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Production of citric acid from food waste using *Aspergillus Tubingensis* and *Aspergillus Niger*

Aabida Parveen[a], Tooba Naveed[b], Suraiya Jabeen[*a], Farman Ahmed[c], Nida Saleem[c]

[a]*Institute of Environmental Studies, University of Karachi, Karachi, 75270, Pakistan.*

[b]*Centre for Environmental Studies, PCSIR Labs Complex, Shahra-e-SalimuzzanSiddiqui, Off University Road, Karachi, 75280, Pakistan.*

[c]*Food and Feed Safety Laboratory, Food and Marine Resources Research Centre, PCSIR Laboratories Complex, Shahrah-e-SalimuzzamanSiddiqui, Off University Road, Karachi, 75280, Sindh, 74200, Pakistan.*

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ABSTRACT

Millions of tons of organic waste are produced annually, but no proper disposal method is practiced that minimizes organic waste in an environmentally friendly manner. Such waste has harmful effects on human health and the environment. Organic wastes can be utilized to synthesize industrial products like citric acid. A number of microorganisms have been used in fermentation studies of citric acid. The genus *Aspergillus* is reported as being a prominent species in the production of a variety of enzymes and organic acids. Exploring more productive strains and valorization of biomass are the most crucial steps in promoting economically viable and useful industrial products such as citric acid, etc. Thus, the present work aimed to assess the potential of locally isolated *A. tubingensis* for the first time and *A.niger* for citric acid production. Segregated kitchen waste, crude molasses, and sucrose were used as substrates. Additionally, the effects of pH, carbon, nitrogen, and methanol were also monitored. The findings indicated that the highest yield, i.e., 16.14±0.03g/l, was produced by the *A. tubingensis*in fermentation media containing sucrose, 1.0% ammonium nitrate (AN), and 2.0% methanol, while 15.97±0.01g/l was produced by the *A.niger*in fermentation media containing sucrose, 1.0% ammonium nitrate (AN), and 1.0 % methanol. The addition of methanol in the *Aspergillus tubingens is* fermentation media produced higher yields of citric acid. The findings demonstrated that *A. tubingensis* is a potential contender for the production of citric acid. Furthermore, kitchen waste could be a sustainable and cost-effective substrate for citric acid production and can be managed in an eco-friendly way.

*Corresponding authorTel.: 02199261386
E-mail: sujabeen@uok.edu.pk
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1.Introduction

Organic waste production is a pressing environmental issue. Improper management of solid waste affects the ecosystem and human health. Decomposition of organic waste releases harmful gases and chemicals that can leach into water bodies and spread vector-borne or infectious diseases. Organic waste can be converted into valuable byproducts like citric acid[1]. Citric acid is among the most versatile organic acids, with a global market value of USD 3.6 billion [2]. Currently, it is extensively used in various industries: food and beverage, detergents and cleaners, pharmaceutical, textile, leather and many other industries. It is Generally Recognized as Safe (GRAS) by the Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives [3,4]. Although citric acid can be synthesized by physical and chemical methods [5-7],they are limited due to high cost and low yields [8]. Therefore, citric acid has been extensively produced via fermentation [9,10] as it is simple, has low energy requirements, and is economically viable [11]. A number of microorganisms, including *Aspergillusniger, Bacillus subtilis*, *Trichodermaviride, Aspergillus awamori,* and many others [12-14], have been used in fermentation studies of citric acid. It has been well reported that the genus *Aspergillus* is prominent in the production of a variety of enzymes and organic acids, such as citric acid, itaconic acid, lactic acid, malic acid, etc. [15-18].

The Food and Agriculture Organization (FAO) estimated that worldwide 1.3 billion tons of food is lost annually [19]. The organic fraction of kitchen waste is highly biodegradable in nature and a nutrient rich source that could be managed by gasification, composting, and fermentation [20-23].

Nowadays, searching for potential biomass from agricultural and industrial sources for environmentally sustainable and economically viable production of citric acid is the prime goal of researchers. In earlier studies, researchers used locally available waste biomass in fermentation studies to produce citric acid from pineapple peel [24], rice husk [25], banana peel [26,27], pomegranate peel [28],tangerine peel [29], sugarcane bagasse [30,31], fruit peels[32], cashews, apple juice[33], downgraded dates [34],etc.

Therefore, the present study was planned and executed to explore the potential use of locally isolated *A. tubingensis* and *A. niger*to ferment (submerged) the segregated kitchen waste for citric acid production. Although it has been reported that *A.tubingensis* has been (Ahmadet *al*., [35]) used to produce enzymes and organic acids, this is the first time attempts have been made specifically for the production of citric acid.

2. Material and methods

2.1 Qualitative screening and selection of citric acid producers

In the initial qualitative screening step, previously isolated and identified species of the genus *Aspergillus: Aspergillus tubingensis* APP11 and *Aspergillusniger*APP13(Ahmadet *al*.,[35] were assessed according to (Rao and Reddy,[36]) for citric acid production. The plates were inoculated and incubated for five days at 30°C;ayellow color zone developed during the incubation period. The diameter of the yellow zone produced by the fungus was measured to evaluate the ability to produce citric acid.

2.2 Inoculum preparation and standardization

Spore suspensions were prepared by washing the five-day-old fungal culture slant with sterile distilled water. Spores were counted using a haemocytometer and adjusted to 10^7spores/ml.

2.3 Collection and analysis of substrate

The kitchen waste samples, viz., banana, potato, lemon and grapefruit peels, cooked rice, leftover bread, tomatoes, chickpeas, spinach, lettuce, etc., were collected from the commercial canteen and households. It was then mixed thoroughly and oven dried at 105°C. The dried mixture was ground into 2 mm sized particles and stored in an airtight jar for chemical analysis. Sugar cane molasses was procured from a local sugar industry in a clean, food grade plastic container and stored at room temperature for analysis and further use. The chemical composition is given below in Table 1.

Table1. Chemical composition of kitchen waste.

Parameters	Unit	Composition (%)
Moisture	%	61.3±0.13
Total Solids	%	38.7±0.03
Volatile Solids	%	94.4±0.01
Ash	%	4.8±0.01
Starch	%	42.6±0.05
Total Sugars	%	49.0±0.26

The pH values were determined on a calibrated pH meter (Hanna). Standard methods [37,38] were used, and aseptic conditions were maintained throughout the study.

2.4 Fermentation Studies

A total of 10 grams of powdered kitchen waste was placed in 500 mL Erlenmeyer flasks containing 200 mL of nutrient solution with the following composition (g/L): NH_4NO_3 (10.0), $MgSO_4{\cdot}7H_2O$ (1.0), and KH_2PO_4 (4.0). The medium was sterilized at 15 psi and 121°C for 15 minutes. Flasks containing the sterilized fermentation medium were inoculated with a 1.0% (v/v) spore suspension. The inoculated flasks were incubated in a shaking incubator at 30 ± 2°C and 200 rpm for 10 days. The pH values were measured aseptically on the 1st, 5th, and 10th days.

2.5 Study of factors affecting citric acid production

Citric acid production was compared at different factors: various concentrations of nitrogen source (ammonium nitrate (AN) 0.5, 1.0, 1.5%), a carbon source (sucrose and molasses 15,15%), and an alcohol source (methanol 1.0, 2%). All of the experiments were done in duplicate with *A.niger* and *A.tubingensis*.

2.6 Estimation of Citric Acid

The contents of each incubated flask were subjected to vigorous shaking followed by filtration using Whatman filter paper No.1.The production of citric acid was determined titrimetrically using phenolphthalein as the indicator [38].The titrimetric method was verified by HPLC for the production of citric acid(with a hypesil gold column, dimensions 150 x 4.6um (Litheria)). The sodium dihydrogen phosphate mobile phase was used at pH 2.2. The pH of the mobile phase was adjusted with phosphoric acid. The detection wavelength was 210nm, and the flowrate was 1m/min.

3. Result and Discussion

It has been reported that the species of *Aspergillus* have been successfully used to manufacture organic acids. *Aspergillus* is better than other fungal species for the commercial synthesis of citric acid because of its enhanced production yield, ease of handling, and ability to ferment various cheap raw materials. Microbial fermentation is environmentally friendly, produces high-purity citric acid, and utilizes renewable resources. On the other hand, chemical synthesis uses hazardous chemicals and generates toxic byproducts. Moreover, it requires energy-intensive processes and produces lower-purity citric acid compared to microbial fermentation.

As reported earlier, substrate plays a key role not only obtaining optimal yield but also reducing the cost of the end product. In several studies, a variety of raw materials such as pineapple peels, rice husks, banana peels, pomegranate peels, tangerine peels, sugarcane bagasse, fruit peels, cashews, apple juice, downgraded dates, etc., have successfully been employed as substrate for citric acid production [24-34,39-41].Analysis of kitchen waste revealed a high volatile solids content of 94.4% and a total sugar content of 49.0% (Table 1), indicating its potential as an effective substrate for citric acid production. Similarly, results for the analysis of molasses were recorded as pH of 4.6, Brix of 77°, and density of 1.399kg/l. The obtained value of pH was in disagreement with the findings of Gasmallaet *al*.[42],who reported a pH value of 5.8±0.35. Likewise, a pH value of 5.1 was reported by Osunkoya & Okwudinka[43].

It is well documented that the pH of the fermentation medium changes as a consequence of microbial metabolic activities and the production of a variety of organic acids. Likewise, in the present study, a progressive decrease in pH values was observed across all substrate combinations fermented by both *A. niger* and *A. tubingensis*, reflecting microbial metabolic activity during fermentation. The fermentation process started in the pH range of 4.21-4.83, and as the fermentation time increased, the pH decreased

and showed values in the range of 1.30-4.69(Figure 1,2). Cherguiet *al.*[34] also reported similar findings in their published works. During citric acid production, significant changes in the pH of the fermentation medium at its initial and final stages have been reported numerously. They found that fungal spores started germinating at a pH less than 5.0 and, after germination and once germinated, started producing citric acid, which ultimately resulted in a drop in pH value. Bellaouchiet *al.* [13] also demonstrated in their study that less than a pH of 5 is obligatory for mycelium growth and microbial spore germination in the initial phase. In that study, a maximum biomass weight of 18g was obtained with a 7.78+0.12g/ICA production on the 10th day with FW+0.5%AN by *A.tubingensis*.

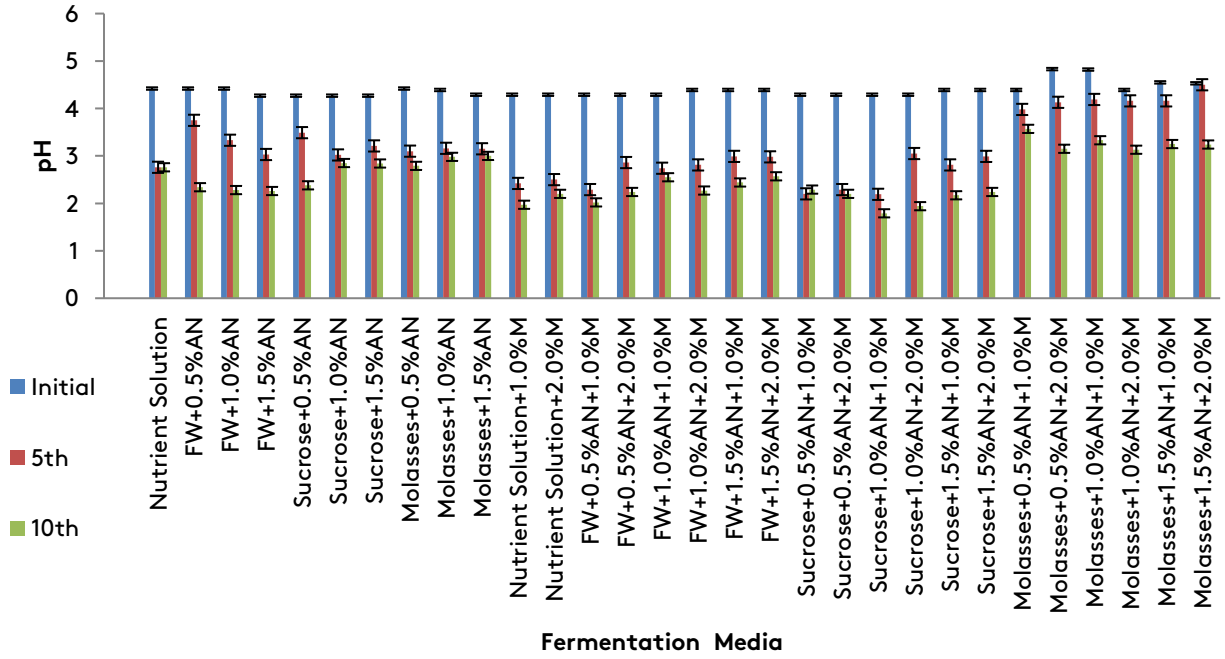


Fig.1. Changes in pH value with respect to time during production of citric acid by *A.niger*.

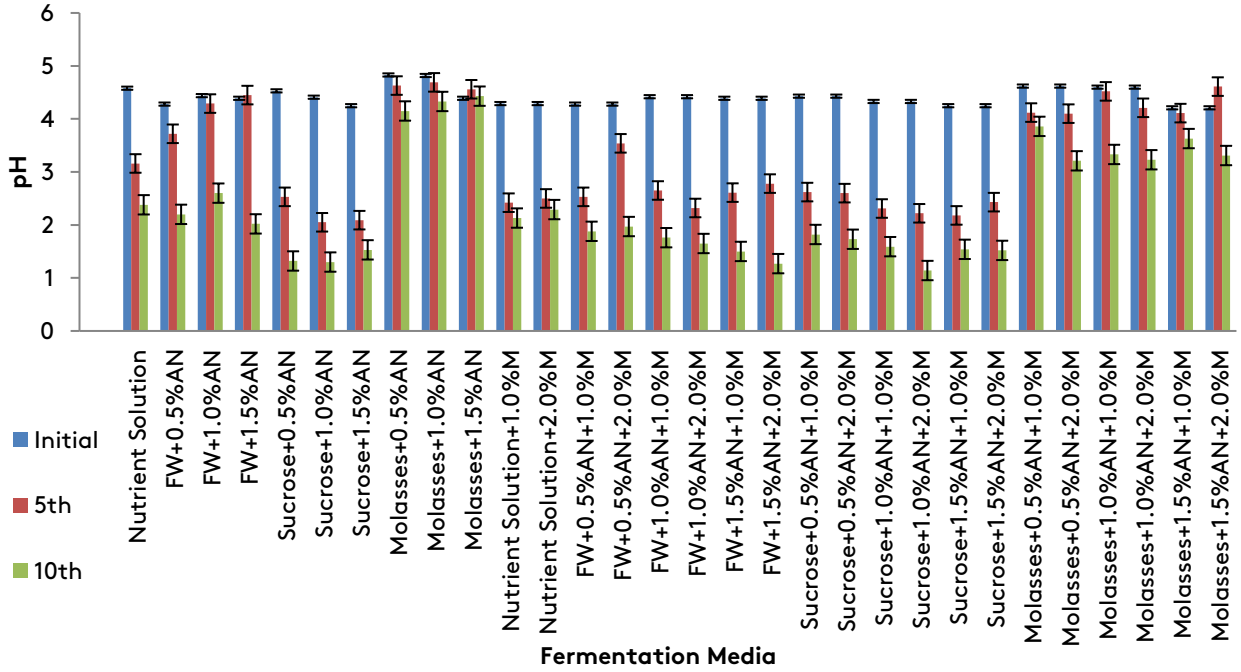


Fig.2. Changes in pH value with respect to time during production of citric acid by *A.tubingensis*.

Munshiet *al.*[44] stated that a long fermentation time period produced a maximum biomass weight. A maximum time period of fermentation increased the fungus mycelium growth and spores that produced the maximum citric acid.

Bellaouchiet *al*. [13] reported that the mycelium dry weight changed during the submerged culture of the Aspergillus species that affected the CA yield.Vidyaet *al.*[32] reported that methanol decreased the mycelia growth that increased the production of citric acid.Many researchers have reported that citric acid production is greatly affected by the type of carbon source. Therefore, in order to evaluate the effect of food waste, sucrose and crude molasses on citric acid production were used. The highest citric acid yields were achieved with fermentation media containing sucrose, followed by food waste and crude molasses, highlighting sucrose's effectiveness as a carbon source.

It has been noted that media containing 15.0% sucrose as a source of carbon gave the maximum yield of 8.13±0.12g/l by*A.niger*(Figure 3) and 10.88±0.05 g/l by *A.tubingensis*(Table 2, Figure 4).Similarly, Aritonanget *al.*,Sarkarand Das,Satheeshkumaret *al.*,and Vidyaet *al*. [32,45-47]claimed that sucrose is the most promising carbon source. They further described that the easily metabolized forms of carbon sources, such as monosaccharides and disaccharides, gave larger yields of citric acid. Likewise, in the study, a10-14% sugar concentration was reported as an optimal concentration at the initial stage of citric acid fermentation by the majority of fungal strains [48]. Cane molasses is a desirable raw material for citric acid fermentation because of its availability and relatively low price. Both fungi gave better yields in fermentation media containing molasses compared to media containing food waste, while they gave lower yields compared to sucrose (Table 2). It may be due to a low concentration of sugar that ultimately resulted in the reduction in the size of mycelium, causing a low accumulation of citric acid [49]. Moreover, citric acid fermentation in a molasses medium is also affected significantly by the presence of some inorganic and organic metabolic inhibitory compounds [50].

In this study, a significant increase in citric acid production was noticed when the fermentation media was supplemented with ammonium nitrate in a 1.0-2.0% concentration. It has been documented that citric acid production is significantly influenced by the nitrogen source because the consumption of ammonium salts by fungi leads to acidic pH, which is favorable for citric acid production. Hanget *al.*[51]reported that an elevated concentration of nitrogen caused an increase in fungal growth, accumulation of oxalic acid, and the consumption of sugars but resulted in low citric acid production.

Previous research has indicated that adding methanol at concentrations ranging from 1% to 4% (v/v) significantly enhances citric acid production during fermentation[46,52,53]. Methanol was added as an additional carbon source to increase citric acid production. In the fermentation media, it also helped dissolve the sucrose and ammonium nitrate in water.

Similar findings were noted in this study, where the addition of methanol sharply enhanced the production of citric acid by both fungi. The optimum concentration of methanol in the fermentation media for both fungi was 2.0%, at which *A.tubingensis*produced 16.14 ± 0.03g/l,whereas,at 1.0%,*A. niger* gave an yield of 15.97± 0.01g/l . The HPLC results also supportedthe highest yield production of CA in each slot with methanol that is calculated by the titration results with methanol (Figure 5-8).

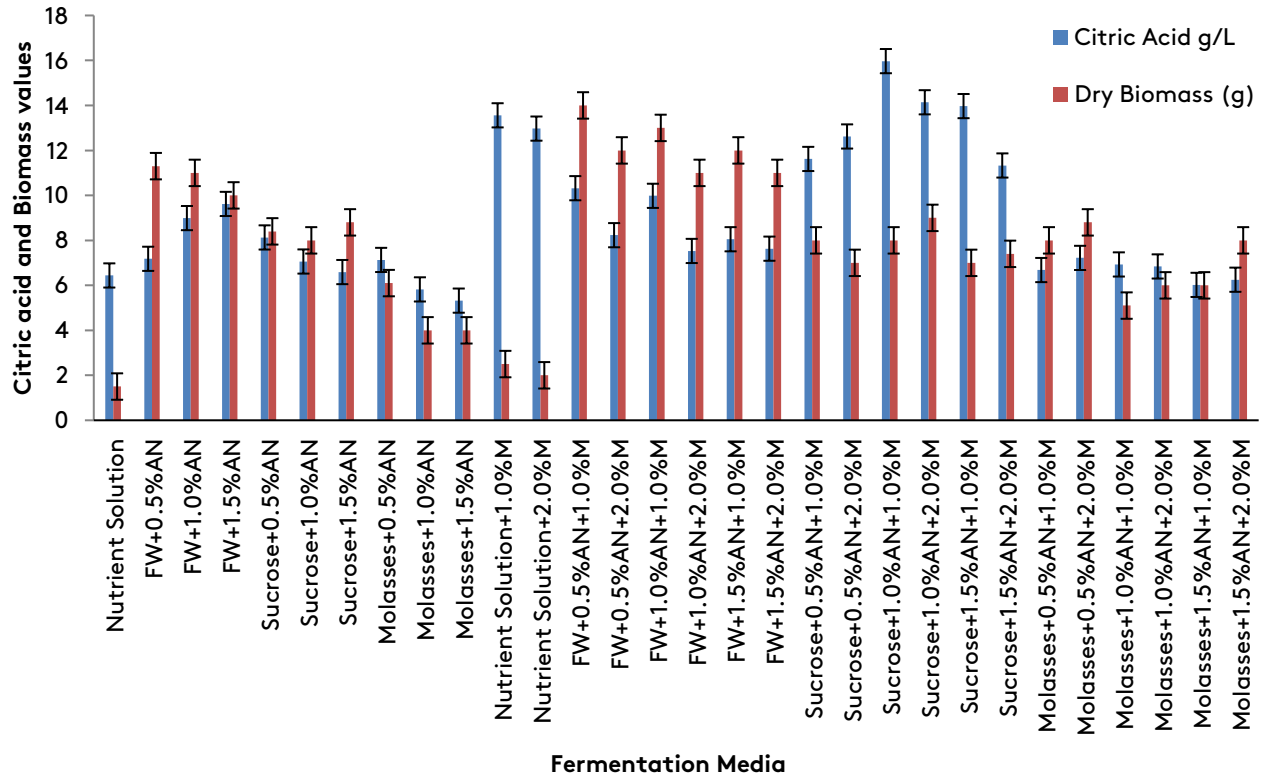


Fig.3. Comparative presentation of citric acid yield produced with *A. niger* Dry Biomass.

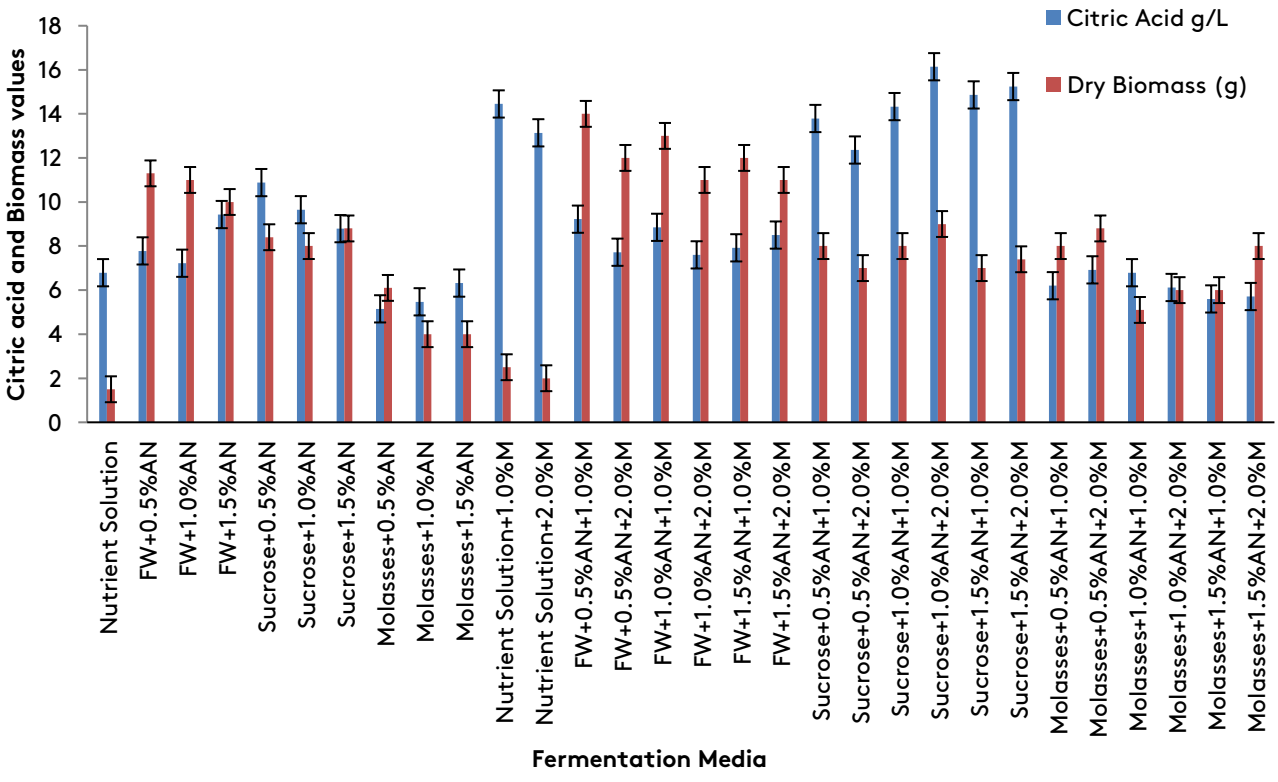
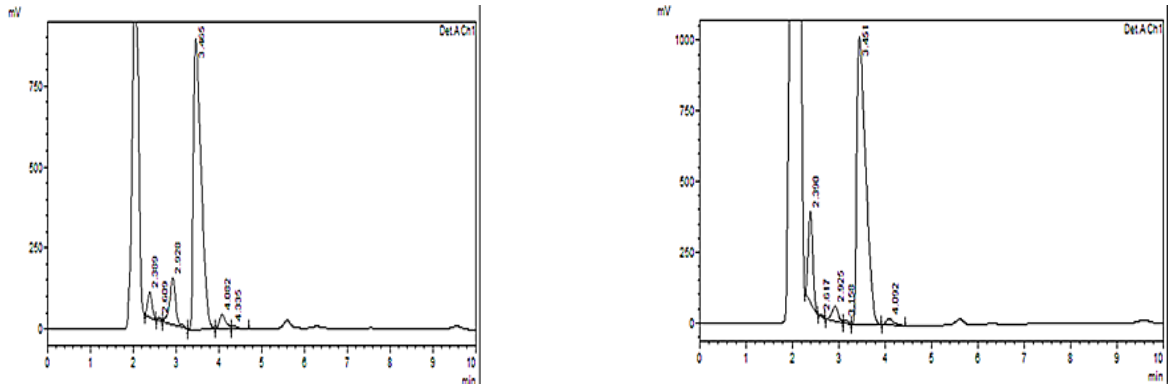


Fig.4. Comparative presentation of citric acid yield produced by *A.tubingensis* with Dry Biomass.

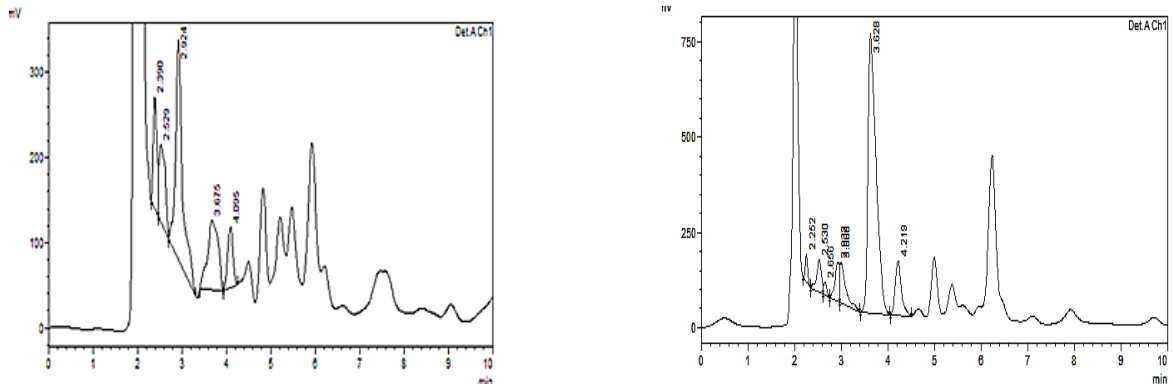
Table 2. Production of citric acid with *A.niger* and *A.tubingensis*.

Fermentation Media	C.A Production by *A.niger*(g/l)	C.A Production by *A.tubingensis* (g/l)	Fermentation Media	C.A Production by *A.niger*(g/l)	C.A Production by *A.tubingensis* (g/l)
Nutrient Solution	6.44 ± 0.02	6.79± 0.31	FW+1.0%AN+2.0%M	7.53±0.12	7.60±0.16
FW+0.5%AN	7.18±0.16	7.78±0.12	FW+1.5%AN+1.0%M	8.05±0.02	7.92±0.16
FW+1.0%AN	8.99±0.01	7.22±0.03	FW+1.5%AN+2.0%M	7.63±0.05	8.50±0.01
FW+1.5%AN	9.62±0.21	9.43±0.11	Sucrose+0.5%AN+1.0%M	11.62± 0.17	13.79±0.07
Sucrose+0.5%AN	8.13±0.12	10.88±0.05	Sucrose+0.5%AN+2.0%M	12.62±0.05	12.36±0.12
Sucrose+1.0%AN	7.06±0.16	9.65±0.05	Sucrose+1.0%AN+1.0%M	15.97±0.01	14.33±0.31
Sucrose+1.5%AN	6.59±0.07	8.79±0.12	Sucrose+1.0%AN+2.0%M	14.14±0.26	16.14±0.03
Molasses+0.5%AN	7.13±0.31	5.15±0.01	Sucrose+1.5%AN+1.0%M	13.97±0.21	14.86±0.16
Molasses+1.0%AN	5.82±0.10	5.47±0.21	Sucrose+1.5%AN+2.0%M	11.33±0.05	15.24±0.13
Molasses+1.5%AN	5.32±0.15	6.32±0.12	Molasses+0.5%AN+1.0%M	6.68±0.11	6.20±0.01
Nutrient Solution+1.0%M	13.56±0.31	14.45±0.04	Molasses+0.5%AN+2.0%M	7.22±0.31	6.92±0.16
Nutrient Solution+2.0%M	12.97±0.01	13.14±0.03	Molasses+1.0%AN+1.0%M	6.93±0.05	6.79±0.17
FW+0.5%AN+1.0%M	10.32±0.05	9.22±0.16	Molasses+1.0%AN+2.0%M	6.84±0.03	6.12±0.21
FW+0.5%AN+2.0%M	8.23± 0.21	7.72±0.08	Molasses+1.5%AN+1.0%M	6.02±0.19	5.60±0.11
FW+1.0%AN+1.0%M	9.98±0.05	8.85±0.24	Molasses+1.5%AN+2.0%M	6.25±0.16	5.71±0.12



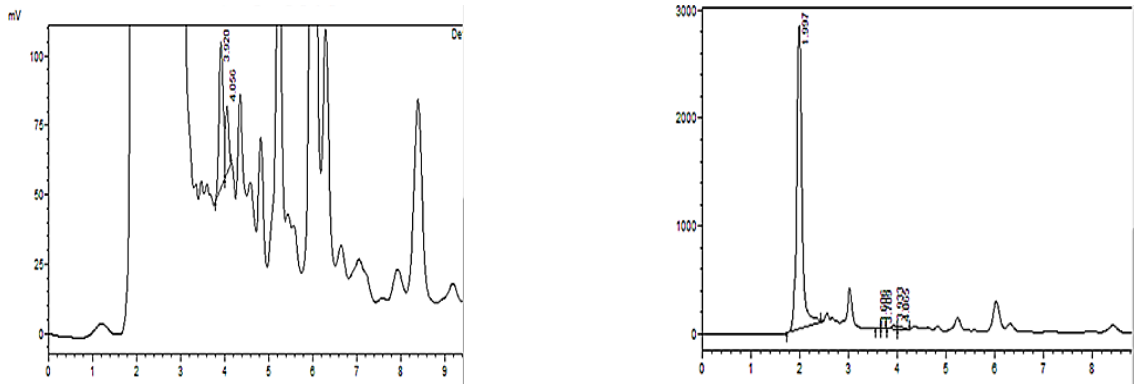
(a) (b)

Fig.5. HPLC peak value of the highest CA yield with *A.tubingensis* (a) sucrose+1.0% ammonium nitrate + 2.0% methanol and *A.niger* (b) with sucrose + 1.0% ammonium nitrate + 1.0% methanol



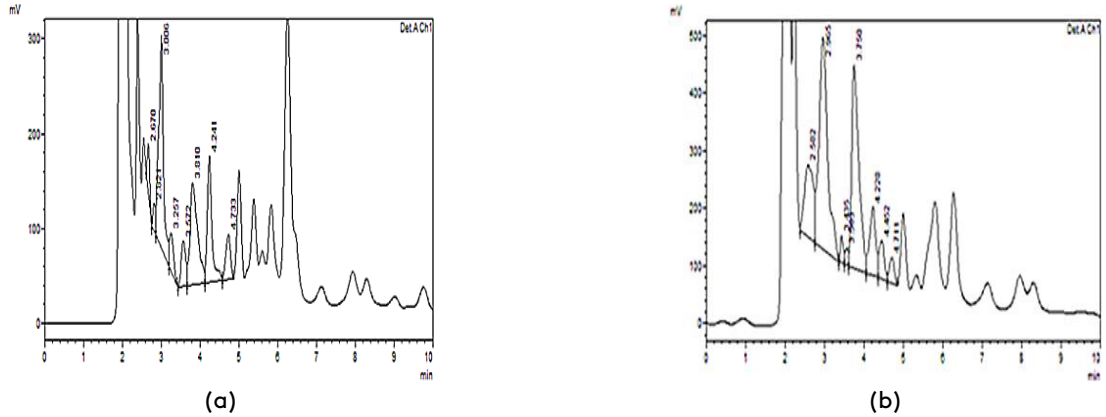
(a) (b)

Fig. 6. HPLC peak value of the highest yield of CA with (a) *A.tubingensis* and (b) *A.niger* with FW+1.5%AN+1.0%M.



(a) (b)

Fig. 7. HPLC Peak value of highest yield of CA with (a) *A.tubingensis* and (b) *A.niger*with Nutrient Solution+1.0%M.



(a) (b)

Fig. 8. HPLC peak value of the highest yield of CA with (a) *A.tubingensis* and (b) *A.niger* with Molasses+0.5%AN+2.0%M.

Likewise, Cherguiet *al*. [34] reported a 4.0% concentration of methanol, while Aduduet *al.*[54] and Roukas and Kotzekidou[28]claimed 3.0% as the most effective. The results (Table 2) revealed that the addition of methanol with food waste provided higher yields. The results of the current study demonstrated that *A. tubingensis* achieved a maximum citric acid yield of 16.14 ± 0.03 g/L when cultured in fermentation media supplemented with sucrose, 1.0% ammonium nitrate (AN), and 2.0% methanol (Table 2). This increase could be attributed to enhanced metabolic activity facilitated by methanol, which serves as an additional carbon source, promoting higher enzymatic activity during fermentation [55]. These findings are consistent with previous studies that reported similar enhancements in citric acid production under optimized nitrogen and carbon conditions[56]. It has also been reported in published works that methanol increases the

membrane permeability by affecting the phospholipids composition of the cytoplasmic membrane or by changing the lipid composition of the cell wall [57,58]. Present findings through Titrimetric and HPLC demonstrated that *A. tubingensis* was a potential candidate for the production of citric acid with methanol. Minute concentration of methanol added to microbial fermentation can invigorate citric acid yield, reduce the harmful effects of trace metals in fermentation, alter the cell membrane permeability, and affect microbial metabolism to accumulate citric acid; however, excessive methanol can be harmful and restrain microbial growth and citric acid production [59].

Instead of conventional methods for the disposal of organic waste, this research work directs the waste to product approach; without expenditure on raw material purchase, free waste can be turned into a valuable resource like citric acid. In many South Asian regions, solid waste management is lacking. This work could lead to eco-friendly production with waste reduction, emphasizing the sustainability aspect by utilizing food waste that contributes to waste management goals and aligns with circular economy principles. The environmental impact can be mitigated by minimizing overproduction and repurposing food waste into valuable products.

4. Conclusions

Solid waste severely damages the environment by creating different types of pollution. The production of solid waste cannot be stopped, but through scientific research, environmental loss can be minimized. Utilization of solid wastes not only minimizes the disposal problems but concomitantly produces valuable organic acids, such as citric acid. Global consumption of citric acid is in an ascending trend due to its vast industrial application. Cost-effective microbial production of citric acid on an industrial scale needs smart strategies, such as the use of leftover biomass as substrates, efficient microbial strains, etc. The findings of the present study demonstrated that *Aspergillus tubingensis* could be a potential candidate for the production of citric acid by using kitchen waste as a cost-effective substrate. The challenge of inconsistent raw supply or substrate can be addressed via proper collection of waste from homes, restaurants, and canteens with the help of authorized vendors. The whole process of waste collection and its utilization will also benefit supply chain management.

Future Prospects

Agriculture waste residue can be utilized for the production of citric acid because crop residue is burnt openly in grassland areas and produces hazardous air pollutants in the South Asian region. In the future, if this waste residue is fermented to produce industrial chemicals, it will help mitigate air pollution. The concerned governmental bodies should consider it when making policies in the future to embrace sustainability.

Limitations of Study

Instead of using a single strain, the use of microbial consortia can produce better results. The fermentation time period can also be shortened; instead of ten days, the same quantity of citric acid can be produced within one week. Environmental conditions like moisture and temperature, along with time, can be optimized in the production of citric acid.

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References

[1] Zhao, J., Madni, G. R., & Anwar, M. A. (2022). Exploring rural inhabitants' perceptions towards food wastage during COVID-19 lockdowns: Implications for food security in Pakistan. Plos one,17(3), e0264534. https://doi.org/10.1371/journal.pone.0264534

[2] Berovic, M., &Legisa, M. (2007). Citric acid production. Biotechnology annual review,13, 303-343. https://doi.org/10.1016/S1387-2656(07)13011-8

[3] Soccol, C. R., Vandenberghe, L. P., Rodrigues, C., &Pandey, A. (2006). New perspectives for citric acid production and application. Food Technology and Biotechnology,44(2), 141-149. https://hrcak.srce.hr/109832

[4] Ruijter, G. J. G., Kubicek, C. P., &Visser, J. (2002). Production of organic acids by fungi. Industrial Applications, 213-230. https://doi.org/10.1007/978-3-662-10378-4_10

[5] Perwitasari, U., Agustina, N. T., Pangestu, R., Amanah, S., Saputra, H., Andriani, A., &Habibi, M. S. (2021). Cacao pod husk for citric acid production under solid state fermentation using response surface method. Biomass Conversion and Biorefinery, 1-9. https://doi.org/10.1007/s13399-021-01690-9

[6] Yin, X., Shin, H. D., Li, J., Du, G., Liu, L., & Chen, J. (2017). P gas, a low-pH-induced promoter, as a tool for dynamic control of gene expression for metabolic engineering of Aspergillusniger. Applied and Environmental Microbiology,83(6), e03222-16. https://doi.org/10.1128/AEM.03222-16

[7] Yu, B., Zhang, X., Sun, W., Xi, X., Zhao, N., Huang, Z., & Ying, H. (2018). Continuous citric acid production in repeated-fed batch fermentation by Aspergillusniger immobilized on a new porous foam. Journal of biotechnology,276, 1-9. http://dx.doi.org/10.1016/j.jbiotec.2018.03.015

[8] Ozdal, M., &Kurbanoglu, E. B. (2019). Citric acid production by Aspergillusniger from agro-industrial by-products: Molasses and chicken feather peptone. Waste and Biomass Valorization,10, 631-640. https://doi.org/10.1007/s12649-018-0240-y

[9] Rehman, K. U., Hollah, C., Wiesotzki, K., Rehman, R. U., Rehman, A. U., Zhang, J., ... &Aganovic, K. (2023). Black soldier fly, Hermetiaillucens as a potential innovative and environmentally friendly tool for organic waste management: A mini-review. Waste Management & Research,41(1), 81-97. https://doi.org/10.1177/0734242X221105441

[10] Kuforiji, O., Kuboye, A. O., &Odunfa, S. A. (2010). Orange and pineapple wastes as potential substrates for citric acid production. International Journal of Plant Biology,1(1), e4. https://doi.org/10.4081/pb.2010.e4

[11] Adeoye, A. O., Lateef, A., & Gueguim-Kana, E. B. (2015). Optimization of citric acid production using a mutant strain of Aspergillusniger on cassava peel substrate. Biocatalysis and Agricultural Biotechnology,4(4), 568-574. https://doi.org/10.1016/j.bcab.2015.08.004

[12] Behera, B. C., Mishra, R., &Mohapatra, S. (2021). Microbial citric acid: Production, properties, application, and future perspectives. Food Frontiers,2(1), 62-76. https://doi.org/10.1002/fft2.66

[13] Bellaouchi, R., Hasnaoui, I., Idrissi Yahyaoui, M., Bentouhami, N., Hasnaoui, A., Taibi, M., & Asehraou, A. (2024). Improving dietary citric acid production by the wild-type Aspergillusniger ASP26 strain isolated from date by-product. Food Science & Nutrition,12(6), 4248-4258. https://doi.org/10.1002/fsn3.4084

[14] Reena, R., Sindhu, R., Balakumaran, P. A., Pandey, A., Awasthi, M. K., &Binod, P. (2022). Insight into citric acid: A versatile organic acid. Fuel,327, 125181. https://doi.org/10.1016/j.fuel.2022.125181

[15] Ghanbartabar, S. A., Najafpour, G. D., &Mohammadi, M. (2016). Comparative studies on citric acid production from cheese why by submerged and immobilized Aspergillusniger. Pakistan Journal of Biotechnology,13(2), 79-85. https://nja.pastic.gov.pk/PJBT/index.php/PJBT/article/download/1655/1628

[16] Karthikeyan, A., &Sivakumar, N. (2010). Citric acid production by Koji fermentation using banana peel as a novel substrate.Bioresource Technology,101(14), 5552-5556. https://doi.org/10.1016/j.biortech.2010.02.063

[17] Lotfy, W. A., Ghanem, K. M., & El-Helow, E. R. (2007). Citric acid production by a novel Aspergillusniger isolate: I. Mutagenesis and cost reduction studies. Bioresource Technology,98(18), 3464-3469. https://doi.org/10.1016/j.biortech.2006.11.007

[18] Mishra, M., Shukla, S., Mishra, A., Zohra, F., Singh, S., Kushwaha, A., &Upadhyay, N. (2023). Biotechnological interventions in the valorization of the organic waste. InBio-based materials and waste for energy generation and resource management(pp. 357-385). Elsevier. https://doi.org/10.1016/B978-0-323-91149-8.00002-8

[19] Silva, P. B., Duarte, C. R., & Barrozo, M. A. S. (2019). A novel system for drying of agro-industrial acerola (Malpighiaemarginata DC) waste for use as bioactive compound source. Innovative Food Science & Emerging Technologies,52, 350-357. https://doi.org/10.1016/j.ifset.2019.01.018

[20] Konkol, I., Świerczek, L., & Cenian, A. (2018). Biogas production from bakery wastes–dynamics, retention time and biogas potential. Journal of Research and Applications in Agricultural Engineering,63(1). https://yadda.icm.edu.pl/baztech/element/bwmeta1

[21] Pour, F. H., & Makkawi, Y. T. (2021). A review of post-consumption food waste management and its potentials for biofuel production. Energy Reports,7, 7759-7784. https://doi.org/10.1016/j.egyr.2021.10.119

[22] Suriapparao, D.V. & Vinu, R., (2021). Biomass waste conversion into value-added products via microwave-assisted Co-Pyrolysis platform. Renewable Energy 170, 400-409. https://doi.org/10.1016/j.renene.2021.02.010

[23] Van Fan, Y., Lee, C.T., Klemeš, J.J., Chua, L.S., Sarmidi, M.R. & Leow, C.W., (2018) Evaluation of Effective Microorganisms on home scale organic waste composting. Journal of Environmental Management 216, 41-48. https://doi.org/10.1016/j.jenvman.2017.04.019

[24] Ayeni, A., Daramola, M. O., Taiwo, O., Olanrewaju, O. I., Oyekunle, D. T., Sekoai, P. T., & Elehinafe, F. B. (2019). Production of citric acid from the fermentation of pineapple waste by Aspergillusniger. The Open Chemical Engineering Journal,13(1). https://doi.org/10.2174/1874123101913010088

[25] Siagian, A. A., Manurung, A., & Martgrita, M. M. (2023, May). Scale-up of citric acid production by utilizing rice husk waste and purification using ion exchange chromatography. InIOP Conference Series: Earth and Environmental Science (Vol. 1187, No. 1, p. 012008). IOP Publishing. https://doi.org/10.1088/1755-1315/1187/1/012008

[26] Chysirichote, T.(2020). Valorization of banana peel for citric acid production under solid state fermentation with Aspergillusniger. Chemical and Biochemical Engineering Quarterly, 34(1), 49-57. https://doi.org/10.15255/CABEQ.2019.1740

[27] .Ajala, A. S., Adeoye, A. O., Olaniyan, S. A., & Fasonyin, O. T. (2020). A study on effect of fermentation conditions on citric acid production from cassava peels. Scientific African,8, e00396. https://doi.org/10.1016/j.sciaf.2020.e00396

[28] Roukas, T., & Kotzekidou, P. (2020). Pomegranate peel waste: a new substrate for citric acid production by Aspergillusniger in solid-state fermentation under non-aseptic conditions. Environmental Science and Pollution Research,27(12), 13105-13113. https://doi.org/10.1007/s11356-020-07928-9

[29] Kumar, P. G., Paul, S. K., Khobragade, C. B., Bania, B. K., &Sengupta, D. K. (2020). Requirements of mixed tangerine (Citrus tangerine) and pineapple (Ananascomosus) powdered peel wastes fermentation for citric acid production. Agricultural Engineering International: CIGR Journal,22(2), 194-207. http://orcid.org/0000-0001-9475-0627

[30] Yadav, P., Chauhan, A. K., Singh, R. B., Khan, S., &Halabi, G. (2022). Organic acids: microbial sources, production, and applications. In Functional foods and nutraceuticals in metabolic and non-communicable diseases(pp. 325-337). Academic Press. https://doi.org/10.1016/B978-0-12-819815-5.00053-7

[31] Campanhol, B. S., Silveira, G. C., Castro, M. C., Ceccato-Antonini, S. R., &Bastos, R. G. (2019). Effect of the nutrient solution in the microbial production of citric acid from sugarcane bagasse and vinasse. Biocatalysis and Agricultural Biotechnology,19, 101147. https://doi.org/10.1016/j.bcab.2019.101147

[32] Vidya, P., Annapoorani, A. M., & Jalalugeen, H. (2018). Optimization and utilisation of various fruit peel as substrate for citric acid production by Aspergillusniger isolated from orange and carrot. Pharma Innovation J,7(6), 141-146. https://www.researchgate.net/profile/Padmanabhan-Vidya/publication/332718238

[33] Adeoye, A. O., &Lateef, A. (2022). Improving the yield of citric acid through valorization of

cashew apple juice by Aspergillusniger: mutation, nanoparticles supplementation and Taguchi technique. Waste and Biomass Valorization,13(4), 2195-2206. https://doi.org/10.1007/s12649-021-01646-0

[34] Chergui, D., Akretche-Kelfat, S., Lamoudi, L., Al-Rshaidat, M., Boudjelal, F., & Ait-Amar, H. (2021). Optimization of citric acid production by Aspergillusniger using two downgraded Algerian date varieties. Saudi Journal of Biological Sciences,28(12), 7134-7141. https://doi.org/10.1016/j.sjbs.2021.08.013

[35] Ahmad, A., Moin, S. F., Liaqat, I., Saleem, S., Muhammad, F., Mujahid, T., &Zafar, U. (2023). Isolation, solubilization of inorganic phosphate, and production of organic acids by individual and co-inoculated microorganisms. Geomicrobiology Journal,40(1), 111-121. https://doi.org/10.1080/01490451.2022.2124329

[36] Rao, P. R., & Reddy, M. K. (2013). Production of citric acid by Aspergillusniger using oat bran as substrate. International Journal of Chemistry and Chemical Engineering,3(3), 181-190. https://www.academia.edu/download/111498960

[37] APHA (2017). Standard Methods for the Examination of Water and Wastewater. 23rd Edition Washington DC: American Public Health Association. https://srjcstaff.santarosa.edu/~oraola/Assets/APHA_SM_20.pdf

[38] AOAC (2019).Official Methods of Analysis of the Association of Official Analytical Chemists: Official Methods of Analysis of AOAC International. 21st Edition, AOAC, Washington DC. https://www.aoac.org/official-methods-of-analysis/

[39] Dutta, D., Rautela, R., Gujjala, L. K. S., Kundu, D., Sharma, P., Tembhare, M., & Kumar, S. (2023). A review on recovery processes of metals from E-waste: A green perspective. Science of the Total Environment,859, 160391. https://doi.org/10.1016/j.scitotenv.2022.160391

[40] Sawant, O., Mahale, S., Ramchandran, V., Nagaraj, G., &Bankar, A. (2018). Fungal citric acid production using waste materials: a mini-review.The Journal of Microbiology, Biotechnology and FoodSciences,8(2),821. https://doi.org/10.15414/jmbfs.2018.8.2.821-828

[41] Zafar, M., Bano, H. S., & Anwar, Z. (2021). Orange Peels Valorization For Citric Acid Production Through Single And Co-Culture Fermentation. Jordan Journal of Biological Sciences,14(2). https://jjbs.hu.edu.jo/files/vol14/n2/Paper%20Number%209.pdf

[42] Gasmalla, M. A. A., Yang RuiJin, Y. R., Nikoo, M., & Man Su, M. S. (2012). Production of ethanol from Sudanese sugar cane molasses and evaluation of its quality. http://dx.doi.org/10.4172/2157-7110.1000163

[43] Osunkoya, O. A., &Okwudinka, N. J. (2011). Utilization of sugar refinery waste (molasses) for ethanol production using Saccharomyces cervicae. American journal of scientific and industrial research,2(4), 694-706.

[44] Munshi, M. K., Hossain, F., Huque, R., Rahman, M. M., Khatun, A., Islam, M., & Khalil, M. I. (2013). Effect of biomass and sugar in citric acid production by Aspergillusniger using molasses and jackfruit as substrates. The American Journal of Food and Nutrition,1(1), 1-6. https://www.academia.edu/download/72977693

[45] Aritonang, A. Y. A., Julianti, E., &Sinaga, H. (2021, June). The production of citric-acid from Bilimbi (Averrhoabilimbi) fruits through the fermentation process using Aspergillusniger. InIOP Conference Series: Earth and Environmental Science(Vol. 782, No. 3, p. 032100). IOP Publishing. https://doi.org/10.1088/1755-1315/782/3/032100

[46] Sarkar, D., & Das, K. (2017). Optimization of citric acid production from aspergillusniger using pineapple waste as feedstock in submerged fermentation. World Journal of Pharmaceutical Research,6(17), 810-818. https://doi.org/10.20959/wjpr201717-10389

[47] Satheeshkumar, S., Sivagurunathan, P., Muthulakshmi, K., & Uma, C. (2019). Utilization of fruit waste for the production of citric acid by using Aspergillusniger. Journal of Drug Delivery and Therapeutics,9(4-A), 9-14. https://doi.org/10.22270/jddt.v9i4-a.3487

[48] Angumeenal, A. R., & Venkappayya, D. (2013). An overview of citric acid production. LWT-Food Science and Technology,50(2), 367-370. https://doi.org/10.1016/j.lwt.2012.05.016

[49] Papagianni, M., Mattey, M., & Kristiansen, B. (1999). The influence of glucose concentration on citric acid production and morphology of Aspergillusniger in batch and culture. Enzyme and Microbial Technology,25(8-9), 710-717. https://doi.org/10.1016/S0141-0229(99)00102-7

[50] Varshney, A. S. (2016). Production, comparative and quantitative analysis of citric acid by Aspergillusniger using food waste as a substrate. Journal of Experimental Food Chemistry,2(4), 1-6. https://doi.org/10.4172/2472-0542.1000121

[51] Hang, Y. D., Splittstoesser, D. F., Woodams, E. E., & Sherman, R. M. (1977). Citric acid fermentation of brewery waste. Journal of Food Science,42(2), 383-384. https://doi.org/10.1111/j.1365-2621.1977.tb01504.x

[52] Shetty, V. G. (2015). Production and optimization of citric acid by Aspergillusniger using molasses and corncob. Int J Pharm PharmSci,7(5), 152-157. https://www.academia.edu/download/110497957

[53] Varshney, A. S. (2016). Production, comparative and quantitative analysis of citric acid by Aspergillusniger using food waste as a substrate. Journal of Experimental Food Chemistry,2(4), 1-6. https://doi.org/10.4172/2472-0542.1000121

[54] Adudu, J. A., Arekemase, S. O., Abdulwaliy, I., Batari, M. L., Raplong, H. H., Aronimo, B. D., &Sani, Y. (2019). Production of citric acid from corn stalk through submerged fermentation using Aspergillusniger. Journal of Applied Sciences,19(6), 557-564. https://doi.org/10.3923/jas.2019.557.564

[55] Smith, A. B., Jenior, M. L., Keenan, O., Hart, J. L., Specker, J., Abbas, A., ... & Zackular, J. P. (2022). Enterococci enhance Clostridioides difficile pathogenesis. Nature,611(7937), 780-786. https://doi.org/10.1038/s41586-022-05438-x

[56] Jones, R. J., Massanet-Nicolau, J., & Guwy, A. J. (2021). A review of carboxylate production and recovery from organic wastes. Bioresource Technology Reports,16, 100826. https://doi.org/10.1016/j.biteb.2021.100826

[57] Orthofer, R., Kubicek, C. P., & Röhr, M. (1979). Lipid levels and manganese deficiency in citric acid producing strains of Aspergillusniger. FEMS Microbiology Letters,5(6), 403-406. https://doi.org/10.1111/j.1574-6968.1979.tb03368.x

[58] Ingram, R. E. (1984). Toward an information-processing analysis of depression. Cognitive therapy and research,8, 443-477. https://doi.org/10.1007/BF01173284

[59] Yaykaşlı, K. O., Demirel, G., & Yaşar, A. (2005). Influence of alcohols on citric acid production by Aspergillus niger A-9 entrapped in polyacrylamide gels. Journal of Food Engineering,70(4), 518-522. https://doi.org/10.1016/j.jfoodeng.2004.10.006

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