Thermodynamic and kinetic determinants of citric-acid crystallization: upstream influences, process integration and industrial design implications

by

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Abstract

Citric acid is produced industrially through *Aspergillus niger* fermentation, but the efficiency and predictability of its downstream crystallization are strongly governed by upstream decisions that determine the composition and thermodynamic character of the mother liquor. This report develops an integrated framework linking substrate choice, fermentation conditions, in-situ product-removal strategies and recovery pathways to the crystallization behavior of citric acid. Each upstream operation establishes a distinct ionic and organic load that shifts activity coefficients, alters solubility and defines realistic supersaturation trajectory.

Fundamental thermodynamic principles (chemical potential, supersaturation, solubility, and hydration–dehydration equilibria) are combined with kinetic concepts such as Classical Nucleation Theory (CNT), Ostwald's Rule of Stages, the terrace–step–kink mechanism and interfacial poisoning to explain observed solid-form outcomes and sensitivity to feed variability. The citric-acid-water T-RH stability diagram provides the basis for controlling monohydrate-anhydrate transitions during crystallization, drying and storage.

The analysis shows that crystallizer performance, to include form selection, metastable-zone width (MSZW), nucleation suppression and particle-size control, depends on how well upstream composition has been stabilized. High-ionic-strength or impurity-rich feeds require slower supersaturation generation and higher seed masses, whereas polished or membrane-conditioned feeds support tighter control and more reproducible growth. This integrated perspective connects process decisions across the entire manufacturing chain and provides a comprehensive basis for improving solid-form control and consistency between batches in industrial citric-acid production.

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List of Abbreviations

Abbreviation or Acronym	Full Text
A	Debye-Hückel Theory Constant
a_w	Water Activity
C	Solute Concentration
C*	Temperature-Dependent Solubility at Equilibrium
Ca	Calcium
CAGR	Compound Annual Growth Rate
cm	Centimeters
CNT	Classical Nucleation Theory
CO_2	Carbon Dioxide
ED	Electrodialysis
Fe	Iron
g	Growth Order
G	Growth (Power-Law Growth Model)
H_2O	Water
ISPR	In-Situ Product Recovery
k_g	Crystal Growth Coefficient
Mg	Magnesium
Mn	Manganese
MSZW	Metastable Zone Width
NF	Nanofiltration
O_2	Oxygen
^oC	Degrees Celsius
p	Water Vapor Pressure
pK_a	Dissociation Constant
R	Ideal Gas Constant
r*	Critical Nucleus Radius
S	Supersaturation
SF	Surface Fermentation
SmF	Submerged Fermentation
SSF	Solid-State Fermentation
T	Temperature
TOC	Total Organic Carbon
T-RH	Temperature-Relative Humidity
T-WA	Temperature-Water Activity
UF	Ultrafiltration
UV	Ultraviolet
V/L	Vapor-Liquid Ratio

vvm	Vessel Volume/Minute
x	Mole Fraction
z_i	Charge Number of Ion Species i
γ	Activity Coefficient
γ_{sl}	Solid-Liquid Interfacial Free Energy (Surface Tension)
ΔG^*	Critical Nucleation Free-Energy
μ	Chemical Potential

Introduction

Citric acid is one of the most widely produced and commercially significant organic acids, playing a major role across food, beverage, pharmaceutical, detergent, cosmetic and biotechnology industries. Its global economic relevance is sustained by increasing demand for acidulants, chelating agents, buffering components and environmentally friendlier processing additives and disinfecting products. As industrial applications expand and production volumes rise, the efficiency and controllability of citric-acid manufacturing depend increasingly on understanding how upstream biological and chemical operations influence downstream purification and crystallization, the final and most thermodynamically sensitive stage of the process.

Although fermentation using *Aspergillus niger* is a mature technology, process yield and product quality are constrained by the composition of the mother liquor exiting fermentation and the alterations it undergoes during recovery. Fermentation substrates, pretreatments, nutrient regimes, morphological control, acidulation strategy, ISPR adoption and intermediate conditioning steps altogether impact the physicochemical state of the mother liquor entering crystallization. These upstream decisions determine ionic strength, activity coefficient behavior, impurity load, water activity and the balance of species that define the thermodynamic and kinetic environment in which nuclei form and crystals grow. As a result, crystallization performance cannot be optimized without taking all the above-mentioned subprocesses into consideration.

A thermodynamic analysis is therefore essential for evaluating and controlling solid-form selection, nucleation behavior, metastable- zone width (MSZW) and growth kinetics in citric-acid systems. The citric acid-water phase diagram, hydrate-anhydrate transition boundary,

solubility behavior, activity-coefficient effects and kinetic factors described by Classical Nucleation Theory (CNT) provide the reference frame together for understanding how industrial crystallizers behave under real operating conditions. This report develops that reference frame and applies it directly to the process-integration challenges seen in industrial production.

The objectives of this work are to establish the general thermodynamic and kinetic principles within the citric-acid crystallization process, to analyze how upstream process decisions alter the solution behavior that governs nucleation, growth and solid-form stability and to translate these thermodynamic insights into operational guidance for crystallizer design, seeding strategy, cooling pathways and in drying and storage conditions. By integrating solution thermodynamics with process-specific considerations, this report clarifies why crystallization variability arises in industrial processes and identifies the most critical parameters for achieving reproducible, high-quality crystalline product.

Although the individual elements of this analysis are well represented in existing literature, they are treated in isolation. Fermentation studies focus on maximizing citric acid yields without evaluating the consequences of the resulting mother liquor composition for downstream crystallization. Recovery literature compares pathways on the basis of yield and cost but rarely considers how residual ions or organics alter activity coefficients, nucleation barriers or metastable zone width in the crystallizer. Crystallization studies typically assume a defined feed composition and do not trace the origin of non-ideality back to the specific upstream decisions. No published framework integrates these stages through a unified thermodynamic analysis for citric acid production. This report addresses that gap by developing an integrated perspective that connects upstream process decisions to crystallization outcomes

through the thermodynamic variables that govern solid-form selection, nucleation behavior and crystal growth.

Citric Acid: Overview and Properties

Citric acid (2-hydroxypropane-1,2,3-tricarboxylic acid) is a weak organic tricarboxylic acid ubiquitous in nature, serving as an intermediate in the Krebs or tricarboxylic (TCA) cycle of aerobic metabolism. It exists commercially as a colorless, odorless, white crystalline powder in two primary forms: the anhydrous form ($C_6H_8O_7$) and the monohydrate form ($C_6H_8O_7 \cdot H_2O$). The chemical structure of the anhydrate form is shown in Figure 1 below. The transition between these forms is thermodynamically governed by a critical temperature of 36.6 ± 1.5 ºC. The monohydrate crystallizes from aqueous solutions below this temperature, while the anhydrous form precipitates at higher temperatures (Behera, 2020).

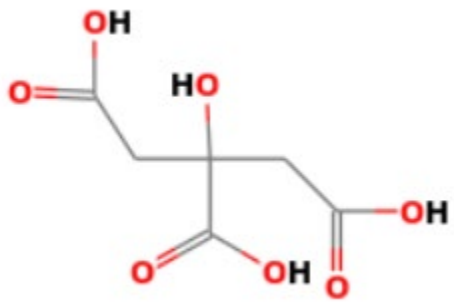

Figure 1: Chemical Structure of Citric Acid Anhydrate

Chemically, citric acid is tribasic, possessing three carboxylic functional groups with distinct dissociation constants ($pK_{a1} = 3.13,\ pK_{a2} = 4.76,\ pK_{a3} = 6.39\ at\ 25℃$). This dissociation behavior is critical for its solution thermodynamics, enabling it to act as an effective buffer (pH 2.0–7.0) and a potent chelator of metal ions such as calcium, copper, and iron (Araújo, Coelho, Balarini, Miranda, & Salum, 2017) (Satish, 2023).

History of Citric Acid

The history of citric acid production is a progression from extraction from natural sources to chemical synthesis attempts, and finally to the establishment of large-scale microbial fermentation, which dominates the industry today.

The discovery of citric acid is attributed to the Swedish chemist Karl Wilhelm Scheele, who first isolated the compound from lemon juice in 1784. By treating lemon juice with calcium hydroxide, Scheele precipitated calcium citrate, which was then reacted with sulfuric acid to release citric acid in the liquid phase (Berovic & Legisa, 2007). Following this discovery, commercial production was established in England around 1826, relying on calcium citrate imported from Italian lemons (Behera, 2020). For nearly a century, the extraction from citrus fruits remained the monopoly for commercial citric acid production (Show, et al., 2015).

Efforts to produce citric acid synthetically were made in the late 19th century. In 1880, Grimoux and Adam successfully synthesized citric acid from glycerol as a starting material (Behera, 2020). However, this chemical synthesis proved to be economically undesirable as only a few reports have been published showing value-added product formation using this substrate. Therefore, research is lacking to support further chemical synthesis development with glycerol as a substrate (Singh & Kaur Brar, 2011).

The potential for microbial production was first identified in 1893 by the German botanist C. Wehmer. He observed that a "sugar fungus," which he named *Citromyces* (now classified as *Penicillium*), accumulated citric acid when cultured in a culture medium containing sugars and inorganic salts (Singh & Kaur Brar, 2011). Although two strains were isolated for this purpose, industrial trials were largely unsuccessful due to long fermentation times and susceptibility to contamination (Latif, et al., 2025).

A very influential finding for industrial citric acid production occurred in 1917 with the work of James Currie. Currie discovered that specific strains of the filamentous fungus *Aspergillus niger* could produce significant amounts of citric acid in a nutrient medium with high sugar concentrations (Soccol, Vandenberghe, Rodrigues, & Pandey, 2006). He found that maintaining a low initial pH (2.5–3.5) suppressed the formation of unwanted by-products like oxalic and gluconic acids while favoring citric acid accumulation (Show, et al., 2015). This discovery laid the foundation for modern industrial biotechnology. Based on Currie's findings, the pharmaceutical company Pfizer established the first successful commercial fermentation plant in the United States in 1923, utilizing surface fermentation techniques (Singh & Kaur Brar, 2011). While submerged fermentation was later developed and currently accounts for the majority of global production, the principles established by Currie regarding *Aspergillus niger* remain the cornerstone of the industry (Show, et al., 2015).

Applications and Market Demand

Citric acid is widely used in many industries. It is approved as a "generally recognized as safe" (GRAS) material due to its non-toxicity. Table 1 shows a summary of industries, applications and uses for citric acid.

Table 1: Applications of Citric Acid

Industry	Application	Uses	References
Food and Beverage	Carbonated Beverages	Acidulant providing tartness and complement fruit flavors	Soccol et al. 2006
		Adjusts pH	
	Jams, Jellies and Frozen Fruits	Preservative	Show et al. 2015
		Lowers pH to inhibit microbial growth and inactivate oxidative enzymes	
	Ice Cream and Dairy Products	Emulsifying agent to improve texture and stability	Behera et al. 2021
	Confectionery	Buffering capacity used to control sugar inversion	Satish 2023
		Masks aftertaste of artificial sweeteners	
Pharmaceutical	Antacids and Soluble Aspirin	Effervescent properties combined with bicarbonates	Behera 2020
	Anticoagulant	Complexes calcium ions to preserve blood	Behera et al. 2021
	Kidney Stone Prevention	Binds urinary calcium, reducing calcium crystal formation	Latif et al. 2025
Detergents and Cleaning Products	Laundry Detergent and Dishwashing Cleaner	Replaces phosphates due to biodegradability and less eutrophic effect	
	Chelating Agent	Softens water and enhances foam formation by binding calcium and magnesium ions	Latif et al. 2025
	Industrial Metal Cleaning	Removes oxidation from metal surfaces	Latif et al. 2025
Cosmetics and Personal Care	Buffering and Chelating Agent	Slows degradation of active ingredients in antioxidant systems	Show et al. 2015
	Antioxidant and Exfoliating Agent	Mitigates influence of free radical damage to skin	
		Promotes skin toning and firmness	
Biomedical Engineering	Tissue Engineering	Used in hydroxyapatite to fabricate bio-ceramic scaffolds	Behera et al. 2021
Environmental Remediation	Chelating Agent	Heavy metal and radionuclides contamination remediation	
Disinfection	Antimicrobial Agent	Used with alcohol to deactivate viruses on surfaces	Behera 2020

Industrial Applications

Citric acid is valued for its versatility, non-toxicity, and multifunctionality, allowing it to serve as a fundamental ingredient across numerous industrial sectors. Its applications are primarily derived from its physicochemical properties, including its acidity, buffering capacity, pleasant taste, and ability to chelate metal ions (Latif, et al., 2025).

- **Food and Beverage Industry:** This industry commands the largest share of the market, accounting for approximately 65–75% of global citric acid consumption (Berovic & Legisa, 2007). In carbonated beverages, soft drinks, and syrups, citric acid acts as an acidulant to provide tartness and complement fruit flavors while also adjusting pH (Soccol, Vandenberghe, Rodrigues, & Pandey, 2006). It serves as a preservative in jams, jellies, and frozen fruits by lowering pH to inhibit microbial growth and inactivating oxidative enzymes (Show, et al., 2015). Also, it is employed as an emulsifying agent in ice cream and dairy products to improve texture and stability (Behera, Mishra, & Mohapatra, 2021). Its buffering capacity can be utilized to control sugar inversion in confectionery and to mask the aftertaste of artificial sweeteners like aspartame in diet beverages (Satish, 2023).
- **Pharmaceutical Industry:** This industry accounts for roughly 10–12% of the total citric acid demand, playing a critical role in drug formulation and delivery (Berovic & Legisa, 2007). Its effervescent property, when combined with bicarbonates, is essential for the production of antacid and soluble aspirin tablets (Behera, 2020). Trisodium citrate is widely used as a blood preservative and anticoagulant, preventing clotting by complexing calcium ions (Behera, Mishra, & Mohapatra, 2021). Additionally, sodium citrate combined with acetic acid helps prevent kidney stone formation by binding urinary calcium, thereby reducing calcium crystal formation in the kidneys (Latif, et al., 2025).

- **Detergents and Cleaning Products:** The detergent industry utilizes approximately 22% of citric acid production (based on U.S. usage) (Soccol, Vandenberghe, Rodrigues, & Pandey, 2006). Due to its biodegradability and less eutrophic effect, citric acid has effectively replaced phosphates in laundry detergents and dishwashing cleaners (Behera, Mishra, & Mohapatra, 2021). Its strong chelating ability allows it to soften hard water by binding calcium and magnesium ions, which enhances foam formation and cleaning efficiency. It is also employed in industrial metal cleaning to remove oxides from ferrous and non-ferrous metal surfaces, including the cleaning of power station boilers (Latif, et al., 2025).
- **Cosmetics and Personal Care:** In this sector, citric acid is used to adjust pH, act as a buffering agent, and function as a metallic-ion chelator in antioxidant systems to slow down the degradation of active ingredients (Show, et al., 2015). It also acts as an antioxidant and exfoliating agent, mitigating the influence of free radical damage to skin and promoting skin toning and firmness (Latif, et al., 2025).

Biomedical and Environmental Applications

Recent advancements have expanded the use of citric acid into high-value biomedical and environmental fields.

- **Biomedical Engineering:** Citric acid is increasingly used for biomedical applications. For instance, it is used in hydroxyapatite to fabricate bioceramic scaffolds for tissue engineering. It is also employed in the cross-linking of ultrafine protein fibers for biomedical applications (Behera, Mishra, & Mohapatra, 2021).
- **Environmental Remediation:** Citric acid is utilized in the bioremediation of soils contaminated with heavy metals and radionuclides due to its chelating efficacy. It has also

been shown to enhance the desorption of hydrophobic organic compounds from soils (Behera, 2020).

- **Disinfection:** In response to global health concerns, such as the COVID-19 pandemic, citric acid has seen increased demand as an antimicrobial agent in disinfectants (Latif, et al., 2025). Additionally, citric acid in combination with alcohol has the ability to deactivate a large variety of viruses on surfaces (Behera, 2020).

Global Market Demand

The global production and consumption of citric acid have witnessed consistent growth. Figure 2 shows both the production volume estimates and projections (in millions of tons) and the market value estimates and projections (in billions of US dollars) from 2007 to 2032 based on literature values discussed below.

Figure 2: Global Market Growth of Citric Acid

(Market Values for 2015, 2020 and 2026 have been linearly interpolated for graph continuity, Values for 2026 and beyond are projections)

Production volumes rose from approximately 1.6 million metric tons (1.8 million tons) in 2007 (Show, et al., 2015) to over 2 million metric tons (2.2 million tons) by 2015 (Behera, Mishra, & Mohapatra, 2021). Recent estimates indicate that global production reached 2.17 million metric tons (2.39 million tons) in 2020 and is projected to climb to 2.64 million metric tons (2.91 million tons) by 2026 (Latif, et al., 2025).

Economically, the market value of citric acid was estimated at approximately $3.52 billion in 2022 and is projected to reach $5.12 billion by 2032, expanding at a compound annual growth rate (CAGR) of 3.82% (Latif, et al., 2025). Other projections suggest the market could expect to reach a CAGR of 5.1% during the forecast period of 2015 to 2026 according to a market analysis by Market Report World, as cited by Behera et al. (2021).

Geographically, China dominates the global market, accounting for approximately 59% of world production and 74% of exports as of 2015 (Behera, Mishra, & Mohapatra, 2021). This dominance has historically driven competitive pricing, influencing market dynamics globally (Show, et al., 2015). Consumption is highest in Western Europe, the United States and China, which together account for 65–70% of global consumption (Berovic & Legisa, 2007). Future growth is expected to be driven by the rising demand for processed foods, beverages and convenience products in developing regions, as well as the increasing adoption of bio-based and biodegradable products in industrial applications (Behera, Mishra, & Mohapatra, 2021).

Background

Production Process

The industrial production of citric acid via fermentation is a sophisticated bioprocess that has largely superseded chemical synthesis due to its superior economic efficiency and yield. A block flow diagram of the general process is shown in Figure 3. The process can generally be divided in three phases, which include preparation and inoculation of the raw material (or substrate), the fermentation stage and downstream recovery of the product (Soccol, Vandenberghe, Rodrigues, & Pandey, 2006). While the specific technologies, surface (SF), submerged (SmF), and solid-state fermentation (SSF), differ in operation, they all share stringent requirements for process control, particularly regarding sterility, temperature, pH and aeration, to maximize citric acid accumulation yield (Show, et al., 2015).

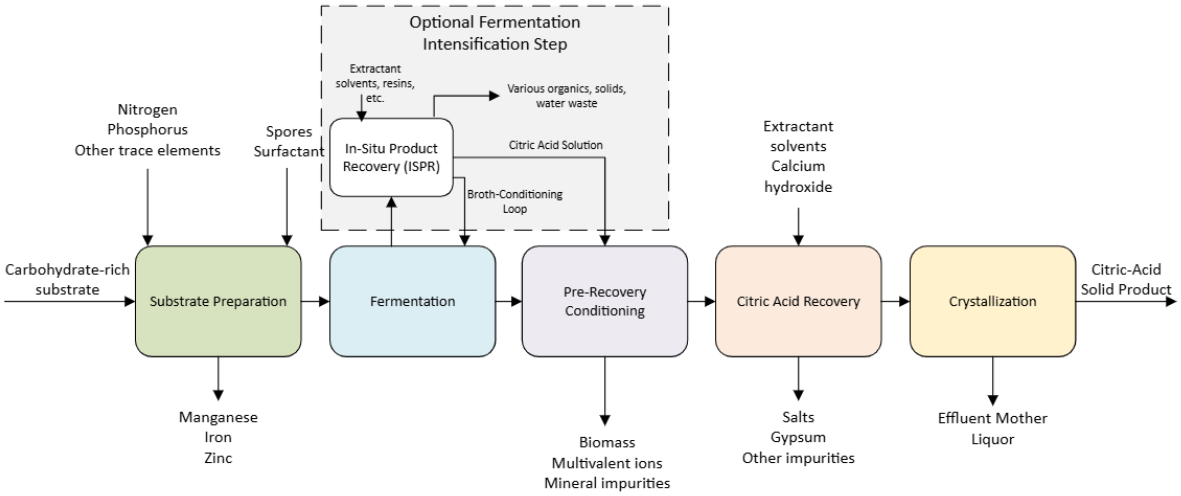

Figure 3: Block Flow Diagram of Citric Acid Production

(including inputs and outputs of various choices of pretreatment and recovery process steps)

Preparation of Raw Materials

The preparation of the fermentation medium is the most critical upstream operation, as the quality and composition of the substrate directly dictates the yield and formation of by-products.

Substrate Selection and Pretreatment

Industrial operations primarily utilize carbohydrate-rich substrates. While pure sucrose and glucose syrups offer the highest yields and purity, economic factors drive the industry toward less expensive agro-industrial by-products, such as molasses, which most industrial processes are based on. Whichever substrate is chosen, its cost and preparation requirements are important aspects of the technology in citric acid production (Berovic & Legisa, 2007). Table 2 has a list of some of the substrates discussed in this paper, along with typical pretreatments needed for each type and some thermodynamic and crystallization considerations which will be discussed in more detail in the discussion section.

Table 2: Substrate Materials with Pretreatment, Thermodynamic and Crystallization Considerations

Substrate Material	Typical Issues in Raw Feed	Typical Pretreatment Required	Thermodynamic Impact	Crystallization Impacts
Refined sucrose and glucose syrups	Low ash and metals Low color High yield/purity High cost	Sterile clarification/polishing Carbon decolorization Minimal de-ashing	Conductivity/Ionic strength Metal impurities Trace color units	Close to nominal intrinsic citric-acid-water thermodynamics/behavior Wider metastable zone width (MSZW) Predictable nucleation Fewer growth poisons (higher k_g at given S)
Molasses (cane or beet)	High ash (Ca/Mg) Inhibitory levels of trace metals (Mn/Fe) Color bodies Variable impurities High level of fermentable sugars Low cost	Potassium ferrocyanide dosing and filtration to remove unwanted metals Clarification Decolorization Tight pH control for consistent fermentation morphology	Conductivity/Ionic strength Metal impurities Trace color units Turbidity	Impurities (metal and color) increase non-ideality Narrow MSZW Heterogenous nucleation Step/kink poisoning (slower growth)
Starch-based feeds (corn, wheat, potatoes or cassava)	Need saccharification to convert starch into fermentable sugar Residual proteins Suspended fines Variable minerals Low cost	Liquefaction and saccharification Solids removal Deproteinization	Large carbohydrate chain Impurities (proteins, Ca/Mg) Clarity	Impurities and chain length increast non-ideality Heterogeneous nucleation Organics inhibit crystal growth Unclear liquor has less predictable supersaturation behavior

Citrus wastes	Pectins Proteins Oils Color Variable ash/metals Low cost	Enzymatic pectinase/cellulase solids removal Milling to increase surface area Solids removal Decolorization De-ashing as needed	Pectin/protein high molecular weight load (viscosity/TOC) Impurities (Ca/Mg, oils) Color	Interfacial penalties Step/kink poisoning Low oxygen transfer due to viscosity affects upstream morphology, inhibiting crystal growth
Other agro-waste residues (apple pomace, coffee husk or sugar cane)	High solids Tannins Metals Variable moisture Heat removal constraints for SSF Low cost	Milling to increase surface area Moisture control Decolorization as needed Partial hydrolysis Strict thermal management (SSF)	Organic impurities (tannins) Metal impurities Moisture Fines	Narrow MSZW SSF - additional byproducts that complicate recovery/crystallization if heat/moisture control is lacking

Molasses (Cane and Beet): Molasses is the most used substrate in industrial citric acid production, typically containing 40–55% fermentable sugars and offering low cost according to a journal article by Downawat in 1995 as cited by P.L. Show et al (2015). However, agro-industrial substrates like molasses often contain inhibitory levels of trace metals, pretreatment is required to ensure metabolic flux toward citric acid. The most common industrial method for metal removal involves the addition of potassium ferrocyanide to precipitate interfering ions (Show, et al., 2015). Controlling these levels is essential because excess manganese, iron and zinc can disrupt the desired fungal morphology and lead to the accumulation of unwanted oxalic and gluconic acids (Tomlinson & Campbell, 1950).

Starch and Hydrolysates: Substrates derived from corn, wheat, potato or cassava require hydrolysis to convert starch into fermentable sugars (glucose). This is achieved through enzymatic liquefaction followed by saccharification (Berovic & Legisa, 2007).

Agro-Industrial Residues: *Aspergillus niger* can be processed also from citrus wastes (Sharma, Mahato, Cho, & Lee, 2017) and other inexpensive solid agro-waste residues, such as apple pomace, coffee husk and sugarcane bagasse. These substrates often require physical pretreatment, such as milling to increase surface area, but generally require less chemical pretreatment than molasses depending on the method of fermentation (Singh & Kaur Brar, 2011).

Nutrient Supplementation

To ensure rapid fungal growth and high acid yields, the medium must be nutritionally balanced.

Nitrogen: While molasses provides some nitrogen, supplementation with ammonium salts (KNO_3 or NH_4NO_3) or urea is often necessary for substrates lacking the optimal levels to promote citrate formation (Angumeenal & Venkappayya, 2013). The concentration is maintained between 0.1 to 0.4 gN/L to prevent excessive biomass formation which lowers product yield (Show, et al., 2015).

Phosphorus: Phosphate in the form of K_2HPO_4 or KH_2PO_4 is typically added, if needed, to achieve concentrations of 0.5–5.0 g/L (Show, et al., 2015).

Trace Elements: Despite the need to remove excess metals, trace amounts of zinc and copper are sometimes added back into the medium to balance inhibitory effects of manganese to allow fermentation (Tomlinson & Campbell, 1950) (Berovic & Legisa, 2007).

Spore Production and Inoculum Preparation

The preparation of a suitable inoculum is a fundamental step in the citric acid production process, as the physiological state and density of the starter culture directly influence the fermentation kinetics and final yield.

Inoculum Source and Preparation: The process is typically initiated using a suspension of fungal spores (conidia) or pre-cultivated mycelia. When spores are used, a surfactant is often added to the medium to ensure the hydrophobic spores are adequately dispersed and do not clump. The spores are generally produced by growing the fungus on solid media substrates (Vandenberghe, Soccol, Pandey, & Lebeault, 1999). The optimum temperature for *Aspergillus niger* growth is typically between 25ºC and 30ºC (Angumeenal & Venkappayya, 2013).

Strain Selection and Improvement: To ensure high productivity, industrial strains are subjected to improvement techniques. Common methods include the "single-spore technique" or the "passage method" to select the most productive populations. Furthermore, hyper-producing mutants are often developed using physical mutagens, such as ultraviolet (UV) treatment, or chemical mutagens to enhance yield (Vandenberghe, Soccol, Pandey, & Lebeault, 1999).

Germination Conditions: The conditions in the early fermentation stage are critical for spore viability. Specifically, the spores require a pH greater than 5 to germinate successfully. Following germination, the uptake of ammonia by the biomass naturally releases protons, which lowers the pH to the acidic levels required for the production phase (Papagianni, 2007).

Inoculum Concentration and Morphology: The density of the spore inoculum is a decisive factor in controlling fungal morphology. Research indicates a clear transition in growth form based on concentration: lower inoculum concentration levels tend to produce compact "pellets," while high inoculum concentration levels result in dispersed, filamentous mycelia. Controlling this morphology is important, as pellet forms are correlated with higher citric acid productivity. The formation itself is not necessarily, but the morphology allows for a lower energy requirement for mixing (in submerged fermentation processes) and the dissolved oxygen levels are typically higher (Mattey & Kristiansen, 2002).

Industrial Fermentation Processes

The industrial production of citric acid by *Aspergillus niger* is executed through three primary fermentation techniques: surface, submerged, and solid-state fermentation. The selection of a specific process is governed by thermodynamic constraints, substrate availability, and capital investment requirements. The generalized aerobic fermentation reaction expression is shown by:

$$Glucose + O_2 \rightarrow Citric\ Acid + Biomass + CO_2 + H_2O$$

Table 3 shows the three main fermentation methods and some considerations due to various parameters, such as contamination, cost, thermodynamic and crystallization impacts downstream. The thermodynamic and crystallization impacts will be discussed in further detail in the discussion section.

Table 3: Fermentation Method Comparison

	Surface Fermentation (SF)	Submerged Fermentation (SmF)	Solid-State Fermentation (SSF)
Contamination	High risk	Less risk	Less risk
Waste	Moderate waste generation	Less waste generation	Low waste generation
Cost	High labor and maintenance costs Low energy cost	High capital cost Less maintenance cost Greater energy cost	Less operating cost
Operational Parameters	Labor intensive Sensitive to changes in substrate composition	Specialty equipment required Requires rigorous process control and sophisticated instrument controls	Difficult to scale up Requires more sophisticated control parameters Poor heat transfer
Raw Material	Glucose syrup Beet molasses	Glucose syrup Beet molasses Can allow for wider variety of substrates than SF	Agro-industrial waste Can allow for wide variety of substrates
Citric Acid Production	Low yield	High yield	High yield (with high impurity)
Thermodynamic Impact	Heat removal by aeration requires large surface area for trays	Heat accumulation Agitator heat dissipation	Heat accumulation
Crystallization Impact	Lower yield of citric acid means higher yield of other organic acids and organic impurities leading to non-ideal behavior	Lower contamination leads to more intrinsic citric-acid-water thermodynamic behavior	High impurity profiles lead to non-ideal behavior

Surface Fermentation (SF)

Historically the first industrial method employed, SF relies on the growth of a mycelial mat on a static liquid medium (Mattey & Kristiansen, 2002).

Procedural Setup and Steps

Tray Configuration: The process utilizes shallow trays made of high-purity aluminum or stainless steel, typically filled with a molasses-based medium to a short depth between 5 and

20 cm. This shallow depth is critical to ensure oxygen diffusion reaches the lower liquid layers since there is no mechanical agitation (Mattey & Kristiansen, 2002).

Inoculation and Mat Formation: The substrate is inoculated with a spore suspension and incubated. After 24 hours, germinated spores form a mycelium mat and fermentation is completed in 8 to 12 days (Behera, 2020).

Thermodynamic Considerations

Heat removal in this system is inefficient compared to stirred tanks. The metabolic heat generated by the fungus must be removed by circulating air over the trays (Vandenberghe, Soccol, Pandey, & Lebeault, 1999). The large surface area required for the trays acts as a heat exchanger. Temperature control is also assisted by evaporative cooling of the solvent (Max, et al., 2010).

Pros and Cons

Pros: Surface fermentation is a static process that does not require any mechanical agitation or high-pressure aeration, keeping energy costs low (Behera, Mishra, & Mohapatra, 2021).

Cons: Surface fermentation requires a large area for operation and high labor and maintenance costs since trays and pipes must be cleaned often. It is also more sensitive to variations of substrate composition and provides a lower yield of citric acid than other fermentation methods (Latif, et al., 2025).

Submerged Fermentation (SmF)

SmF is the dominant global technology, accounting for approximately 80% of production (Soccol, Vandenberghe, Rodrigues, & Pandey, 2006). It involves the growth of the fungus in a liquid medium within an aseptic bioreactor with agitation (Behera, 2020).

Procedural Setup and Steps

Inoculum and Morphology: The process begins with a vegetative seed culture. Maintaining "pellet" morphology rather than filamentous growth is essential to lower broth viscosity, which leads to increased oxygen mass transfer and citric acid production (Behera, 2020).

Process Control: The temperature is strictly maintained at 28–30°C. High aeration rates (0.5–1.5 vvm) are required to maintain dissolved oxygen, while agitation ensures mass transfer (Mattey & Kristiansen, 2002).

Thermodynamic Considerations

SmF is thermodynamically intensive. The rapid metabolic rate of *Aspergillus niger* generates significant heat. In addition, literature reviews report that the mechanical energy input from agitation dissipates into thermal energy. Because of this, heat transfer within the bioreactors must be adequate to maintain isothermal conditions (Van Der Merwe, 2002).

Pros and Cons

Pros: Submerged fermentation provides higher yields and lower contamination risk than surface fermentation. Also process parameters are much easier to maintain with better automation capability, which allows for a wider range of substrates that are able to be used (Behera, Mishra, & Mohapatra, 2021).

Cons: With submerged fermentation, there is specialty equipment required, such as bioreactors and more sophisticated instrument controls, making capital costs considerably higher than surface fermentation (Behera, Mishra, & Mohapatra, 2021). Additionally, there is a significant generation of liquid effluent (citric acid effluent), which poses environmental treatment challenges (Wang, et al., 2020).

Solid-State Fermentation (SSF)

SSF involves the cultivation of microorganisms on moist solid supports in the absence of free-flowing water, mimicking the natural habitat of fungi.

Procedural Setup and Steps

Substrate Preparation: Agro-industrial residues (e.g., citrus peel, coffee husk, cassava bagasse) are dried, ground, and moistened to about 70% water content (Soccol, Vandenberghe, Rodrigues, & Pandey, 2006).

Bioreactor Design: Fermentation occurs in "koji" rooms, tray bioreactors, or rotating drums.

Thermodynamic Considerations

The most critical challenge in SSF is metabolic heat accumulation. Solid substrates have low thermal conductivity, creating temperature gradients within the bed. If the core temperature exceeds 30°C, citric acid production is inhibited due to an increase in oxalic acid production (Behera, Mishra, & Mohapatra, 2021). Therefore, forced aeration is used not only for oxygen supply but as the primary thermodynamic mechanism for evaporative cooling (Raimbault, 1998).

Pros and Cons

Pros: Solid-state fermentation is environmentally favorable as it can use a variety of waste substrates (Soccol, Vandenberghe, Rodrigues, & Pandey, 2006). This method also allows for a higher yield of product, better oxygen circulation and less susceptibility to trace metals, while creating less amount of waste after recovery (Behera, Mishra, & Mohapatra, 2021).

Cons: Maintainability of a constant temperature and moisture content at the same time in a larger scale solid-state fermentation process is difficult since water evaporation is widely accepted as the most efficient heat transfer method (Raimbault, 1998).

In-Situ Product Removal (ISPR)

In-situ product removal (ISPR) refers to the integration of separation steps within the fermentation process in order to remove citric acid as it is produced, mitigating product inhibition, reducing downstream load and potentially improving overall process efficiency. In Figure 3, the block flow diagram shows an optional fermentation intensification step that represents ISPR. In this figure, the inputs and outputs are shown to accommodate various types of ISPR. However, typically the ISPR method used would either produce a "cleaner" citric acid solution to go downstream to the next unit process or it would condition the mother liquor and recycle it back to the fermentation reaction step, while removing various organics, solids, etc. Although ISPR is not categorized as a recovery operation, various ISPR concepts have been investigated as a process-intensification approach that modifies the composition of the mother liquor entering downstream processing. Because of this, ISPR is better understood as an upstream enhancement that influences but does not replace classical recovery techniques.

Several ISPR modes have been discussed in broader organic-acid literature, including in-situ extraction, in-situ adsorption and membrane-integrated fermentation. For citric acid, the most commonly discussed ISPR method is reactive extraction coupled to fermentation, where a tertiary amine or quaternary ammonium salt extractant is circulated externally and contacted with the mother liquor to remove acid as it accumulates (Lopez-Garzon & Straathof, 2014). These systems aim to maintain low citric-acid concentrations in the fermenter, but also introduce challenges similar to downstream extraction, such as solvent toxicity, emulsification and the risk of third-phase formation.

Membrane-coupled ISPR has also been used in organic-acid fermentation, where electrodialysis or nanofiltration run in parallel with fermentation to remove accumulating acid.

This strategy can maintain favorable pH and ionic conditions in the fermenter and reduce neutralization requirements. Electrodialysis-integrated fermentation has been demonstrated for several organic acids is and is conceptually applicable to citric acid. Also electrodialysis has the ability to generate acidified product streams with low mineral content, highlighting it as a modern "green process" (Wang, et al., 2020). Although it is not widely used in industrial citric-acid production, membrane ISPR has proven to yield lower downstream salt content and reduce (or eliminate) gypsum generation when compared to the classical precipitation/acidulation route.

Another ISPR mode, in-situ adsorption, uses resins suspended in the fermenter or circulated through and external loop to selectively bind citric acid. This approach parallels downstream adsorption but is applied during fermentation to limit product inhibition from acid accumulation. General ISPR reviews show that in-situ solid phase adsorption has been successfully used across multiple fermentation systems to increase product titers, or yield within the mother liquor, and simplify downstream purification (Phillips, et al., 2013). Although citric-acid-specific adsorption studies are primarily on downstream recovery, they provide clear information that polymeric and weak-base resins bind citric acid effectively and with high affinity, supporting the feasibility of adsorption-based ISPR conceptually (Peng, et al., 2020). Recent studies of in-situ adsorption in organic-acid bioprocesses further confirm that integrating an adsorbent loop can significantly improve yield and space-time productivity, showing that the mechanism of this ISPR mode could be relevant to citric-acid recovery (Pastoors, et al., 2023). Despite challenges such as resin fouling, regeneration requirements, and interactions between resins and fungal morphology, in-situ adsorption remains a straightforward and widely discussed ISPR strategy for organic-acid fermentation processes.

With each of these ISPR modes, the purpose of the approach is not to replace downstream recovery sequences, but rather to alter fermentation-stage conditions to reduce complexity in the downstream recovery. ISPR influences the thermodynamic state of the mother liquor entering into the crystallization stage by lowering impurity levels and shifting the acid concentration profile entering into other conditioning and recovery unit operations. For example, ISPR strategies minimizing the addition of neutralizing components reduce ionic strength in the mother liquor, allowing for more predictable crystallization behaviors closer to intrinsic citric-acid-water thermodynamics. In this way, ISPR serves as a fermentation-stage intensification tool with outputs connecting directly with recovery and crystallization considerations discussed in the following sections.

Recovery and Purification: Process Routes and Thermodynamic Considerations

Overview of Citric Acid Recovery Pathways

The recovery of citric acid from fermentation broths is one of the most critical process steps in regard to energy usage and overall cost of the production process. Regardless of whether citric acid is produced via submerged fermentation, surface fermentation, or solid-state fermentation, the intermediate product typically consists of a dilute aqueous solution containing various amounts of other impurities, such as mineral salts, proteins, other organic acids, etc. (Gluszcz & Ledakowicz, 2002).

Recovery strategies differ depending on the technology and substrate used. However, recovery strategies can be broadly classified into three categories: chemical precipitation routes, solvent extraction routes, adsorption pathways (Singh & Kaur Brar, 2011). Additionally, membrane-based operations may be used as a pre-recovery conditioning process step or with

more recent studies used as a stand-alone recovery strategy to clarify the broth, remove proteins and a significant percentage of residual sugars and salts (Van Der Merwe, 2002).

From a thermodynamic perspective, nearly all recovery routes are finished with crystallization as the final purification step, since citric acid is commercially traded as a crystalline product. Any upstream recovery subprocesses will strongly influence how crystals form and develop (Gluszcz & Ledakowicz, 2002). Table 4 summarizes the key engineering and thermodynamic distinctions between the primary citric-acid recovery routes, emphasizing how each method modifies the composition of the stream entering crystallization. The thermodynamic and crystallization impacts will be discussed in further detail in the discussion section.

Table 4: Comparison of Recovery Strategies

	Membrane	**Chemical Precipitation**	**Solvent Extraction**	**Adsorption**
Key Parameters	Membrane type (UF/NF/ED) Pressure/voltage pH Temperature Fouling control	pH Calcium ion activity Temperature Ionic strength	Solvent choice pH Mixing	Adsorbent material choice pH Contact time
Principle	Separation by size/charge using a semipermeable membrane	Formation of solid crystals from solution	Transfer of solute between two immiscible liquids	Attachment of solute to solid surface
Recovery Efficiency	Moderate to high depending on membrane and feed purity	High to moderate	Moderate to high	Varies depending on adsorbent
Selectivity	Moderate	Varies	Varies	High selectivity possible
Ease of Operation	Moderate	Simple	Requires solvent, mixing and separation	Simple
Product Purity	High	High	Moderate	High
Equipment	Membrane modules (UF/NF/ED) Pumps	Reactors Filters Centrifuges	Liquid-liquid extraction equipment Mixers Settlers	Adsorption column or packed bed
Scalability	Generally scalable	Generally scalable	Scalable	Scalable
Waste	Concentrate stream with salts, organics and rejected contaminants	Large quantities of gypsum Spent acid/neutralization water	Solvent losses Raffinate purge Wash water	Spent regenerant solutions Resin fines
Thermodynamic Impact	Reduces ionic strength and multivalent ions Improves predictability of solubility and activity coefficients	Residual sulfates and calcium can alter activity coefficients	Organic impurities and solvent traces can change activity coefficients	Removes organics and color bodies Lowers interfacial work Reduces non-ideality

Crystallization Impact	Produces cleaner feed Wider metastable zone width (MSZW) Fewer heterogeneous nucleants	Impurities can alter crystal nucleation kinetics May narrow MSZW	Solvent residues or co-extracted organics can inhibit crystal growth and alter habit	Removes impurities to reduce step/kink poisoning Supports seed-dominated growth and predictable size distribution

Pre-Recovery Conditioning Overview

Following the fermentation stage, and after any process-intensification approaches such as ISPR, the citric-acid stream typically undergoes a set of pre-recovery conditioning operations. These operations are not considered part of the classical recovery sequences seen in foundational citric-acid literature. Instead, conditioning operations serve to prepare the mother liquor so that it enters downstream operations with a composition that supports efficient and controllable recovery.

Process Description

Membrane operations can typically be found as ultrafiltration, nanofiltration and electrodialysis. These operations are clarification and concentration steps that help to manage solution purity and reduce mineral salt formation. Using one of these techniques yields a broth that is cleaner, more uniform and easier to purify downstream in downstream recovery. Ultrafiltration removes biomass, allowing the product to pass through and the retentate stream to collect most of the peptides and proteins from the broth. Nanofiltration can partially concentrate citric acid by allowing it to permeate, while rejecting multivalent ions (Gluszcz & Ledakowicz, 2002). Electrodialysis is a membrane-based process using an electrical driving force to transport ions from one solution into another to produce an acidified product stream with lower additional mineral impurities (United States Patent No. 5532148, 1995).

These unit operations can be used in series to pre-condition the fermentation broth prior to a recovery step or, in the case of electrodialysis, can be used as the primary means of recovery and purification. Electrodialysis also boasts itself as a "green" process, allowing for elimination of gypsum waste (United States Patent No. 5532148, 1995).

Thermodynamic Considerations

The thermodynamic relevance of membrane-based concentration arises from the way each method alters ionic strength, counter-ion composition and overall solution concentration. These all govern the solubility behavior and solution properties of citric acid. Unlike other recovery methods, membrane processes do not introduce new chemical components. Instead, they change the solution composition in a manner that improves predictability and controllability of downstream operations by creating a purer intermediate product to work with.

In ultrafiltration (UF), the removal of biomass and colloidal particles eliminates high-molecular-weight organics and solids that could influence viscosity and filtration resistance. Although UF does not modify ionic equilibria, producing a clarified broth has thermodynamic implications because it removes macromolecular species that interact with citric acid through nonspecific binding or physical entrapment. The result is a solution whose activity profile is more accurately represented by the dissolved solutes alone.

Nanofiltration (NF) exerts stronger thermodynamic influence. By reducing concentrations of multivalent ions and inorganic salts, NF modifies the ionic strength of the solution, which in turn affects activity coefficients and solubility of citric acid. Literature describes NF primarily as a tool for salt reduction. This has a practical thermodynamic effect of decreasing any interaction of citric acid with counter-ions and also produces a solution that behaves more predictably during downstream purification and/or crystallization steps.

Electrodialysis (ED) introduces ion selectivity through electrically driven migration. By removing specific ions and producing acidified product streams, ED shifts citric acid toward forms with fewer associated inorganic species. This reduction in ionic load changes the effective activity coefficients of citric acid and reduces the tendency toward salt formation, especially compared with precipitation-based routes (Wang, 2020). Because ED removes counter-ions directly rather than through neutralization, it produces citric-acid streams whose solubility characteristics and concentration behavior reflect the intrinsic thermodynamics of the acid–water system more closely than solutions regenerated through acidulation due to the residual calcium and sulfate ions in the mother liquor in regenerated solutions.

Precipitation

Process Description

The conventional industrial recovery method involves neutralization of the fermentation broth with calcium hydroxide or calcium carbonate to precipitate insoluble calcium citrate, followed by filtration and subsequent hydrolyzation with sulfuric acid to regenerate citric acid in solution and calcium sulfate as a by-product (Bauer, Marr, Rueckl, & Siebenhofer, 1989).

Thermodynamic Considerations

The precipitation of calcium citrate is governed by solubility product equilibria, with supersaturation controlled by pH, calcium ion activity, temperature, and ionic strength. While robust and well established, this route introduces significant thermodynamic inefficiencies due to irreversible salt formation and generation of large quantities of gypsum waste (Gluszcz & Ledakowicz, 2002).

Moreover, the regenerated citric acid solution entering the crystallization step often contains residual sulfate ions and trace calcium, which can alter activity coefficients and impact crystal nucleation kinetics.

Solvent Extraction Processes

Process Description

Solvent extraction using tertiary amines, similar to Alamine 336, dissolved in organic diluents has been investigated as an alternative recovery route to avoid calcium salt formation. In this process, citric acid is selectively transferred from the aqueous fermentation broth into an organic phase via acid-amine (or acid-base) complexation, followed by stripping into water at elevated temperatures (Wennersten, 1983).

Thermodynamic Considerations

Solvent extraction using long-chain tertiary amines has been widely studied as an alternative to salt-based citric-acid recovery, with particular emphasis on the thermodynamics of acid–amine complex formation and the temperature-dependent distribution equilibrium between aqueous and organic phases. In these systems, citric acid is transferred from the fermentation broth into an organic phase via formation of an acid–amine complex, which is governed by the distribution coefficient**,** which is strongly influenced by temperature, diluent polarity and acid concentration (Wennersten, 1983).

Thermodynamically, the extraction step is driven by the acid–base neutralization reaction between citric acid and the tertiary amine, forming an ion-pair complex in the organic phase. The equilibrium position of this reaction varies significantly with temperature. Wennersten demonstrated that distribution coefficient decreases at elevated temperatures, meaning that extraction is favored at lower temperatures while stripping into water becomes

thermodynamically favorable at higher temperatures due to a shift in the complex dissociation equilibrium. This temperature dependence allows extraction and regeneration to be effectively decoupled, improving process control and reducing energy demand relative to processes requiring complete solvent evaporation (Wennersten, 1983).

The choice of diluent plays a major role in the thermodynamic characteristics of extraction. Studies have shown that the polarity and hydrogen-bonding capacity of the diluent influence the stability and solubility of the acid–amine complex. More nonpolar diluents, such as paraffinic hydrocarbons, yield lower water solubility of amine and reduced co-extraction of impurities but also exhibit lower solvency for the complex. In order to avoid a third-phase formation while using a non-polar diluent, a high phase ratio, V/L, must be maintained in the extractor. The formation of a viscous third phase, which occurs when the organic phase splits into two immiscible layers, becomes thermodynamically incapable of dissolving additional complex, is especially detrimental, as it disrupts both phase separation and the thermodynamic reversibility required for efficient stripping (Wennersten, 1983).

Impurities originating from the fermentation broth influence extraction thermodynamics as well. Organic impurities, trace metals, and residual nutrients alter activity coefficients within the aqueous phase and can participate in weak interactions with the amine, shifting equilibrium behavior and increasing solvent losses. Such impurities not only affect the extraction step but carry through the stripping and crystallization stages, where they influence solubility, supersaturation, and nucleation behavior. Consequently, solvent extraction must be considered not only as a separation operation but as a thermodynamic pre-conditioning step for subsequent crystallization.

Temperature-dependent equilibrium also determines the selectivity of tertiary amines toward citric acid in the presence of other organic acids and fermentation by-products. Studies show that the citric acid-amine complex becomes more stable when the amine is larger and more branched. However, this molecular weight increases past a certain point have diminishing thermodynamic benefits (Wennersten, 1983). This observation aligns with broader thermodynamic principles of complexation with low- or non-polar diluents.

Overall, solvent extraction presents a thermodynamically favorable route for citric-acid recovery when appropriately designed around the temperature dependence of the distribution coefficient, the complex-solubility limits of the chosen diluent, and the activity coefficients within both phases. These parameters must be managed to maintain a stable extraction–stripping cycle and to deliver a regenerated aqueous stream with predictable thermodynamic behavior entering the crystallization step.

Adsorption and Ion Exchange

Process Description

Adsorption and ion exchange have been widely explored as alternative recovery and purification methods for citric acid because they avoid the formation of mineral by-products and require fewer chemical inputs than precipitation–acidulation. Laboratory experiments show that ion exchange, particularly with weakly-basic anionic resins, can effectively remove citric acid from aqueous solutions and synthetic broths (Gluszcz, Jamroz, Sencio, & Ledakowicz, 2004).

Adsorption and ion-exchange processes recover citric acid by contacting the aqueous solution with a solid sorbent material that selectively binds the acid. In typical operation, the citric-acid stream is passed through a packed bed of adsorbent or ion-exchange resin. The resin contains functional groups that interact with citric-acid molecules, allowing the acid to be retained on the solid phase while the remaining components of the solution pass through the bed.

For citric-acid systems, weak-base anion-exchange resins are commonly used because their functional groups can effectively bind the acid in its dissociated or partially dissociated forms (Gluszcz, Jamroz, Sencio, & Ledakowicz, 2004). In more recent studies, polymeric adsorbents, including modified hyper-cross-linked resins, are used to enhance interactions between citric acid and the sorbent surface (Peng, et al., 2020).

The process generally consists of three main steps: loading (adsorption), rinsing and elution (desorption). The fermentation broth flows through the resin bed during loading. Citric acid is taken up by the resin and exiting liquid is depleted of citric acid. Rinsing is an optional step to apply water to remove impurities or displace interstitial liquid. Then citric acid that is bound to the resin is released using an appropriate eluent during desorption.

In a continuous or semi-continuous system, multiple beds may be operating in a cycle of the steps previously to provide an uninterrupted operation. This process does not generate

gypsum or require organic solvents, making adsorption/ion exchange potentially "green" in comparison to precipitation or solvent extraction (Ghorbanian, Davoudinejad, Khakpay, & Radpour, 2015).

Thermodynamic Considerations

The performance of adsorption and ion exchange in citric-acid recovery is governed by acid–base equilibria, ionic interactions, and binding energetics that control how citric acid partitions between the liquid phase and the resin.

Because citric acid is a triprotic acid, its degree of dissociation depends on pH, and this directly affects how strongly it interacts with ion-exchange resins. Weak-base resins show higher affinity for citric acid. This behavior is supported by comparative resin studies showing weak-base resins perform more effectively than strong-base resins for citric-acid adsorption (Gluszcz, Jamroz, Sencio, & Ledakowicz, 2004).

Temperature also affects adsorption thermodynamics. Experiments involving fixed-bed systems show that changes in temperature alter the rate at which citric acid diffuses into the resin (Ghorbanian, Davoudinejad, Khakpay, & Radpour, 2015).

Additionally, resin composition influences the thermodynamics of binding. Modified polymeric adsorbents containing nitrogen-containing functional groups show enhanced adsorption performance for citric acid due to favorable molecular-level interactions (Peng, et al., 2020).

Together, these thermodynamic factors, pH-dependent speciation, ion-exchange equilibria, resin–solute interaction energies, and temperature effects, define the equilibrium capacity, selectivity, and desorption behavior observed during adsorption and ion-exchange recovery of citric acid.

Crystallization

Process Description

After recovery, the standard finishing process steps include cooling and/or evaporative crystallization, then crystal isolation by centrifugation or filtration, washing and drying to preserve the solid product form.

The fundamental process of crystallization involves crystal nucleation and growth. In practice, a production process must target the form of citric acid that is required for the product. In aqueous systems, monohydrates are usually obtained at lower crystallization temperatures and anhydrous product is favored at higher temperatures. Because of this, an industrial plant may align the cooling target and rate with seeding crystals of the desired form (monohydrate vs. anhydrous), thus allowing for crystal growth predominantly, rather than new nucleation (Ma, et al., 2023).

Both batch and continuous crystallization are used in industrial applications. Batch units are common for citric acid production, while continuous equipment is highlighted for stabilizing product attributes, such as purity, shape, size, through tighter supersaturation control. Industrial operating practices typically include conditioning of the mother liquor, charging the crystallization unit (or first-stage of the crystallization process), seed addition, controlled cooling/evaporation, residence-time management for growth, and discharge to solid–liquid separation (Ma, et al., 2023).

Crystals are isolated via centrifugation or filtration, washed to minimize mother-liquor entrapment, then dried at the temperature and conditions based on the targeted form. Drying steps need to be planned carefully to ensure that dehydration or form change is not induced because the solid form depends on temperature and relative humidity (Lafontaine, Sanselme, Cartigny, Cardinael, & Coquerel, 2012).

Thermodynamic Considerations

The thermodynamic behavior of citric acid during crystallization is governed by a combination of equilibrium phase relationships, solute-solvent interactions, and kinetic pathways. The combination of these determines which solid form will appear and how the crystals will grow during industrial operations.

The citric-acid–water phase diagram and the hydrate/anhydrous transition data establish the equilibrium conditions at which each solid form (monohydrate or anhydrous) is stable. These references identify the peritectic/transition temperature and the boundaries where hydration or dehydration will occur as temperature and relative humidity change. Because these boundaries show exactly where each solid form is favored, they are used in practice to choose the crystallization temperature range, determine which crystal form should be used as seed, and set drying and handling conditions that keep the product within the stability region of the intended form (De Kruif, et al., 1982).

The solubility curve for citric acid in water defines the equilibrium concentration at each temperature. Crystallization proceeds only when the solution is taken above a point on this curve. The difference between the actual concentration and the solubility value is what creates supersaturation. This supersaturation is the immediate driving force for both nucleation and crystal growth. Because the method by which supersaturation is generated strongly shapes the resulting solid, industrial cooling or evaporation schedules are planned so that supersaturation increases gradually and in a controlled manner. Doing so encourages the solution to deposit material on the crystals already present in the crystallizer (especially when seeded) rather than forming many new crystals through uncontrolled nucleation.

Although equilibrium references show the conditions at which the monohydrate or anhydrous form is expected to be stable, the actual solid that forms during crystallization also

depends on the kinetics of nucleation and growth and on the solution's speciation. Experimental crystallization studies demonstrate that anhydrous citric acid can nucleate across a wide temperature range, including temperatures where the monohydrate would normally be the stable form, when the kinetic pathway favors anhydrous nuclei or when the solution composition shifts the relative rates of formation. Form-specific is used in industry to push the system to the desired solid product form (Macaringue, Li, Li, Gong, & Tang, 2020).

The upstream composition of the mother liquor also greatly influences the crystallization process. Ionic strength, the presence of counter-ions such as sulfate or calcium, residual organics, and the solution's acid–base speciation all influence apparent solubility and nucleation behavior. For example, recovery routes involving precipitation and acidulation introduce sulfate and may leave trace calcium, while solvent extraction introduces its own set of impurities unless followed by polishing. Electrodialysis produces an acidified stream with fewer inorganic ions. These compositional differences affect how easily the liquor departs from its equilibrium solubility during cooling or evaporation. Upstream steps that reduce the inorganic or organic load generally produce a liquor whose crystallization behavior more closely reflects the intrinsic thermodynamics of the citric-acid–water system.

Industrial reviews of citrate crystallization consistently highlight the same general controls for achieving consistent purity, particle size, and product form: management of supersaturation, strategic seeding, and selection of appropriate crystallization equipment. These operational choices are guided by the equilibrium and kinetic behavior summarized above, but can be analyzed in more detail in a dedicated thermodynamics and kinetics discussion. Continuous crystallization is increasingly emphasized for its ability to keep supersaturation

within a narrow, controlled range, while batch processes rely more heavily on careful scheduling and seeding (Ma, et al., 2023).

Thermodynamics

Since crystallization is the final and most thermodynamically sensitive step in citric-acid production, the thermodynamic principles outlined in this section establish the foundation for the entire analysis in this report. These concepts provide the framework needed to understand how upstream decisions shape solution behavior, how supersaturation and solid-form stability are controlled and why crystallizer performance depends on the combined effects of equilibrium, non-ideality and kinetic pathways.

General Thermodynamics Background

Crystallization is governed by a combination of phase equilibria, solute–solvent interactions, and kinetic pathways that determine how a solution transitions from the liquid state to a solid crystalline phase. Before examining the behavior of citric acid specifically, it is useful to establish the core thermodynamic concepts that frame all crystallization processes. These principles define how equilibrium conditions, supersaturation, nucleation, growth, and solution composition interact to determine the solid form and crystal properties obtained under industrial operating conditions.

A basic understanding for any crystallization system is its equilibrium phase diagram, which identifies the conditions of temperature, composition, and, where relevant, water activity at which specific crystalline forms are stable. For the citric-acid–water system, detailed equilibrium maps have been constructed using solubility measurements, vapor-pressure data,

calorimetry, and in-situ structural analyses (De Kruif, et al., 1982). Such diagrams illustrate, in general terms, how solids appear as the solution crosses a boundary into a region where the crystalline phase is favored. These maps provide the thermodynamic reference frame within which crystallization operations must be designed.

Solubility describes the equilibrium concentration of a solute in solution at a given temperature and provides the baseline from which supersaturation is defined. Supersaturation is the amount by which the actual solute concentration exceeds solubility and is the driving force for both nucleation and crystal growth. Under practical conditions, supersaturation is generated by cooling, by evaporating solvent, or by altering solution composition, and its magnitude and rate of change determine whether crystallization proceeds primarily by controlled growth on existing crystals or by formation of new nuclei. Reliable solubility data serves as essential input for developing cooling or evaporative trajectories that produce stable, predictable crystallizer operation.

Nucleation and growth are governed by molecular processes that depend on interfacial energies, local speciation, and mass-transfer rates. As a result, crystallization may yield metastable or unexpected forms, especially under steep supersaturation or when multiple crystal forms are accessible. This interplay between thermodynamic stability and kinetic accessibility is formalized in Ostwald's Rule of Stages, which states that crystals often form via the phase with the lowest nucleation barrier rather than the most thermodynamically stable phase. Although not universally obeyed, the rule provides a useful conceptual framework for understanding why crystallization outcomes may deviate from equilibrium predictions (Macaringue, Li, Li, Gong, & Tang, 2020).

Ostwald's Rule of Stages provides even more context for interpreting how crystallization pathways can diverge from equilibrium predictions. The rule states that when multiple solid forms are accessible, a system often nucleates the phase with the lowest kinetic barrier rather than the phase that is thermodynamically most stable at equilibrium. This concept is important because the likelihood of forming a critical nucleus depends strongly on the interfacial free energy and molecular-level requirements, which may not align with the relative free-energy stability of the final solid-form phases (Macaringue, Li, Li, Gong, & Tang, 2020). Importantly, Ostwald's Rule describes a kinetic tendency, not a thermodynamic requirement, and is therefore best viewed as a guideline for anticipating how real solutions behave under practical supersaturation generation rather than as a statement of equilibrium phase behavior. This distinction becomes especially relevant for the citric-acid system, where solution structure and speciation can shift kinetic accessibility between multiple solid forms.

Crystal growth itself is controlled by molecular-scale surface features that determine where incoming solute units can most readily incorporate. According to the Kossel model (shown in Figure 4) described in classical crystal-growth theory, growth units diffuse across broad flat terraces and attach preferentially at step edges and especially at kink sites, where the local bonding environment provides the greatest stability for incorporation (Mullin, Crystal Growth, 1976). Since kink sites offer the highest coordination, they govern the rate at which a crystal face can advance under a given supersaturation, whereas flat terraces contribute very little unless supersaturation is sufficiently high to support two-dimensional nucleation. Impurities can adsorb selectively to step and kink positions, blocking these favorable incorporation sites and thereby reducing effective growth rates or producing irregular, inhibited growth.

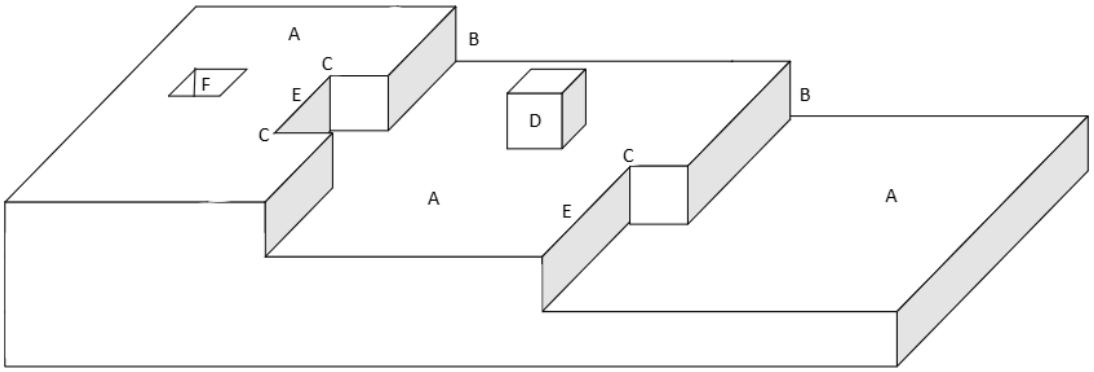

Figure 4: Kossel model of a growing crystal surface
showing (A) terraces, (B) steps, (C) kinks, (D) surface-adsorbed growth units, (E) edge vacancies and (F) surface vacancies

The composition of the mother liquor entering a crystallizer further modifies its thermodynamic and kinetic behavior. Ionic strength, the presence of counter-ions, organic impurities, and the acid–base speciation of the solute all influence activity coefficients, apparent solubility, and nucleation tendencies. Changes imposed upstream, through the chosen unit processes, alter these variables and therefore affect how supersaturation develops and how readily particular solid forms nucleate (Show, et al., 2015).

Even when the underlying equilibrium phase diagram is unchanged, solution non-ideality leads to practical differences in supersaturation generation, form selection, and growth behavior.

Finally, industrial crystallization requires aligning these thermodynamic principles with operational constraints. Process design focuses on establishing supersaturation profiles, temperature schedules, and seeding strategies that remain within controlled regions of the phase space where growth dominates over uncontrolled nucleation. The use of batch or continuous crystallizers, the method of generating supersaturation, and the conditions used for drying and handling are all guided by these equilibrium and kinetic considerations (Ma, et al., 2023).

Together, these equilibrium, kinetic, and compositional principles, along with the foundational concept illustrated by Ostwald's Rule, provide the context for evaluating citric-acid-specific crystallization behavior, which is developed in the following section.

Citric-Acid-Specific Thermodynamic Behavior

The crystallization behavior of citric acid displays the interaction of its unique molecular characteristics along with the thermodynamic properties defined by temperature, relative humidity, solubility and solution speciation. Since citric acid exhibits complex hydration behavior, has multiple solid forms and temperature-dependent transitions that provide distinct regions of stability for the monohydrate and anhydrous phases, it is considerably more complex to understand crystallization than a simpler inorganic salt. To understand this system, one must interpret general crystallization principles from a perspective using the citric acid-water phase diagram (as shown in Figure 5), the hydration/dehydration equilibria and the solution chemistry of this triprotic organic acid.

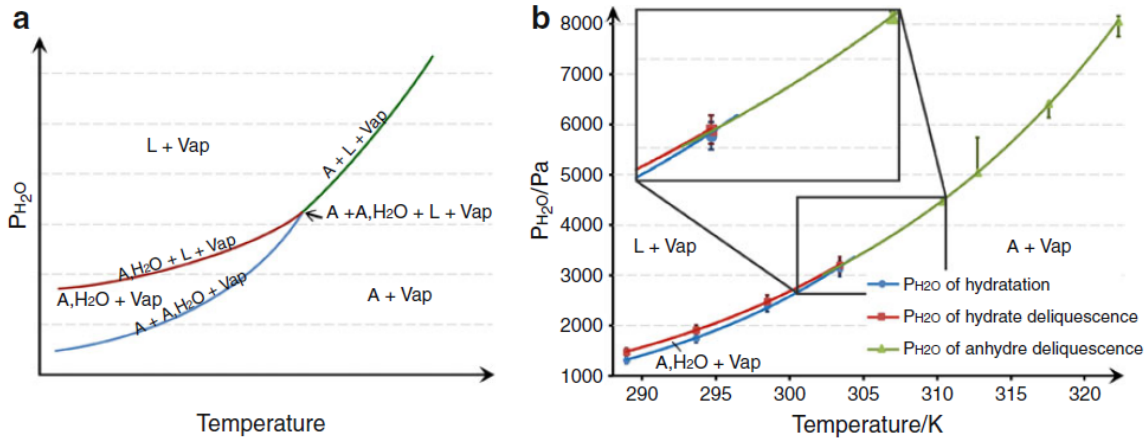

Figure 5: a. Theoretical, and b. experimental stability domains of monohydrate and anhydrate as a function of temperature and water partial pressure.

(Lafontaine, Sanselme, Cartigny, Cardinael, & Coquerel, 2012) (Used with permission from Springer Nature)

In these figures, the behavior of the citric-acid-water system and the temperature-concentration conditions for which solid forms are thermodynamically favored are shown. In Figure 5a, the solubility curves for the monohydrate and anhydrous forms define two distinct stability regions. To the left of the intersection and at lower temperatures, the monohydrate is the stable form. To the right of the intersection and at higher temperatures, the anhydrous form is favored. The point at which the curves intersect represents the solid-form transition boundary where the relative stability of the two solids can change. Figure 5b presents this information as a simplified phase diagram, showing the monohydrate stability field occupying the lower-temperature, lower-concentration region and the anhydrous stability field occupying the higher-temperature region. These diagrams demonstrate that crystallization at low temperatures or modest supersaturation naturally yields the monohydrate, while operating in the higher-temperature domain promotes formation of the anhydrous solid. As a result, cooling profiles, evaporation methods and seeding strategies must be aligned with the appropriate phase field to achieve the desired solid form consistently.

Thermodynamic analyses using calorimetry, vapor-pressure measurements and solubility data show that the monohydrate is the stable aqueous solid phase at lower temperatures, while the anhydrous form becomes the thermodynamically-favored phase above an experimentally measured transition, or peritectic, temperature of near 36ºC (Lafontaine, Sanselme, Cartigny, Cardinael, & Coquerel, 2012). Hydration and dehydration reactions shift, influencing the transition boundary, depending on relative humidity. This creates distinct regions in the temperature-relative humidity (T-RH) space where the monohydrate or anhydrous solids are stable. Lafontaine et al (2013) quantified these boundaries and produced a citric-acid solid-phase diagram showing the critical relative humidity for hydrate formation and the conditions under

which dehydration becomes spontaneous. These data form the equilibrium basis for selecting crystallization temperatures and planning drying conditions to preserve the desired solid form.

Although the phase diagram defines which solid form is thermodynamically stable, the rate at which each form develops depends on molecular-level features of its crystal surfaces. As described in classical crystallization texts, crystal growth occurs primarily at step and kink sites along incomplete molecular layers, where incoming solute has the most favorable bonding environment for incorporation (Mullin, Crystal Growth, 1976). The monohydrate and anhydrous lattice differ in hydrogen-bonding geometry, resulting in different step densities and face-specific growth rates. Because kink sites are the most active incorporation positions, surface-active impurities such as colored organics, proteins or fermentation-derived metabolites can adsorb at these sites and block growth, even when supersaturation is unchanged. This sensitivity at the surface level helps to explain why citric-acid crystallization is strongly affected by upstream composition and why seemingly small differences in impurity load can yield different habits, growth rates or even solid forms.

The solubility behavior of citric acid further supports the solid-form distinctions defined by the T-RH stability boundaries. Reliable solubility datasets for citric acid monohydrate in water are available to provide precise correlations of solubility versus temperature (Laguerie, Aubry, & Couderc, 1976). These curves show the equilibrium concentration from which supersaturation is determined as the solutions cools or evaporates. Since the monohydrate and anhydrous forms differ in stability across the temperature range, the shape of the solubility curve and the temperature trajectory used to generate supersaturation have direct implications for which solid form crystallizes. At temperatures below the hydrate/anhydrate transition, cooling

tends to generate supersaturation with respect to the monohydrate, while evaporation at higher temperatures may push the solution into regions where anhydrous crystallization is possible.

Kinetic factors and solution speciation also have a critical role in determining which solid form is observed. Citric acid is a tripotic acid, and its distribution among protonated forms changes with pH. These shifts in speciation alter the intermolecular interactions and have an affect on both nucleation barriers and relative growth rates. Macaringue et al. (2020) demonstrated that anhydrous citric acid is able to nucleate across a far broader temperature range than what is predicted by equilibrium alone. This includes temperatures below the nominal transition point where the monohydrate is thermodynamically favored. This behavior shows differences in nucleation energy barriers based on solution structure. When looking at this behavior with Ostwald's Rule of Stages in mind, the system is either consistent or deviates from the rule, depending on which form has the lower nucleation barrier under a given composition and supersaturation. Actual crystallization outcomes depend strongly on the kinetic pathway in these cases, while hydrate/anhydrate stability is described by the thermodynamic endpoints. The observed nucleation behavior of citric acid illustrates this kinetic-thermodynamic relationship. Although the monohydrate is the stable solid form at equilibrium just below 36ºC, experimental work shows that the anhydrous phased can nucleate across a much broader temperature range, to include conditions where it is not the thermodynamically stable phase (Macaringue, Li, Li, Gong, & Tang, 2020). This behavior is consistent with Ostwald's Rule of Stages, in which the phase appearing first is the one with the lower nucleation barrier, not necessarily the one with the lowest Gibbs free energy. In citric-acid solutions, any shift in pH and speciation alter intermolecular interactions and can lower the kinetic barrier for anhydrous nucleation relative to the monohydrate. As stated in the general thermodynamics section,

Ostwald's rule in this context reflects a kinetic pathway, not a thermodynamic driver, and its influence depends on how supersaturation is generated and how upstream composition modifies the solution behavior. Therefore, hydrate/anhydrate outcomes often display the kinetic conditions during cooling or evaporation, even when the equilibrium phase diagram predicts a different outcome.

The composition of the solution entering crystallization also directly influences solubility, nucleation tendencies, and form stability. Upstream recovery steps can leave the mother liquor enriched in specific ions or organic impurities. Precipitation introduces sulfate and may leave residual calcium. Solvent extraction requires polishing to remove traces of organic components. Electrodialysis generates acidified, low-salt streams. Adsorption or ion-exchange can remove organics, color bodies, and mineral ions. These compositional differences alter activity coefficients and shift the effective solubility curve, changing the supersaturation profile that develops during cooling or evaporation. Streams with lower ionic content and fewer complexing impurities tend to behave more predictably and more closely follow the intrinsic citric-acid–water thermodynamics established by equilibrium and solubility measurements (Show, et al., 2015).

Finally, industrial crystallization of citric acid displays the relationships between these thermodynamic and kinetic system characteristics. Recent industrial reviews discuss the need to align crystallizer design with the full set of conditions required for stability of the specific solid form that is desired (Ma, et al., 2023). Continuous crystallizers can maintain more stable supersaturation profiles, while seeded batch crystallizers must have tight control on temperature, concentration and agitation to push the system toward the intended solid form. Because citric acid solid-form stability depends on both temperature and relative humidity, the T-RH phase

diagram defines the regions of where the monohydrate or anhydrous form is favored (Lafontaine, Sanselme, Cartigny, Cardinael, & Coquerel, 2012). In practical usage, drying and storage conditions must avoid areas of the T-RH phase diagram where hydration or dehydration may occur to avoid any changes in the crystalline structure of the desired solid form.

Discussion

Thermodynamic Analysis of Citric-Acid Crystallization

Crystallization of citric acid is governed by a combination of thermodynamic driving forces, solution non-ideality, solute-water interactions and kinetic barriers that together determine when a stable nuclei form and grow, which solid form appears and the speed in which nuclei and crystals develop. This section expands on the foundational concepts introduced earlier by providing the mathematical framework necessary to understand and quantify these behaviors. These principles are quantified in CNT and form the basis for understanding solid-form selection, nucleation behavior and crystallizer performance. Table 2, Table 3 and Table 4 can be referenced from the background sections for information regarding upstream thermodynamic impacts.

Thermodynamic Driving Force in Solution: Chemical Potential and Supersaturation

In solution crystallization, the fundamental driving force is the difference in chemical potential between the solute in solution and in the solid phase. Crystallization becomes favorable when the chemical potential (μ) of citric acid in solution exceeds that of the solid phase:

$$\Delta\mu = \mu_{solution} - \mu_{solid} < 0 \qquad \text{(spontaneous crystallization)}$$

The chemical potential of the solute in solution is:

$$\mu_{\text{solution}} = \mu^{\circ} + RT\ln\, a$$

where activity is defined as:

$$a = \gamma x$$

and:

γ = activity coefficient

x = the mole fraction of the solute

This expression and its role in supersaturated systems are well established in nucleation theory discussions and thermodynamic treatments of phase change.

Supersaturation is defined as:

$$S = \frac{C}{C^*}$$

where:

C = solute concentration

C* = temperature-dependent solubility at equilibrium

For crystallization to proceed:

$$\Delta\mu = RT\ln\ S$$

This expression and its role in supersaturated systems are well established as a central result in nucleation theory discussions and thermodynamic treatments of phase change, which identify supersaturation as the fundamental thermodynamic driving force (Zhou, Zhou, & Tang, 2022).

Because Δμ depends on activity, upstream operations that alter ionic strength or impurity content modify thermodynamic driving force through changes in γ.

Influence of Activity Coefficients and Ionic Strength

In electrolyte-containing organic acid systems, the activity coefficient γ depends on ionic strength *I*. While citric-acid solutions are not dilute, the qualitative dependence described by Debye-Hückel theory remains instructive:

$$\ln(\gamma_i) = -Az_i^2\sqrt{I}$$

where:

z_i = the charge number of ion species i

A = a constant that depends on the solvent (i.e. for water at 25ºC, it is 1.172)

It is important to note that since the ions in solution act together, the activity coefficient in this equation represents a mean activity coefficient (ChemEurope, 2026).

This relationship illustrates how sulfate, calcium, ammonium and other ions introduced in upstream processes change apparent solubility and shift supersaturation trajectories.

Solubility and Solid-Form Stability

Citric-acid solubility is strongly temperature-dependent. Classical datasets, such as Laguerie et al, provide the reference solubility curve for the monohydrate form. Above and below the transition temperature, ~36ºC, the stable solid changes, as demonstrated by calorimetry and vapor-sorption studies (Lafontaine, Sanselme, Cartigny, Cardinael, & Coquerel, 2012). These thermodynamic boundaries are shown by the areas on the T-RH phase diagram where each solid form is stable.

Therefore, solubility curves and stability maps both govern the crystallization operating window.

Classical Nucleation Theory

CNT provides a quantitative description of how nuclei form in supersaturated liquids. The critical nucleation free-energy barrier is defined by:

$$\Delta G^* = \frac{16\pi\gamma^3}{3(\Delta\mu)^2}$$

This represents the maximum in the Gibbs free-energy curve that must be overcome for a stable crystal nucleus to form.

The critical nucleus radius (r*) is the radius of the smallest stable nucleus. Clusters smaller than r* tend to dissolve, while clusters larger than r* tend to grow. This value is defined as:

$$r^* = \frac{2\gamma_{sl}}{\Delta\mu}$$

where:

γ_{sl} = solid-liquid interfacial free energy (surface tension) (Sear, 2007).

As the driving force increases, supersaturation becomes stronger. This means that r* decreases, ΔG* decreases, nucleation becomes more likely and there is a kinetic bias towards phases with a lower surface tension. This is where Ostwald's Rule becomes relevant.

Ostwald's Rule and Nucleation-Barrier Asymmetry in Citric-Acid Systems

Ostwald's Rule of Stages predicts that the phase with the lowest nucleation barrier appears first. With this prediction, the first solid-form is not always the most stable one, resulting in polymorphs. Although originally empirical, modern interpretations show it arises naturally from CNT and intermolecular interactions (Black, et al., 2018).

For citric acid, anhydrous nuclei have a lower nucleation barrier than monohydrate nuclei under specific solution-speciation and supersaturation conditions. This occurs even below the nominal transition temperature as discussed by Macaringue et. al (2020). This phenomenon supports that kinetic factors outweigh the thermodynamic preference and supports the critical nucleation free-energy barrier relationship where:

$$\Delta G^* \propto \frac{\gamma_{sl}^3}{(\Delta\mu)^2}$$

where the anhydrous form has a lower γ_{sl} or higher $\Delta\mu$ under certain solution speciation conditions.

Growth Kinetics and Morphology

Once nucleated, crystals grow according to the power-law growth model:

$$G = k_g(S-1)^g$$

where:

k_g = growth coefficient

g = growth order (typically 1-2 for organic acids)

Zheng et al. studied growth models in a 2023 study showing that empirical data collection for growth rates increased with supersaturation and temperature, consistent with the power-law model (Zheng, 2024).

Solution impurities can reduce k_g, change g and alter habit through selective adsorption. These effects explain why upstream purification is critical to reduce growth inhibition and improving crystal-size distribution control.

Metastable Zone Width

MSZW represents a temperature and/or a concentration interval between the solubility curve and the point where spontaneous nucleation is favorable. It is affected by many of the same control issues we see commonly across citric-acid production processes, such as cooling rate, impurities, agitation, ionic strength and seed presence. A larger MSZW means that nucleation is suppressed and the system must be cooled further before crystallization will begin (Mullin, 2001). This is a large consideration in controlling hydrate versus anhydrate forms of citric acid.

Figure 6 (shown below) illustrates the general solubility-supersolubility relationship that defines the MSZW. The region between the dissolution line and the nucleation line constitutes the metastable zone, within which the solution is supersaturated but spontaneous nucleation does not occur. This is the operating window for controlled, seeded crystallization. Seed crystals are introduced at an appropriate point within the metastable zone grow at a rate governed by the local supersaturation without triggering uncontrolled primary nucleation. Above the nucleation line, the system enters the region where spontaneous nucleation proceeds rapidly and uncontrollably. Below the dissolution line, the solution is undersaturated and crystals dissolve rather than grow. In citric-acid systems, the width of this metastable zone is sensitive to the ionic strength, organic impurity load and colloidal content of the mother liquor, as discussed in the following sections. Upstream decisions that alter these solution properties effectively shift the nucleation line relative to the solubility curve, compressing or expanding the MSZW and therefore changing the cooling or evaporation strategy required to maintain seed-dominated growth.

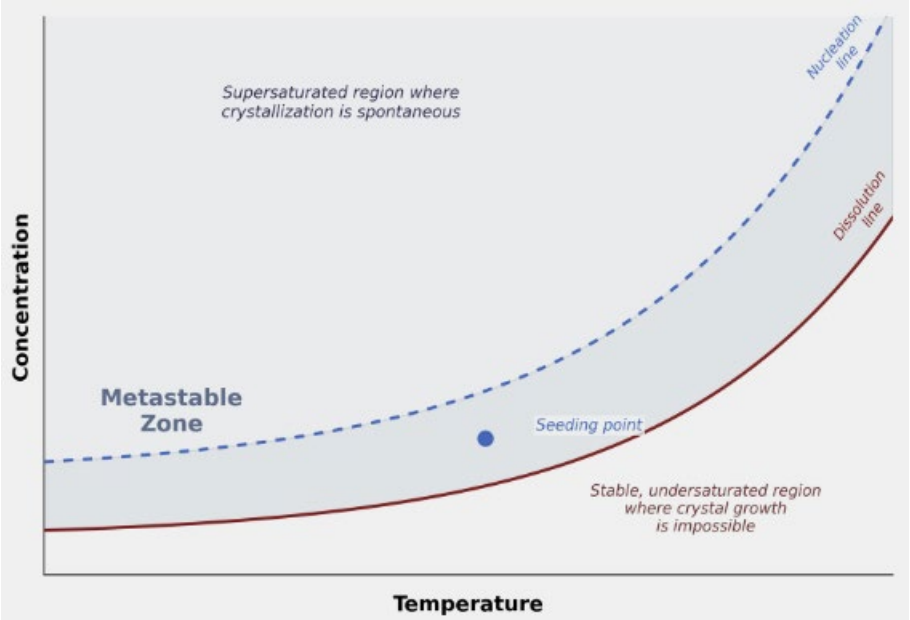

Figure 6: Generalized solubility-supersolubility diagram illustrating the metastable zone width (MSZW)

Adapted from Mullin (2001)

Water Activity Effects in Hydrate-Anhydrate Stability

The citric-acid-water system exhibits strong dependence on water activity (a_w):

$$a_w = \frac{p}{p_0}$$

where:

p = water vapor pressure of a substance

p_0 = water vapor pressure of pure water

Studies have quantified the minimum RH for hydration and the critical RH for deliquescence, producing a complete T-RH (or T-WA) phase diagram (Lafontaine, Sanselme, Cartigny, Cardinael, & Coquerel, 2012).

Thermodynamic Integration with Process Design

As we have discussed the overall production process backgrounds, thermodynamic considerations were taken into effect for individual unit processes. Each of these unit processes must take into account the effects that will be passed downstream, all the way into crystallization, drying and even packaging and storage. Thermodynamic behavior and operational strategy in the crystallizer is the most critical of these downstream processes to plan and design for.

These preceding sections establish the driving force ($\Delta\mu$), the molecular interactions, supersaturation pathways and kinetic preferences that ultimately decide which solid form appears and how crystals grow. This section will connect those thermodynamic principles directly to practical process-design considerations, emphasizing the influence of upstream operations on solution behavior and the operations strategies that affect crystallizer performance.

Influence of Upstream Composition on Crystallization Process Thermodynamics

The upstream process flow sets the physicochemical state of the mother liquor that enters crystallization. These processes include substrate selection and pretreatment, nutrient regime and morphological control in *Aspergillus niger* cultivation, acidulation and pH strategy, adoption of in-situ adsorption, pre-recovery conditioning and recovery. Thermodynamic effects from the upstream processes are repeatedly documented as the principal drivers of complexity in downstream separations and variability in crystallization for citric-acid manufacturing.

Activity coefficients and ionic strength

The first constraint the crystallizer encounters is non-ideality of the solution. Ionic strength and the presence of counter-ions shift activity coefficients, which also shifts the apparent solubility curve and the supersaturation that can be realized at a given concentration and temperature. Substrate quality and pretreatment dictate the baseline for ash and multivalent cation content through trace metals that accumulate through fermentation. Acidulation and pH control then add a characteristic counter-ion signature, normally sulfate, that further elevates ionic strength. In practice, sulfate-rich mother liquors reach the homogeneous nucleation boundary earlier using the same cooling trajectory, narrowing the MSZW and forcing a more controlled cooling strategy in order to keep crystallization behavior in control. Targeted ion management immediately upstream of the crystallizer, such as ion-exchange polishing or electrodialysis, can reduce ionic strength, moving the mother liquor closer to the intrinsic citric-acid-water behavior and improving batch-to-batch reproducibility of nucleation onset.

In practice, there are a few design considerations that can reduce complexity going into the crystallizer. Higher-purity carbohydrate substrate selection and effective clarification can reduce initial ash and trace metal impurities. Discipline during acidulation process steps can curb counter-ion accumulation. Lastly, late-stage ion-exchange or electrodialysis restores a wider operating window for supersaturation generation in the crystallizer.

Organics and interfacial energy

Colored organics, proteins and surface-active metabolites complicate the work needed to form a critical nucleus and can poison step and/or kink sites on the surface of solid forms. This makes nucleation more erratic and slows crystal-face growth, even with unchanged

supersaturation. These species originate in the substrate and are amplified by fermentation by-products. Adsorptive steps upstream of crystallization, such as activated carbon decolorization and protein removal by thermal or pH shifts, lower the interfacial penalty and reduce growth poisoning. Even earlier in the process flow, in-situ adsorption can remove inhibitory or hydrophobic compounds while simultaneously increasing product yield, which typically delivers a cleaner, less non-ideal mother liquor to the crystallizer and a wider, more stable MSZW in operation.

There are some process techniques to consider for simplifying the effects of organic and interfacial energy complexities. Those include selecting cleaner substrates and pretreatments, applying ISPR during fermentation to limit the build-up of inhibitory organics and finishing with deproteinization/decolorization so the feed supports seed-dominated growth instead of new-nuclei formation.

Fines, macromolecules and heterogeneous nucleation

Fine particles and large biological molecules suspended in the solution provide surfaces that crystals can start growing on, causing nucleation to occur earlier than it should, at higher temperatures and lower supersaturation, than would be expected in a clean, particle-free solution. In *Aspergillus niger* systems, medium composition (to include micronutrient levels), inoculum strategy and agitation drive morphology toward pellets or dispersed mycelia. Dispersed forms shed more fines and larger biomasses, which then carry into recovery and crystallization. Robust solids removal, through centrifugation or filtration, and clarification prior to crystallization reduce heterogenous nature to the solution and makes the nucleation point more predictable, while improving effective growth rates on the intended seed at the same supersaturation.

Practically, there are several process levers that can be utilized to simplify crystallization. Minimizing fines by following nutrient and morphology regimes, fines removal before crystallization and verifying that residual macromolecules are lowered to reduce unwanted surface-mediated nucleation are some of the techniques that will ensure thermodynamic effects due to non-citric-acid solids in the mother liquor.

Water activity and solid-form stability

Citric acid exhibits a well-defined transition between monohydrate and anhydrous forms near 36ºC. Water activity, or relative humidity, has a role in which solid form is stable along a given thermal path. Concentration steps, solvent exchange, washing strategy and dryer humidity all perturb water activity around the crystal. When upstream decisions concentrate salts or humectants, the effective water activity at the crystal surface can shift toward regions that destabilize the target solid form during growth or in the dryer. Aligning seed selection, set-points and washing/drying media with the citric-acid-water phase map, as well as verifying the solid form in situ during both crystallization and drying, maintains the product in the intended phase field from nucleation through storage (Lafontaine, Sanselme, Cartigny, Cardinael, & Coquerel, 2012).

By controlling concentration factors and wash composition, avoiding drying paths that cross into the opposite form's stability field and using in-line Raman spectroscopy to confirm the solid form within the solution during critical transitions, the process can guarantee better crystallization behavior downstream.

Metastable zone width and the supersaturation pathway

The combined non-ideality from ions and organics sets how deep into supersaturation the liquor can be driven before spontaneous nucleation initiates (see Figure 6). Impurities or sulfate-rich feeds typically narrow the MSZW, forcing gentler early cooling and evaporation to suppress uncontrolled primary nucleation. Feeds polished by adsorption and ion control can widen the MSZW, enabling a more aggressive cooling rate without losing seed control. Operationally, crystal growth rates are fitted to the standard power-law expression $G = k_g(S-1)^g$ using the actual mother liquor produced by the process. When upstream changes alter the fitted parameters k_g or g, those changes must be reflected in how the cooling trajectory is designed, how much seed is added and what particle-size distribution can be expected.

In order to optimize crystallizer operations downstream, operations for the crystallizer will need to be adjusted based on the MSZW. If MSZW is compressed due to high ions and organics, the cooling ramp will need to be slowed initially and seeding should be increased. If the MSZW is widened by polishing or ISPR upstream, cooling ramps can be shortened while holding form and size targets. If possible, measure and refit k_g and g after upstream changes to keep crystallization trajectories predictable.

Process integration and feed stabilization

When substrate quality and utilities vary, closed-loop water/effluent management stabilizes both ionic backgrounds and organics load. Demonstrated schemes combining anaerobic digestion and air stripping with electrodialysis reuse deliver steady fermentations and lower contaminants going downstream to crystallization. This ensures that the same cooling and evaporation strategy yields the same supersaturation history and the same nucleation and growth

response across all batches. Direct recycling of extraction effluent, when paired with upfront conditioning, has shown similar stability over multiple batches (Wang, et al., 2020).

In practice, if variability cannot be eliminated at the source, an engineered recycle and pretreatment process keeps ionic strength and organics within a narrow band. By doing this, the upstream variable mother liquor content becomes a predictable crystallizer feed.

Implication for design

Understanding upstream influences through their thermodynamic effects clarifies the specific variables that must be controlled before crystallization begins. Managing counter-ions through acidulation strategy or ion-exchange polishing establishes predictable activity coefficients, while adsorptive steps limit the organic and surface-active species that increase interfacial penalties and inhibit growth rates. Similarly, upstream morphology control and consistent solids-removal practices reduce the fines and macromolecules that otherwise trigger heterogeneous nucleation at higher temperatures and lower supersaturation. Water-activity management ensures that operating conditions remain within the stability field of the intended solid form. When these considerations and practices are aligned, the crystallizer receives a mother-liquor-feed stream whose behavior reflects the intrinsic thermodynamics of the citric-acid-water system, allowing supersaturation to be utilized primarily into controlled growth on the desired seed rather than being consumed by uncontrolled nucleation.

Operational Crystallization Considerations

The operational goal in the crystallizer is to convert the incoming mother liquor's solute into a solid form through controlled trajectory of supersaturation, nucleation suppression and

growth of the intended solid form. Since the hydrate-anhydrate stability boundary and the mother liquor's non-ideality are fixed before crystallization begins, operational strategy must work within these constraints to encourage a growth-dominated pathway. Table 2, Table 3 and Table 4 can be referenced from the background sections for information regarding crystallization impacts from upstream processes.

The targeted solid form must be determined and designed for, as the monohydrate and anhydrous crystals differ in stability, surface structure and acceptable temperature and relative humidity conditions. Selecting the correct seed and introducing it within the proper thermal and humidity environment establishes the initial growth pathway. In citric-acid systems near the 36ºC hydrate-anhydrate boundary, small deviations in temperature or water activity can cause a conversion between forms. For this reason, seeding must occur inside the stability field of the target form and analysis techniques are often used to verify that the solid form remains unchanged during both growth and drying.

Supersaturation generation determines whether growth proceeds or if unplanned nucleation occurs. Since nucleation rates depend exponentially on the free-energy barrier, modest differences in ionic strength or impurities, inherited from upstream processes, can shift nucleation behavior largely at fixed operating conditions. Impurity-rich or sulfate-rich feeds generally require a gentler, slower cooling ramp initially to maintain seed control, while polished and conditioned feeds can tolerate a more aggressive ramp without excessive nucleation. Designing the supersaturation profile with seed addition ensures that the chemical potential of the solute in solution is driven towards growth on existing surfaces rather than creating new nuclei.

To quantitatively express solution conditions into operating trajectories, growth rates are commonly fitted to the power-law model, where $G = k_g(S-1)^g$. Here, the exponent g reflects the sensitivity of growth to supersaturation and changes when surface integration becomes more or less hindered, while k_g represents the combined effects of temperature, hydrodynamics and mother liquor composition. When upstream composition shifts, the fitted k_g and g values change as well. Those changes must be reflected in the cooling-profile design, amount of seeding and expected particle-size distribution.

Hydrodynamics has a controlling influence on how uniformly supersaturation is seen throughout the crystallizer. Adequate suspension, controlled micromixing and proper feed introduction are utilized to minimize localized spikes in supersaturation that can inhibit the designed outcome of crystallization, leading to excessive fines or habit changes. When upstream variability cannot be fully mitigated, robust hydrodynamics are often the most cost-effective buffer against inconsistent nucleation and growth.

Finally, drying and storage can be regarded as extensions of the crystallization process. If the thermal or humidity conditions cross into regions where the alternate solid form is favored, hydration or dehydration can occur post crystallization. Aligning dryer set-points and storage conditions with the citric-acid T-RH phase diagram and confirming form integrity using the appropriate analytical tools ensures preservation of the target hydrate or anhydrate.

Together, these operational strategies can be used to optimize the thermodynamic and compositional characteristics of the incoming mother liquor into consistent crystallization performance. By controlling supersaturation generation, seeding, hydrodynamics and post-crystallization conditions, the system can reliably produce (and store) the desired solid form and particle-size distribution even under citric acid's complex solution behavior.

Conclusion

Citric-acid crystallization is shaped by the combined influence of equilibrium phase behavior, solution non-ideality, kinetic accessibility and the composition of the mother liquor produced upstream. This report demonstrates that variability in crystallizer performance is rarely due to crystallization conditions solely. Rather, that variability comes from thermodynamic constraints established much earlier in the process. Substrate impurities, trace-metal loads, nutrient strategies, morphological outcomes from fermentation, counter-ion addition during acidulation, ISPR usage and conditioning steps like UF, NF, adsorptions, ion exchange or ED each can modify ionic strength, activity coefficients and impurity profiles. These modifications determine how supersaturation develops, which solid form nucleates, how growth proceeds at step and kink sites and how wide or narrow the metastable zone becomes during operation.

This integrated perspective represents the primary contribution of this work. While the thermodynamic principles applied here are individually well established, no prior work has applied them systematically across the citric acid manufacturing chain to explain how upstream variability propagates to the crystallizer. The framework developed in this report fills that gap by identifying five specific thermodynamic pathways: activity-coefficient shifts from ionic-strength changes, interfacial-energy penalties from organic impurities, heterogeneous nucleation from suspended fines, water-activity perturbations affecting solid-form stability and the combined effect on MSZW. Each of these pathways affect crystallizer performance through decisions determined by upstream decisions. This analysis also reveals several areas where the existing literature remains incomplete: quantitative activity-coefficient data for citric acid in realistic multi-component industrial mother liquors, direct experimental measurements linking

ISPR adoption to crystallization quality metrics and growth-rate parameters fitted to process solutions rather than idealized binary systems.

By linking crystallizer behavior with the thermodynamic driving force $\Delta\mu$, interfacial-energy requirements and the solubility and stability boundaries defined by the citric-acid-water system, this report provides a comprehensive framework for interpreting hydrate-anhydrate outcomes and for designing crystallization operations aligned with industrial product specifications. The analysis illustrates how controlling upstream composition is often the most effective way of stabilizing crystallizer performance. Lowering counter-ion loads reduces non-ideality, removal of organic impurities improves interfacial conditions for growth and managing solid fines and macromolecules limits heterogeneous nucleation. When upstream variability cannot be fully eliminated, crystallizer operation must be tailored to compensate through supersaturation profiles, strategic seeding, enhanced hydrodynamics and careful drying and storage to maintain the intended solid form.

Successful citric-acid crystallization is fundamentally an exercise in thermodynamic integration across the entire manufacturing process. When crystallization is not seen as an isolated unit operation, but as the thermodynamic endpoint of fermentation, recovery and conditioning, the specific variables that must be stabilized to achieve predictable solid-form selection, controlled crystal growth and consistent product quality are more apparent. Viewing the process through this thermodynamic lens helps guide practical optimization, supports better equipment-design decisions and highlights key areas for future work, such as improving real-time monitoring of solid forms, refining growth-rate models using more realistic process solutions and strengthening upstream conditions steps so the mother liquor entering the crystallizer behaves more like the ideal citric-acid-water system.

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