

Overview across green production of citric acid**Abstract**

Citric acid has a predominant position between the most highly relevant biotechnological products in industrial demand. Multi-functional citric acid is well known of its applications, mainly in the food and nanotechnology drug delivery sectors. Improper disposal of the agro-waste causes a serious ecological threat by encouraging growth of microbial pathogens. The alternatives for utilization of such cellulosic biomass are therefore of great importance for cleaner production to reduce the cost of bioactive compounds production. Hence, pulps, seeds and peels wastes were used as feedstocks carbon sources for a citric acid-production. This review provides a brief summary describing microorganisms, morphology, self-immobilization, bioreactors variability, metabolic engineering, parameter optima and product recovery in order to highlight microbial citric acid production.

Keywords

A. niger, glycolytic flux, biological carriers, rotating biological contactor (RBC), immobilization, genetic engineered strains.

Introduction

Citric acid (CA) possesses many important qualities as a green solvent, palatable, highly soluble and an extremely low toxic to human and mammals. Chemical synthesis of citric acid is more costly than fermentation, the second largest fermentation product. Industrial

citrus processing or cashew apple juice extraction for example generates tons of wastes of peels and segment membranes that produce odor and result in pollution from harmful chemicals which were used conventionally. (1), mAHATO(2) and hence its handling is the most demanding target (3,5)atheeshkumar2019 **Ajala, A.S2020 Zafar, M.; Bano .**

Substrates for citric acid production

Lignocellulosic biomass is the most abundant raw material on earth and could be used as renewable substrate for citric acid fermentation because it contains proteins, fats, fibers, and polysaccharide necessary for good acid fermentation like wheat bran for example(6)**Hussain, A.M.** 2019. The chemical hydrolysis of cellulose and hemicellulose or the use of cellulolytic enzymes for the degradation of lignocellulosic biomass is an effective approach. The cellulolytic liberation of sugars or pretreatment of solid substrate release peptides and amino acids from proteins and a mixture of fermentable sugars including glucose, mannose, xylose and arabinose (7) **Khosravi, D.K. and Zoghi, A. (2008)**, which could promote growth but dislike Ly generate in some substrates furfurals that inhibit biomass production.

Fruit wastes like shea nut shell Kobomoje(8), Yam, Chijioke Elijah et al 2022,(9) sweet lime, banana and pineapple peels contain soluble sugar and pectin. Groundnut pod, pomegranate, rice bran, (10,11)T Roukas · 2020), cassava,Campanhol et al., 2019) white star apple(12) Dienye et al.2018, , cotton waste, pretreated milled peel moistened with co-substrate sucrose medium, banana pseudostem (...etc(13-19,) are other examples for potential substrates using *A. niger* for fermentation (Naveen Prasad B. S.1 13 2020eena et al., 2022 14TP West · 202315 Maharani,16 V.ukana, Ukana, et al 17 Dauda, A.O.18

(2014), , Milesh Laltha, B.S., Kareem, et al 201019. Similarly, *A. niger* as a world's leading choice attractive production host excrete citric acid after growth on corn substrates because it is good source of nutrient sugary substrates for citric acid. Many microorganisms are able to ferment bagasse and blackstrap molasses in liquid-solid phase culture. (6) Khosravi, D.K. and Zoghi, A. (2008 but fermenting beet and cane molasses result in lower citric acid production mostly due the presence of furfural and hydroxymethyl furfural that have been shown to be the main important fermentation inhibitors. Furfural inhibits central enzymes in glycolysis, e.g. hexokinase, phosphofructokinase and triosephosphate dehydrogenase (Modig et al. 2002)(20) . Also, molasses contains high level of impurities so, pretreatment by centrifugation, potassium ferrocyanide, cation exchange resins or activated charcoal in order to compete with cultures depend on utilizing simple refined sugars efficiently in glucose or sucrose synthetic medium.

Certain conditions of drastic nutrient imbalance in fungi, yeast, and bacteria produce CA in excessive amounts (Soccol et al. 2006; hu et 2019(21,22) Traditionally, *A. niger*, *Penicillium janthinellum*, *P. restrictum*, *Trichoderma viride*, *Mucor piriformis* and *Ustilina vulgaris*, produce the acid when cultivated on various traditional biobased feedstocks for fermentation. both natural and transformed strains of *Yarrowia lipolytica* able to produce CA using transesterification of oils obtained from seeds and glycerol-containing wastes of biodiesel industry Imandi, S.(23) Among other species that are reported to produce CA are *Bacillus licheniformis*, and *Corynebacterium* sp. Among Solid state bioconversion(SSF) agricultural processing coproducts are coconut husk, coconut cake, sunflower cake, wet corn distillers' grains,

or autoclaved grape pomace ...etc **Maharani, V., Reeta, D.24**, Xie and West 2006(25-26) Behera et al., 2021).SSF aim to energy saving, and lower wastewater generation. G. Xie, T.P. Wes 2009(27)*OKAREH O.T., 10.D. ENESI and 2R. OLAWOYIN2016 (28)Chysirichote(29), 2020) taking into consideration low moisture levels reduced substrate availability to the fungus causing citric acid reduction while the high moisture content resulted in susceptibility to microbial attack. Enhanced aeration facilitates O₂ and CO₂ exchange between substrate matrices. Despite turnout on *A. niger* solid-state fermentation, commercial synthesis of citric acid has relied on submerged or surface fermentation west (15) .

Scale up processing of fermentation

As the submerged fermentation process is commonly employed technique for citric acid production, stirred tank bioreactor and airlift bioreactor both are generally preferred for this process mahatoAGHERA2019(30). However, different types of SSF bioreactors, including tray, packed-bed, rotating/stirred-drum, fluidized-bed, rocking-drum, and stirred-aerated bioreactors, are used X. Ge, J. Vasco-Correa, Y. Li *2017(31).

In order to work up product isolation immobilization was used. The main methods employed in fungi immobilization are self-immobilization in pellets and mycelium adhesion to a fixed or porous surface. The first method is based on the natural tendency that some filamentous fungi species in submerged cultures develop morphology in pellets (Gibbs et al. 2000)(33). In other fungal species, the fixation to a surface or supporting material has a predilection due to exopolysaccharides acting as an adhesive material. Immobilized cell techniques in a supporting material are also performed by encapsulating the fungal mycelium in the pores or intersections of fibrous materials as calcium alginate gel,

polyacrylamide gel or on polyurethane foam using generally leads to minimize their main drawback of central autolysis caused by diffusional limitations, thus enhancing nutrient and oxygen transfers which makes repeated batch and continuous processes possible. Immobilization physically trapped within porous solids or matrices allowing repetitive use of the immobilized biofilm without loss of bioactivity is also performed. Such techniques have several advantages, as higher volumetric production and, protects the cells from shear forces and imparts a special stability to the microorganism against environmental stresses in the waste stream. Mycelial pellets biocarriers are used for enhanced large-scale fermentation to decrease culture viscosity and improve oxygen and mass transfer efficiency. As biomass density is inhomogeneous and is inversely proportional to pellet porosity leading to a limited accessibility for nutrients and consequently hinder substrate uptake, the diameter of the mycelial pellets or clumps should be controlled within an appropriate range to prevent cell lysis.

Electrostatics, hydrophobicity and interactions between spore wall components are main triggers for pellet formation, Lukas et al 2018(32) **immobilized *A. nigr* cells on using date palm syrup, Al-Shehri (33).**

When the process was operated in a packed-bed reactor solid-state fermentation, the bed loading, the airflow rate, substrate and particle size were the most important operational parameters. Output in pilot citric acid production is controlled by the mixing characteristics of the bioreactor. In tray type bioreactor or bio-fermenter, large quantity of inoculum is required but increasing biomass concentrations result in highly viscous fermentation media, resulting in issues with gas-liquid mass transfer, liquid mixing resulted in a complex rheology in *A. terreus* (Porcel(35) et al. 2005). Using rotating biological contactor

that obtained with a stirred-tank fermenter improve stirrer rotational speed, and oxygen flow rate. Jianlong,w.(2000). 36

RBC-PUF is a bio-functions integrating filter system capable of retaining considerable amounts of attached *A. niger* biomass with self-immobilized and biofilm growth. RBC disks coupled to PUF porous biomass support when coupled with a good oxygen transfer capability of the system, could provide successful performance jiang wang 2000 (35). RBC provides both acceptable effluent quality and a removal efficiency at a comparatively very low cost. Short contact periods are required because the large active surface biomass generally has good settling characteristics,

The fluidized bed biofilm reactor (FBBR) represents a recent innovation in biofilm processes. When biomass was repeatedly used, the immobilized filamentous fungal pellet as small fluidized particles of the medium results in higher liquid throughputs and absence of biomass wash-out Shieh, W.K., Keenan, J.D. 1068,(37)

A significant number of engineered strains have been developed showing higher performance Adeoye *et al*,(1) With the development of molecular biology techniques, it is possible to control the mycelial morphology at the desired molecular level. Isato Yoshioka, Kohtaro Kirimura2021(38). Nowadays, more sophisticated techniques as genomic editing have been explored In order to improve their special capabilities or metabolic of *A. niger* to endure stressful conditions Metabolic engineering has emerged as a powerful and widespread method for genome editing due to its advantages of transient genome editing and reduced off-target effects, ribonucleoprotein-based (CRISPR-Cas9) complex genome editing system consisting of Cas9 protein

YANG (40) has been shown to revolutionize strain construction faster and more reliable: Isato Yoshioka, Kohtaro 2021(38) . Also, genetically engineered strains by knocking out genes or gene silencing Mores a, Vandenberghe2021(39). The silencing of chitin synthase gene (chsC) in *A. niger* interfer with morphogenesis and citric acid production as it was demonstrated that chsC silencing caused the compactness of the mycelial pellets was obviously reduced in the morphological mutants, with lower proportion of dispersed mycelia, leading to improvement in citric acid production. Thus, genetic manipulation of the chitin synthase gene chsC might represent an effective method to obtain excellent traits for improving the production of the desired product during industrial filamentous fungal fermentation of economic significance. Sun et al 2018(41)

Nutritional parameter for citric acid fermentation by *A niger*

Citric acid concentration will rise to appreciable amounts only under conditions of substantive metabolic imbalances, membrane transport in *A. niger* emphasizing the roles of glycolytic flux and its control excretion of citric acid from the mitochondria and the cytosol, within critical fermentation variables. (Modig et al. 2002.(20). Statistical models predict optimization under Response Surface Methodology (RSM) is widely used for maximizing production. The highest levels of the extraction parameters from single factor experiments were considered to be the basal point for the design of the final citric acid production and the fermentation optimization experiment through Box-Behnken design (BBD) Mustafa Germec].(42-44) Kusuma et al., 2022 **Yueyang Fu*2021**. Models main advantage is ability to reduced number of experimental runs needed to provide sufficient information for statistically acceptable results due to multitudinous

factors that jointly determine the acid yield, and their complex interactions using Response Surface Methodology (RSM) .

Central composite design and RSM were applied to determine the effects nutritional limiting factors C, N, P sources, time and their reciprocal interactions for acquiring better fermentation efficiency, beside other physicochemical factors as volume, age of inoculum, incubation temperature, moisture process, and surface area to volume ratio. Additionally, the biotechnological processes such as, agitation which induce acid production, type of inoculum, and culture pH to boost mycelium formation in submerged cultures hence the morphology of the fungus.

However, in industrial mass scale certain nutrients must be in excess as sugar and oxygen. Some studies report that limitation of the nitrogen source and deficiency of manganese or phosphate in the fermentation medium will inhibit the anabolism of *A. niger* resulting in protein degradation which, in turn, leads to a higher concentration of ammonium ion.

Biomass production by the fungus can affect its citric acid production because the fungus consumes large quantities of sugar to support cell multiplication. The decrease in citric acid production was likely because of oxidation of citric acid upon exhaustion of the fermentable sugars. Amengown2023(45) Stated that sucrose can be readily attacked by the microbial intracellular enzymes, because of its low molecular weight in nutrient medium recording maximum citric acid production than the glucose added medium. **Ukana, D.A.,3012 (17)**. Divalent metal ion, pretreatment of fermentation medium and the addition of different low molecular weight alcohols affect the yield of citric acid.

Ethanol resulted in inactivate crucial glycolytic enzymes and affect mitochondrial functionality. Furthermore, it also known to increase the permeability of the cell membrane and thus higher secretion of CA due to morphological changes and damaging the cell wall. Methanol addition stimulate a dehydrating action on the substrates, intensifies the outbound permeability of cellular walls for secretion of citric acid and reduce the inhibitory effects of metal ions. Kiel Nadeem, A., et al 2010 (46).

Many research reported the use of ANN in intelligent modeling for approximate media constituents in pilot scale citric acid production harma and Mishra (47), A. N. Amenaghawon (45) and it is thought that ANN and ANFIS have the capability to model and precisely predict the rapid changes and the final product yield (**Kusuma et al. (2022)(43)**) Ak Kumar,.

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Rheological behavior of *A. niger*

A. niger growth was found to be diauxic, spore germination being followed by an exponential growth phase, then a period of growth disturbance, and last of all a phase of secondary growth, characterized by a constant coefficient of growth decline appeared. The chief increase in citric acid concentration took place in the last fermentation phase. . The transient element act as nutrients should be in limiting concentrations Zn, Mn, Fe, ions and so are the heavy metals. Mn deficiency result in accumulation of citric acid by interfering with phosphofructokinase enzyme of TCA cycle. Oligo- elements contents

affect mold morphology, e.g., copper ions have a direct influence on mycelial morphology, provoked pellet formation and increased volumetric productivity. Addition of some ions produce an antagonistic effect on deleterious action of iron and manganese ions.

Extract recovery of citric acid

Although classical method is the most used technique on large scale processes, solvent extraction has been developed for the separation of citric acid

The high energy cost, complex process, and heavy use of reagents hazardous to the environment in the conventional industrial separation, extraction, and production strongly call for modern technological innovation, Rong Ye et al 2022 (48). Recovery technologies are processed by precipitation by typical downstream processing using calcium hydroxide to get calcium citrate and subsequent sulfuric acid to regenerate citric acid which then recovered and crystalized. Solvent extraction method as an alternative to citric acid classical large scale precipitation process has been developed. The reactive solvent extraction method from dilute aqueous broth by tri-n-butylphosphate (TBP), tri-n-octylamine (TOA), and the surfactant Aliquat 336 (A336), is quite familiar. thaker et al 2018 M. Pazouki, T. Panda 1998. (49,50). Supercritical CO₂ is another recommended solvent as a reactive extractant. Capturing supercritical CO₂ used to convert calcium citrate to citric acid. Ion exchange, macro-porous adsorption process and the biological electrodialysis with bipolar membrane and ultrafiltration techniques are used for citric acid recovery to eliminate calcium sulfate Wang J 2020.(51). Minimizing cost, saving experimental time, adjusting combined effect of the extraction process parameters; shaking speed, solvent ratio, and shaking time were done statistically with the help

of experimental design BBD under RSM and a second order regression model. A. Saltelli et al.(52)

Crystallization of citric acid

The evolution of the crystal size and shape distribution during crystallization processes is an important task, as the latter decisively influences the physical and solid-state properties of crystalline material, i.e., Crystallization kinetics nucleation and growth are the major steps which control the process. The microstructures of crystalline citric acid from seeding in large seed population and the measuring the dimension change of a single crystal in a microscopic cell at different supersaturation levels were reported using a vibrated bed evaporative crystallization. Both the measured de-supersaturation profiles and the time variations of the crystal size dimension were explained by the simultaneous occurrence of nucleation and crystal growth. This involves two different populations of particles: seed crystals and particles issued from sustained secondary nucleation Leila Nemdili et al 2016.(53). The yield of citric acid crystals estimated gravimetrically and predictive models were characterized using X- ray diffraction ,attenuated total reflection (ATR)-Fourier transform infrared (FTIR) spectroscopy, TIR Naveen Prasad B. S.1*, Selvaraju Sivamani 2021,(13)

References

1-Adeoye, A. O., & Lateef, A. (2022). Improving the yield of citric acid through valorization of cashew apple juice by *A. niger*: Mutation, nanoparticles supplementation and Taguchi technique. *Waste and Biom- Valoriz*, 13(4): 2195-2206.

2- Mahato, N., M. Sinha et al. (2019). Modern extraction and purification techniques for obtaining high purity food-grade bioactive compounds and value-added co-products from citrus wastes. *Foods*, 8, 523.

3- Satheeshkumar, S., Paramasivam, S. et al. (2019). Utilization of fruit waste for the production of citric acid by using *A. niger*. *J of drug deliv and therap*, 9(4-A):9-14.

4- Ajala, A.S.; Adeoye, A.O.; Olaniyan, S.A.; Fasoyin, O.T. (2020). A study on effect of fermentation conditions on citric acid production from cassava peels. *Sci. Afr.*, 8, e00396.

5- Zafar, M.; Bano, H.S.; Anwar, Z. (2021). Orange peels valorization for citric acid production through single and co-culture fermentation. *Jord J. Biol. Sci.* 14, 261–266.

6- Hussain, A.M. (2019). Citric acid production using wheat bran by *A. niger*. *Ind J. Pub Health Res. Dev.*, 10, 1213–1217.

7- Khosravi-Darani K, Zoghi A. (2008). Comparison of pretreatment strategies of sugarcane bagasse: experimental design for citric acid production. *Biores. Technol*, 99, 15, 6986-93.

8- Kobomoje, O.S., Mohammed, A.O. and Omojasola, P.F. (2013). The Production of citric acid from Shea Nut Shell (*Vitellaria paradoxa*) using *Aspergillus niger*. *Adv in Appl Sci Res*, 4, 25-28.

9- Onu C E , Nweke C N, Nwabanne J T (2022). Modeling of thermo-chemical pretreatment of yam peel substrate for biogas energy

production: RSM, ANN, and ANFIS comparative approach. App surface. sci adv .11.

10-Roukas, T.; Kotzekidou, P. (2020). Pomegranate peel waste: A new substrate for citric acid production by *A. niger* in solid-state fermentation under non-aseptic conditions. Environ. Sci. Poll. Res. 27, 13105–13113.

11-Campanhol B.S. and G.C. Silveira et al. (2019). Effect of the nutrient solution in the microbial production of citric acid from sugarcane bagasse and vinasse, Biocat and Agric Biotechnol, 19, 101147.

12-Dienye B. N et al. (2018). Citric acid production potential of *A. niger* using *Chrysophyllum albidum* peels. Adv in Bios and Biotechnol, 9, 190-203.

13-Naveen P. B, Selvaraju S. et al(2021).Chemical route for synthesis of citric acid from orange and grape juices. Chem Ind and Chem Eng Quart 28(00).

14-Reena R. Sindhu R, et al. (2022).Insight into citric acid: a versatile organic acid. Fuel 327,1,125181.

15- West T P. (2023). Citric acid production by *A. niger* using solid-State fermentation of Agricultural processing coproducts Appl. Biosci., 2, 1–13.

16--Maharani, V., Reeta, D., et al, (2014) Isolation and characterization of citric Acid producing *A. niger* from spoiled coconut. Inter J of Curr Microbiol and Appl Sci, 3, 700-705.

17-Ukana, D.A., Aniekan, E.A. and Godwin, N.E. (2012). Evaluation of proximate compositions and mineral elements in the star apple peel, pulp and seed. *J of Bas and Appl Sci Res*, 2, 4839-4843

18-Dauda, A.O. (2014) Physio-chemical properties of nigerian typed african star Apple fruit. *Inter J of Res in Agric and Food Sci*, 2.

19-Milesh Laltha, Y. Sewsynker-Sukai, E.B. Gueguim Kana,(2022). Simultaneous saccharification and citric acid production from paper wastewater pretreated banana pseudo stem: Optimization of fermentation medium formulation and kinetic assessment ,*Biores Technol*, 361, 127700.

20-Modig T, et al. (2002). Inhibition effects of furfural on alcohol dehydrogenase, aldehyde dehydrogenase and pyruvate dehydrogenase. *Biochem J* 363(Pt 3):769-76.

21- SOCCOL C.R. et al. (2006): Citric Acid production, *Food Technol. Biotechnol.* 44 (2) 141–149.

22-Hu, W.; Li, W.; Yang, H.; Chen, J (2019). Current strategies and future prospects for enhancing microbial production of citric acid. *Appl. Microbiol. Biotechnol.* 103, 201–209.

23-Imandi, S.B., Bandaru, V.V.R., Somalanka, S.R. and Garapati, H.R. (2007) Optimization of Medium Constituents for the Production of citric Acid from by product glycerol using Doehlert experimental design. *Enz and Mic Technol*, 40, 1367-1372.

24-Maharani, V., Reeta, D., Sundaramanickam, A., Vijayalakshmi, S. and Balasubramanian, T. (2014) Isolation and characterization of citric acid

producing *A. niger* from spoiled coconut. *Inter J of Curr Microbiol and Appl Sci*, 3, 700-705.

25- Xie G., West T.P. (2006). Citric acid production by *A. niger* on wet corn distillers grains. *Lett in Appl Microbiol* 43, 3 , 269-273.

26-Behera, B.C. (2020) Citric acid from *A. niger*: A comprehensive overview. *Crit. Rev. Microbiol.* 46, 727–749.

27-Xie, G.; West, T.P.(2009). Citric acid production by *A. niger* ATCC 9142 from a treated ethanol fermentation co-product using solid-state fermentation. *Lett. Appl. Microbiol.*, 48, 639–644.

28-Okareh O.T., O.D. Enesi & R. Olawoyin (2016). Production of citric acid from solid state fermentation of sugarcane waste using *A. niger* and indigenous sugarcane microflora. *AJSD* 6 1.

29-Chysirichote, T. (2020). Valorization of banana peel for citric acid production under solid state fermentation with *Aspergillus niger*. *Chem. Biochem. Eng. Q.* 34, 49–57.

30- P. Aghera, N Bhatt (2019). *Citric Acid: biosynthesis, properties and application*, publisher: Lambert academic publishing ISBN: 978-620-0-09567-1.

31- Ge X., Vasco-Correa J., Li Y (2017). Solid-state fermentation bioreactors and fundamentals in current developments in biotechnology and bioengineering: *Bioproc, Bioreact and Cont* (381-402).

32-Veiter L, Rajamanickam V, Herwig C.(2018). The filamentous fungal pellet-relationship between morphology and productivity. *Appl Microbiol Biotechnol.*;102(7):2997-3006.

33-Al-Shehri, M.A. and Mostafa, Y.S. (2006) Citric acid production from date syrup using immobilized cells of *A. niger*. *Biotechnol*, 5, 461-465.

34- Gibbs, P. A., Seviour, R. J. & Schmid, F. (2000). Growth of filamentous fungi in sub-merged culture: Problems and possible solutions, - *Crit Rev Biotechnol*;20(1):17-48.

nology 20(1): 17–48.

35-PORCEL EMR, LOPEZ JLC. (2005). Effects of pellet morphology on broth rheology in fermentations of *Aspergillus terreus* .*Bioch Eng J* 26(2-3):139-144.

36-Jianlong, w. (2000). Production of citric acid by immobilized *A.niger* using a rotating biological contactor (RBC), *Biores Technol*,75,3,245-247.

37- Shieih W. K., KENNAN J. D. (2005). Fluidized bed biofilm reactor for wastewater treatment book: *Bioprod* (131-16).

38-Isato Yoshioka 1, Kohtaro Kirimura(2021). Rapid and marker-free gene replacement in citric acid-producing *A. tubingensis* (*A. niger*) WU-2223L by the CRISPR/Cas9 system-based genome editing technique using DNA fragments encoding sgRNA. *J Biosci Bioeng*2021 Jun;131(6):579-588.

39- Mores, S., de Souza Vandenberghe, et al (2021). Citric acid bioproduction and downstream processing: Status, opportunities, and challenges. *Biores Technol*. 320, B, 124426.

40-Yang, L., Henriksen, M.M., Hansen, R.S. et al.(2020) Metabolic engineering of *Aspergillus niger* via ribonucleoprotein-based CRISPR–Cas9 system for succinic acid production from renewable biomass. *Biotechnol Biofuels* 13, 206 .

41-Sun, X., Wu, H., Zhao, G. et al. (2018). Morphological regulation of *A. niger* to improve citric acid production by *chsC* gene silencing. *Biop Biosyst Eng* 41, 1029–1038.

42-Mustafa Germec and Ali Demirci and Irfan Turhan (2020). {Biofilm reactors for value-added products production: An in-depth review *Biocat and Agric Biotech*,27 ,101662.

43-Kusuma H.S. et al. (2022)A comparative evaluation of statistical empirical and neural intelligence modeling of *Manihot esculenta*-derived leaves extract for optimized bio-coagulation-flocculation of turbid water .*Ind. Crops and Prod.* 186, 115194.

44-Yueyang Fu (2021). Intelligent numerical computing methods for determining the concentration of citric acid by accurate measurement and calculation .2nd Inte Conf on Appl Phy and Comp. Ser. 2083 032089.

45- Amenaghawon A. N. and Omoruyi B. O. et al (2023). Biotechnological conversion of yam peels for enhanced citric acid production: Data-driven machine learning modeling and global sensitivity analysis of the impact of metabolic stimulants, *Ind Crops and Prod*, 191,11602.

46-Nadeem, A., Syed, Q., Baig, S., Irfan, M. and Nadeem, M. (2010) Enhanced production of citric acid by *A. niger* M-101 using lower alcohols. *Turk J of Bioche*, 35, 7-13.

47- Sharma R. and Mishra, R. (2014) "A Review of evolution of theories and models of technology adoption," *Indore Manag. J.*, 6(2), 17–29.

48-50-Rong Ye, Zirui Z., et al (2022). Conversion of Calcium Citrate to Citric Acid with Compressed CO₂. *ACS Omega* 7 (1), 683-687.

49-Thakre N, Prajapati A.K. et al (2016). Modeling and optimization of reactive extraction of citric Acid. *J of Chem & Eng Data* .61(7).

50- Pazouki M., Panda T. (1998). Recovery of citric acid. *Biopr Engi*, 19(6) ,435-439.

51-Wang J, Cui Z, Li Y, Cao L, Lu Z. (2020) Techno-economic analysis and environmental impact assessment of citric acid production through different recovery methods. *J Clean Prod.*; 249: 119315.

52-Saltelli A. et al. (2021). Sensitivity analysis: a discipline coming of age. *Environ. Model. Software*. 137, 104954.

53- Nemdili L., Koutchoukali, Q. et al (2016). Crystallization kinetics of Citric Acid Anhydrate *J of Crystal Growth* 451.