waste+ 5% glucose	0	14.8	15.7	16.9	19.9	20.7	21.3	21.9
Pineapple waste+ 10% glucose	0	16.6	20.9	22.8	25.2	26.7	27.2	27.4
Pineapple waste+ 15% glucose	0	16.7	19.8	23.2	25.5	29.4	29.9	30.2
Pineapple waste+ 5% sucrose	0	12.6	16.4	17.7	19.8	22.2	22.8	23.0
Pineapple waste+ 10% sucrose	0	16.3	19.6	22.4	25.8	29.7	30.5	31.6
Pineapple waste+ 15% sucrose	0	20.8	23.3	27.7	30.4	36.6	37.5	39.6
Mean of triplicate determinant ; SD±0.5								

It was noticeable that citric acid production and biomass values were increased along with steady decrease in sugar with respect to the incubation time. Kubicek (1998) also reported that the final yield of citric acid in fermentation by *A. niger* is strongly related not only on the type but also dependent on the concentration of carbon source.[19] It may be suggested from our result that the major regulatory points at the level of hexose transport and phosphorylating activities of regulatory enzymes involved in sugar metabolism are the prime cause by which the carbon source and it concentration influence citric acid accumulation.

Effect of nitrogen supplements

Effect of nitrogen sources on citric acid productivity by *A.niger* was shown in Table 2. Supplementation of the basal medium with ammonium nitrate (0.25% w/v) gave an increase in citric acid production from 39.63 g/kg to 44.10 g/kg compared to ammonium sulphate(41.8 g/kg).Nitrogen had been reported to be an important factor in fermentation processes due to an increase in C:N ratioas per Pandey (2003).[20-23] Any increase or decrease other than(0.25% w/v) concentration, clearly found that there is a disturbance of fungal growth as well as subsequently citric acid production. However, addition of ammonium nitrate (0.25% w/v) gave maximum biomass value of 11.20 g/kg while maximum biomass 12.20 g/kg was obtained at 0.75 % w/v concentration in case of ammonium sulphate. Nitrogen constituent has an enormous effect on citric acid production because nitrogen is not only important for metabolic rates in the cells but it is also play role in basic part of all cellular proteins. Our findings supported with Grewal and Kalra (1995)[8,20] that fermentation media for citric acid biosynthesis should consist of necessary substrates such as carbon, nitrogen and phosphorus for the growth of microorganism.

Table 2: Effect of Nitrogen supplements on citric acid production from pineapple waste medium.

Concentration(% w/v)	Citric acid (g/kg)	Biomass (g/kg)
Control: Pineapple waste supplemented with sucrose (15g)	39.63 ± 0.5	8.4 ± 0.2
Ammonium nitrate		
0.25	44.10 ± 0.5	11.2 ± 0.5
0.50	39.10 ± 0.5	13.8 ± 0.5
0.75	34.80 ± 0.5	9.8 ± 0.5
1.0	29.50 ± 0.5	7.7 ± 0.5
Ammonium sulphate		
0.25	41.8 ± 0.5	9.6 ± 0.5
0.50	38.62 ± 0.5	11.7 ± 0.5
0.75	33.4 ± 0.5	12.2 ± 0.5
1.0	27.2 ± 0.5	10.4 ± 0.5
Mean of triplicate determinant ; SD±0.5		

Effect of incubation temperature

Effect of temperature on the production of citric acid was shown in Table 3. A temperature of 30 °C was found to bethe best for citric acid production (44.5 g/kg) in the present study. The mould produced only a small amount of citric acid at 40 °C in seven days. Sporulation however, was more marked at 30 °C than at lower temperatures. At low temperature due to low enzyme activity citric acid production was too low. Our findings supported Hang and Woodams (1986)[9] that the temperature of a fermentation medium is one of the critical factors which have a significant effect on the production of citric acid by using solid state fermentation after using several agro wastes.

Table 3: Effect of temperature on fungal production of citric acid from pineapple peel.

Temperature °C	Citric acid (g/kg)
20	28.5 ± 0.5
25	31.6 ± 0.5
30	44.5 ± 0.5
35	33.3± 0.5
40	22.4± 0.5
Mean of triplicate determinant ; SD±0.5	

Effect of moisture content

Result shown in Table 4 indicated that a maximum citricacid value (54.11 g/kg) was obtained when the initial level of moisture was 60%. Moisture level under solid state has influential role on the biosynthesis of microbial metabolites by changing the physical properties of solid particles. Lower moisture level shows a lower degree of swelling and higher water tension, and then ultimately reduces the solubility of nutrients.[23-26] On the other hand, high moisture level shows reduce porosity, changes particle structure, enhance stickiness. It also create slower oxygen transfer due to reduces gas volume and exchange and decreases diffusion (Lonsane *et al*., 1985).[20]

Table 4: Effect of moisture level on fungal production of citric acid from pineapple peel.

Moisture level (%)	Citric acid (g/kg)
30	28.20 ± 0.5
40	32.20 ± 0.5
50	46.51 ± 0.5
60	54.11 ± 0.5
70	30.41 ± 0.5
Mean of triplicate determinant ; SD±0.5	

Effect of methanol

Effect of methanol on citric acid production was shown inTable 5. Maximum citric acid production (63.1 g/kg) was obtained at 4 % concentration. An increase in citric acid production at 4% methanol concentration was inagreement with Hossain *et al*. (1984),[21] who explained that the presence of methanol in fermentation media may increase citric acid production by *A. nige*r. The inductive effect of methanol for citric acid production may be due to pulling down the negative inhibitory effects of metal ions (Kiel *et al*.,1981).[18] Moyer (1953)[22] suggested the use of low molecular weight alcohols i.e. methanol, isopropanol if added tothe culture medium, leads to increase citric acid production in both surface and submerged cultures and thus make it possible to ferment directly crude carbohydrate substrates.

Concentration (% v/v)	**Citric acid (g/kg)**	**Biomass (g/kg)**
Control: Pineapple waste supplemented with sucrose (15g) and NH_4NO_3 (0.25% w/v)	49.1 ± 0.5	9.2 ± 0.5
1 %	52.3 ± 0.5	10.4 ± 0.5
2 %	56.6 ± 0.5	9.2 ± 0.5
3 %	59.3± 0.5	7.4 ± 0.5
4 %	63.1 ± 0.5	7.0 ± 0.5
5 %	57.2 ± 0.5	6.7 ± 0.5
6 %	49.7± 0.5	6.0± 0.5
7 %	45.5± 0.5	5.4± 0.5
Mean of triplicate determinant ; SD±0.5		

CONCLUSIONS

In conclusion we must say that the use of pineapple peel for fungal production of citric acid might represent an efficient method of minimizing pineapple waste disposal problems and for producing other organic acids, pineapple peel would be the important feed stock for fermentation media in near future.

REFERENCE

1. AOAC. Official Methods of Analysis.16th edn. Association of Official Analytical Chemist, Washington D.C., 1995.
2. Adachi, D. M., Toyama, H., Yamada, M., Shingawa, E.and Matsushita, K. New developments inoxidative fermentation. Applied Microbiology and Biotechnology, 2003; 60: 643-653.
3. Archer, D. B., Mackenzie, A. and Jeenes, D. J. Genetic engineering; Yeasts and filamentous fungi.In: Basic Biotechnology. 2nd edn. Ratledge, C. andKristiansen, B. (eds.). Cambridge University Press, Cambridge, 2001; 95-126.
4. Crolla, A. and Kennedy, K. J. Optimization ofcitric acid production from Candida lipolyticaY-1095using n-paraffin. Journal of Biotechnology, 2001; 89: 27-40.
5. Drysdale, C. R. and McKay, M. H. Citric acid production by Aspergillus niger on surface culture oninolin. Letters of Applied Microbiology, 1995; 20: 252-254.
6. El-Holi, M. A. and Al-Delamy, K. S., Citric acidproduction from whey with sugars and additives byAspergillus niger. African Journal of Biotechnology, 2003; 2: 356-359.
7. Fernando, A. V., Carlos, G. A. and Torres, N. V. Metabolism of citric acid production by Aspergillusniger.Biotechnology and Bioengineering, 2000; 70: 82-108.
8. Grewal, H. S. and Kalra, K. L. Fungal productionof citric acid. Biotechnology Advances, 1995; 13: 209-234.

9. Hang, Y. D. and Woodams, E. E. Utilization of grape pomace for citric acid production by Solid state fermentation. American Journal for Enology and Viticulture, 1986; 37: 141-142.
10. Hang, Y. D. and Woodams, E. E. Microbial production of citric acid by Solid-state fermentation of Kiwi fruit peel. Journal of Food Science, 1987; 52: 226-227.
11. Haq, I., Ali, S. and Qadeer, M. A. Fed-batchculture studies during citric acid fermentation by Aspergillus nigerGCMC-7. Biologia, 2001; 45: 32-37.
12. Hossain, M., Brooks, J. D. and Moddax, I. S. The effect of the sugar source on citric acid production by Aspergillus niger. Applied Microbiology and Biotechnology, 1984; 19: 393-397.
13. Haq, I., Ali, S., Qadeer, M. A. and Iqbal, J. Citricacid production by mutants of Aspergillus nigefromcane molasses. Bioresource Technology, 2004; 93: 125-130.
14. Kamzolova, S. V., Shishkanova, N. V., Morgunov, I. G. and Finogenova, T. V. Oxygen requirements for growth and citric acid production of Yarrowialipolytica. Federation of European Microbiological Societies FEMS Yeast Research, 2003; 3: 217-222.
15. Kapoor, K.K., Chaudry, K., Tauro, P. Citric acid. In: Prescott and Dunn's Industrial Microbiology. Reed, G.(ed.). UK: MacMillan Publishers Ltd. 1983; 709-747.
16. Karaffa, L. and Kubicek, C. P. Aspergillus nigercitric acid accumulation: Do we understand this well working black box? Applied Microbiology and Biotechnology, 2003; 61: 189 196.
17. Khare, S. K., Krishna, J. and Gandhi, A. P. Citricacid production from okara (soy-residue) by solidstatefermentation. Bioresource Technology, 1995; 54: 323-325.
18. Kiel, H., Gurin, R. and Henis, Y. Citric acid fermentation by Aspergillus nigeron low sugar concentration and cotton waste. Applied Environmental Microbiology, 1981; 42: 1-4.
19. Kubicek, C. P. The role of sugar uptake and channeling for citric acid accumulation by Aspergillusniger. Food Technology and Biotechnology, 1998; 36: 173-175.
20. Lonsane, B. K., Ghildyal, N. P., Budiatman, S., Ramakrishna, S. V. Engineering aspects ofsolid state fermentation. Enzyme and Microbial Technology, 1985; 7: 258-265.
21. Mattey, M. and Allen, A. Metabolic accumulation in Aspergillus species. Biochemical Society Transaction, 1990; 18: 1020-265.
22. Moyer, A. J. Effect of alcohols on the mycologicalproduction of citric acid in surface and submergedculture. Applied Microbiology, 1953; 1: 1-7.
23. Pandey, A. Solid-state fermentation. Biochemical Engineering Journal, 2003; 13: 81-84.

24. Raimbault, M. General and Microbiological aspects of solid substrate fermentation. International Training Course. Solid-State fermentation. Curitiba-Parana, Brazil, 1997.
25. Schuster, E., Dunn-Coleman, N., Frisvad, J. C. and Van Dijek, P. W. On the safety of Aspergillusniger–A review. Applied Microbiology Biotechnology, 2002; 59: 426-435.
26. Torres, N. V., Lopez, J. C., Rivero, M. G. and Rojas, M.G. Kinetics of growth of Aspergillus nigerduring submerged, agar surface and solid-state fermentations. Process Biochemistry, 1998; 33: 103-107.
27. Xu, D. P., Madrid, C. P., Rohr, M. and Kubicek, C. P. The influence of type and concentration ofcarbon source on production of citric acid by Aspergillus niger. Applied Microbiology Biotechnology, 1989; 30: 553-558.