



PRODUCTION OF CITRIC ACID FROM BANANA PEEL USING *ASPERGILLUS NIGER*

¹ J.Damari priscilla, M.Gnaneel

¹Damari Priscilla.J , Gnanavel .G² , M.Tech, Dept. of Biotechnology, Karunya Institute of science and technology, Karunya nagar, Coimbatore-641114, Tamilnadu.

²Assistant prof, Dept. of Biotechnology, Karunya Institute of science and technology, Karunya nagar, Coimbatore-641114, Tamilnadu.India.

Abstract:

Municipal waste has become a severe problem in the developed and developing countries during the last century. Among these are various waste materials from food, packing, commercial materials ect; however the main constituent of municipal wastes is organic wastes ect. Citric acid commercially important product used in several industrial processes. Production of citric acid by *Aspergillus niger* from different varieties of banana peel such as green banana and yellow banana in surface culture process was studied. It was found that the maximum amount of citric acid (87%) was produced from yellow banana peel used culture. Lower citric acid (45%) was obtained from green banana peel used culture. By the extension of the fermentation (upto 25 days) resulted in an increase in citric acid concentration and biomass. The potential of Banana peels as a substrate for citric acid production by *A.niger* was investigated. Addition of supplements significantly enhanced the yield of citric acid. Of the three cultures examined, *A.niger* UABN 210 was found to produce the highest amount of citric acid (82.0g/Kg dry weight) after 96h of growth at 30°C in the presence of methanol (1%v/v) and copper ions(10ppm) . The yield of citric acid was over 90% based on the amount of sugar consumed. The study presents banana peel as an inexpensive medium for the production of citric acid by *Aspergillus niger*.

KEYWORDS- Solid state fermentation, citric acid, banana peel, filtration, *Aspergillus niger*.

1. INTRODUCTION

CITRIC ACID:

Citric acid is a 6-carbon containing tricarboxylic acid which was first isolated from lemon juice. It is a natural component of many citrus fruits, and was crystallized from lemon juice by scheele in 1784. Approximately 70% of citric acid produced is used in the food and beverage industry for various purposes, 12% in pharmaceuticals and about 18% for other industrial uses (yigitolu, 1992) . Commercial production of citric acid is generally by submerged fermentation of sucrose or molasses using the filamentous fungus *Aspergillus niger* or synthetically from acetone or glycerol (adachi *et al.*, 2003; haq *et al.*, 2004). in the recent times solid state fermentation (SSF) is considered as an alternative to submerged fermentation in the production of microbial metabolites because of higher yields, low water requirement and lower operating costs.

Many microorganisms have been evaluated for the production of citric acid including bacteria, fungi and yeast (yigitolu, 1992). However, fungal strains of *Aspergillus niger* remained the organism of choice for citric acid production due to ease of handling, its ability to ferment a variety of cheap raw materials, and high yields (schuster *et al.*, 2002). a cost reduction in citric acid production can be achieved by using cheap agricultural wastes such as apple and grape pomace, orange peel, kiwifruit peel, cotton waste, okara soy-residue and cane molasses (hang and woodams,1986,1987, yigitolu,1992,khare *et al.*,1995, haq *et al.*,2004,kareem *et al.*,2010).

Citric acid exists in greater than trace amounts in a variety of fruits and vegetables, most notably citrus fruits. Lemons and limes have particularly high concentrations of the acid; it can constitute as much as 8% of the dry weight of these fruits (about 47 g/l in the juices . The concentrations of citric acid in citrus fruits range from 0.005 mol/l for oranges and grapefruits to 0.30 mol/l in lemons and limes. Within species, these values vary depending on the cultivar and the circumstances in which the fruit was grown.

Industrial-scale citric acid production first began in 1890 based on the italian citrus fruit industry, where the juice was treated with hydrated lime (calcium hydroxide) to precipitate calcium citrate, which was isolated and converted back to the acid using diluted sulfuric acid. in 1893, c. wehmer discovered penicillium mold could produce citric acid from sugar. However, microbial production of citric acid did not become industrially important until world war I disrupted italian citrus exports. Lemons, oranges, limes, and other citrus fruits possess high concentrations of citric acid.

1.1 BANANA PEEL UTILIZATION:

Bananas are a popular fruit consumed worldwide with a yearly production of over 145 million tonnes in 2011. Once the peel is removed, the fruit can be eaten raw or cooked and the peel is generally discarded. Because of this removal of the banana peel, a significant amount of organic waste is generated .Banana peels are sometimes used as feedstock for cattle, goats, pigs, poultry, rabbits, fish and several other species, typically on small farms in regions where bananas are grown. There are some concerns over the impact of tannins contained in the peels on animals that consume them.

The nutritional value of banana peel depends on the stage of maturity and the cultivar; for example plantain peels contain less fibre than dessert banana peels, and lignin content increases with ripening (from 7 to 15% dry matter). On average, banana peels contain 6-9% dry matter of protein and 20-30% fibre (measured as ndf). Green plantain peels contain 40% starch that is transformed into sugars after ripening. green banana peels contain much less starch (about 15%) when green than plantain peels, while ripe banana peels contain up to 30% free sugars. banana peels are also used for water purification, to produce ethanol, cellulase, laccase, as fertilizer and in composting.

Banana peel is an example of a solid substrate that can be used for SSF. Nigeria is the fourth largest producer of banana in sub-Saharan Africa. The processing of banana into products such as chips, puree/pulp, powder, jams, juice, bar, biscuits, wine etc results in the generation of solid banana wastes particularly the peels which account for about 40% of the total weight of the fresh banana fruit. These wastes do not have any important use apart from being used as animal feed. Furthermore, they are typically disposed of inappropriately particularly in less developed countries thereby contributing to environmental problems. As the waste material is rich in ideal substrate for citric acid production via solid state fermentation. The production of citric acid has been reported to be influenced by fermentation conditions such as type and composition of fermentation medium, substrate type and concentration, agitation rate, aeration, temperature, pH etc. The performance of the SSF process can be significantly improved if these conditions are optimised. Experimental design methods coupled with response surface methodology (RSM) have been reported to be very effective in achieving this and it has been successfully applied to the optimisation of many bioprocesses. Hence the objective of this study was to optimise the fermentation conditions for the production of citric acid from banana peels using *Aspergillus niger* in batch reactor.

AIM AND OBJECTIVE

1. The objective of this study was to adopt the use of banana peel for the production of citric acid by *Aspergillus niger*.
2. The aim of the present work was production of citric acid by *A. niger* using different varieties of banana peel as substrate.
3. Banana waste is currently posing disposal problem in the tropics.

MATERIALS AND METHODS:

PRE-TREATMENT OF BANANA PEELS:

Banana peels were collected and oven-dried at 60°C for 2h and cut into 2mm mesh size.

PRODUCTION OF CITRIC ACID:

The basal medium was prepared by introducing banana peels (30g) into 200mL Erlenmeyer flasks. The medium was supplemented with nitrogen supplements was studied by adding Ammonium phosphate; Potassium hydrogen phosphate Peptone (0.5%) to the basal medium and moistened to 60% moisture content. Effect of trace elements was also studied by adding copper, iron, manganese and zinc ions (10ppm) The flask was cotton plugged

and autoclaved at 121°C for 15 minutes. After cooling at room temperature, each medium was inoculated with the *Aspergillus niger* (6.0×10^6) suspension and incubated at 30°C in a rotary shaking incubator for 5 days. Methanol (0-3%) 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.

ISOLATION OF ASPERGILLUS SPP:

Soil samples from sugarcane growing areas of Boko, Kamrup District (Rural), Assam, India during 2013-14. The soil samples were taken by means of sterilised spatulas and collected sterile sealed polythene bags. The samples were brought to the Research laboratory, Department of Botany, Cotton College, Guwahati, Assam, India and stored at room temperature for microbiological study. 1 gram of soil sample was taken in a 250 ml conical flask containing 100 ml of sterile distilled water and shaken well for 20 minutes. From the stock, various dilutions were prepared from (10^{-2} , 10^{-4} and 10^{-6}) using sterile distilled water. 1 ml of the serially diluted sample was poured in the sterile petridishes containing Potato Dextrose Agar (PDA) medium. Streptomycin was added in the molten PDA medium in the petridishes and were incubated at $30 \pm 1^\circ \text{C}$ for 3-5 days for the isolation of *Aspergillus* spp. After incubation, different fungal colonies grow on the PDA medium. Preliminary identification of the fungi was done based on their morphological characteristics, with the help of "A Manual of Soil Fungi" A total of 10 different *Aspergillus* isolates were obtained, 6 belonging to *Aspergillus niger* and 2 belonging to *Aspergillus fumigatus*. The dominant isolates of *Aspergillus niger* and *Aspergillus fumigatus* were then sub-cultured in PDA medium. These isolates have been tentatively named as *Aspergillus niger* S 1 to 6 (*Aspergillus niger* S-1, S-2, S-3, S-4, S-5 and S-6) and *Aspergillus fumigatus* S-7 and S-8. The isolated *Aspergillus* spp were purified by re-streaking on the medium and the pure cultures were maintained on the PDA slants and stored at 5°C in the refrigerator.

BASAL SCREENING FERMENTATION MEDIUM:

The isolate *Aspergillus* spp were screened quantitatively for the production of citric acid in liquid culture medium containing soluble

TABLE: 2 SCREENING TEST FOR ASPERGILLUS NIGER.

starch	10 gm/L
(NH ₄) ₂ SO ₄	2.2 gm/L
K ₂ HPO ₄	10 gm/L
K ₂ HPO ₄	0.05 gm/L
K ₂ HPO ₄	0.05 gm/L

The basal screening media was autoclaved at 121°C for 15 minutes. The basal medium was inoculated with 1ml of the spore suspension of the concentration (2×10^8) spores/ml prepared from 5 days old cultures of the isolated *Aspergillus* strains. A total of 8 *Aspergillus* strains were screened for citric acid production. Based on the screening results for citric acid yield, only *Aspergillus niger* S-6 was selected for further study.

FERMENTATION CONDITION:

Fermentation was carried out in the laboratory in 250 ml Erlenmeyer flasks as small scale laboratory fermentor containing 100 ml of fermentation medium in each flask. *Aspergillus* strains were inoculated separately to the fermentation medium at an inoculum concentration of 1 ml containing the spore suspension of the concentration (2×10^8) spores/ml. Experiments were carried out at $30 \pm 1^\circ\text{C}$ in the incubator.

FEEDSTOCK PREPARATION

Banana peels were obtained from a local market and used as solid carbon substrate in this study. The chemical composition of banana peels has been previously reported in a study. The peels were washed with clean water to remove any adhering dirt after which they were dried in an oven at 60°C for 2 hours. The dried peels were then milled to obtain 2 mm particles.

Table 3: CHEMICAL COMPOSITION OF BANANA PEELS

Component Composition (wt %)

Carbohydrate	59.0
Protein	0.9
Crude fibre	21.7
Phytate	0.28
Crude lipid	1.7
Ash	8.5
Moisture	6.7

Microorganism and Inoculum Preparation *Aspergillus niger* ATCC 9142, obtained from the Biotechnology division of the Federal Institute of Industrial Research Oshodi (FIRO), Lagos, Nigeria was used throughout the study as the fermenting organism. Conidia suspensions of fungal strains were obtained from cultures grown on potato dextrose agar slants at 30°C for 5 to 7 days. The spores were washed with sterilised 0.8% Tween 80 solution by shaking vigorously for 1 minute. Spores were counted with a haemocytometer to obtain the desired number of spores.

SOLID STATE FERMENTATION[SSF]

Solid state fermentation was carried out in 250 mL Erlenmeyer flasks with a working volume of 100 mL. The solid substrate was weighed into the flask and to this a supplemental salt solution was added to achieve the desired moisture level. The composition of the salt solution was as follows (g/L):

TABLE :4 CHEMICALS ADDED TO THE BASAL MEDIUM

MgSO ₄ ·7H ₂ O	0.15
CaCl ₂	0.015
ZnSO ₄ ·7H ₂ O	0.002
MnSO ₄ ·H ₂ O	0.006
FeCl ₃ ·6H ₂ O	0.015

Yeast extract was taken as nitrogen source. The contents were thoroughly mixed, cotton plugged and autoclaved at 121°C (15 psi) for 20 min. After cooling, the sterilised substrate including media was inoculated with 2 mL of inoculum and then incubated at varying conditions in a rotary shaking incubator for 5 days. The solid substrate loading, pH and the inoculum density were varied according to the experimental design. At the end of fermentation, the medium was diluted with distilled water (1:4 w/v) and then filtered and the filtrate was used for analysis.

ANALYTICAL METHODS

The concentration of citric acid produced during fermentation was determined using the pyridineacetic anhydride method as reported by Marrier and Boulet. This was accomplished by adding 1 mL of the filtered fermentation broth along with 1.30 mL of pyridine and 5.7 mL of acetic anhydride in a test tube. The test tube was then placed in a water bath at 32 °C for 30 min. The absorbance of the sample was measured at 405nm using a UV-Vis spectrophotometer (PG Instruments model T70). The concentration of citric acid in the sample was determined from a citric acid calibration curve which was prepared from known concentrations of citric acid. The pH of the sample was determined using a Unicam 9450 model pH meter.

MEASUREMENT OF CITRIC ACID:

The concentration of citric acid in the fermentation medium was estimated titrimetrically (AOAC, 1995) as reported by different investigators. The readings were taken at regular intervals of 4 hours from the time of inoculation of the medium with the fungal spores. The amount of citric acid produced by the different isolates of *Aspergillus* spp.

3.1.0 OPTIMISATION OF FERMENTATION MEDIUM:

The next phase of the study conducted was optimisation of the culture conditions taking four different parameters i.e. Temperature, pH, Carbon sources and Nitrogen sources, for citric acid production, using *Aspergillus niger* S – 6, as it showed the maximum levels of acid production so far. The citric acid production was estimated after 60 hours of incubation of the inoculated medium.

TEMPERATURE:

Different temperature ranges were selected i.e. 25°C, 30°C, 35°C, 40°C and 45°C and the fermentative media were allowed to produce citric acid under these temperature ranges. The amount of citric acid produced at different temperatures by *Aspergillus niger* S-6 .

pH:

The pH of the fermentative medium was another criteria that was worked upon. A range of pH starting from (5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0)were selected for citric acid production by *A niger* S-6.

CARBON SOURCES:

Three Carbon sources are chosen and tested for their suitability as substrates for citric acid production, among starch, sucrose and maltose. Different concentrations of solutions containing starch, sucrose and maltose are used to find out the suitable concentration at which the citric acid production is the optimum.

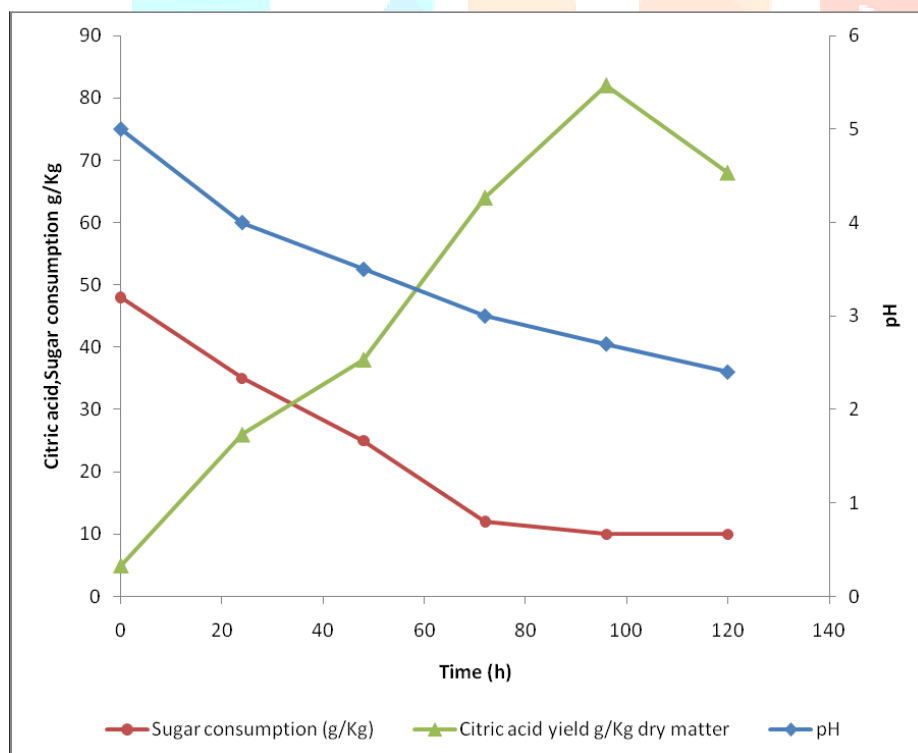
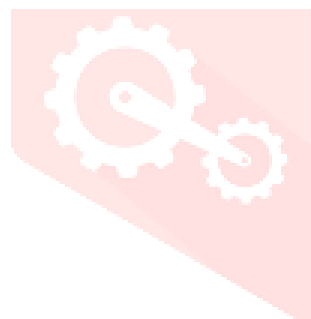
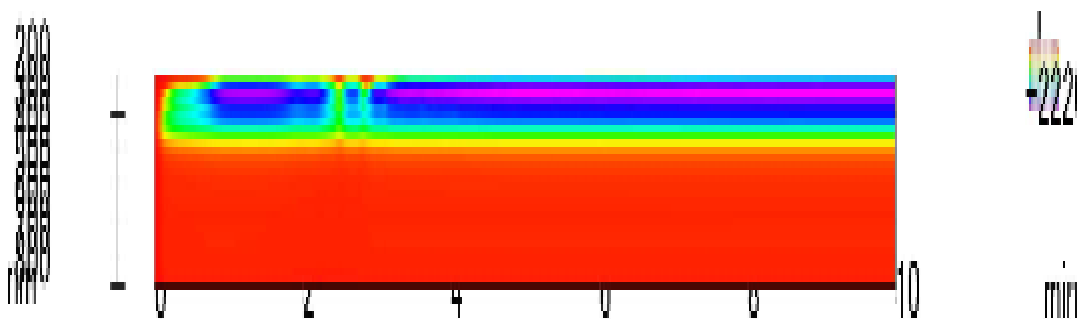
GRAPH : 1 CITRIC ACID SUGAR CONSUMPTION g/kg (VS) TIME(h)

TABLE: HPLC –CHROMATOGRAM OF CITRIC ACID.

CITRIC ACID:

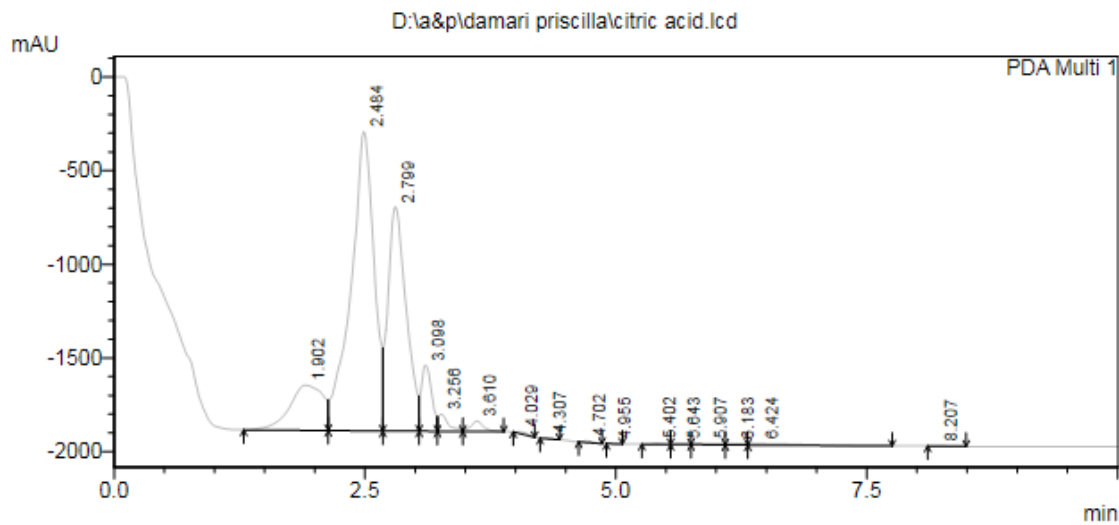
Name	Ret. Time	Area	Area %	Conc	Height	Units
RT2.484	2.484	25656507	50.3827	0.00000	1596161	mg/L
RT2.799	2.799	15220940	29.8900	0.00000	1198794	mg/L
RT3.098	3.098	2521782	4.9521	0.00000	354767	mg/L

GRAPH : PDA MULTI 1/254nm 4nm**LCSOLUTION ANALYSIS REPORT**

IJCRT

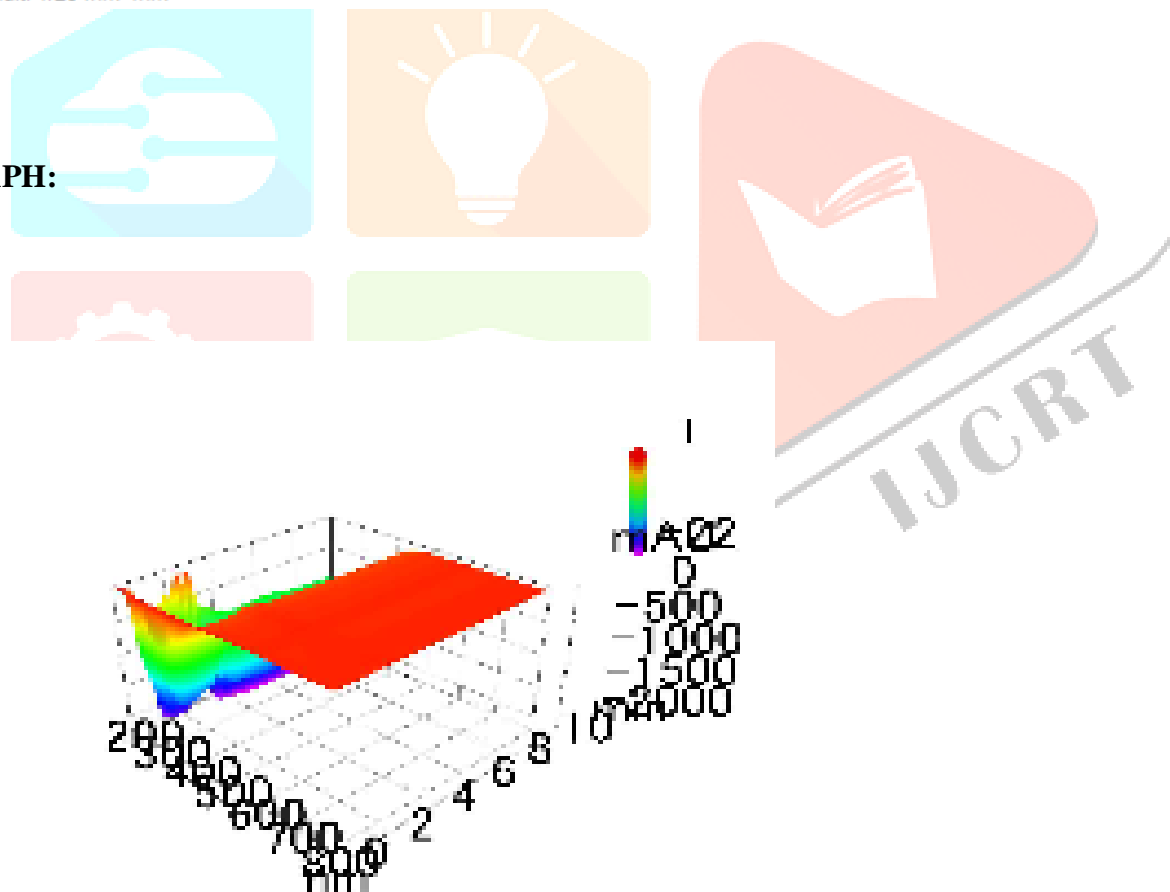
GRAPH: HPLC

<Chromatogram>



1 PDA Multi 1/254nm 4nm

3D GRAPH:

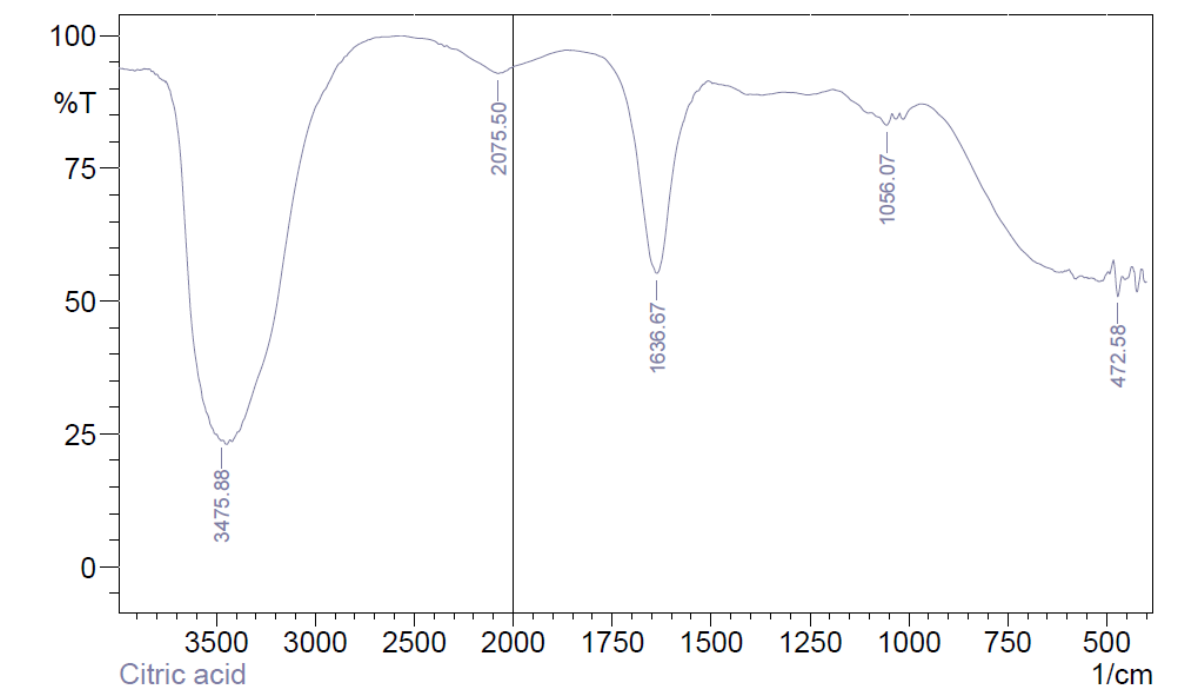


GRAPH: UV-SPECTROPHOTOMETER READINGS:

DATA POINTS: PEAK HEIGHTS

nm	Abs
236.0	0.504

GRAPH: FT-IR



FTIR READINGS

Citric acid

1/cm

No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	472.58	50.823	5.371	483.19	461.97	5.772	0.457
2	1056.07	83.121	2.213	1095.61	1042.57	3.934	0.292
3	1636.67	55.211	38.072	1803.52	1518.04	28.269	20.234
4	2075.5	92.844	0.444	2292.5	2061.03	5.231	0.279
5	3475.88	23.683	0.293	3499.99	3472.02	17.246	0.099

DISCUSSION:

Production of citric acid by *A.niger* on banana waste with or without supplements. A minimum citric acid production (15.48g/kg) was obtained in banana waste while supplementation with nitrogen sources led to an increase in citric acid secretion. Among the supplements, peptone gave a maximum citric acid yield (30.8g/kg) followed by ammonium phosphate (42.32g/kg). Nitrogen had been reported to be an important factor in fermentation processes due to an increase in C/N ratio. Nitrogen constituent has a 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. The fermentation media for citric acid biosynthesis should consist of substrates necessary for the growth of microorganism primarily the carbon, nitrogen and phosphorus sources. Effect of methanol on citric acid production. Among the three strains of *Aspergillus niger*. Maximum citric acid production (64.20g/kg) was exhibited by *A.niger* UABN 210 at 1% methanol concentration.

The addition of methanol to banana peel waste medium led to an increase in citric acid production by *Aspergillus niger*. The inductive effect of methanol for citric acid production may be due to reduction of the inhibitory effects of metal ions. The addition of lower alcohols, methanol, ethanol, n-propanol, to crude carbohydrate raw materials could increase the yield of citric acid. Optimal concentration of methanol, which was said to be more effective than ethanol, varied from 1 to 4% by volume. However addition of methanol to highly

purified, high yielding substrates is deleterious to acid yields. The presence of methanol at a concentration of 3% in grape pomace led to an increase in the citrate yield. The exact mechanism of the alcohol effect however is unexplained, though it is postulated that addition of methanol increases the tolerance of fungi to Fe²⁺, Zn²⁺ and Mn²⁺. Effect of trace elements on citric acid production as showed that copper and zinc favored citric acid production while iron and manganese exhibited deleterious effect on bioaccumulation of citric acid. Trace element nutrition is one of the most important factors affecting the yields of citric acid fermentation (Yigitolu 1992).

RESULTS:

The results showed that scaling up of this process would be helpful to produce the costliest acid like citric acid. Best utilization of waste, for citric acid production solved the problem of waste management and pollution in the environment. The effects of fermentation period on citric acid production, pH and sugar consumption. A steady increase in citric acid production was observed along the fermentation period with optimum yield (82.2g/kg) after fermentation for 96h while a decrease in the consumption of sugar was observed. Previous reports have shown that the production of citric acid on pineapple waste approximately paralleled the consumption of sugar. Based on the amount of fermentable sugar consumed, the yield of citric acid was 90.62% under optimum solid-state fermentation conditions. A decrease in pH values from 5.0 to 2.8 was noted after 96h of fermentation. The pH value maintained at the beginning of fermentation was important for a specific biomass formation. The progressive decrease in pH was noted as incubation time increased which was due to the formation and accumulation of citric acid.

REFERENCE:

1. AOAC(1995):Official Methods of Analysis,16th ed.Association of Official Analytical Chemist, Washington D.C.
2. Adachi,D.M., Toyama,H., Yamada,M., Shingawa,E.and Matsushita K.(2003):New developments in oxidative fermentation. *Applied Microbiology and Biotechnology*,60:643-653
3. El-Holi M.A.and Al-Delamy K.S.(2003): Citric acid production from whey with sugars and additives by *Aspergillus niger*. *African Journal of Biotechnology* 2(10):356-359
- Grewal,H.S. and Kalra ,K.L.(1995): Fungal production of Citric acid. *Biotechnology Advances* 13:209-234
4. Hang,Y.D. and Woodams E.E.(1986):Utilization of grape pomace for citric acid production by Solid state fermentation. *American Journal for Enology and Viticulture* 37(2):141-142
5. Hang,Y.D. and Woodams E.E.(1987):Microbial production of citric acid by Solid-state fermentation of Kiwifruit peel. *Journal of Food Science* 52:226-227
6. Hossain M, Brooks J.D. and Moddax ,I.S.(1984):The effect of the sugar source on citric acid production by *Aspergillus niger*. *Appl. Microbiol.Biotechnol.*19:393-397

7. Haq,I., Ali,S.,Qadeer,M.A.and Iqbal,J.(2004):Citric acid production by mutants of *Aspergillus niger* from cane molasses. *Bioresource Technology* 93(2):125-130
8. Kareem,S.O., Akpan,I. and Oduntan S. B.(2009) Cowpea waste- A novel substrate for the solid state production of amylase by *Aspergillus oryzae*. *African Journal of Microbiology Research* 3(12):974-977
9. Kareem,S.O.,Akpan,I., and Alebiowu,O.O.(2010): Production of citric acid by *Aspergillus niger* using pineapple waste. *Malaysian Journal of Microbiology* 6(2)161-165
10. Khare S.K., Krishna J.and Gandhi A.P.(1995): Citric acid production from okara (soy-residue) by solid-state fermentation. *Bioresource Technology* 54: 323-325
11. Maddox,I.S. and Brooks,J.D.(1998): Application of a multilayer packed bed reactor to citric acid production in solid-state fermentation using *Aspergillus niger*. *Process Biochem.*,33(2):117-123 .
12. Schuster,E., Dunn-Coleman, N., Frisvad, J.C. and Van Dijek,P.W.(2002):On the safety of *Aspergillus niger* –A review, *Appl. Microbiol.&Biotechnol.*,59(4-5):426-435 .
13. Xu D.P., Madrid C.P., Rohr M.,and Kubicek C.P.(1989): The influence of type and concentration of carbon source on production of citric acid by *Aspergillus niger*.*Appl. Microbiol.Biotechnol.*30:553-558.
14. Soccol, C. R. and Vanderberghe L. P. S (2003) Overview of Applied Solid–State Fermentation in Brazil. *Biochemical Engineering Journal* 13:205-218.
15. Lofty, W. A., Ghanem, K. M. and El-Helow, E. R. (2007) Citric Acid Production by a Novel *Aspergillus niger* Isolate II. Optimization of Process Parameters through Statistical Experimental Designs. *Bioresource Technology* 98: 3470-3477.
16. Grewal, H. S., Kalra, K. L. Fungal production of citric acid, *Biotechnol. Adv.*13 (1995) 209-234.
17. Ikeno, Y., Masuda, Y. M., Tanno, K., Oomori, I., Takahashi, N. Citric acid production from various raw materials by yeasts, *J. Fermentat. Technol.* 53 (1975) 752-756.
18. Kubicek, C. P., Röhr, M. Citric acid Fermentation, *Crit. Rev. Biotechnol.* 3 (1986) 331-373.