

ENHANCED CITRIC ACID PRODUCTION BY *ASPERGILLUS NIGER*

Dr. Sai Subhashini¹ M.Sc., Ph.D. M. Lakshmipriya²

Assistant Professor¹ PG Scholar²

Department of Microbiology¹

Dr. MGR Janaki College of arts and science for women¹ Chennai, India

Abstract:

Citric acid is an important organic acid that has extensive applications in food, pharmaceutical, cosmetic, detergent and several other industrial sectors. Although it occurs naturally in many fruits, large-scale production is mainly achieved through microbial fermentation using microorganism such as *Aspergillus niger*. Among the microorganisms investigated for citric acid synthesis, *Aspergillus niger* has become the major industrial organism because of its strong acid-producing ability, adaptability to different substrates and capacity to accumulate citric acid in high amounts. Different fermentation approaches, including submerged fermentation, solid-state fermentation and surface fermentation, have been explored to improve productivity and reduce manufacturing costs. The efficiency of citric acid production is strongly influenced by the type and concentration of carbon source, nitrogen availability, pH, temperature, inoculum level, oxygen transfer, trace elements, moisture and fermentation duration. Conventional substrates such as sucrose, glucose, molasses and syrups are commonly used, while increasing attention has been given to low-cost agricultural residues and agro-industrial by-products. Materials such as sugarcane bagasse, cassava bagasse, fruit peels, coffee husk, pineapple waste and molasses can provide economically useful alternatives to refined substrates. Recent studies have also demonstrated that optimization of fermentation conditions and suitable pretreatment of substrates can substantially improve citric acid formation. This review summarizes the important characteristics of citric acid, the role of *Aspergillus niger*, biochemical aspects of production, fermentation techniques, substrate selection, major process parameters, agricultural waste utilization, product recovery, industrial applications, current limitations and future opportunities for enhanced citric acid production.

Keywords: Citric acid, *Aspergillus niger*, Microbial fermentation, Submerged fermentation, Solid-state fermentation, Sugarcane molasses, Agricultural waste.

Introduction:

Citric acid is a naturally occurring organic acid belonging to the group of tricarboxylic acids. It is widely distributed in plants and animals and is particularly abundant in citrus fruits such as lemon, lime and orange. Because of its acidic taste, buffering capacity, metal-chelating ability and compatibility with many food and pharmaceutical formulations, citric acid has become an important commercially produced organic acid (Ksiazek, 2024). Its applications extend beyond

the food sector to pharmaceutical preparations, cosmetics, detergents, chemical processing and other industrial areas. (Behera *et al.*, 2021; Ksiazek, 2024).

Historically, citric acid was obtained from citrus fruits, especially lemon juice. However, extraction from natural sources was not sufficiently economical for meeting the increasing industrial demand. Chemical synthesis was also investigated, but microbial production gradually became the preferred commercial approach because fermentation offered a more practical and economical route. The development of *Aspergillus niger*-based production played a major role in establishing modern citric acid biotechnology. (Show *et al.*, 2015; Behera *et al.*, 2021).

The discovery that *Aspergillus niger* could accumulate large quantities of citric acid under suitable nutritional and environmental conditions created the foundation for industrial production. Since then, extensive research has focused on improving fungal strains, identifying inexpensive substrates, optimizing fermentation conditions and developing efficient recovery methods. (Show *et al.*, 2015).

At present, submerged fermentation is the predominant industrial approach, although solid-state fermentation has received considerable research attention because of its potential to utilize inexpensive agricultural residues. Reviews have emphasized the importance of fermentation conditions, substrate composition, oxygen availability, trace elements and downstream processing in determining the final productivity of citric acid. The increasing cost of conventional raw materials and concerns related to agricultural waste disposal have encouraged researchers to explore alternative substrates. Sugarcane molasses, bagasse, cassava residues, fruit peels, coffee husk and other agro-industrial materials have therefore become important candidates for low-cost citric acid production (Show *et al.*, 2015; West, 2023).

2. Chemical and Physical Properties of Citric Acid:

Citric acid is chemically known as 2-hydroxypropane-1,2,3-tricarboxylic acid. It contains three carboxylic acid groups and therefore exhibits triprotic acid behaviour. It is generally obtained as a colourless or white crystalline compound and is highly soluble in water. Citric acid can occur in anhydrous and monohydrate forms. The anhydrous form has a molecular weight of approximately 192.12 g/mol, whereas the monohydrate form has a molecular weight of approximately 210.14 g/mol. It has a melting point around 153°C and shows useful buffering and chelating properties (Ksiazek, 2024).

The ability of citric acid to bind metal ions is particularly important for its industrial applications. Its chemical characteristics allow it to function as an acidulant, preservative, stabilizer, buffering agent and sequestrant. (Behera *et al.*, 2021; Ksiazek, 2024).

3. Historical Development of Citric Acid Production:

The early commercial production of citric acid relied mainly on extraction from citrus fruits. However, this approach was associated with limitations related to raw-material availability, seasonal variation and production cost. Microbial production began to attract attention after observations that certain fungi could synthesize citric acid from sugar-containing media. The work of James Currie in 1916 demonstrated the strong potential of *Aspergillus niger* for citric acid production. The organism's ability to grow under acidic conditions and accumulate citric

acid made it particularly suitable for industrial development. Subsequently, commercial fermentation processes were established, and improvements in fermentation technology, strain selection and product recovery gradually increased the efficiency of production. (Show *et al.*, 2015)

4. *Aspergillus niger* as a Citric Acid Producing Microorganism:

4.1 General Characteristics

Aspergillus niger is a filamentous fungus widely distributed in soil, plant materials and organic substrates. It is capable of utilizing a broad range of carbon sources and can grow under acidic conditions.

The organism has several characteristics that make it suitable for industrial citric acid production:

- High citric acid accumulation ability
- Ability to utilize inexpensive carbon sources
- Adaptability to different fermentation systems
- Relatively simple cultivation requirements
- Good tolerance to acidic conditions
- Availability of improved production strains
- Ability to grow on agro-industrial residues

Among the microorganisms investigated for citric acid production, *Aspergillus niger* remains the most extensively used industrial organism. (Show *et al.*, 2015; Behera *et al.*, 2021; Ksaizek, 2024; Papagianni, 2007).

4.2 Isolation and Screening

Citric-acid-producing strains can be isolated from soil and other environmental sources. In one study, soil samples were serially diluted and plated on potato dextrose agar, followed by purification and morphological examination of fungal colonies. The isolates were subsequently screened for citric acid production (Almoussa *et al.*, 2018).

Screening is important because different *Aspergillus niger* isolates can exhibit considerable variation in their acid-producing capacity. The study by Almoussa and colleagues reported differences among isolates and selected a productive local strain for further optimization (Almoussa *et al.*, 2018).

5. Biochemical Basis of Citric Acid Production:

Citric acid formation in *Aspergillus niger* is closely connected with the tricarboxylic acid cycle. Fermentable sugars are first metabolized through glycolysis, generating pyruvate. Pyruvate is subsequently converted into oxaloacetate, while acetyl-CoA is generated through oxidative metabolism. Citrate synthase catalyses the condensation of oxaloacetate and acetyl-CoA, resulting in citrate formation (Papagianni, 2007; Karaffa & Kubicek, 2003).

Under conditions favouring citric acid accumulation, citrate is produced at a rate that exceeds its subsequent utilization in cellular metabolism. This allows citric acid to accumulate and eventually be released into the fermentation medium. The production process is not controlled

by a single factor. Instead, carbon availability, oxygen supply, trace-metal concentration, pH, nitrogen limitation, phosphate availability and fungal morphology interact with one another to determine the final yield. (Papagianni,2007; Behera *et al.*, 2021)

6. Substrates Used for Citric Acid Production:

The selection of substrate is one of the most important considerations in fermentation because the carbon source directly influences fungal growth and acid accumulation.

6.1 Conventional Substrates

Commonly investigated carbon sources include:

- Sucrose
- Glucose
- Fructose
- Molasses
- Sugar syrups
- Starch hydrolysates

Molasses from sugar-processing industries is particularly attractive because it is inexpensive and contains substantial amounts of fermentable carbohydrates (Show *et al.*, 2015; Ksaizek,2024).

6.2 Agricultural and Agro-Industrial Wastes

Increasing attention has been given to agricultural residues because they can reduce substrate costs while simultaneously providing a useful route for waste utilization.

Reported materials include:

- Sugarcane bagasse
- Cassava bagasse
- Coffee husk
- Banana peel
- Pineapple peel
- Orange peel
- Apple pomace
- Grape pomace
- Rice bran
- Wheat bran
- Corn residues
- Vegetable processing wastes

The literature indicates that fruit-processing residues can sometimes support substantial citric acid formation, whereas some cereal-processing residues may provide lower productivity because of differences in available sugar content (Vandenberghe *et al.*,2000; West,2023).

7. Sugarcane Molasses as a Low-Cost Substrate:

Sugarcane molasses is an important alternative substrate because it is readily available as a by-product of the sugar industry and contains considerable quantities of carbohydrates. However, untreated molasses can contain metal ions and other components that interfere with citric acid

biosynthesis. Therefore, pretreatment or clarification may be required before fermentation. Studies have examined treatments involving ferrocyanide, acids, lime and ammonium oxalate to reduce inhibitory substances and improve fermentation performance. A recent study using sugarcane molasses investigated several factors including magnesium sulphate, potassium ferrocyanide and ammonium oxalate. The reported results demonstrated that optimization of these components influenced citric acid production by *Aspergillus niger* (Ksiazek,2024; Khurshid *et al.*, 2024).

8. Fermentation Techniques:

Three major fermentation approaches are reported for citric acid production:

Submerged fermentation, Solid-state fermentation, Surface fermentation (Show *et al.*, 2015; Behera *et al.*,2021).

8.1 Submerged Fermentation

Submerged fermentation is the most commonly used industrial technique. In this process, *Aspergillus niger* is cultivated in a liquid nutrient medium containing a suitable carbon source and other required nutrients.

Advantages include:

- ❖ Better control of pH
- ❖ Easier temperature regulation
- ❖ Efficient mixing
- ❖ Improved oxygen transfer
- ❖ Easier monitoring of fermentation parameters
- ❖ Suitability for large-scale production

Most current commercial production is associated with submerged fermentation using *Aspergillus niger* (Show *et al.*, 2015; Papagianni,2007).

8.2 Solid-State Fermentation

Solid-state fermentation involves fungal growth on moist solid materials with relatively limited free water. Agricultural residues can function both as nutrient sources and as physical support for fungal growth.

This approach has received attention because it can convert low-value agricultural residues into a useful industrial product.

Important advantages include:

- Lower water requirement
- Reduced energy demand
- Reduced wastewater generation
- Possibility of using inexpensive residues
- Potentially lower operating cost
- Suitable oxygen transfer for filamentous fungi

However, difficulties can occur in controlling temperature, moisture and other process parameters during scale-up (West,2023; Vandenberghe *et al.*,2000).

8.3 Surface Fermentation

Surface fermentation is an older method in which fungal growth occurs at the surface of the liquid medium. Although historically important, it requires larger space and may be more difficult to control compared with submerged fermentation (Behera *et al.*, 2021).

9. Comparison of Submerged and Solid-State Fermentation:

Submerged fermentation and solid-state fermentation differ in their physical characteristics, process control requirements and substrate utilization.

Table 9: Comparison of Submerged and Solid-State fermentation

Parameter	Submerged Fermentation	Solid-State Fermentation
Medium	liquid medium	moist solid substrate
Water requirement	relatively high	relatively low
Process control	easier	more difficult
Mixing	easier	limited
Oxygen transfer	controlled through agitation and aeration	dependent on substrate structure and aeration
Agricultural waste utilization	possible	particularly suitable
Heat transfer	easier	difficult
Waste generation	comparatively high	lower

Submerged fermentation remains the major industrial approach because it allows relatively effective control of pH, temperature, agitation and aeration (Show *et al.*, 2015; Behera *et al.*, 2021).

Solid-state fermentation offers opportunities for using inexpensive agricultural residues and can have lower water requirements, but control of heat, moisture and process uniformity can become difficult during scale-up (West, 2023; Latif *et al.*, 2025).

10. Factors Affecting Citric Acid Production:

Citric acid fermentation is highly sensitive to environmental and nutritional conditions. The major parameters are discussed below.

10.1 Carbon Source

Carbon is the primary substrate for citric acid formation. Sucrose, glucose, molasses and starch-derived substrates have been extensively investigated. The concentration of carbon should be optimized because both insufficient and excessively unsuitable substrate conditions can reduce productivity (Papagianni,2007; Ksaizek,2024).

10.2 Nitrogen Source

Nitrogen is necessary for fungal growth, but excessive nitrogen availability may favour biomass formation rather than citric acid accumulation. Therefore, nitrogen limitation under appropriate fermentation conditions can support citric acid production (Papagianni,2007; Behera *et al.*,2021).

10.3 Initial pH

pH has a major effect on fungal metabolism and by-product formation. *Aspergillus niger* is capable of growing under acidic conditions, and acidic fermentation environments are generally associated with citric acid accumulation. The Almousa study identified an initial pH of 6.5 under its specific experimental conditions, while other studies have reported different optimum values depending on strain and substrate. This difference demonstrates that an optimum pH should not

be assumed to be universal for every strain or substrate combination (Almousa *et al.*, 2018; Papagianni, 2007).

10.4 Temperature

Temperature influences fungal growth, enzyme activity and metabolic rate. For example, Almousa *et al.* reported 28°C as the optimum temperature under their experimental conditions. In contrast, the solid-state study using cassava bagasse obtained its maximum production at 26°C. Thus, temperature optimization should be performed according to the organism, substrate and fermentation system (Almousa *et al.*, 2018; Ksaizek, 2024).

10.5 Inoculum Size

The amount of fungal inoculum influences the initial growth pattern and morphology of the organism. Too little inoculum may result in slow colonization, whereas excessive inoculum may increase biomass formation and alter nutrient consumption. An appropriate spore concentration is therefore necessary for consistent fermentation (Almousa *et al.*, 2018).

10.6 Oxygen and Aeration

Citric acid formation by *Aspergillus niger* is strongly associated with aerobic metabolism. Adequate oxygen transfer is therefore essential for maintaining fungal activity and obtaining satisfactory production.

Aeration and agitation become especially important in submerged systems because oxygen availability is influenced by mixing and culture viscosity (Papagianni, 2007).

10.7 Trace Elements

Trace metals can have either beneficial or inhibitory effects depending on their concentration. Elements such as iron, zinc and manganese are required in small amounts but excessive concentrations may negatively influence citric acid accumulation.

Therefore, controlling trace-element concentration is an important part of substrate pretreatment and medium optimization (Kubicek *et al.*, 1980; Show *et al.*, 2015).

10.8 Fermentation Time

The duration of fermentation determines the amount of citric acid accumulated before nutrient depletion or metabolic changes begin to reduce productivity.

In the Almousa study, maximum production under the tested conditions was observed at five days.

The optimum time differs among fermentation systems and substrates (Almousa *et al.*, 2018).

11. Optimization of Fermentation Conditions:

Optimization is essential because citric acid production is affected by several interacting parameters.

Important parameters that can be optimized include:

- ✓ Carbon concentration
- ✓ Nitrogen concentration
- ✓ Initial pH
- ✓ Temperature
- ✓ Inoculum concentration
- ✓ Agitation

- ✓ Aeration
- ✓ Fermentation period
- ✓ Phosphate concentration
- ✓ Magnesium concentration
- ✓ Trace metals
- ✓ Moisture content
- ✓ Substrate pretreatment

A study using a local *Aspergillus niger* isolate reported substantial improvement after optimizing temperature, pH, inoculum concentration, carbon source, nitrogen source, phosphate and methanol. Similarly, research using sugarcane molasses demonstrated that changes in magnesium sulphate, potassium ferrocyanide and ammonium oxalate affected citric acid yield. These findings indicate that optimization should be treated as a combined process rather than focusing on a single parameter (Show *et al.*, 2015; Behera *et al.*, 2021; Almousa *et al.*, 2018).

12. Pretreatment of Low-Cost Substrates:

Agricultural residues may contain complex carbohydrates, minerals and inhibitory compounds. Pretreatment can improve the accessibility of fermentable nutrients.

Different pretreatment approaches include:

- ✚ Acid treatment
- ✚ Alkali treatment
- ✚ Heat treatment
- ✚ Enzymatic hydrolysis
- ✚ Clarification
- ✚ Ferrocyanide treatment
- ✚ Lime treatment

For sugarcane bagasse, studies summarized by West reported improved citric acid production after different pretreatment methods compared with untreated material. Molasses may also require treatment because metal ions such as iron, zinc and manganese can interfere with the fermentation process (Ksiazek, 2024; West, 2023).

13. Recovery and Purification of Citric Acid:

After fermentation, citric acid must be separated from the fungal biomass and other components of the fermentation broth.

A general downstream sequence includes:

Fermentation broth → Biomass separation → Filtration → Clarification → Citric acid recovery → Purification → Crystallization → Drying → Final product

The recovery stage is important because the fermentation broth contains residual sugars, fungal biomass, proteins, minerals and other metabolites.

Different recovery approaches have been investigated, and the selection depends on the type of fermentation medium and desired product purity (Mores *et al.*, 2020; Behera *et al.*, 2021; Latif *et al.*, 2025).

14. Applications of Citric Acid

Citric acid has a broad range of applications because of its acidity, buffering ability, chelating capacity, flavour characteristics and compatibility with different formulations (Behera *et al.*, 2021; Książek, 2024).

It is extensively used in food and beverages and also has important roles in pharmaceutical, cosmetic, detergent, chemical and environmental applications (Behera *et al.*, 2021; Książek, 2024).

14.1 Food and Beverage Industry

Citric acid is extensively used as:

1. Acidulant
2. Flavour enhancer
3. Preservative
4. pH regulator
5. Buffering agent
6. Stabilizer

It provides a characteristic sour taste and can balance sweetness in beverages and food products (Behera *et al.*, 2021; Latif *et al.*, 2025).

14.2 Pharmaceutical Industry

Citric acid is used in pharmaceutical formulations as:

1. pH regulator
2. Antioxidant
3. Effervescent component
4. Stabilizing agent
5. Component of citrate preparations

Its buffering and chelating properties make it useful in several pharmaceutical applications (Behera *et al.*, 2021; Ksiazek, 2024).

14.3 Cosmetic Industry

Citric acid is incorporated into several cosmetic and personal-care formulations. It can contribute to pH adjustment and formulation stability (Ksiazek, 2024).

14.4 Detergent Industry

Citric acid and citrate salts can function as chelating agents and have been investigated as alternatives to phosphate-containing detergent components (Latif *et al.*, 2025).

14.5 Textile and Chemical Industries

Citrate compounds are used in chemical processing and textile-related applications. The ability of citrate to interact with metal ions contributes to its usefulness in industrial processes (Show *et al.*, 2015).

14.6 Environmental Applications

Citric acid has attracted interest in environmental applications because its chelating ability can assist in the mobilization and treatment of certain metal contaminants (Behera *et al.*, 2021).

Its biodegradability and relatively low toxicity also support its investigation in environmentally oriented applications (Książek, 2024).

15. Recent Developments in Enhanced Citric Acid Production:

Recent research has moved beyond conventional medium optimization toward a combination of strain improvement, metabolic engineering, substrate utilization and process optimization (Behera, 2020; Behera *et al.*, 2021).

The availability of the *Aspergillus niger* genome has provided opportunities to investigate genes involved in primary metabolism, regulation and citric acid accumulation (Behera, 2020).

Metabolic engineering has therefore become an important research direction for improving citric acid titre and productivity (Behera, 2020; Latif *et al.*, 2025).

CRISPR/Cas9 and other molecular approaches have also been investigated for modifying *Aspergillus niger* and improving its suitability as an industrial production organism (Behera, 2020).

Another important development is the increasing use of low-cost substrates such as agricultural residues and food-processing wastes (West, 2023).

Recent research also emphasizes integrated optimization of fermentation parameters rather than optimization of only one factor at a time (Latif *et al.*, 2025).

16. Challenges in Citric Acid Production:

Despite the extensive industrial use of citric acid, several challenges remain in improving production efficiency.

One major challenge is the high sensitivity of *Aspergillus niger* fermentation to nutritional and environmental conditions (Papagianni, 2007).

The carbon source, nitrogen concentration, pH, temperature, trace metals, oxygen transfer, morphology and fermentation time can interact with each other and influence the final citric acid yield (Behera *et al.*, 2021).

The use of agricultural wastes can reduce raw-material costs, but differences in composition between batches may affect fermentation reproducibility (West, 2023).

Pretreatment of lignocellulosic materials may also increase processing requirements and overall production cost (Behera, 2020).

Another challenge is the scale-up of solid-state fermentation because temperature distribution, moisture control and oxygen transfer can become difficult in larger systems (West, 2023; Latif *et al.*, 2025).

Downstream recovery is another important consideration because the separation and purification of citric acid can contribute substantially to overall processing cost (Mores *et al.*, 2020; Latif *et al.*, 2025).

17. Future Perspectives

Future research on citric acid production is expected to focus increasingly on sustainable substrates, improved fungal strains and efficient fermentation technologies (Behera, 2020; Latif *et al.*, 2025).

The use of agricultural and food-processing wastes may provide an opportunity to reduce dependence on refined sugars while simultaneously addressing waste-management problems (West, 2023).

Metabolic engineering and genome-editing technologies may provide new possibilities for redirecting carbon flux toward citric acid accumulation (Behera, 2020).

Further development of fermentation systems with improved monitoring and control could help maintain optimal pH, oxygen transfer, temperature and nutrient conditions during production (Latif *et al.*, 2025).

Integration of fermentation with downstream recovery and waste-treatment systems may also improve the overall economic and environmental performance of the process (Mores *et al.*, 2020).

Future studies should therefore consider not only citric acid yield but also substrate cost, energy consumption, water use, waste generation, product recovery and overall environmental impact.

18. Research Gap

Although many studies have investigated citric acid production by *Aspergillus niger* differences in strain characteristics, substrates and fermentation conditions make it difficult to define a single optimum production condition for all systems (Papagianni, 2007; Behera *et al.*, 2021). There is a need for systematic comparison of different low-cost substrates under standardized fermentation conditions.

More research is also required to determine how substrate pretreatment affects both citric acid productivity and overall process economics.

Another important research area is the development of agricultural-waste-based fermentation systems that provide reproducible production despite variations in the composition of raw materials.

The integration of metabolic engineering with low-cost substrate utilization represents another promising area for future investigation (Behera, 2020; Latif *et al.*, 2025).

Further work is also required to develop scalable solid-state fermentation systems with improved control of temperature, moisture and oxygen transfer (West, 2023).

19. Conclusion:

Citric acid production using *Aspergillus niger* remains an important example of industrial microbial biotechnology because the organism combines high citric acid-producing ability with flexibility in substrate utilization (Show *et al.*, 2015; Behera *et al.*, 2021).

The efficiency of production depends on the interaction between fungal strain, carbon source, nitrogen availability, pH, temperature, inoculum, oxygen transfer, trace elements and fermentation time (Papagianni, 2007; Behera *et al.*, 2021).

Submerged fermentation remains an important industrial technology because of its controllability, whereas solid-state fermentation provides an attractive route for utilizing agricultural and agro-industrial residues (Show *et al.*, 2015; West, 2023).

The use of sugarcane molasses and other low-cost substrates can potentially reduce raw-material costs while supporting waste valorisation (West, 2023; Książek, 2024).

At the same time, strain improvement, metabolic engineering and modern molecular technologies provide opportunities for further enhancement of citric acid production (Behera, 2020; Latif *et al.*, 2025).

Overall, future development should combine efficient strain selection, sustainable substrate utilization, optimized fermentation, improved product recovery and environmentally responsible processing to achieve economically viable and sustainable citric acid production.

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