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Comprehensive Review on Citric Acid Fermentation

Pratiti Ghosh*

Department of Physiology, Associate Professor & Head, West Bengal State University, Kolkata-700126.

ABSTRACT

The increasing demand for citric acid in food processing industries and its multifarious usage as a weak organic acid in cosmetics and other industries has always prioritized its production. The acid is present in nature especially in fruits and also as a biochemical product in all living beings. The latter is tapped for overproduction as a secondary metabolite by mutated strains of microbes. This review work aims to analyze the huge array of substrates used in different kinds of citric acid fermentation under a variety of physical conditions while considering the economy of the procedure. The process parameters optimization has been tried for more than hundred years and is still ongoing. The search for ambient physical and chemical environment by a strain of microbe unfortunately changes with time as yet-unknown random mutations affect the stability of the fungal or bacterial strain. Hence researchers with success of overproduction opt for patent and thus nondisclosure of data enforces near repetition of the work.

Keywords: *Citric acid; Fermentation; Ambient conditions; Production; History.*

Corresponding author

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INTRODUCTION

Citric acid or 2-hydroxypropane 1,2,3-tricarboxylic acid [77-92-9] (MW 192.13) is also known as p-hydroxy tricarballylic acid. It is a weak organic acid occurring in high concentrations in citrus fruits. It is ubiquitous in nature as it forms an intermediate in Kreb's cycle, whereby carbohydrates are oxidized to CO_2. The widespread presence of citric acid in animal and plant kingdom is an assurance of its non- toxic nature and it has long been used as an acidulant in manufacture of soft drinks, as an aid to setting jam and in other ways in confectionery industry.

The history of citric acid dates back to 1784 when it was isolated and crystallized by Scheele, by adding lime to lemon juice [1]. The presence of three carboxyl and one hydroxyl group was first recognised by Liebig in 1838 [2]. Citric acid was first prepared from calcium citrate in 1860 in the U.K. By 1880, it was being thus produced from juice of unripe fruits in France, Germany and the U.S. [3]. About hundred years after isolation of citric acid, it was thus synthesized from glycerol by deducing it as a tribasic acid [4].

Citric acid is found as colorless translucent crystals, odorless, with strongly acid taste. The hydrate is efflorescent in dry air. 1 gram anhydrous acid dissolves in 0.5 ml water, 2 ml alcohol and 30 ml ether [5].

Citric acid is found in large quantities in citrus fruits. Lime contains 7 gm citric acid per 100 ml juice Lemon, raspberry, black currant, tomato, pineapple, strawberry, cranberry, apple, etc. contain the free acid in descending order. Mixed with malic acid it is found in gooseberry etc. and the calcium salt in wood, potatoes, beetroot, etc. In addition to fruits, citric acid is found in all animal species. The citric acid cycle, also known as the Kreb's cycle is pivotal in the oxidation of sugars and acetates to CO_2 and water, releasing energy for physiological functions.

Citric acid has multifarious uses. In beverages it is used as flavor enhancer and as preservative. It eliminates haze due to trace metals, prevents color and flavor deterioration. It prevents turbidity in wine, inhibits oxidation and adjusts pH. In soft drinks it gives the cool taste maintaining carbonation. In food and candy, it inverts sucrose, prevents oxidation, produces darker color in candies, jams and jellies. Citric acid neutralizes residual dye in frozen fruits, protects ascorbic acid from oxidation, inactivates trace metals and oxidative enzymes by lowering pH. In dairy products it acts as an antioxidant and as emulsifier in cheese, cream etc. In pharmaceutical industry it acts as a solvent and flavoring agent, producing effervescence when combined with bicarbonate. Citric acid acts as antioxidant and as synergist in cosmetics. It is also used for treatment of boiler water, in metal plating, in detergents as builder, in tanning and in textiles. Due to its biodegradability, this organic acid has also found acceptance in detergent industry in place of phosphates and in the removal of sulfur in stack gases in power stations and elsewhere where sulfur is to be removed [5].

The manifold uses of citric acid obviate its demand, which exceeds the production rate. India is not self-sufficient in this aspect. Western European countries, viz., UK, France, Netherlands, Belgium, Austria, Germany and Ireland produce the major part of world

production. USSR, Canada, Japan, Czechoslovakia, Australia, Poland and Israel also contribute a major share [7]. The different modes of citric acid are discussed here.

1.1 Chemical Synthesis

While biotechnologists were exploring all aspects of their arena, chemists tried to find out few ways of citric acid synthesis. The classic laboratory synthesis beginning with acetone has not been used on an industrial scale as usage of bromine is hazardous [5]. There are many other methods but none of them is yet economically feasible [8].

1.2 Natural Source

Citrus fruits contain citric acid in large quantities from 5% in the fruit to 9% in the juice. Citric acid content (in mg /100 ml) of various common fruits is as follows: lime, 7000; lemon, 5630; raspberry, 2480; black currant, 1170; tomato, 1018; .pineapple, 605; strawberry, 580; cranberry, 202; apple, 14; etc. In addition to fruits it is found in all plants and animals. The citric acid cycle, also known as Kreb's cycle or tricarboxylic acid cycle is pivotal in oxidation of sugars and acetates to carbon dioxide and water, releasing energy for physiological functions. Acid extracted from these products is "natural citric acid" in contrast to "fermentation citric acid". Minute quantity of the acid is obtained from animal tissues by homogenization in appropriate buffer [9, 10].

For about fifty years, citric acid was obtained exclusively from juice of unripe fruits, especially lemons, limes and pineapples, produced chiefly in Italy, and also in certain parts of USA including Hawaii and West Indies. The juice was mixed with lime (CaO) to precipitate calcium citrate which was further treated-with sulfuric acid to form a solution from which citric acid was crystallized. The yield of purified product was 2-3 weight % of the fruit.

In 1922, Italy contributed to 90% world's supply of calcium citrate, the raw material for citric acid manufacture. Bulk of this calcium citrate was exported to the US, England and France.

Since 1927, import of citric acid or calcium citrate in the US was practically ceased. This was attributed in part to the following factors, (a) production of citric acid by development of fermentation, (b) decrease in the number of lemon bearing trees, (c) high import duties on calcium citrate and citric acid. Gradually, fermentation process developed in many other countries of Europe and large quantities of fermentative acid were being produced in the US, England, Belgium, Czechoslovakia, Germany, Russia and other countries [3].

Though citric acid can be prepared chemically it cannot meet the demand to cost ratio and thus has to be prepared by microbial fermentation within narrow and stringent conditions for maximum production by a mutated microbe, especially fungi.

1.3 Biochemical Synthesis

Early ^{14}C tracer experiments giving only moderate yield of citric acid resulted in 40% production by a C_2,C_2 condensation and 60% via a C_1,C_3 condensation. Moreover, @ 40% was produced from recycled C_4 acids. Using glu-3,4-^{14}C under good yielding conditions, it was shown that a near quantitative recovery of the labeling was obtained in C_4 of citric acid [11]. C_6 had a specific activity of @15% of original. None of the other carbons was labeled to any significant degree. So there was no recycling of C_4 acids and citric acid was formed by an initial symmetrical 3:3 split of glucose and that decarboxylation of one fragment produced a 2-C fragment. The other 3-C fragment was carboxylated to give a 4-C compound and finally citric acid was produced by condensation of 2 and 4-C moieties. C introduced by carboxylation was C_6, the specific activity having been diluted by the CO_2 pool in hyphal cell [12]. The other modes of obtaining citric acid are:

1.3.1 Embden-Meyerhoff Pathway

The enzymes of this pathway have been well attested by Damodaran et al, 1955, [13] in *A. niger.* The aldolase requires Zn for activity in fermentation medium. Citrate is feedback inhibitor of PFK and mitigation of this inhibition is required for citric acid accumulation [14]. Bowes found that enzymes from low yielding strains were greatly inhibited by addition of 5.5mM citrate, whereas those from high yielding strain were slightly activated under the same conditions [15].

1.3.2 Citric Acid from Pyruvate

Citric acid is formed by condensation of acetyl CoA and oxaloacetate under citrate synthase. When citric acid accumulates, the cycle is to a greater or lesser degree blocked and another so called anaplerotic reaction i.e. carboxylation of pyruvate by pyruvate carboxylase is needed to supply oxaloacetate. Since both citrate synthase and pyruvate carboxylase are not effective regulators, the rate of formation of citric acid is related to the speed at which carbohydrate is changed to pyruvate [16].

1.3.3 Citric Acid from Carbohydrates, Fats and Amino Acids as a common intermediate.

1.3.4 Citric Acid from Glyoxylate Cycle

In plants and some micro organisms living on acetate as sole carbon source for synthesis of carbohydrates, a variation of the citric acid cycle, the glyoxylate cycle comes into play and make possible the citrate formation. [16]

1.3.5 Kreb's bi-cycle

There is a link between citric acid cycle and urea cycle in ureotelic animals, both being discovered by Hans Kreb, have been jointly referred to as Kreb's bi-cycle. [16]

1.4 Microbial synthesis

The development of citric acid fermentation may be divided into many phases.

The first phase fermentation route was indicated by Wehmer in 1893 [17]. He discovered that certain species of *Penicillium* were able to accumulate significant quantities when grown on solutions containing 10 - 20% sugar, calcium carbonate "may be added" and ammonium nitrate, potassium phosphate and magnesium sulfate were added. Yields up to 50 % had been obtained at 15 - 20°C requiring 4 -6 weeks. The process received broad patent coverage in Germany and elsewhere but was later abandoned in 1903. The *Citromyces pfefferians* and *C. glaber* used by Wehmer (later considered by Charles Tom [18] as true *penicillia*), was isolated from air of different localities and was also found to be produced by *P. lacteum* and by strains of *Mucor pyriformis* and *M. recemosus* of class phycomycetes.

In 1904, Maze and Perrier [19] isolated and used Penicillium-like strains for citric acid production with bean extracts, 10% glucose and organic nitrogen source as preferable medium Addition of calcium carbonate was essential for high yields. The medium was incubated at 16 - 22° C for 57 days to obtain 4.47 gm citric acid per 100 ml from 11.63 gm glucose. They showed that increased yields of more than 50% sugar used was obtained after mycelial growth was essentially complete.

In 1909, Buchner and Wustenfeld [20] used strains of fungi from Wehmer and Maze with yields up to 69% sugar utilization in 66 days. Herzog and Polotzky (1909) [21] using *"Citromyces"* (citric acid producing *Penicillium,* named by Wehmer) obtained 28% yield on 5% glucose in 8 weeks.

The second phase of citric acid fermentation started with the excellent publication by Currie in 1917 [22] who found a strain of *Aspergillus niger* with better yield on static medium held in trays housed in ventilated rooms. At first sucrose and inorganic salts, then beet molasses was used as media [22]. Wehmer [17] did not discover that *Aspergilli* especially those belonging to *A. niger* group were potent. He believed that oxalic acid formation was its characteristic. Actually, citric acid was already recognised to be formed in substantial amounts by the black *Aspergilli* [18][23] but Currie laid the foundation of the current knowledge of the process. During a study of oxalic acid production by *Aspergilli* he observed that titrable acidity sometimes greatly exceeded the oxalate produced which was identified as citric acid and Currie's work followed. His discovery was that many strains of *A. niger* when grown at low pH values in surface culture on concentrated (up to 15%) sucrose solutions, in synthetic media containing optimal concentrations of other nutrients, gave yields of citric acid up to 55% of sucrose present in the media [18]. Oxalic acid formation recognised by Wehmer, could be considerably reduced by adjusting the nitrogen supply and initial $[H^+]$ of the fermentation media.

Bernhauer et al (1914) [24] using beet sugar found that 22.5% solution is the highest concentration readily fermented. Molliard in 1919 chose NH_4NO_3 as the nitrogen source [25]. Amelung in 1930 used 9.8% sucrose as carbon source and $(NH_4)_2SO_4$ as the nitrogen source [26],

though in 1927 he said that citric acid production by living fungus did not require presence of inorganic salts excepting so far as they were contained in the mycelium itself [27]. Bernhauer and Iglauer found peptone unsatisfactory [28], though in 1930 Sumiki used the same [29]. So far citric acid production was confined to species of *Penicillium* and *Aspergillus* under stationary or surface culture conditions.

The development of submerged fermentation process began in the 1930s. In this, the nutrient media after inoculation was subjected to vigorous and controlled aeration and agitation in large fermentors. The time involved was much shorter (3-5 days). Amelung in 1930 [26] obtained promising results with *A. japonicus,* though not as high as surface culture. Detailed systematic studies on this aspect was available in 1938 from Perquin's report in Dutch version which consequently did not receive required attention [30].

It was found that higher initial pH produced oxalic acid excessively, so it was kept at low level. pH 2 was maintained by Frey (1931) [31]. Kostuichev & Chesnokov [32] found citric acid in acidic, whereas, oxalic acid in alkaline medium. In 1932, Quilico & Dicapua [33] found that effect of iron on citric acid depended on strain of *A. niger* used. Luz in 1934 [34] said that in solution of high sugar concentration, greater conversion to citric acid occurred. Doelger & Prescott (1934) [35] recommended 9.8% sucrose, K_2HPO_4 as P source in medium at pH 1.6-2.2, incubated at 26°-28°C. In the following year, Chrzaszcz & Peyros [36] used 20% sugar and NH_4NO_3 as the nitrogen source, KH_2PO_4 as the P source and reported that Zn salts were injurious. Fulner et al in 1935 [37] used NH_4Cl in the medium.

In solid state fermentation 1935 was first described by Cahn [38]. The fermentation medium was impregnated in porous solid materials viz., sugarcane bagasse, potato or beet pulp, pineapple pulp etc. in an appropriate ratio, sterilized and then inoculated with a suspension of fungal spores before incubation in trays [39][40]. After fermentation the mash was extracted with water, concentrated and then processed for citric acid precipitation.

Vasilev (1935) [41] used 0.01% $ZnSO_4$ and Bolcato [42] added Na-acetate to increase yield. Kresling and Stern [43] used UV rays to increase the acid production. Kirsanova (1936) [44] showed that maximum yields of acid was obtained by increasing sugar concentration up to 25%, Bernhauer and Iglauer [28] used KNO_3 as nitrogen source, while Chrzaszcz and Zakornorny [45] used asparagine and tyrosine. Nussbaum [46] used asparagine too. Publication of Porges [47] showed 12-20 % sugar was required with KNO_3 On the basis of sugar consumed, yields amounting to about 100 % was obtained by Butkevich and Gaevskaya [48] ; Clutterback et al [49] got 87 % yield on semi-large scale fermentation. Weiland's patent [50] in 1936 was more of a passing interest as he used yeast for inoculation, chose solution of sodium or calcium acetate or acetic acid as substrate. A great part of the acid disappeared after 12 hours and citric acid and succinic acid were obtained. In 1938, Nakazawa and Simo [51] irradiated *A. niger* with radium for higher production. The use of chemicals such as potassium ferrocyanide to reduce the concentration of Fe^{2+} in the medium was first described by Mazzadroli [52] in 1938. Jacobi and Schwartz, 1939 [53] found that small amounts of gelatin, colloidal sulfur, methyl cellulose, egg albumin and agar accelerated citric acid production. In 1940, Nebe [54] observed that addition of

certain quarternary ammonium salts (1-2 %) accelerated citric acid production when molasses were used. Knoblock and Sellman [55] found that Zn, Fe, Mn in proper proportion wasimportant in a particular strain [56-58]. Szucs patent (1944) [59] described the essence of submerged fermentation. Mycelia was grown on a medium of " growth solution " which was apparently phosphate deficient and then mycelia was transferred to a fermentation solution containing no phosphate in which citric acid was produced. Growth phase comprised of 3 days and fermentation phase of more than 4 days. Fermentation solution was agitated and then stirred by oxygen gas (unmixed oxygen or mixture of oxygen with nitrogen or air) with or without pressure at 25°C. He added gelatin in the medium. In 1948 Szucs [60] proposed a modified procedure with phosphorous deficient medium with pure sucrose and milk powder. Shu and Johnson [61] grew *A. niger* in shake flasks with sucrose as carbon source. In 9 days, 72 gm anhydrous citric acid was obtained from 100 gm sucrose. They also observed that carry over of Mn in the spore inoculum could be detrimental to the yields obtained in submerged culture. Karow in 1942 [62] and Karow and Waksman, 1947 [63] met with success using *A. wentii* on pure sugars. Kovats, 1946 [64] used sucrose at 28° C for the first three days, then reducing to 21°C. To remove contaminating metal ions, ion-exchange resins were used by many [65][66][58]. $Al(OH)_3$ treatment was done by Shu and Johnson [61]. Specific precipitants like morpholine [65][66], camphor or tannic acid [67] and lower aliphatic alcohols [68] (Nadeem et al, 2010)[69] were used.

Shortly after World War II, in the National Research Laboratories in Ottawa, investigation began on shallow pans and submerged production of citric acid from beet molasses was undertaken. Significant increase in the yields was reported by Martin and Waters, 1952 [70] using *A. niger.* In tower-type fermentors using ferrocyanide-treated beet molasses, yield increased with decreasing time to 6 days and 0.93 % conversion per hour was observed.

UV treatment was used by some [71, 72] X-ray by Diller,1950 [73]; UV rays followed by X-ray by Gardner, 1956 [71] on microbes to increase yield. Regarding fermentation medium, Moyer in 1953 [68] used glucose, corn steep liquor, citrus molasses, date pulp (Assadi & Nikkhah, 2002 [74] and beet molasses. Ozaki and Takeshita [75] in 1955 used cane juice. Martin and Steel [76] observed that increased phosphate concentration sometimes increased citric acid production in beet molasses but excess phosphate decreased citric acid production increasing gluconic acid, 5-keto gluconic acid, oxalic acid and malic acid.

EDTA was used by Chaudhary and Pirt (1966) [77] to reduce trace metal content of molasses. Kovats in 1960 [78] used 0.04 - 0.6 % ferrocyanide at pH 2.2, while Clark [79] (1963) and Leopold and Valtr (1969) [80] suggested a concentration of 0.005 - 0.020 % for molasses clarification. Use of quaternary compounds, viz., diisobutyl phenoxy ethyl dimethyl benzylammonium chloride to molasses, increased yield of citric acid by 92% (Miles Lab, 1969) [81]. Wendel [82] in 1967 carried out electrical conductivity measurements. Different additives have been used, viz., mild oxidising agents such as hydrogen peroxide or napthoquinone or methylene blue have been used by Bruchmann [83] to stimulate production. Few other examples were chemical additives like aromatic amides, esters of dichloroacetic acid, sodium sulfide, crysyllic acid [84]. Millis [85] reported that addition of fatty acids of chain length of 15 carbon atoms or fewer or natural oils containing high amounts of unsaturated fatty acids, viz.,

corn oil, almond oil, linseed oil, peanut oil, etc. increased yield by 20%. Complex components like mycelial digests [86], baker's yeast [87], and alkanes [88] were pioneered during this period.

Coming to the seventies, a continuous multi-stage process was patented [89][90]. Caustic alkali was added to neutralize 1/3 citric acid produced. Dhankar et al [91] proposed 0.4% $NaNO_3$ as superior to NH_4NO_3. Khan et al [92] proposed that a high concentration of phosphate promoted more growth and less production. Fedoseev et al [93] observed that addition of Cu^{2+} at 0.1-500 ppm, counteracted the deleterious effect of iron (as low as 0.2 ppm), According to Wold and Suzuki [94], 1976, Zn^{++} (1-2 M) allowed continuation of growth phase but restricted growth at lower concentrations Addition of cAMP during production phase enhanced yield, whereas Zn^{2+} retarded it. Other trace elements viz., Mn^{2+}, Ba^{2+} Al^{3+} etc., have been reported to affect fungal morphology and citric acid production at concentrations that did not inhibit growth [95, 96]. Pretreatment of molasses with ferrocyanide had been done by many workers [97]. Further polyethyleneamine [98] reduced the metal content of molasses. Mints et al [99] reported that addition of Trilon B (100-500 mg / L) to a molasses media improved production by *A. niger.* Regarding pH, when sucrose or glucose or clarified molasses or relatively pure material was used, a low pH (3.0) was desirable. A higher initial pH led to accumulation of oxalic acid [100]. Besides, the age of inoculum was explored to find that a three day old culture was as good as a eight day old one [101]. Again, interruption of aeration during fermentation under submerged condition affected acid production; however, the extent of damage depended on the duration of interruption and phase of fermentation e.g. 30 minute gap of a 24 hour old fermentation decreased the acidity of the medium by 13%, a 60 minute interruption by 20%, a 7.5 hour interruption by 60% [102].

Addition of methanol after 24 hr or later was not found to be beneficial [101] but probably increased the tolerance of fungi to trace elements viz., Fe, Mn, Zn, etc. The derivation of mutants that tolerated a high concentration of trace metals but still responded to methanol addition was inconsistent with this assumption [103] The effect of chemicals with no nutritional value, e.g. the inhibitors of metabolism viz., CaF, NaF, KF at a concentration of 10^{-4}M have been found to accelerate production [104]. Similarly, malic hydrazide (1g / 2.5 Kg molasses) under submerged conditions increased production by about 40% [105]. Short chain carbohydrates, viz., glycerol or lipid materials or other metabolizable complex compounds have been examined. Addition of glycerol to a molasses medium at a rate of 30-50 g / L has been observed to have increased yield by 30% [106]. Sorbitol, mannitol or erythritol have been used too. Use of refined peanut oil in molasses or cane juice medium improved the citric acid yield [107]. Wold and Suzuki [108] reported a strain of A *niger* which accumulated citric acid in the medium when cyclic AMP concentration was 10^{-6} M or higher. Adenosine, ATP and / or cGMP also stimulated production at 10^{-3} M. AMP had no effect while GMP and guanosine slightly inhibited production, ADP strongly inhibited whereas, addition of theophylline along with cAMP increased the effect. It was suggested that citric acid production resulted from an abnormal cAMP metabolism. Halama in 1974 [109] used antiseptics viz., pentachloro phenolate, formic acid, 5, nitro-3-furaldehydesemicarbazone, tetracycline, etc. to reduce bacterial contamination.

Variation in organism was brought about by yeasts like *Hansenula anomala* in glucose media containing 3 % calcium carbonate at 30° C rotating at 110 rpm , using KH_2PO_4 (0.05 %), $MgSO_4$ (0.025%), NH_4Cl (0.1 %) for a six day fermentation period. 46% citric acid was produced [110]. Ishi et al [111] fermented on waste glucose (32.6% glucose, 3% fructose) after the addition of corn steep liquor, KH_2PO_4, $MgSO_4$, $MnSO_4$ and $CaCO_3$. Inoculation was done with *C. oleophila.* Again, using C. *guillermondii* and C. *lipolytica,* a continuous process for citric acid production has been described [112]. Various alcohols viz., methanol, butanol, ethanol and C_{12-16} alcohols served as carbon source for production by strains of C. *fibrae, C. subtropicalis, Pichia, Farinosa, Hansenula sp.* and *Torulopsis xylinus* [113-115]. Production of citric acid using fatty acids, natural oils and fats have been tested using various genera of yeasts viz., *Candida, Hansenula* and *Pichia* [116,117]. Tallow, coconut oil, palm oil, olive oil, soybean oil, linseed oil, rapeseed, fish, corn oil and free fatty acid have been tested for the purpose. Strains of *C. lipolytica* yielding a high amount of citric acid without the formation of isocitric acid have been developed by mutagenic treatment [118-122]. Suzuki et al [118] used yeasts viz., *Candida, Brettanomyces, Debaromyces, Hansenula, Kloeckers, Torulopsis, Pichia, Saccharomyces* or *Trichosporon.* Optimum pH <7.5 or >9.5, 0.05% yeast extract, 0.2% NH_4Cl, 0.1% KH_2PO4, 0.05% $MgSO_4.7H_2O$, 0.05% $FeSO_4.7H_2O$ and 1% $CaCO_3$ was used in shake culture for four days at 28°C.

Several processes for citric acid production from n-paraffin have been described [119-120,123-126].The process involved shake culture at 28-30°C for 4-6 days with media containing hydrocarbons, 40-60 g; NH_4Cl, 2g; KH_2PO_4, 0.5g; $MgSO_4$, 0.5 g; cornsteep liquor 1g and $CaCO_3$, 30 g / L [127]. As with *A. niger,* fermentation concentration of Fe^{3+} ion affected the accumulation of ratio of citric and isocitric acids. Since the maintenance of low concentration of iron in complex media is difficult, fluoroacetate sensitive strains of C. *lipolytica* with low aconitase activity have been developed which produced increased citric acid from n-paraffins [120]. With sufficient thiamine a large amount of citric acid was produced while its restriction caused α-keto glutarate accumulation [128], A unique medium containing a lead salt (0.5 - 1.5 gm) ; cerelose, 150 gm ; $CaCO_3$, 10 g ; NaCl, 4g ; yeast extract, 5 g ; peptone, 15 g was seeded with an inoculum of C. *guilliermondii* [129].

Some bacteria, e.g. *Bacillus licheniformis* produced citric acid [130]. Under aerobic conditions, at a pH of 7, fermentation was carried out. Yields up to 42 g / L have been reported. Kyowa Fermentation Industry, 1970 [131] have patented a process by *Arthrobactor* using paraffins containing poly (oxyethelene) glycol monostearate 0.05 % in medium. Fukuda et al [132] patented a process involving various species of *Corynebacterium* in paraffin containing media. Alternate methods, by growing bacteria viz., *Klebsiella, Aerobactor, Pseudomonus, Micrococcus, Bacillus, Brevibacterium, Corynebacterium* and *Arthrobactor* in media containing isocitric acid (5-6 %) have been reported [133-134]. pH of 5-8 for about 2 days at 32-37° C under shake culture was performed to convert isocitric acid to citric acid. A mutant of *Brevibacterium flavum* with media constituents : glucose, 3.6 % ; urea, 0.2 % ; KH_2PO_4, 0.01 %; $MgS0_4$, 0.04 % ;sodium L-glutamate, 0.05 % and $CaCO_3$, 1 % in addition to soybean hydrolysate (3 ml / L) and minor amounts of vitamin B_{12}, $FeSO_4$ and $MnSO_4$ yielded citric acid up to 50 mg / ml [135].

Citric acid fermentation of potato pulp by *A. niger* and *A. usamii* have been observed [136]. Addition of defatted rice bran decreased the production showing that N/C ratio played a role. Gradel [137] found that addition of oxidants viz., H_2O_2 to molasses solution, accelerated spore germination and mycelial growth but its addition or that of oleic acid to synthetic media had no influence. Brehr [138] showed that intensity of citric acid production was different with 21 various sources of *Botrytis cinerea.* Among them, isolates from *Linum usitatissimun* produced the most (22.05 g /100 g maltose). Fresh isolates of *B. tulipae, B. allii, B. paeoninae and Botryotinia convoluta* also produced citric acid. Nagashima et al [139] observed the effect of methyl alcohol and $K_4[Fe(CN)_6]$ from the view of aconitase activity. By 1970, genetics of citric acid producing strains were probed into. llczuk et al [140] observed citrate synthesis by auxotrophic mutants of *A. niger* induced with UV rays. Tanaka et al, [141] (1970) by using *Penicillium* increased citric acid yield in media containing paraffins, NH_4NO_3, $MgS0_4$, KH_2P0_4, Na_2HPO_4, $CaCl_2$, $MnSO_4.4H_2O$, $ZnSO_4.7H_2O$, corn steep liquor, Nonion OT 221, $CaCO_3$, H_3BO_3: $Na_2MoO_4.2H_2O$, $CuSO_4.5H_2O$, $CoCl_2.2H_2O$ and $FeSO_4.7H_2O$. Sanchez-Marroquin [142] developed a strain of *A. niger* that tolerated high concentrations of trace metals viz., $MgSO_4.7H_2O$, 0.025 g /100 ml ; $FeSO_4$, 0.15-0.75 ; $ZnSO_4$, 0.10 and $CuSO_4$ 0.01 mg / L. Fukuda [125] used dry yeast for production with *Candida, and Corynebacterium Kromsr* [143] used 10^{-3} mole EDTA / L media in sugar beet molasses. Shcherbakova [144] observed the influence of chemical mutagens viz. H_2O_2, HNO_2, HCHO, nitrosomethylurea on *A.* nigerto increase citric acid yield. Ol' Shanskii [145] used cyclohexane to increase the yield and quality of citric acid and also to simplify the process. Suzuki et al [146] utilized *Candida, Brettanomyces, Debammyces, Hansenula, Klockera, Trichosporon, Torulopsis* or *Pichia* for accumulating citric acid. Kimura et al [147] fermented citric acid in the presence of hydrocarbons as main carbon source and an alcohol viz., ethyl alcohol or methyl alcohol or lauryl alcohol or amyl alcohol or CH_2FCO_2Na or $K_4Fe(CN)_6$. 0.05% corn steep liquor was added too. Divekar et al in 1971 [148] investigated with sugar cane juice as raw material for submerged fermentation. He used refined groundnut oil to stimulate yield and also to use as antifoam agent.

Pomar in 1969 [149] observed the effect of temperature on the assimilation of urea and other nitrogen sources by an *A. phoenicis* strain. Baras [150] measured the daily kinetics of biosynthesis of the acid by determining organic matter, nitrogen, phosphorous ash content, sucrose consumption and citric acid formation. He.also assessed phosphorous metabolism. Kovats et al [151] tested for bacterial contamination with sulfonamide, nitrofurantoin, nitrofurazone and furazolidone. Qadeer et al [152] tested with chelating agents viz., EDTA, CTDA, NTA and DTPA to observe citric acid yield. Stopczyka [153] observed the effect of strontium on the growth and mineral uptake of *A. niger,* Baras [154] observed the possibilities of increasing yield with amino acids. Kahlon et al [155] found that malic, palmitic, malonic, orotic, oleic and fumaric acid and tripalmitin had no beneficial effect, Leopold [156] observed that glycerol, erythritol, mannitol and sorbitol markedly increased fermentation in molasses media but weakly in sugar solutions. Kumarmoto [157] added Ba^{2+} ion to enhance production.

In 1972, Fried [158] obtained increased yield by adding minute quantity of a tricarboxylic acid viz., n-hexadecyl citric acid or trans-aconitic acid in the media. Suzue [159] used corn steep liquor and $FeSO_4$ (10 ppm) in the media and adjusted pH with $CaCO_3$. In 1972, Kovats [160]

controlled bacterial contamination with streptomycin, detreomycin and oxyterracin. Su Yuan Chi [161] used starch waste from dry sweet potato, cassava and defatted rice bran. Baig et al [162] added peanut or soybean or olive or almond oil to stimulate production. Nakamishi et al [119] added aconitase inhibitors to increase production.

Wold et al in 1976 [94] considered Zn as essential for growth. They found no effect of Fe or Mn on citric acid. They further stated that cAMP enhanced growth at early stages but inhibited at high concentrations. Uchio of Aginomoto Co. [163] cultured *A. awamori* in $Ba(OH)_2$ and lecithin among others. Akiyama et al [164] found that addition of monofluoro acetate increased citric acid yield, decreasing isocitrate. These strains had low aconitase activity, grew only on glucose, n-hexadecane, acetate and pyruvate. The parent strain grew on all citric acid cycle intermediates except malate and fumarate. Jayaraman et al in 1971 [165] observed that K_2S0_4 at 3% levels, at 35°C produced citric acid. Addition of 2,4- dinitro phenol inhibited isocitric acid formation [166]. Ceuci and Cavazzini [167] observed that the acaricide N-methyl N-(1-napthyl) monofluroacetamide inhibited ethyl alcohol and lactic acid production and stimulated citric acid production. Volkov et al [168] found that the strain utilized glutamic acid, aspartic acid, alanine, leucine and isoleucine from molasses containing medium. Serine and glycine decreased production during first five days to increase again to original level whereas lysine and threonine increased yield by two to three times the original level. Polyhydric alcohols viz., 1% 2,3-propanediol and 1% glycerol showed beneficial effect [169].

Tabuchi et al found that the activities of aconitase and NAD or NADP-linked ICDH decreased markedly during the stage of citrate accumulation. Fe^{2+} inhibited aconitase activity [170]. In a thiamine restricted medium, *C. lipolytica* accumulated large amount of α-ketoglutarate which intensified isocitrate dehydrogenase activity for which 1-2 % methyl alcohol or ethyl alcohol or butyl alcohol was used at the beginning [171]. Takayama [172] added 0.5 % glycerol and 50 µg/L thiamine to the medium inoculated with *Brevibacterium, Corynebacterium, Arthrobacter, Nocardia, Streptomyces* or *Saccharomyces* for fermentation in a patent work by Pfizer Co. [173], solution from NZ Amine YTT (peptones from casein degradation), 1-dodecene, 1-tetradecene, 1-hexadecene and 1-octadecene were used. Hitachi Co. [174] grew *Klebsiella, Aerobacter, Pseudomonas, Micrococcus* or *Bacillus* in a medium containing d-isocitric acid or ICA lactone or by incubating cells grown on such medium with an aqueous solution of either of these compounds. Addition of lipids shortened the yeast growth lag time [175]. They added oleic acid and antifoam agent, $CaCO_3$, corn steep liquor and vitamin B_1. Citric acid was also produced by *Trichosporon, Hansenula* or *Rhodotorula* from alcohols, yeast extract, $CuSO_4.5H_2O$ etc. [176].

Srivastava et al [177] proved the superiority of Ca-ammonium nitrate, nitrobenzene and urea over KNO_3 as nitrogen source. Chaudhary et al [178] successfully used N-Methyl-N'-nitro-N-nitrosoguanidine for mutation. Tachibana in his patent in 1974 added schizoflavine and thiamine [179]. Ikeno et al produced citric acid from natural oils, fatty acids, glycerol, ethanol and n-paraffin; especially, palm oil yielded 146 % acid of interest [180]. Addition of borax and casamino acids improved fermentation [181]. Maleic hydrazide (0.1%) showed 70% yield on sugar consumption basis [182]. Cyanoacetic acid inclusion after 24 hr in yeast culture [183] and pineapple juice or defatted rice bran showed good fermentation, the latter in a semi-solid

medium [184]. Shintolex (detergent) as defoamer was used along with $CaCO_3$ and thiamine-HCI to obtain remarkable results [185].

In 1977, Singh et al [186] experimented with endrin, DDT, lindane and heptachlor in fungus medium. Kikuchi et al used Ca-acetate in the medium [187] and 2,4-DNP by Hustede and Siebert [188]. Introduction of trace elements viz., Cu, Co, Mo, I and B were done to prevent formation of other acids [189]. Uchio et al [190] cultured a medium containing lysine, methionine, biotin, thiamine-HCI and Ca-acetate, Maldonado et al [183] observed remarkable increase in production with 15% quinaldic acid. Kubicek & Roehr [191] studied the influence of Mn on enzyme synthesis and citric acid accumulation, Gorbataya et al [192] used Me p-hydroxybenzoate in molasses medium to inhibit Penicillin growth. Hanissa [193] observed that ethyl acetate and chloroform had no significant effect but PrOH or formaldehyde had a toxic effect. Addition of methyl or ethyl alcohol stopped sporulation in surface cultures. Banik in 1976 [194] worked with NaCl, $CaCl_2$, Cu, Co, Mo, Ni, and V to observe mineral nutrition of *A. niger.* Hang et al in 1977 [195] used spent grain liquor, a brewery waste. Production varied from 3.5-12.3 g / L (42-58% on sugar consumption basis). Maslova et al [196] added Trias preparation (alkyl sulfate + Na-tripolyphosphate + Na-silicate + calcined soda +Na_2SO_4) to decrease the negative effect of bacterial impurities. Abraham and Chaudhury [197] experimented with effects of illumination and darkness on production. Intermittent alterations showed higher productivity.

Habison et al [198] observed a correlation between elevated levels of NH_4^+ and enhanced citrate formation. Zecin et al (1978) [199] found that addition of $NaNO_2$ inhibited production by *A. niger.* Mycelium development was retarded. Products of nitrite decomposition had a more toxic effect. From a patent literature of El-Sayed [200], production was first observed on whey permeate by *E. coli.* In the second stage, *Hansenula wickerhamii* was cultured on it. Fishkova et al [201] found that yield was increased and use of substrate reduced when poly(vinyl) alcohol or polyacrylonitrile ion-exchange fibres treated with antimicrobial activity was used. Zecin et al [199] found that nitrite producing bacteria viz., *E. coli, Klebsiella, Alcagenes, Pseudomonas* and *Citrobacter* inhibited growth and citric acid production by *A.niger.* Al-Obaidi and Berry [202] prolonged the active phase of fermentation by an exchange procedure in which about 50% of the medium was replaced by fresh one every 10 days up to 30 days with daily production of 9 -10 g / L. Nowakowska et al [203] added 0.1-0.2 µg/ml Brilliant Green after inoculation, decreasing the mycelium weight by 2-40 % and stimulating rate of acid formation by 20-70 % and shortening the fermentation cycle by 1-1.5 days. 5-10 % increase was observed by treatment of submerged cultures with ionized air [204]. In 1979, Finogenova et al [205] grew *C.lipolytica* on acetate, ethyl alcohol, glycerol, glucose or hexadecane.

Srivastava and De in 1980 observed that ultrasonic waves alone improved yield and in presence of NaCI decreased yield [206]. Hydrolysed cellulose increased production from 12-8 mg/ml [207]. Dimecron inhibited yield by *A. niger* but this was reversed in part by increasing the ATP concentration [208]. Soybean oil, peanut oil, PLP, linseed oil and oleic acids in order decreased citrate yields. Stearic acid, cotton seed oil and ergosterol were ineffective [209]. Constancy in production was observed for about 100hr, using n-paraffins and methyl alcohol

inoculated with C *lipolytica,* The citrate production was governed by Michaelis-Menten kinetics and depended on pO_2[210].

Vaija et al [211] entrapped *A. niger* mycelium in Ca-alginate beads and employed an air-lift completely stirred reactor for continuous production, maximum efficiency was 40%. Lai and Srivastava [212] found that glutamine and aspartic acids stimulated production by 79.6% and 76.7%, lysine by 62% and serine by 50.4%. Cysteine had a detrimental effect. A patent by Showa Oil Co. [213], worked with *Candida* sp. on paraffins to suppress production of isocitric acid by addition of non ionic surfactants viz., Span 30 to increase yield from 23 to 35g/L. Cu^{2+} and BO_3^{3-} inhibited isocitrate to increase citric accumulation. The strain possessed higher citrate synthase and lower aconitate hydratase activity than parent [214]. Eysmond in his patent work [215] replaced 15-20% of the medium with fresh one after 4-6 days when sucrose concentration decreased from 0.1-0.5% with 90% yield.

Bolach & Lesniak [216] liquefied starch with H_2S0_4 before saccharification with amyloglycoside and fermentation. NH_4OH, $Ca(OH)_2$ and Na_2CO_3 were then used for neutralization. Asenjo et al studied fermentation of C. *guilliermondii* on an enzymic hydrolysate of Solka Floe. Production was increased by limiting the level of nitrogen in the media [217]. Kahlon & Vyas [218] used cane sugar as carbon source and organic metabolites viz., napthol, resorcinol, cresol and benzaldehyde increased yield when present in 1000, 2000, 3000 and 4000 ppm respectively. A mixture of corn starch and wheat bran was mixed with water; extruded, granulated and inoculated as solid culture for 6 days to obtain citric acid [219]. Cell immobilized in polyacrylamide, carrageenam or citric acid arginate gel carrier was aerated replacing the solution every 24 hr to obtain yield of about 430 mg/dl per run [220]. Varosek et al [221] fermented molasses with *A. niger* to increase yield by adding 0.1-0.5 ml H_2O_2 / L medium in presence of glycerol and / or H_3PO_4 to decrease decomposition of H_2O_2 in sterilization.

Maddox and Kingston [222] found that immobilization of S. *lipolytica* in polyacrylamide gel caused no loss of activity for 14 days producing 50 mg / L^{-h}. Elimer [223] in his patent used soyabean, rape or sunflower oil in stirred and aerated culture to obtain 92.7% yield with the first. He supplied the medium with oleic, rape , soyabean oil and other carbon sources. All lipids were utilized and better growth was observed on vegetable oils. Nikiforova et al [224] grew *A. niger* on wort agar, stimulated by 15% formose to yield 54.8% acid, i.e., 75 mg/L medium. Yield increased by 23%.

Tan et al (1984) [225] used a thermophilic citric acid producing mutant (84.4% production). Sanyo Kokusaku Pulp Co. Ltd. [226] utilized waste water from wood pulp plants, ultrafiltered to increase sugar. 15 mg of citric acid/ml was obtained by *C. guilliermondii.* Kirimura et al [227] cultivated *A. niger* in synthetic media containing 2 g gelatin / L and glass wool as carrier. Hang and Woodams [228] obtained 88 g yield from apple pomace in the presence 4% MeOH. Cultivation of C. *lipolytica* on glucose or glycerol preferentially produced citric acid whereas isocitric acid was favoured by ethyl alcohol [229]. Solinsky [230] carried out surface culture on plastic plates coated with steelon fabric. The degree of hydrolysis of starch affected both yield and fermentation time. Highest yield was approximately 75% with respect to starch at

the degree of fluidization 5-15 [231]. Two fold increase in production was obtained by addition of 0.1% phytin along with rice bran, its oil and extract [232]. Grape pomace in presence of 3% MeOH, was a good medium [228]. Addition of malt sprouts to the culture stimulated conidiation of fungus, p-amino benzoic acid had the same effect and was used as inoculum [233]. Protoplast fusion of *Candida* produced acid from xylose [234]. Citric acid by aerobic fermentation of palm oil consuming yeast [235] was observed.

Gum et al [236] produced citric acid by sexually compatible heterothallic haploids and diploids of *Saccharomycopsis lipolytica.* Glycerol accumulation was an early event in the sequence of reactions leading to citric acid accumulation [237]. Citric acid was produced from agricultural wastes to yield 58.2, 48.7 and 54.4% [238]. Effect of dithiocarbamates viz., tetramethylthiuram disulfide (25µg/ml) showed 74.2% increase, Na-dimethyldithiocarbamate (2.5 µg/ml) gave 19.6 % increase and 33.1% with 0.6 µg/ml Zn-dimethyldithiocarbamate [239]. Feeding oxygen instead of air decreased isocitric acid by cultivating *Candida* in media containing palm olein [240], Tamarind seed powder was used as raw material for enzymic studies on *P. chrysogenum* for citric acid and lipid production [241], Fukuda and Tanaka [242] manufactured this organic acid by immobilized bacteria also i.e. by contacting organic acid producing *Aspergillus* or *Rhizopus* which has been immobilized on bacteria-holding material.

Addition of increased levels of Mn^{2+}, Fe^{3+}, Zn^{2+}, Co^{2+}, citric acid, Na-monofluoroacetate and MeOH during citric acid fermentation of spent grain liquor by *A. niger* showed good production [243]. Sugarcane was used as carbon source by Rumba et al [244]. The conversion of cellulose hydrolysate into citric acid by addition of fluoroacetate and coconut oil was also studied [245]. Podgorski et al added rape seed oil to a medium which was aerated to study foam content in the fermentation [246]. Elimer [247] cultured *A. niger* on media containing 4g low erucic rape oil. Shevtsova et al [248] suggested usage of limestone 1 : 4 and water to neutralize citric acid fermentation broth to facilitate the filtration and minimize loss of acid.

Maximum production was observed when *A. niger* was grown on polymers (polyamide, polyether, fluoroplast) after freeze drying and storage for 1.5 year [249]. Solid state fermentation was carried out on sugarcane bagasse fortified with sucrose [245]. Ethanol, coconut oil (3%) and fluoroacetate increased citric acid. Fermentation of Kraft black liquor for bioconversion of acetate to citrate was achieved when no nitrogen was supplemented. Substrate uptake and product formation rates were lower with respect to synthetic media. Utilization of immobilized biomass improved process parameters and enhanced fermentation capabilities [250].

Berovic and Cimerman [251] realized that for an effective fermentation the values of redox potential peaks, their time relationships were important. Deviations greater than 10 mV predicted too low yield of product, the most crucial period being the first phase. Georgieva and Aleksieva [252] studied the dynamics of citric acid biosynthesis and morphological changes of *A. niger* in media containing starch hydrolysate and 3 % MeOH. Glucose liquor from the manufacture of glucose by acid enzymic hydrolysis of starch was used, so was byproducts of potato processing industry [253]. Fatty acid wastes from vegetable oil refining as carbon energy

source was used. Elimer [254] produced the acid on sucrose and vegetable (soybean oil). Mandarin orange peel was also used [255].

Geogieva et al [256] biosynthesized citric acid on medium containing corn starch hydrolysate ethyl alcohol (6%), etc. Yield for a strain was greater in pure than mixed cultures. However, low yielding strains in combination often augmented the yield [257]. The concentration of dissolved oxygen was stressed upon to increase production and cellular growth. Production was optimum at approximately 60 ppm though cellular growth decreased to about half of that at 6 ppm [258], Zhang [259] tried with orange juice and 2 % MeOH for citric acid manufacture. The effect of anthraquinone 2,6-disulfonic acid (10^{-5} M) in release of H_2O_2 to inhibit aconitase and stimulate citric accumulation was observed by Graf and Muller in 1989 [260]. Glycerol functioned as an osmoregulator in early stages of *A.niger* growth [261]. Hydrazine was detrimental to citric production but hydroxylamine alloxan and colchicine favored growth and production [262]. The effect of initial starch concentration on citric acid production simulated the Luedeking-Piret type ratio taking residual starch into consideration [263]. Solid state fermentation of wheat bran gave low yields, though supplementation of C,N, P sources along with 3 % MeOH resulted in 13.1 fold increase [264]. Marked decrease was observed with banana extracts in shake culture [265]. Usage of growth factor in the medium to form vigorous stationary phase with immobilized *S. lipolytica* was also done [266]. Kirimura et al, 1992, [267] developed mutants of *A. niger* resistant to 2-deoxy-D-glucose which were of two types depending on growth and carbon utilization. Yang in 1991 [268] produced citric acid from corn starch - wheat bran - peanut oil combination. Of two different inocula of *Y. lipolytica,* one in full growth medium, another in N deficient one, the latter yielded higher citric acid production [269]. Using ion exchange treatment for molasses adding K4[$Fe(CN)_6$] activated with MeOH or peanut oil stimulated the yield. Na_2EDTA and phytin decreased fermentation [270].

Citric acid was manufactured from wheat flour with culture $Co_8$6o, after washing the flour to prepare starch solution, removing excess proteins and incubating with a-amylase [271]. Citric acid yield was independent of dissolved O_2 consumption during initial phase but volume productivity (gm citric acid/L^{-h}) increased sharply with dissolved oxygen concentration. In production phase, yields increased by 50% but productivity similarly decreased due to loss of cell viability under prolonged N deficient conditions [272]. Rugsaseel et al 1993 [273] selected mutants on a modified starch-methyl red agar plate by higher amylolytic activity and acid formation. Their glucoamylase activities were measured. Citric acid alginate immobilized cells were distinctly desensitized to metal ions especially Mn^{2+}, Zn^{2+}, Fe^{2+} or Cu^{2+} but free cells were severely affected. This did not apply if metal ions were added during production phase [274].

Sugarcane pressmud, proved a novel substrate. Potassium ferrocyanide improved the conversion and lowered fermentation time by 24 hr, with *A. niger* [275].

Jin et al [276] bred thermotolerant citrate overproducing strain especially in 20% sweet potato flour medium. Production was possible on cassava meal without nitrogen source [277]. Barnidate coat sugar extract as carbon source and date-seed hydrolysate as nitrogen (amino acid) source were used for fermentation by *Y. lipolytica.* On increasing oxaloacetic acid

concentration in the medium, the citrate yield was also increased [278]. Nguyen et al in 1992 [279] used low concentrations of glucose or corn starch. In the latter case, aeration efficiency and amylase formation were critical factors.

Drsysdale and Mckay (1995) [280] worked on production from inulin, though it was 20-30 % lower than from sucrose. Yields may be improved by air flow. Researchers repeated the fermentation conditions of the production from cheese whey (El-Samragy et al, 1991) [281], Nica & Woinaroschy, 2010)[282], pineapple waste (Kareem et al, 2010)[283], cotton waste (Kiel et al, 1981)[284], pumpkin (Majumder et al, 2010)285], undersized semolina, (Alben and Erkmen, 2004) [286], cassava (Prado et al, 2005) [287], corn starch hydrolyzate (Amenaghawon & Aisien),[288] etc. by *A. niger.* El-Holi & Al-Delaimy in 2003 [289] repeated the production by *A. niger* from whey with sugars and additives. Despite experimenting with newer substrates, molasses and jackfruit bagasse still (Munshi et al, 2012) [290] continue to be the carbon source of choice.

Both showed a high ability to utilize lactose. In 1997, Pera and Callieri [291] reported that Ca^{2+} addition lowered biomass and increased PO_4^{3-} and sucrose uptake resulting in higher production. The cell walls featured vesicles, the cell membrane and many inclusions.

Watanabe et al in 1998 [292] worked on citric acid production from cellulose hydrolysate by a 2-deoxyglucose-resistant mutant strain of *A. niger.* They also worked on improvement of citric acid production by *A. niger* with addition of phytate to beet molasses resulting in 2.4 fold higher production [293]. Roukas [294] for the first time showed carob pod as a new substrate for the acid production by *A. niger.* Specific citric acid production rate was 0.18/g/d and specific sugar uptake rate was 0.358/g/d at an initial sugar concentration of 200 g/L at pH 6.5. He also [295] observed the production of citric acid at different pH with the same substrate. Thangavelu & Murugaiyan in 2011, [296] introduced novelty in citric acid production by *Aspergillus niger* using *Gelidiella acerosa, a marine* alternative to sugar substrate.

The high commercial potentiality of citric acid has eventually been stepped up into intensive interest in the domain of industrial fermentation. A vast amount of research work has been carried out in different countries and the problem has been dealt from diverse angles by different investigators but the imposition of voluntary restrictions governed by secrecy in industrial development and practices operative in highly competitive chemical and pharmaceutical industry has not divulged the intricacies of the technique. Moreover, there prevails a significant lack of uniformity and reproducibility of the methodology. The process parameters and thus the yield differ greatly with each strain of the microbe. Citric acid synthesis has not made considerable progress since its first industrial production (Haq et al, 2002) [297]. Accounting for the economy of the process, surface culture by *Aspergillus niger* on carbohydrate rich inexpensive and readily available substrate of the country is of prime choice. Despite its increasing usage in pharmaceuticals and cosmetics industry, its worldwide production has not increased with time as random mutation affects strain maintenance over generations. It is hoped that biotechnology will aid in unraveling the altered secondary metabolism ongoing in the microbe for its citric acid overproduction and the genetics responsible for it, so that it can be replicated in any strain of that species under similar conditions.

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