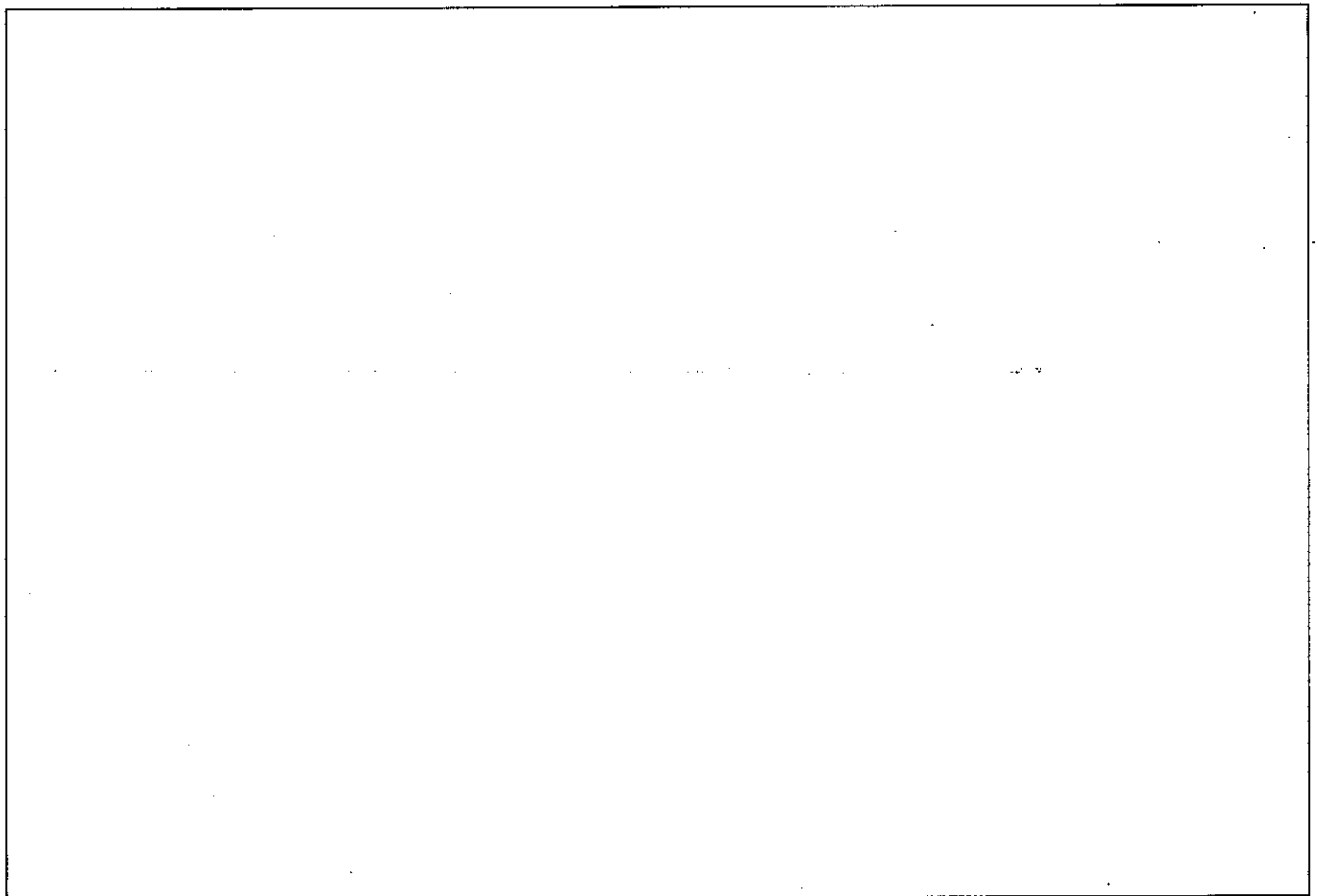


The technical production of citric acid on the basis of molasses

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**Reprint from ChED,
Vol. 2, Issue 9, pp. 309-316
Georg Thieme Verlag, Stuttgart**

Among the biotechnical processes, the alcoholic fermentation for the production of beer and wine is widely known; regarding the less familiar fact that a multitude of products is produced by microbial processes, rich material may be found in literature (only the papers¹⁻⁷ shall be indicated as being associated with the following report); Fig. 2 shows a selection of the variety of raw materials and products of these processes.

Biotechnology⁵⁻⁷ is closely related to biology (microbiology), chemistry (biochemistry) and process engineering. It is based on microorganisms, such as viruses, bacteria, fungi or microalgae, which serve as reaction media⁶ for desired chemical conversions. But also the biomass itself may be the desired product. Biotechnology deals with technical processes and industrial production methods which are based on living cell or cell components.

1. Introduction

Some products can only be produced with difficulties and uneconomically with respect to their chemical composition. To this, the classic chemical synthesis of citric acid shall be indicated for introduction into the theme of this work:

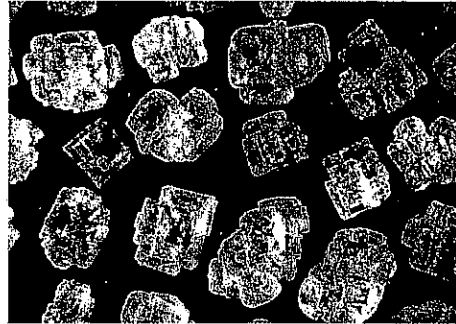
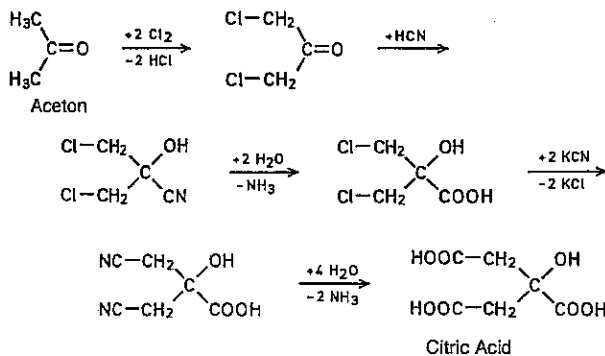
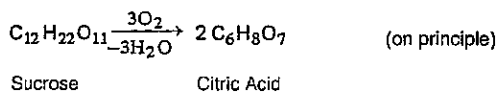


Fig. 1 Citric acid monohydrate (crystals from running production)

Industrial using of microbiological processes resulted in the development of an industrial microbiology. The economic aspect as shown on table 1 — will more and more increase in importance in future⁸. Biotechnical processes are particularly positiv for environment or are used for environment protection (e.g. reconditioning of waste water); the waste products can be used economically in many cases.

The synthesis obviously passes through a series of intermediate stages which partly result in low yields only, and is not an economic process in large-scale production.

Although the biochemical conversion of sucrose to citric acid by a microorganism takes place in a much more complicated way than the summary equation represents, it is — from the point of view of process engineering — only a single-stage process (fermentation): The living cell itself effects the control of the individual part reactions.



2. The Citric Acid

Among the fruit acids⁹ used in the beverage and food industry the citric acid holds a cardinal place; besides, it finds application in the pharmaceutical and generally in the chemical industry (table 2).

Its economic importance has increased in the same measure as the industrial production of this acid became feasible.

Citric acid widely occurs in nature and its name is derived from its most frequent occurrence in citrus fruits, from which Scheele isolated the citric acid by calcium salt in 1784 for the first time. From these fruits — especially from lemon juice which contains 7-9% of citric acid — the acid was technically brought to crystallization after cleaning operations and concentration still to the end of the first quarter of this century. But since more than 30 t of lemons (about 300.000 fruits) had to be pressed for the production of 1 t citric acid, the industrial production was rather limited; only countries as Italy disposing of corresponding raw materials, were able to do this.

Table 1. World production of various biotechnical products⁸ in ton per year

Product	World production (tons per year) rounded resp. estimated
Beer	54 000 000
Wine	28 500 000
Baker's yeast	600 000
Albumen yeast	800 000
Citric acid	200 000
Antibiotics	8 000
Glutamic acid	80 000
Vitamine B ₁₂	3

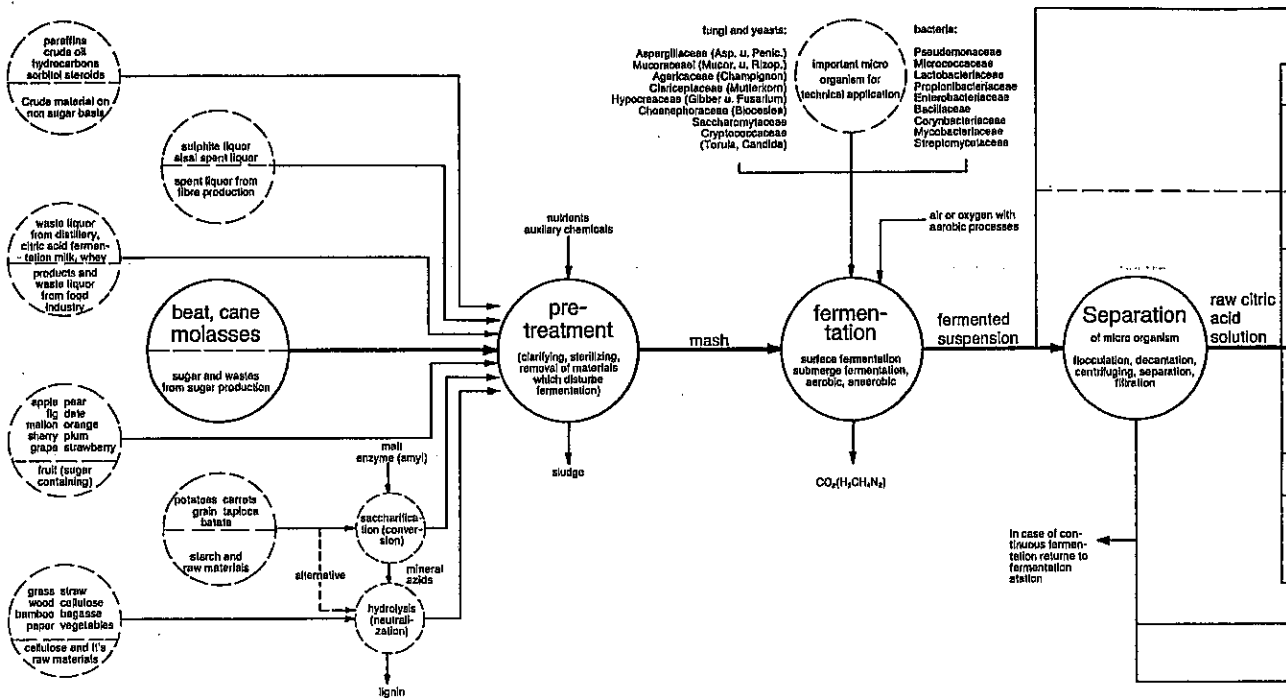


Fig. 2 Flow diagram of biotechnical processes

Table 2. Split up of the use of citric acid

Branch	approx. Percentage (%)	Examples
Food and beverage industry	60	Lemonades, sweets, jellies, ice cream, fruit essences, baking powder, preservatives
Chemical industry	15	Artificial resins, plasticiz., citric acid esters
Textile and leather industry	10	Fining of textiles
Pharmaceutical industry	5	Ferrum citricum, blood preservation, tablets, salve
Metal industry	5	Cleaning of metal surfaces, metal pickling
Others	5	Buffer substance, hair cosmetics, feed additions, rinses

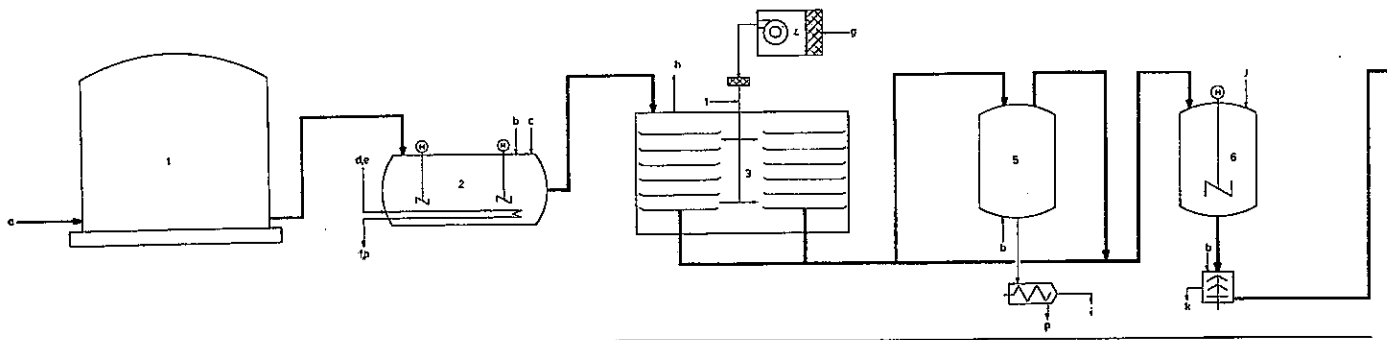
Already towards the end of the last century, Wehmer had discovered the formation of citric acid through certain moulds. When — in the twenties — this discovery could be used on industrial basis, the production of acid from citrus fruits rapidly became insignificant.

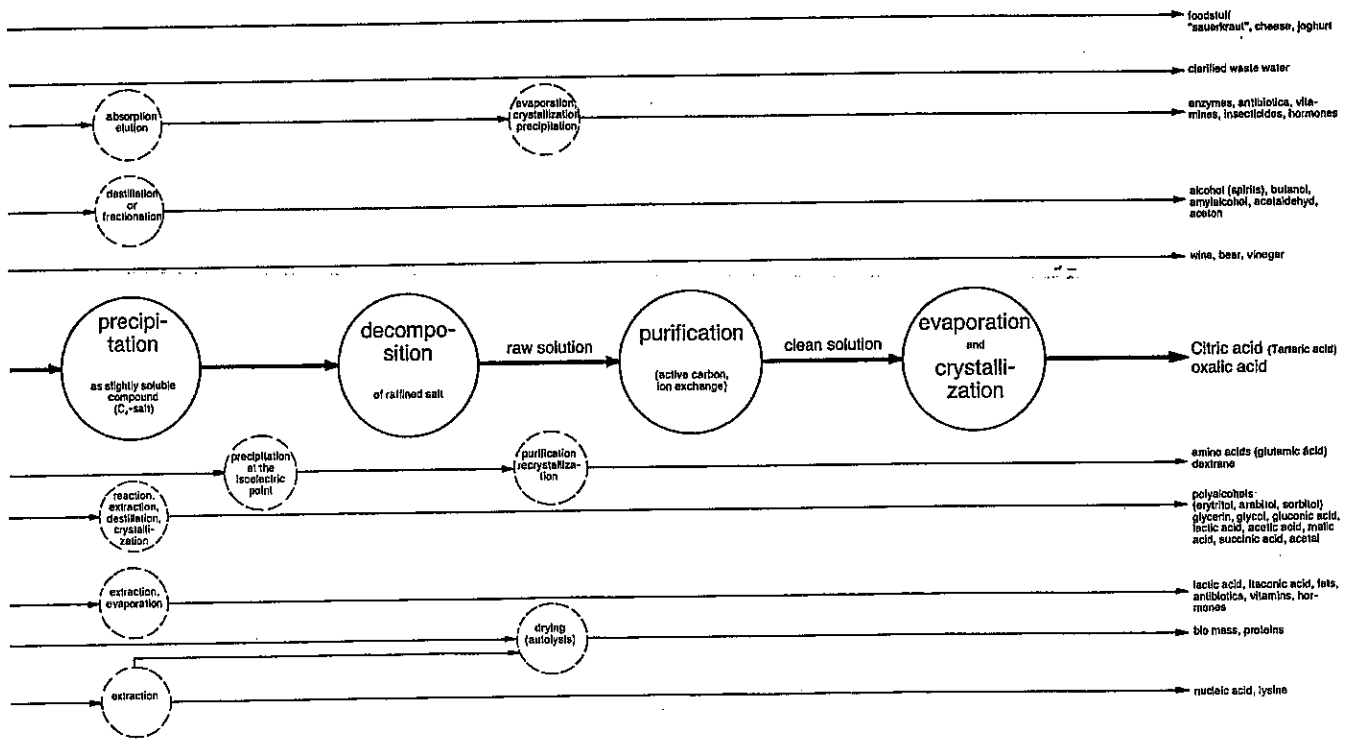
Now, citric acid could be produced by fermentation of carbohydrate-containing nutrient substrates (see Fig. 2). As most suitable substrates have proved best the molasses, saccharine waste products of the sugar industry, which, however, are processed to a variety of products (alcohol, yeast, etc.) but nevertheless are available in excess in many countries thus being an inexpensive raw material basis. For the production of 1 t citric acid, abt. 3 t molasses are required; the use of other carbohydrate-containing substrates, such as sugar itself, is not economical in many cases.

Recently, also processes with hydrocarbons as basic material have been found in literature; however, only insufficient industrial experience are available, compare e.g.¹⁰

Fig. 3. Flow diagram of citric acid production

1 storage of molasses; 2 molasses pre-treatment; 3 fermentation station; 4 air-conditioning station; 5 mycelium washing station; 6 oxalate station; 7 citrate station; 8 decomposition station; 9 activated carbon station; 10 cation exchanger; 11 anion exchanger; 12 evaporation; 13 crystallization; 14 separator; 15 drier; 16 continuous bagging station; a molasses; b utility water; c fermentative additions; d steam; e cooling water; f condensate; g fresh air; h reaction gases; i mycelium; j lime; k Ca-oxalate; l sludge; m H₂SO₄; n CaSO₄·2H₂O; o regenerates; p waste water; q vapours; r ClH₃-solution to be returned; s product; t sporous suspension





Nowadays, more than 90 % of citric acid is produced by fermentation. In 1974, the world production was estimated at more than 200.000 tons annually (compare: 5000 t/a in 1929, 50.000 t/a in 1953).

Nearly 80 % of the world production is effected in plants operating in U.S.A. and Europe. The sizes of plants vary between 10.000 and 30.000 t/a in most cases.

Recently, industrially developing countries have intensified their efforts to get independent of importing this relatively expensive and more and more required chemical (annual rate of increase abt. 7 %) by establishing industrial plants on their own territory. Here, sizes of 2.000 to 6.000 t/a are usual, by which their own requirements can be fulfilled and eventual excesses can be exported to the neighbouring countries.

In view of the prevailing application in food industry, the product must comply with the pharmaceutical standards¹¹. Citric acid normally appears as crystalline product — either in an anhydrous modification ($C_6H_8O_7$) or as monohydrate ($C_6H_8O_7 \cdot H_2O$) with one molecule crystal water. Above 36,5 °C, the monohydrate loses its water molecule; under adequate storage conditions, however, both product shapes are stable.

3. Process description of the biotechnical production of citric acid

The process for the citric acid production of carbohydrate-containing substrates can be divided into two sections:

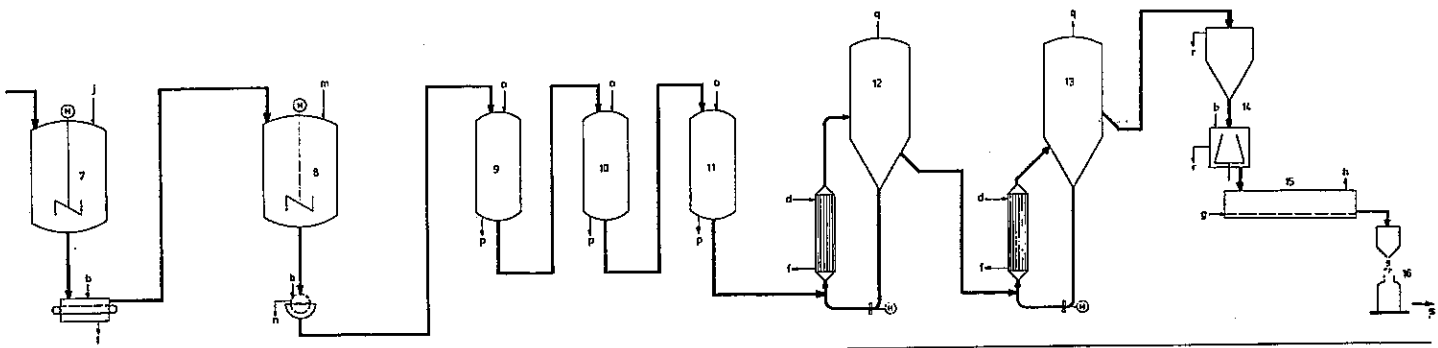
- a) fermentation of raw materials;
- b) reconditioning of the impure fermented solution to pure, mostly crystalline product

Fig. 3 shows the flow of the process as a scheme.

3.1 Fermentation

3.1.1 Raw material

The molasses used for fermentation are distinguished according to their provenance into beet and cane sugar molasses^{12, 13}. Both raw materials are of different composition and therefore require different pre-treatment processes. The sugar content is about 50 %.



Generally, the molasses are transported from the sugar mills by truck, rail or ship to the citric acid factory. To keep down transport costs, the citric acid factory should be situated near the sugar mill. The molasses are stored in big tanks, out of which the highly-viscous medium can be pumped into the plant, as required.

3.1.2 Surface and Submerged Process

At first, the molasses were processed exclusively by surface fermentation: On the surface of the substrate, a mat of mycelium grows after inoculation with spores, this mycelium converting most of the sugar of the molasses to citric acid.

In the fifties, the submerged process has been developed, where the fungus effects conversion within a moved molasses solution.

Nowadays, both processes are applied: The submerged process considered "modern" has the advantage with respect to expenditure on civil works and staff requirements, and is therefore partly applied in Europe and the U.S.A.; the expenses on machinery, however, are higher than with surface fermentation. Moreover, the energy consumption is considerably higher and fermentation itself is more susceptible to faults.

That is the reason why the surface fermentation in total is preferred.

In view of the obtainable yields (i.e. conversion of sugar to citric acid) both processes are to be valued equally. The amounts of yield depend on many factors — such as the species of molasses (origin), the chemical treatment, the filling height of the substrate in the trays, the activity of the microorganism, and the air conditioning.

Stoichiometrically, by conversion of 100 g sucrose with oxygen, 123 g citric acid monohydrate could be obtained; however, the yields obtained in practice are considerably lower, since the mycelium growth and other by-reactions as well (oxalic acid formation) consume sugar. Thus, also part of the carbon from the sucrose is converted to carbon dioxide during fermentation. Fermentation of citric acid is a time-consuming discontinuously operated process. In practice, fermentation will be stopped when prolongation of the fermentation time increases the yield only unessentially.

3.1.3 The microorganism

Prerequisite of high yields is a highly activated microorganism. The mycomycetes of the *Aspergillus niger* species have proved best. The citric acid formation by this fungus is the consequence of incomplete respiration; it therefore runs only to the optimum under particular conditions.

For inoculation of the pre-treated substrate, an aqueous suspension of fungal spores is used. In the fermentation process, this means that the microorganism is continuously required in relatively large quantities. In practice, spores of the strain are transferred onto bigger culture flasks and thus "multiplied" at choice. All process steps require sterile conditions and high care. The covered nutrient substrates produced in laboratories are stored in refrigerating chambers.

It is essential that the performance of the strain is maintained by adequate biological measures (selection, artificial or natural mutation). The figures 4 and 5 show single spores isolated from a selection which have multiplied at incubation and are tested and analysed for their fermentative activity.

Criteria for the selection of these "single spores" which are then cultivated in large-scale, are:

- amount of yield
- fermentation rate
- mycelium weight
- production of by-products

3.1.4 Fermentation of beet molasses according to the surface process

The molasses is diluted with water to a sugar content of 12 to 20 %, and sterilized by heating after a special chemical treatment. The chemical pretreatment depends on the raw material and on the microorganism used; with beet molasses it comprises the addition of potassium hexacyanoferrate (II), a pH-adjustment and the additional dosing of nutrient salts. The required chemical quantities depend on the respective raw material and are to be determined empirically in laboratories.

According to the respective culture, raw material and treatment oxalic acid might be formed as by-product. *Aspergillus niger* strains which bring out high yields of citric acid, generally tend to produce oxalic acid when beet molasses are used.

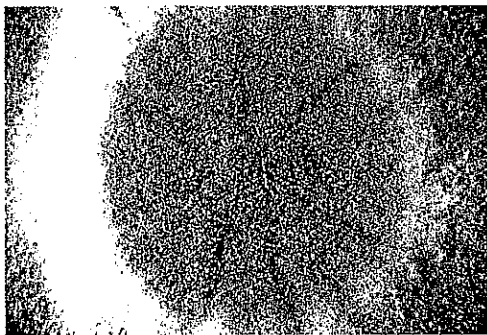


Fig. 4. *Aspergillus niger* (top view on a Petri dish, which was inoculated with a single spore in the centre, after incubation).



Fig. 5. *Aspergillus niger* (lateral view, cut through the spore colony of Fig. 4).

A careful pH-adjustment to the optimum results in high yields in relatively short fermentation time. Besides, it is possible to restrict the oxalic acid production by the addition of chemicals. After cooling down to abt. 40 °C, the sterilized solution is filled into the sterile, shallow trays of the fermentors, and then inoculated with a sporous suspension of the *Aspergillus niger* (Fig. 6). According to the respective plant size, the complete pre-treatment of the molasses is carried out several times either batch-wise or continuously. For the design of the trays, the ratio surface to volume is decisive, since with increasing s/v-ratio, also the conversion rate increases.

The fermentors are to be aerated with particular care, when a thin mycelium layer is formed within 24 hours (germinating period): Along with aeration, there exists danger of infection with foreign germs, which subsequently might result in a complete breakdown of the batch.

In the following period (production period), the mycelium gets stronger, and the temperature of the fermented solution rises; the exothermic citric acid production starts. Simultaneously, the air quantity supplied is increased in order to keep the temperature constant by evaporation of water. The design of the chamber- and air system must correspond with these conditions of temperature regulation and sterility¹⁴. Till the end of fermentation, sugar content and acidity of the solution are controlled.

Fig. 7 shows the rise in yield (referred to the sugar in the molasses) in dependence of the fermentation time. In the germination period no acid is produced; when finally the whole of sugar is converted, a decline of the curve by conversion of the citric acid might be caused.

The fermentation rate depends on many factors, such as fermenting organism, height of substrate in the trays, species of raw material, chemical adjustment and air conditioning.

Cane sugar molasses require a different pretreatment. Possible are either the use of ion exchangers, organic and anorganic constituents being removed, or the differentiated chemical treatment of the molasses solutions.

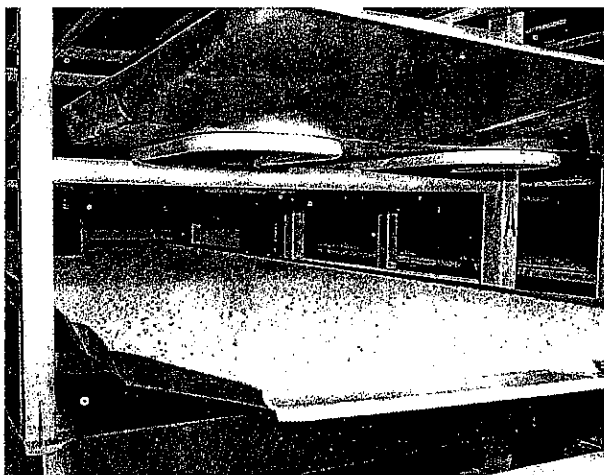


Fig. 6. View into a fermentor (the mycelium is floating on the fermented solution in the tray).

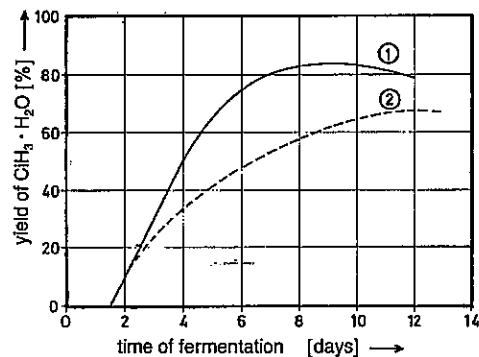


Fig. 7. Citric acid production in dependence of fermentation time; 1 Turkish beet molasses; 2 Brazilian cane sugar molasses

Once the fermentation being terminated, the trays are discharge and the mycelium is isolated from the solution. By water evaporation during fermentation, the volume only amounts to abt. 60 % of the molasses solution used at first; concentration of the acid ranges between 150 and 200 g/l.

The fermentation chambers are to be cleaned afterwards and to be sterilized for the next batch.

3.1.5 Mycelium washing

The sponge-like mycelium separated from the fermented solution still contains considerable citric acid quantities and must be washed to avoid losses. This is to be done most carefully, since by breakage of the cells, substances might penetrate into the solution which raise difficulties in the subsequent cleaning of the crude solution. In order to reduce the high water requirements, a fractional washing is recommended. The washing water joins the fermented solution separated from the mycelium and is conveyed to the reconditioning station.

The mycelium freed from the acid has high proteine contents and is not toxic. After the squeezing and drying, it is therefore an excellent by-food for animals and can be distributed as by-product. According to the respective species of molasses and chemical treatment, 150 - 200 kg dry mycelium per ton of citric acid are formed during fermentation.

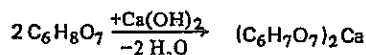
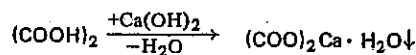
3.2 Reconditioning of the crude acid solution

The solutions out of fermentation are strongly impure. Such impurities have got into resp. have been found in the fermented solution through molasses, the addition of chemicals and finally as metabolites of the fungus and as cell substances as well. Since the final product must comply with the required quality standards, a direct crystallization of the citric acid, however, is not possible, a series of cleaning stages is necessary. By precipitation of the citric acid as calcium citrate from the fermented solution, quantitatively the biggest part of impurity is taken out of the process. This process has proved most efficient.

By such separation only however, the necessary purity grades are not obtainable. In the course of time the purification steps have been improved and brought to an optimum with respect to process engineering; in particular, the point of view of a continuous processing has been taken into consideration.

3.2.1 Separation of solid impurities and of oxalic acid

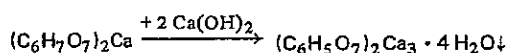
Prior to the citrate precipitation, at first all the non-dissolved impurities and the dissolved oxalic acid are to be extracted from the fermented solution. Oxalic acid is precipitated completely and continuously as calcium oxalate at low pH-values with lime milk; citric acid remains in the solution, since the mono calcium citrate being formed is well soluble:



The non-dissolved impurities and the precipitated oxalic acid are separated from the fermented solution (e.g. in separators). As was already described, the occurring quantity of oxalic acid depends on the adjustment of the molasses prior to fermentation, on the microorganism and on the species of raw material. E.g. the quantity accrued with beet molasses may fluctuate between 50 and 200 kgs oxalic acid per ton $\text{C}_6\text{H}_8\text{O}_7$. In general, the separated Ca-oxalate is stored. A further reconditioning seems, however, expedient at least for higher plant capacities.

3.2.2 Precipitation of calcium citrate

The solution which has been purified from solid matters, is filled into agitator vessels and precipitated with lime milk at higher temperatures and pH-values of abt. 6,5 - 7.



The calcium citrate precipitation is the greatest loss source in the reconditioning process, since tricalcium citrate has a relatively high solubility in fermented solutions. The citrate solubility and thus the source of loss declines with increasing temperature — as is illustrated on Fig. 8. Therefore, the precipitation reaction must be carried out under as hot conditions as possible.

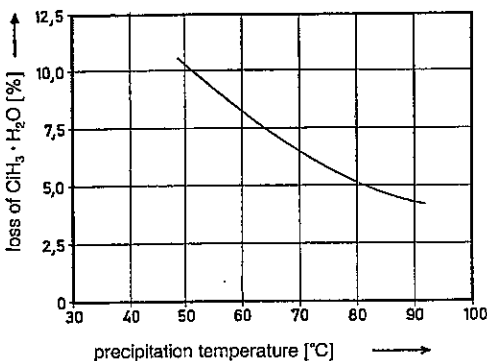


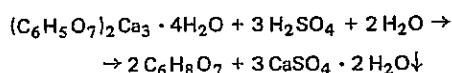
Fig. 8. Loss of citric acid in dependence of the precipitation temperature (temperature of the washing water in all cases 90 °C, pH 6,7)

Apart from the temperature, a number of other factors influence the losses in citrate precipitation: To obtain complete conversion of citric acid to tricalcium citrate, the retention time must be long enough; through as high as possible concentration of $\text{C}_6\text{H}_8\text{O}_7$ in the fermented solution, the solution quantity which is to be separated from the citrate and to be removed from the process, can be kept small.

Especially important is the accurate adjustment of the pH-value. With too high pH-values, the quality of citrate is deteriorated, and the further reconditioning to citric acid is considerably disturbed. With too low pH-values, however, the losses increase perceptibly, since reaction takes place incompletely. Another source of loss is finally the contents of impurities (e.g. MgO) in the lime milk, since those partly form soluble citrates. Therefore the lime to be used must be selected accordingly. After finished reaction, the citrate is separated from the filtrate, e.g. by means of vacuum belt filters. Impurities which still adhere to the citrate are displaced by hot water. The filtrate, the so-called sludge, is rejected out of the process. It contains the non-fermented molasses constituents, and therefore is a valuable by-product for the feeding stuffs industry after a reconditioning procedure by evaporation.

3.2.3 Decomposition of the calcium citrate

The calcium citrate reacts in agitating tanks with diluted sulphuric acid to citric acid and gypsum:



To ensure a complete reaction in this process stage and so to avoid losses, it is run with a little excess of H_2SO_4 . The process control limits this excess to 1 - 2 g/l, in order to prevent from overloading of the subsequent ion exchange station. It is to be paid special attention that during reaction, temperature will not exceed 60 °C to keep colouring into its bounds.

Gypsum and citric acid solution are separated e.g. on vacuum belt or rotary filters, the gypsum being carefully washed. From this process stage, special attention is to be paid to the water consumption, since the water getting into the solution will have to be taken out of process due to the evaporation which will later on become necessary.

A conditioning of the gypsum which accrues in a ratio of abt. 1,4 t $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (dry) per ton $\text{C}_6\text{H}_8\text{O}_7$ is feasible, in most cases, however, dumping is effected.

3.2.4 Decolorization and deionization

The citric acid solution separated on the gypsum filter, contain apart from the indicated H_2SO_4 -excess inorganic impurities — mainly dissolved — and traces of other ions such as Fe^{3+} etc.

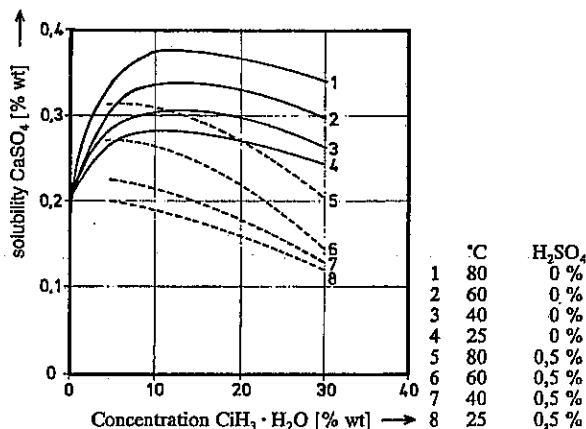


Fig. 9. Solubility of calcium sulphate in citric acid solutions

Fig. 9 shows that the solubility of CaSO_4 is in general higher in $\text{C}_6\text{H}_8\text{O}_7$ -solution than in water. Besides, the solubility depends on concentration: Excessive sulphuric acid diminishes the solubility of CaSO_4 . In practice, the concentration of the solution is between 20 and 25 % $\text{C}_6\text{H}_8\text{O}_7$ by weight.

Remainders still are organic impurities which effect a colouring of the solution.

All the components indicated would contaminate the product to an inadmissible extent. Moreover, $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ would cause undesired incrustations in the evaporation station.

Whilst in former times, the solutions were gradually decolorized batch-wise by addition of activated carbon, and the anorganic impurities were precipitated by reaction with chemicals (in both cases with subsequent laborious filtering processes, e.g. with filter presses), nowadays, the impurities can be removed continuously (Fig. 10): The citric acid is decolorized continuously running through towers which are cascade-connected and filled with activated carbon.

When the activated carbon in one tower is exhausted, the solution to be decolorized is conveyed by changing-over through different towers. The activated carbon charged can be chemical regenerated in the tower itself; this makes unnecessary an expensive thermal regeneration, for which the carbon would have to be removed from the tower.

Hereafter, the decolorized solutions flow through cation and anion exchangers. With a very acid cation exchanger, the cations present in the solution (Ca^{2+} , Fe^{3+} , etc.) are at first exchanged against H^+ ; subsequently, the solution is freed from the still present anions (SO_4^{2-} , Cl^- , etc.) with a medium strong anion exchanger. The resin absorbs the citrate ion which exists in high concentration. The sulphate ions then displace, however, the citrate ions, thus forming a selective separation.

By a similar connection as with the activated carbon towers, charged exchanger towers are taken out of the process and are chemically regenerated. The residues out of regeneration contain salts such as CaCl_2 , Na_2SO_4 in highly diluted form and are abandoned.

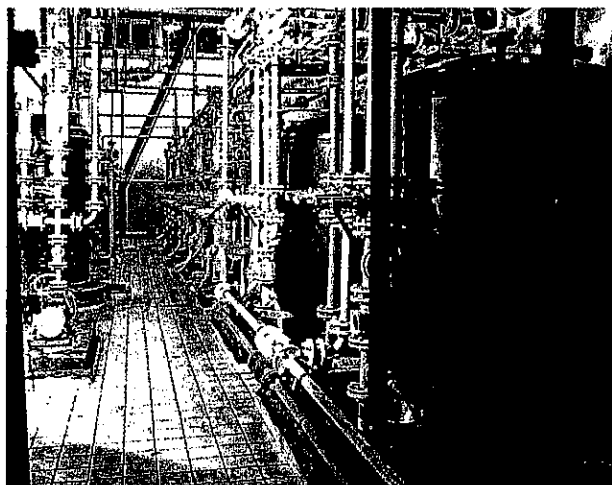


Fig. 10. Cleaning station. Activated carbon filter and ion exchanger

3.2.5 Evaporation and crystallization

The solution coming from the ion exchangers is clear like water and its citric acid concentration normally is abt. 22 % by weight. If the final product citric acid monohydrate shall be produced, crystallization must be carried out at temperatures below the conversion point. When, e.g. a crystallization temperature of 25 °C is chosen, the saturation concentration is abt. 62 % by weight, as is shown on the solubility diagram (Fig. 11). Therefore, the pure citric acid solution is to be evaporated accordingly prior to crystallization.

Evaporation and crystallization take place continuously. Simple control circuits keep down the expense on operation and manual control. Moreover, the expense on machinery and space is considerably lower compared with the discontinuous method with repeated recrystallization, which is still often applied.

The diluted solution coming from the ion exchangers is fed into the first stage of a 2-stage vacuum evaporation (Fig. 12). For the feeding of heating steam, this 2-stage type is suitably used for saving live steam. The waste steam of the first stage is conveyed into the exterior heat exchanger of the second stage. Normally, evaporator types with forced circulation are used.

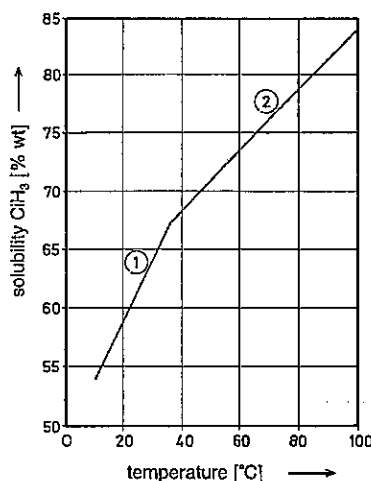


Fig. 11. Solubility of citric acid in water in dependence of the temperature
2 solid phase $\text{C}_6\text{H}_8\text{O}_7$
1 solid phase $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$

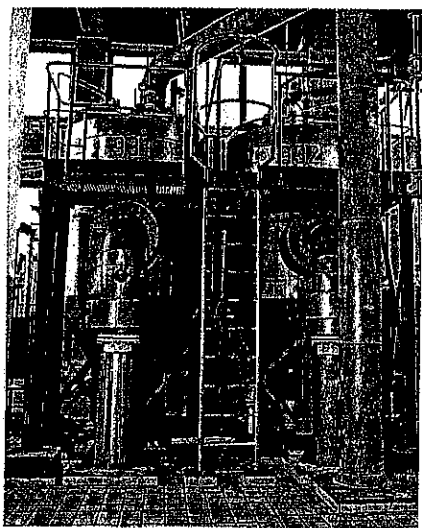


Fig. 12. Two-stage evaporation plant

From the first stage, the concentrated solution is fed into the colder second stage, where it is evaporated to a nearly saturated state.

In order to avoid caramelisation, the evaporation is to be so designed that the solution in the various stages is not exposed to high temperatures and long retention times; a colouring of the solution hinders the production of a white final product.

The concentrate leaving the second stage of evaporation is continuously fed into an evaporation crystallizer (with forced circulation of the crystal suspension) for the production of $C_6H_8O_7 \cdot H_2O$ -crystals. Through the solution from evaporation, whose temperature is higher than the crystallization temperature and additionally through the heat exchanger in the circulation line, heat is supplied to the circulating suspension. Thereby, the suspension reaches a higher temperature than corresponds with the vacuum formed in the crystallizer: the suspension is overheated.

This overheating is reduced in the crystallizer by evaporation of water on the surface of the solution. The supersaturation of citric acid obtained by evaporation of water forms the driving force for crystallization of citric acid. Through controlled heat supply, evaporation and circulation, a stable operation and thus controlled crystallization is possible, through simple regulation of the flow rate, the continuity of crystallization is ensured.

3.2.6 Separation and drying

In the same measure as solution is fed, crystallized suspension leaves the crystallizer and gets into the separation station. Here, the crystallized product is separated from part of the solution by sedimentation in a pre-thickener. The concentrated suspension is then supplied into a continuously working centrifuge — e.g. a sieve centrifuge — which separates the crystallized product from the solution to a few percent by weight.

The separated solutions are reconveyed to the process. The humid product is dried in suitable machines — e.g. in fluid bed driers — in consideration of the conversion temperature, and then conveyed to the bagging machine.

3.2.7 The product

The crystallized product is colourless and has a pleasant acid taste. In most cases, it enters the market in units which are usual in trade — e.g. in 50-kg paper bags with polyethylene insert. Fig. 1 illustrates the crystalline structure of $C_6H_8O_7 \cdot H_2O$, whose contents of impurities is limited in virtue of the high quality demands.

Conclusion

All over the world, the need of citric acid in the miscellaneous branches has in the passed years brought into prominence the question of production and conditioning of the acid. Nowadays, this fruit acid is almost exclusively produced by fermentation. Although the principles have already been discovered in the passed century, the investigation of detailed problems still required a considerable time. The "know-how" of these particularities today enable the plant constructor to establish and the users of such equipments to be masters of the overall process¹⁴.

published in: Chem. Exp. Didakt. 2, 309-316 (1976)
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Bibliography

- ¹ H. J. Rehm in: Ullmanns Encyklopädie der technischen Chemie, Vol. 8. 4th Edition, P. 521-524. Verlag Chemie, Weinheim, 1974
- ² K. Buchta, Chemiker-Ztg. 98, 532/538 (1974)
- ³ W. Dimmling, CZ-Chemie-Techn. 2, 425/429 (1973)
- ⁴ G. Neseemann u. W. Dimmling, Chemiker-Ztg. 98, 523/532 (1974)
- ⁵ H. J. Rehm: Industrielle Mikrobiologie. Springer Verlag, Berlin - Heidelberg - New York 1967. — Einführung in die industrielle Mikrobiologie. Springer Verlag, Berlin 1971
- ⁶ H. Kretzschmar: Technische Mikrobiologie. Parey, Berlin - Hamburg 1968
- ⁷ An extensive bibliography is found under the catchword "Mikrobiologie" in *Römpps Chemie-Lexikon*. 7th Edition., Franckh'sche Verlagshandlung, Stuttgart 1974 (pages 2166/2167 in particular)
- ⁸ Biotechnologie — Eine Studie über Forschung und Entwicklung, elaborated on behalf on the Bundesministerium für Forschung und Technologie, Frankfurt a. M. 1974.
- ⁹ H. Rudy: Fruchtsäuren. Dr. Alfred Hützig Verlag, Heidelberg 1967
- ¹⁰ G. Schulz u. J. Rauch in: Ullmanns Encyklopädie der technischen Chemie (see ¹), volume 9; p. 624/636
- ¹¹ compare e.g. Deutsches Arzneibuch, 7th Edition, Stuttgart 1968
British Pharmacopoeia, London 1973
- ¹² J. Kovats u. Z. Niestrawski, Branntweinwirtschaft 113, 373/381 (1973)
- ¹³ H. Olbricht: Die Melasse. Institut für Gärungsgewerbe, Berlin 1956
- ¹⁴ R. Schmitz, Chemie-Technik 5, 271/272 (1976) (a further report for this journal on the technological details is being prepared)