Citric acid bioproduction: the technological innovation change

Citric acid bioproduction~~,~~: ~~a patent review~~the technological innovation change

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Abstract

~~Considering its several application fields, t~~The citric acid is considered one of the most valuable weak organic acid on the market and its production by biotechnological approaches is a very interesting topic. Despite the related scientific research, the literature still lacks a state of art of the technological innovation change, necessary for the ~~identification~~ study of the inventions designed for the real scale implementation. In this context, the present review took into account more than 100 worldwide patents (1929-2018~~9~~), necessary for the identification of the innovative markets and the most promising fields for the economic investments. ~~To fill this gap, the present review took into account more than 100 worldwide patents (1929-2019).~~ The deepened study identified an increasing invention number, combined with the current worldwide citric acid export flows, ~~confirmed the~~with China as the leader (with an economic contribution of 75%, in 2017). In order to satisfy the requests of the market which has moved towards a circular economy, the possibility to use waste substrate represents one of the main options considered in the recent patents. ~~Furthermore, the new technology study identified the most critical aspects on which the innovation has invested: the alternative substrates, mainly scraps, and the~~The discussion highlights the sustainability improvement, achieved by the conversion ~~towards~~ from a submerged technology to a solid-state fermentation (*koji* process).~~These advancements could increase the process sustainability combining the waste exploitation with a reduction of both the consumptions and the wastewater production, in agreement with the circular economy pillars.~~ The showed results are essential for both a scientific audience and the stakeholders citric acid production, in order to have a complete and updated overview about this topic.

Keywords: citric acid; patent~~ review~~; technological evolution; bio-production; *Aspergillus niger*; fermentation~~; innovative substrates~~

1. Introduction

The citric acid (CA) is~~ considered~~ the most valuable weak organic acid~~and~~, widely used ~~weak organic acid~~ on the market for many applications [1]. ~~Initially crystallized from lemon juice, citric acid~~It is a tricarboxylic acid with an essential role for the metabolism of aerobic organisms [2]~~[2], [3]~~. Studies about its production dates back many years when it was ~~Its production is one of the most thoroughly studied and it dates back many years,~~ crystallized from lemon juice (18th century). ~~thanks to the several applications, as confirmed by Curie in 1916, which describes a filamentous fungal fermentation process [3].~~ Currently, the ~~citric acid~~CA biotechnological production is mainly located in China and ~~, with amounts that have increased from 0.5 to~~ 2 million tonnes of product for year are expected to~~, in the last twenty years~~2020 [2]~~, a quantity that far exceeds the production of every other organic acid made by fermentation~~. The use~~s~~ of this agent includes ~~different~~ several fields, ~~like:~~including food, textile, chemical and pharmaceutical industries [3]. ~~More in detail, about 70% of the citric acid on the market is used for food and beverage products, of which the 50% of the total for carbonated beverages [4]. This percentage is further growing for the expansion of the developing countries markets. On the other hand, pharmaceutical industry employs citric acid as a preservative for stored blood, tablets, ointments, and cosmetic preparations [5]. In the chemical sector, it is used as an antifoam agent and for the textile treatment.~~

Nowadays, ~~almost the entire~~the experimental world production ~~is manufactured using~~of ~~citric acid~~CA mainly involves fungi and yeasts: *Aspergillus niger, A. wentii, A. clavatus, Penicillium luteum, P. citrinum, Mucor piriformis, Candida guilliermondii, Saccharomycopsis lipolytica, Trichoderma viride* and *Arthrobacter parafineus*. However, only *A. niger* and the closely related strain of *A. wentii* are chosen for the commercial production [4]. Overall, ~~Two main phases characterize~~ the processes include~~:~~ the previous fermentation ~~, which needs high productivity and~~

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yield values, anfollowed bydthe consecutive recovery and purification. The main critical parameters for the fermentation are: the high carbohydrate concentration, the maintenance of high dissolved oxygen, the constant agitation and the low pH value [5]–[9]. As concern the further recovery and purification steps, the most common approaches are On the other hand, two common processes for citric acid recovery and purification are: the limeCaCO$_3$/sulphuric acidH$_2$SO$_4$ precipitation (with calcium hydroxide) [10]–[12] and the liquid solvent extraction (by a solvent mixture) [10]. Both options are characterized by a preliminary filtration or centrifugation for the separation of the fermentation liquor and the solid biomass. In the lime/sulphuric acid process, the fermentation liquor is treated by calcium hydroxide for the calcium citrate precipitation. The filtered product is washed for the impurity elimination and dissolved with sulphuric acid. The produced insoluble calcium sulphate is separated from the citric acid solution, which is deionised and concentrated by crystallization to form both anhydrous and monohydrated citric acid [12], [13]. The main process weakness is the production of calcium sulphate, as by-product, with criticalities due to its disposal. On the other hand, the liquid solvent extraction needs a counter-current set-up and it uses a mixture of tri-laurylamine, n-octanol and decane or undecane to extract citric acid from the fermentation broth. This step is followed by the citric acid extraction/back-extraction by water, at high temperature [13], [14]. OtherwiseAlternatively, Ledakowicz et al. (2004) describe the an anion-exchange advanced method can be used for the citric acid recovery from aqueous solution using aby tertiary amine resin, followed by thermal desorption [15].

In this context, the knowledge of the *A. niger* metabolism is an essential step to improve the citric acid fermentation processes and the literature has exhaustively reviewed the related mechanisms, summarized in Figure 1 [4], [15]–[18]. The production of citrate from glucose or sucrose involves many enzymatic steps occurring in two different membrane-bound cellular compartments: the cytosol and the mitochondrion. Glucose is transported into the cell and converted to the three-carbon acid, pyruvate, via the glycolytic pathway in the cytosol. The decarboxylation of one

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~~molecule of pyruvate forms acetyl-CoA by the mitochondrial pyruvate dehydrogenase complex and another is carboxylated to oxaloacetate in the cytosol by pyruvate carboxylase. The oxaloacetate must be taken into the mitochondrion (via malate) and condensed with the acetyl-CoA to form citrate. The product is transported out of the mitochondrion and finally out of the cell [10].~~

Considering the ~~strong interest for~~relevance of the ~~citric acid~~CA for the market, the recent literature has widely discussed ~~about the citric acid~~its production by fungi, mainly *A. niger*, reporting several substrates and operative conditions [16]–[20]. ~~In this regard, m~~Many reviews have deepened~~ned~~ ~~the acid production~~this topic~~, often~~, focusing on specific aspects as~~:~~ the fermentation variables [21]–[24] and the characteristics of the solid--state fermentation [25]–[27]. Recent studies has focused on the possibility of a waste ~~material~~ use as substrate [23], [28], following the circular economy principles~~, the solid stat fermentation [23] [25] and the fermentation variables, more generally [16], [28], [29]~~. Nevertheless, the scientific literature ~~still lacks~~should be integrated by ~~works about~~ the state of art of the technological innovation change, ~~represented by the registered patents,~~ able to create an overview of the inventions designed for the application in ~~a~~ real scale. ~~Currently, t~~This relevant aspect~~the relevance of this kind of information~~, represented by the registered patents, was already highlighted in other application fields (e.g. waste recycling or medical applications) ~~, confirming the possibility~~ to identify the most promising technologies ~~useful~~ for the creation of innovative markets [29]–[33].

~~In order t~~To fill ~~in~~ the gap, the present work~~,~~ (updated to March 2019~~,~~) analyzes 100 patents, between 1929 and 2018, showing the technological evolution of the last decades. The free access Espacenet platform was used as the main information source [34] using the keywords, for the patent search: “citric acid bioproduction”, “citric acid *Aspergillus niger*”, “citric acid *Candida*”, “citric acid *Penicillium*”, “citric acid *Aspergillus*”. This information source, created by the

European patent office, ensuresd a worldwide invention overview [29]. In order to simplify the review reading, the included inventions are organized following the roadmap in Figure 2.

2. *A. niger*, Sugardifferent methods to use raw material- as carbon source

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The main conditions with an effect on Tthe citric acid bioproduction by *A. niger* is influenced by several suitable conditions, mainlyare: low pH, high dissolved oxygen and high sugar concentration [10], [34]. This *microorganism*A. *niger* uses many takes advantage of different substrates for its growth (i.e. maltose, mannose, galactose, fructose, sucrose and glucose).. Nevertheless, glucose and sucrose produce the best results showing both (both the greatest growth and the highest citric acidCA productioproduction)n rate with glucose and sucrose, with a concentration of 10-14% (w/v) [35], [36]. Overall, Tthe fungal fermentation can be carried out by three different fermentation techniques: submerged fermentation, surface fermentation (liquid surface culture) and solid-state fermentation (*koji* process) [27], [37], [38]. Nevertheless, the highest patent number (Figure 1A) identifies the first method as the most common, as also confirmed by the literature which reports around 80% of the world production used theby firsthis approach [21], [39], as also confirmed by the significant number of related patents (Figure 3).

2.1 Immobilization method

The fungal immobilization method was used to improves the citric acidCA production by *A. niger*. The fungal was embedded by calcium alginate to reduce the contact with the produced citric acidCA, decreasing the acid toxicity on the metabolism. This effect is due to the citrate, a strong inhibitor of the glycolytic enzymes 6-phosphofructokinase , a glycolytic enzymes [40], [41]. The patent CN102864184 describes the use of an optimal medium composed of sucrose at with a concentration of 120 g/L, at 30°C and 200 rpm [42]. A similar approach is reported in the patent CN107022541, which involves a pretreated fibrous material, as immobilization medium. An air sparging system improves the citric acid production, with a final yield of 93% (expressed as citric acidCA yield/glucose consumed), a citric acidCA concentration of 898.65 g/L and a fermentation

rate of 1~~.06~~ g/L-·h [43]. The immobilization method allows multiple advantages: the ~~increase of the citric acid~~ production efficiency increase, the ~~reduction of the~~ fermentation time reduction, the decrease of the biomass and the fermentation broth amount and a simplified separation between the ~~final~~ product and the mycelia.

2.2 Multiple step processes

The fungal growths using a multiple step process is an alternative technique ~~to~~ for the production increase ~~the citric acid production~~. ~~In this regard, t~~The invention CN107815421 ~~discloses~~ describes a method where *A. niger* is cultivated ~~by~~ in three different growth phases to prevent the criticality of the spore aggregation, allowing both the highest quantity and a size uniformity of the mycelium pellets.~~;~~ ~~thereafter t~~After this preliminary step, the matured seem liquor is transferred to the final fermentation tank. ~~This approach solves the spore aggregation issue, permitting the highest quantity and size uniformity of the mycelium pellets.~~ Consequently, the glucose is metabolized by the fungal and the conversion rate is improved, with ~~the reduction of the~~lower fermentation time [44]. On the other hand, the inventions CN106868061, CN102181490 describe a method which includes a first step ~~where~~ of *A. niger* ~~grows~~ growth by CO_2 gas sparging~~with CO_2 sparging in a fermentation tank~~. When the fungal biomass is grown, it is transferred to a second section where fungi continue the ~~citric acid~~CA production ~~until~~ decreasing the reducing sugar content ~~of reducing sugar is lower than~~up to 1-3 g/L [45], [46]. ~~Overall, this approach~~These treatments ~~allows both higher~~increase the conversion rate and they reduce~~lower~~ the residual sugar-, ~~than~~compa~~e~~red to the traditional ~~treatments~~approaches. The pH shifting, during the acid production, is proposed ~~An alternative, showed~~ in patents CN104277978, CN102851330~~, includes the solution pH shift during the acid production~~. During the preliminary phase, the pH is ~~maintained between~~ 6.2~~- and~~ 7.2, then its rise increases the production rate [47], [48]. As ~~reported~~ explained in the invention CN102373242, the temperature ~~is another variable which~~modification improves ~~could be modified during~~ the ~~citric acid~~CA synthesis. More in detail, a 35-45°C range is selected during the rise

phase of *A. niger* respiratory quotient and growth; thereafter, the value is reduced~~, up~~ to 30-40°C, during the second step, when the biomass is constant and the respiratory quotient decreases. ~~The~~An additional ~~temperature~~ increase ~~s~~(35-45°C) ~~again~~ is necessary in the last step ~~(35-45°C)~~, when the sugar content is very low [49]. The fungi age change ~~could~~ affects the process efficiency, as reported in patents CN104099253, CN104087624. *A. niger* grows in ~~the~~ a first medium to obtain a mature seed solution; part of the resulting dispersed mycelium is transferred to a fermentation medium (5~~%~~-15% of inoculum concentration), when it reached the maximum growth rate. This second step finishes when the concentration of the reducing sugar ~~concentration~~ in the fermentation medium is lower than 0.5%. The use of the dispersed mycelium seed solution from the second step avoids the repetition of the first step with a consequent continuous production of ~~citric acid~~CA [50], [51]. A different method is described in the invention CN102443611, characterized by the addition (in the same fermentation tank) -of a monosaccharide with 6 carbon atoms to the fermentation liquid, ~~starting~~ from 24 h~~ours~~ after the *A. niger* inoculum to 5 h~~ours~~ before the fermentation conclusion [52]~~. The main achieved advantage is the possibility to use one fermentation tank, thanks to the continuous monosaccharide addition~~.

2.3 Metal ~~utilization~~use

The metal ions have an essential role in the ~~citric acid~~CA biosynthesis ~~The~~ by *A. niger*. Nevertheless, the identification of the best concentration is necessary, since high concentrations, at uncorrected conditions, can be translated into a low efficiency production [10], [53]~~(Christian and Rohr 1986; Magnuson and Lasure 2004) requires a variety of metal ions, at low concentrations, for the citric acid biosynthesis. Too high concentrations could be translated into a low efficiency production, nevertheless, the selection of the best conditions can significantly improve the synthesis, as described in several patents (Christian and Rohr 1986; Magnuson and Lasure 2004)~~. ~~Therefore~~In this regard, the invention US5532148, includes the ~~manganese~~ Mn (II) ~~use~~addition, with a concentration ~~between~~ of 2.5~~- and~~ 20 ppb, at pH 1.5-3.0, achieving the highest ~~citric acid~~CA

production between 4 and 7 days of fermentation~~ days~~. The further~~Thereafter, the~~ broth ~~is~~ decolorize~~d~~ation and ion-exchange~~d~~, ~~to~~ allow the ~~remove the color and the inorganic ions with the~~ production of an aqueous ~~citric acid~~CA solution, with a purity around 98% [54]. ~~As an alternative to the~~Alternatively, ~~manganese~~Mn, ~~zinc~~ Zn (30-250 ppm), ~~[hexacyanoferrate ions~~$Fe(CN)_6]^{-4}$ (100-500 ppm) and ~~copper~~ Cu ~~proved to be useful to~~ increase the acid production, at pH 1.5-2.0~~pH values between 1.5 and 2.0~~, ~~as confirmed by~~in patents~~:~~ US5081025, US3936352, GB1392942, GB1342311 [55]–[58].

2.4 ~~Other~~ *Alternative techniques*

Additional strategies have been optimized ~~Alternative methods, which include different approaches, have been studied~~ to increase the ~~citric acid~~CA production by A. *niger*. ~~In this regard,~~ CN105586366, CN103497977 patents describe ~~the possibility of the~~a stirring speed increase during the ~~first~~ preliminary 6-20 h~~ours~~ of incubation. This condition allows~~,~~ the hypha shape changes with the formation of small and compact pellets, able to increase the oxygen exchange capacity, with the consequent improvement of~~, improving~~ the acid synthesis [59], [60]. On the other hand, CN103695319 patent ~~explains the~~ uses a sugar solution with neutralization wastewater to replace the traditional tap water [61]. The possibility of a waste stream use~~,~~ as ~~nitrogen~~ N and ~~phosphorous~~ P source, ~~produces a double advantage: a process~~combines a significant cost reduction ~~and a~~with the decrease of the environmental load~~.~~ for the wastewater treatment.

Overall, a multi-~~-~~step process represents the main option chosen for the improvement of ~~citric acid~~CA production by *A. niger* with raw materials, as carbon sources. Around 50% of the patents in this section, referred to the last decade, confirmed an efficiency increase achieved by process suitable for the scale-up. On the other hand, the metal use is taken into account in old inventions, due to the high criticality of the element concentration which can decrease the process effectiveness [22], [62], [63].

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3. „*A. niger*, food and Agriculture waste as carbon source

3. Food and Agriculture waste as carbon source

The possible substitution of the traditional glucose (or other sugars) by food and agriculture waste, as carbon source for the fungal metabolism, represents an interesting topic since 1920 , as confirmed by the related patents (Figure 41B2). In this regard, tThe present paragraph takes into accountfocuses on the main streams considered for the technical innovation in this field.

3.1 *Corns as carbon source*

Several kinds of corn have been used, as carbon source, to produce citric acidCA by *A. niger*, mainly: corn cobs, corn wheat, bran, soy, sorghum, corn sugar, wheat straw and rice. Usually, the substrates need a pretreatment to make the cellulose bio-available for the fungal metabolism. Patents CN106119306, CN105524951 In this regard,describe a sulfuric acidH_2SO_4 washing (for 1-3.5 hours), followed by the addition of an enzyme (e.g. cellulase or cellobiase) is used as corn pretreatment, in patents CN106119306, CN105524951, to provide the hydrolysis and the conversion of cellulose into glucose. At the end of this reactionAt the end of this pretreatment, the fugal *A. niger* is inoculated and the fermentation starts the citric acid production [64], [65]. On the other hand, the inventions CN103710397 and CN107815475 describe report a corn liquefied solution use, which needs. In the first patent, the substrate is treated with the preliminary addition of βbeta-cyclodextrin (pH 6.0-6.8 and 15-17% (w/v) of sugar concentration between 15% and 17% w/v) and then sterilized to make it suitable for the fermentation. Thereafter, the fungal is inoculated and the fermentation is carried out at 35-37°C, ensuring the oxygen supply and the necessary inorganic nitrogenN amount of inorganic nitrogen [66]. The two A two-stage fermentation is

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~~described in the second invention. The~~ stages fermentation process~~-~~, in the second invention, is conducted in a ~~fermentation~~ cylinder (16-24 h~~ours~~)~~, where the fungal spores are added to sterile water and the fermentation is carried out for 16-24 hours~~. The first step starts after the corn liquefied addition and it carries out for 2-8 h~~ours~~, at 35-39°C. In the second one a sucrose solution acts as supplementary carbon source and the citrate production continues until the sugar concentration is lower than 5 g/L. This multiply step design allows to increase both the pH and the nutrient concentration with a positive effect on the fungal growth [67]. The possibility of a cornstarch use avoids the pretreatment. More in detail, the ~~fermentation~~ medium described in the patent CN102864182, is prepared as following: cornstarch (20% ~~(~~w/v)~~ of cornstarch~~, $(NH_4)_2SO_4$ (0.2% w/v), KH_2PO_4 (0.2% w/v), $MgSO_4 \cdot 7H_2O$ (0.05% w/v) and ~~methanol~~ MeOH (4% w/v). The fermentation is carried out at: pH 3.0, 30°C, 200 rpm, for 7 days [68]. Alternatively, the invention RU267614 combines the addition of the nutrients to the cornstarch, with the change of both the mixing speed and the aeration rate during the fermentation~~ period~~. ~~. More in detail d~~During the growth phase, the two parameters increase from 120 rpm and ~~8~~480 L/h~~min~~ of the first 6 h~~ours~~, to 250 rpm and ~~32~~1920 L/~~min~~h ~~in~~ of the remaining 18 h~~ours. On the other hand, during the deep fermentation period, the stirring and the aeration conditions rise from 250 rpm and 24-40 L/min of the first day, to 300 rpm and 32-48 L/min of~~n the second one day to reach 400 rpm and 40-56 L/min until at the process conclusion~~ [69]. Another waste carbon source which avoids the pretreatment is ~~Even~~ the corn sugar~~ does not require further process, before the fermentation~~. ~~In this regard, t~~The inventions GB738940, GB742972, CN102851328 describe the ~~citric acid~~CA production by the ~~nitrogen~~ N source addition~~, in ammonia gas form,~~ combined with further salts (i.~~-~~e. KH_2PO_4, $MgSO_4$, morpholine, $CaCl_2$, MoO_3, ~~zinc~~Zn), adjusting the pH ~~value~~ at 2.6 to stimulate the acid synthesis [70]–[72]. On the other hand, the dry corn powder needs a treatment before the fermentation, as described in the invention CN101555497. A water addition produces a paste, which is mixed with amylase and heated at 92-98°C, for a first step, and at 88-90°C, for a second one, maintaining the liquefaction for ~~90-120 minutes~~1 h. About the 90% of the resulting

solution is filtered and used for the inoculation (at 38°C), after the inorganic ~~nitrogen~~ N addition ~~and the sterilization~~ [73]. Alternatively, ~~if bran is chosen as substrate to produce citric acid,~~ *A. niger* is previously grown in a glucose solution for 24-48 h~~ours~~, at 35-38°C and 150-300 rpm. ~~;~~ ~~t~~Thereafter, it is transferred in a seed tank with the bran medium and it is cultured at 36-38°C, for 4-6 days. After the adaptation to the new substrate, it is ~~transferred to a new tank~~mixed with a bran solution ~~to start~~for the fermentation ~~step~~, as described in the patents CN103667372, CN102649971 [74], [75]. Soybean molasses or sorghum powder are chosen as organic source in the inventions CN103614421, CN102864185, without a pretreatment. In both cases the selected conditions are: 26% (w/v) of sugar content, 35°C, pH 4.5, time 4 days and ventilating to supply oxygen [76], [77]. On the other hand, the wheat straw (patent CN1884563) and the rice grain (patent CN1693470) require a preliminary enzyme addition (e.g. cellulase or α~~alfa~~-amilase), at the concentration of 20 units/~~ per~~ substrate gram. The further hydrolysis is conducted at the 40-45°C for 40-80 h~~ours~~, followed by a solution filtration ~~and sterilization,~~ and the fungal inoculation [78], [79].

3.2 Fruit and lignocelluloses as carbon source

The possibility of the ~~-~~kiwi use, as organic source ~~a fruit use~~ is explained in the patent CN105671093, which produces ~~citric acid~~CA by *A. niger* by ~~kiwi as organic source, using~~ a solid-state fermentation set-up. ~~-~~The ~~kiwi~~ fruit ~~offcuts~~ residues are washed and dried, at low temperature, to obtain a water content lower than 6%. Thereafter, the ~~fruit~~ product is ~~cut to achieve a powder where~~pulverized and it is used for the fungal ~~is~~ inoculat~~ed~~ion.~~; t~~ The fermentation starts at 27-33°C and it carries on for 4-6 days~~. At the end of the fungal metabolism, the resulting citric acid is extracted and recovered as a crystal salt~~ [80]. Further patents use grape pomace (US4791058), apple pomace (US4767705) or other kind of fruit (GB302338). In these cases, pretreatments are not required since the glucose is available for the ~~fungal~~ metabolism [81]–[83]. On the other hand, the orange peel, ~~showed~~ in the invention KR930001261, ~~needs~~ is ~~drying~~ dried,~~and~~ ~~pulverization~~ pulverized ~~(to obtain a 15-30 mesh powder), followed by the addition of:~~and mixed with water,

~~ammonium nitrate~~NH_4NO_3 and ~~magnesium sulfate~~$MgSO_4$ ~~(~~to stimulate the fungal fermentation~~)~~. After a pH adjustment ~~up to~~at 4.5~~, and a sterilization~~. ~~At the end of these steps,~~ the submerged fermentation starts at 10-30°C, for 3 days [84]. With the same aim, ~~A~~a pretreatment is ~~necessary~~ carried out on ~~also for~~ the lignocellulose ~~raw materials (as~~ described by CN104805136.~~)~~ ~~to convert the cellulose in glucose~~ thanks to the enzymatic hydrolysis by the enzyme cellulase [85]. Patent CN105506004 uses ~~P~~pulverized konjak~~ is used as carbon source in patent CN105506004~~, obtaining a ~~citric acid~~CA production ~~with a final yields~~ of 150 g/L [86].

3.3 Starchy materials as carbon source

Starchy materials ~~The~~ need a preparation to be used as substrate~~of the substrate is necessary when starchy materials are used as carbon source for *A. niger* metabolism, as reported in the present section~~. ~~In this regard, t~~The invention CN104232699 ~~provides~~ suggests a preliminary solid fraction removal~~solid and liquid separation,~~, followed by the addition of the *A. niger,* previously cultivated in a ~~second~~ broth with untreated starchy material, for the fungal adaptation [87]. ~~As an alternative~~Alternatively, the techniques~~ presented~~ in patents CN103290070, CN103146769 and CN102839203 requires an ~~water supply~~ α~~alpha~~-amylase supply and a high temperature to form a mixed slurry~~;~~. ~~a liquefied liquid is obtained by the continuous injections liquefying technology at 70-85°C.~~ After a solid/liquid separation, the resulting solution is used for the ~~citric~~ acid production, and the ~~fungal~~ fermentation is curried out at 38-40°C. Thereafter, a N~~nitrogen~~ source is added ~~the~~ to the medium~~ is sterilized,~~ and~~ a nitrogen source is added,~~ a ventilation is applied for ~~the first~~ 20-36 h~~ours~~, feeding the culture broth in batch, for 16-22 h~~ours~~. The fermentation finishes when the residual sugar is below 1% (w/v)~~, so the medium is sterilized at 75-85°C and citric acid is recovered from the solution~~ [88]–[90]. ~~Another method, described in p~~Patent CN101942487 describes a method ~~for~~suitable for starchy material, ~~in general,~~ also applied ~~and reported by several patents for~~ with specific substrates (cheap dried sweet potatoes CN102952830, puffed dried sweet potato CN102851329, unhusked CN1415755)~~;~~. It includes the addition of α~~alpha~~-amylase enzyme

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for the reduction to amino acids and the ~~implementation~~ increase of the bio-availability, avoiding~~.~~ ~~The four described techniques avoid the~~ a further liquid/solid separation [91]–[94]. The ~~possible~~ addition of a ~~nitrogen~~ N source, ~~selected~~ in the ~~the~~ patent RU2007125728, ~~further~~ improves the conversion rate of sugar up to 87~~6.7~~-93~~.2~~% [95]. ~~Alternatively, c~~Comparable results are achieved in the invention RU2186850 using metals~~-~~ (i.e. ~~, like~~ Zn~~zinc~~, Fe~~iron~~ or Cu~~copper~~) [96].

3.4 Molasses as carbon source

Same roots, commonly used for the commercial sugar production, are suitable for the ~~citric acid~~CA synthesis~~ by A. *niger* metabolism~~. Among these substrates, cassava is chosen in patents CN103045659, CN102864183, without any pretreatments, ~~in patents CN103045659, CN102864183, where~~carrying out the fermentation ~~is carried out a~~at 39°C, for 3-4 days and 300 rpm [97], [98]. Satisfying results (up to 15% (w/v) of ~~citric acid~~CA, oxalic acid free) are obtained by the molasses from beet sugar manufacturing, as described in the inventions GB951629 and GB799752. In the first one, 0.3% ~~potassium ferrocyanide~~$K_4[Fe(CN)_6]$ and 0.2% ~~potassium dihydrogen~~KH_2PO_4 are added to the molasses, which shows a sugar amount of 6.0-7.5% (w/v) and a pH ~~between~~ 5.7~~ and~~ 5.9. This substrate is inoculated with *A. niger* in the presence of ~~passing~~ sparging air and the resulting fungal pellets are added to a similar ~~sterilized~~ medium, at pH 6.8 and a sugar content around 15% (w/v). The solution is agitated ~~and aerated~~ at 30°C for 40-48 h~~ours~~, the pH is adjusted to 3.0 by ~~hydrochloric acid~~HCl and the air supply is replaced by the oxygen insufflation to stimulate the ~~citric acid~~CA production~~. The resulting solution shows a citric acid content of 15% (w/v), oxalic acid free~~ [99]. In the second patent, the beet sugar molasses, with a sugar amount of 10-15% (w/v), is pretreated with ~~lime~~ $CaCO_3$ ~~or an insoluble hydroxide~~ for the impurity removal. Before the fungal inoculation, nutrient salts are added in the following amount (w/v): 0.15-0.2% of ~~ammonium carbonate~~$(NH_4)_2CO_3$, 0.01-0.02% of ~~potassium acid phosphate~~KH_2PO_4, 0.08-0.15% of ~~hydrate magnesium sulphate~~$MgSO_4$ and 0.0002-0.0004% of ~~zinc~~ Zn~~. The resulting liquor contains about 10-12% of citric acid~~ [100].

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This paragraph encloses around 40% of the patents of the present review. This is due to the high price of glucose (or sucrose), as substrate from raw material, that increases the industrial production cost of the ~~citric acid~~CA [23]. The positive cost effect achieved by the waste use can be further enhance by the use the biomass from the fermentation for both the biogas production and the fertilizers preparation [28]. Overall the main materials selected for the inventions are the corns (around 45%) and the starchy (30%), because they combine the low cost with a great availability. The patents confirm the relevance of the multiple step set-up, where the first one is necessary for the fungal adaptation.

4. *A. niger,* ~~Citric acid production by *Aspergillus niger* -~~ mutagenic strain

Since the sixties, the researches have experimented innovative approaches which have involved the fungal genome mutations to increase the ~~citric acid~~CA production by *A. niger* fermentation. The techniques of UV, γ~~gamma -~~ray-induced and chemical mutagenesis are currently accepted as routine methods, ~~Despite of~~although the patent number is lower than that ~~of the inventions where the~~related to ~~fungal is used as a~~the wild strain~~,~~ use ~~the technique of UV, gamma ray-induced and chemical mutagenesis are currently accepted as routine methods in the citric acid synthesis field~~ [101], [102] ~~. Considering the strong interest for this kind of approach, next paragraph shows the registered patents related to the mutation strain techniques~~ (Figure ~~5~~2~~3~~).

4.1 Sugar as carbon source – mutagenic strain

The ~~patent~~inventions CN106755138 and US2018195052 propose the modification of the succinic semialdehyde dehydrogenase (SSD) gene of the *A. niger* genome to obtain a recombinant ~~*Aspergillus*~~ *niger* strain. The expression of the SSD gene is regulated by the low pH inducible Pgas promoter which initiates the expression of the SSD protein in ~~*Aspergillus*~~ *A. niger*~~,~~. T~~t~~he~~reby~~ final effect is the increase~~sing~~ of ~~citric acid~~CA ~~the~~ production ~~of citric acid~~ by enhancing the GABA pathway [103]. The method, described in the inventions, utilizes the *A. niger* H915-1 as a host, with an efficiency increase of 10% and a time reduction of 10 hours, at 42°C [104], [105].

Alternatively, the patent CN106635847 integrates the *A. niger* low-affinity glucose transporter LGT1 gene into the *A. niger* genome to ~~obtain~~ achieve the *A. niger* recombinant ~~*A. niger*~~. The present ~~invention~~ method ~~utilizes~~ involves a low pH inducible promoter to initiate the expression of LGT1 protein in *A. niger*, increasing the uptake of glucose during the acidogenic phase. The result is an ~~increase~~ improvement of the ~~citric acid~~CA production of 6.5%, at 42°C, with a time decrease of 10 h~~ours~~ [106]. On the other hand, ~~T~~the invention CN108018216 ~~proposes~~ includes the modification of the glucosyltransferase genes to increase both the glucose assimilation and the conversion rate. The new genetic engineering strain ~~permits~~ allows a sugar concentration ~~decrease~~ reduction of 10% and an increase of the ~~citric acid~~CA yield around 10% ~~(w/v)~~ [107]. The *A.~~spergillus~~ niger* RCAM 02149 ~~realized~~ from the strain VKPM F-501~~,~~ ~~described (in~~ patent RU2013151521), is produced by a genetic mutation ~~with~~ by chemical mutagens (ether) and UV-radiation. This method allows ~~to achieve~~ about 90% of sugar conversion ~~to citric acid~~, at 32°C and a pH ~~between~~ 1.7 ~~and~~ 7.0 [108]. In the patents CN103952318, CN103045487 the *A. niger* FY2013 and FYCA8561 strains is prepared ~~starting~~ from the FY2010 strain using a low-dose compound treatment ~~at the following conditions:~~by ~~Co~~^{60} γ-ray treatment, ~~high temperature (~~at 90°C, for 5 minutes~~)~~, adding the nitrosoguanidine (0.~~,~~1 mg/ml). The resulting ~~citric~~ acid production is about 150~~1~~ g/L [109], [110]. The patent CN103194398 is designed to obtain high-yield strains, named *A. niger* TN-A09, with high tolerance levels to both sugar and acid concentration and high-conversion rates. Overall, the production of ~~citric acid~~CA is 18% (w/v), with a fermentation cycle of 60 h~~ours~~ ~~and an almost complete conversion~~ [109]. On the other hand, the patent WO2013082459 provides a genetic enhancement of a LaeA gene or an inactivat~~e~~ion the Alg3 gene of *A. niger* to increase the acid production ability of the fungus ~~to produce more citric acid~~ [111]. ~~The *A. niger* FYCA8561 strain (presented in the patent CN103045487) is produced by a genetic mutation with γ-ray treated with nitrosoguanidine. The use of this new fungal produces an acid concentration of 15% (w/v) and a conversion rate of 95% [111].~~ The *A. niger* CGMCC5342 and CGMCC5343 strains, ~~included~~ in the inventions CN102533570 and CN102399702, respectively,

are obtained by the genetic mutation of *A. niger* Co 827. The method allows a ~~citric acid~~CA production of 15% (w/v), after 52 h~~ours~~ at ~~the applied conditions:~~ 30-40°C and a starting pH ~~between~~ 4.0~~-4 and~~ 5.0 [112], [113]. ~~The~~ A portion of spore suspension is collected, after a grown of 5-6 days, in patent CN102352322 ~~provides the *A. niger* strain mutation, using the cultured spores grown for 5-6 days, eluted with sterile water, shaked for 30-60 minutes. A minimum portion of 0.1 mL of spore suspension is collected, spread on a sterile Petri dish surface and dried at room temperature. Thereafter, the culture dish coated is placed in an ion implanter, in the presence of~~ and mutated by ~~nitrogen~~ N ions. The mutant slant is inoculated ~~in a medium~~ at ~~constant temperature around~~ 40°C, 220 rpm and cultured for 90-96 h~~ours~~. The achieved ~~citric acid~~CA production is 10% higher than that of the original strain [114]. ~~On the other hand, the p~~Patent RU2428481 uses the fungal strain *A. niger* VKPM F-696 ~~which is~~ grown in a solution ~~containing~~ with sugar, in the presence of a water-soluble complex of C60 fullerene (0.5-0.7% w/v), with polyvinylchloride (0.7~~5~~-1.2~~5~~ mg/mL). The use of ~~the present~~this technology ~~allows an~~ increases ~~of~~ the citrate synthesis activity of 30%, from the third day with an ~~increase~~ improvement of the ~~citric acid~~product content ~~of about~~around 11%~~,~~ and a final conversion efficiency ~~around~~ of 98% [115]. The *A. niger* No CCM8210 strain (~~described within the~~ invention WO9710350) is developed from a starting variant obtained by protoplasts isolation from selected *A. niger*. The protoplasts are isolated from the hyphae in a stabilized ~~aqueous~~ solution (0.7 M NaCl + glucose), in the presence of ~~calcium ions.~~Ca^{2+}. The resulting protoplasts, ~~are~~ filtered and washed with water, ~~. The protoplast suspensions~~ are radiated by UV radiations, for 5 minutes. The further fusion is performed with wild strains to increase the growth and the production ability. This *A. niger* strain is subjected to a further mutation by the combination of UV-radiation and chemical mutagens (i.e. 5-bromouracide, 2-aminopurine, diethylsuiphate, ethylethane sulphonates and their combinations). The achieved CCM8210 strain is a high producing mutants, ~~cultivated on sugar media,~~ able to start fermentation at ~~low~~ pH ~~value (~~lower than 2.8~~) and to~~, avoiding the production of undesirable organic acids~~, mainly oxalic acid~~. The strain has ~~a h~~high~~er~~ conversion rate ~~than~~

~~conventional strains~~, about 1.3 g/L·-h of ~~citric acid~~CA ~~per liter of fermentation broth, for each hour~~. Overall, the variant produces up to 93 kg of ~~citric acid~~CA from 100 kg of supplied sugar [116]. The *A. niger* R-3 strain ~~(produced in the patent US4380583)~~ is selected from the *A. niger*-119 strain using ethyleneimine, N-nitrosomethylurea and UV-~~-~~radiation, in the patent US4380583. Its properties include the resistance to antagonist bacteria that may occur ~~in the process of citric acid~~during the fermentation. ~~The~~ Up to ~~95~~ 99% of the whole synthesized acid ~~synthesized by the new strain~~ consists of ~~citric acid~~CA with a yield~~s~~ that reaches the 100% of sugar conversion [117].

4.2 Waste as carbon source - mutagenic strain

Molasses is the principal carbon source from food and agriculture wastes, used as substrate for the metabolism of *A. niger* mutagenic strain ~~in the citric acid production~~. ~~Overall, t~~The described patents ~~described below~~ show two main differences: the genome mutation kind and the ~~final citric acid~~ production yield. The use of *A. niger* F-718 and molasses as substrate is reported in the invention RU2125607. The process includes a first stage ~~with a medium~~ at pH 4.0~~.0~~-5.0~~.0~~ and a fermentation, ~~carried out~~ at 30°C. During the first 30-48 h~~ours~~ of the process, a continuous feeding of both molasses and nutrient salts ~~solution~~ is requested. The second stage uses the microorganism suspension overflowing from the first stage, at 30°C and pH 2.0~~.0~~-3.0~~.0~~ [118]. As an alternative, *A. niger* R-1 and P-1, are involved in the patents~~:~~ FR2361330, SU568677, GB1499093. Both fungi are produced by the combined activity of: UV-radiation, ethylene imine and N-nitrosomethyl urea on *A. niger* EU-119 (*A. niger* R-1) and *A. niger* FY-119 (*A. niger* P-1). The new strains increase the sugar conversion rate from 89% to 99% and the ~~citric acid~~CA production yield from 94% to 97%. Furthermore, these variants show ~~significant advantage as~~ both a high resistance to antagonist bacteria and a low sensitive to ~~chemical~~ the composition of the starting molasses, allowing the ~~decrease of the molasses~~low quality substrate use [119]–[121]. The use of ~~an alternative substrate, which~~a mix of molasses medium ~~with~~ and sugar solution, at different concentrations, is proposed ~~within~~ in the patent RU2203322. ~~-~~More in detail, using the *A. niger*

VKPM-817, the obtained sugar conversions to ~~citric acid~~CA are: 87% with a 30 g/L sugar solution, 66% with 130 g/L and 96% with 150 g/L [122]. Sorghum condensed juice, molasses and sucrose are showed, as substrate, in the invention -RU2192460 that uses *A. niger* VKPM-809 with final conversion rates ~~between~~ of 60-90% [123]. A high conversion ~~rate~~ of sugar to organic acid ~~in the range~~(-9-41%)~~,~~ is ~~obtained~~ described in the patent RU95113067 by the fungal VKPM-713 strain ~~,~~(-from the *A. niger* F-326 strain genetic mutation) by UV, using several ~~raw~~ substrates (i.e. beet and sugar cane molasses sugar, food sugar, crude sugar, glucose and their mixtures, and fermentation conditions) [124]. Alternatively, the microorganism strains 1015, 10577, 11414, 12846, 9142, 13794, 26036, with sugar beet or date molasses as carbon source, are used in patent FR2336477. In this case, the highest acid production rates (95% of ~~citric acid~~CA) is achieved at these conditions: substrate pretreated by ~~potassium ferrocyanide~~$K_4[Fe(CN)_6]$ (to remove ~~heavy~~ metal traces), pH 2.7, 24 h~~ours~~ [125]. A pretreatment is required in the patent GB799752, for the impurity removal from molasses, corn starch hydrolyzed or beet molasses, adding ~~lime or an insoluble hydroxide~~$CaCO_3$. The ~~necessary~~ nutrient salts ~~amount is~~are added to the broth: 0.15-0.2% of ~~ammonium carbonate~~$(NH_4)_2CO_3$, 0.01-0.02% of ~~potassium acid phosphate~~KH_2PO_4, 0.08-0.15% ~~hydrated magnesium sulphate~~$MgSO_4$ and 0.0002-0.0004% of ~~zinc~~Zn, at pH 2.5. The fermentation is carried out at 24-34°C by the *A. niger* mutant strain and the resulting ~~citric acid~~CA production is about 10% (w/v), after 8-12 days [100]. The *A. niger* R-6 and R-5 strains are used with sugar beet molasses (LV11342) and sugar cane molasses (LV11340), as substrates, respectively. The fungal fermentation is carried out with a sugar amount of 15% (w/v), for 9-12 days. The resulting sugar conversion rate reaches the 100% in the first medium and the 85% in the second one, with a the ~~citric acid~~CA content of 95-99%, in both cases [126], [127]. Alternative substrates are used with mutagenic strain, in patents CN106367359, CN108588133 (acorn) and RU2103346 (~~ethanol~~EtOH). In the first invention, *A. niger* AA120, obtained after mutagenic screening by the atmospheric and room temperature plasma (ARTP) technology, allows high ~~citric~~ CAand tannic acids production [128]. In the second one, acorns are pretreated with ~~ethanol~~EtOH,

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to remove phenolic substances, and liquefied before the *A. niger* CICC 2716 inoculum. The acorn pulverized use improves the ~~citric acid~~CA production [129]. In the last patent, *A. niger* VKPM-722 synthesizes the ~~citric acid~~CA with a conversion rate higher than 80%, using ~~ethanol~~ EtOH or a mix of EtOH~~ethanol~~ and sucrose [130].

Considering the deepened study carried out on the traditional strains, the best operative conditions have~~s~~ been identified and further development could mainly focus on the innovative substrates. Therefore, the possibility of mutagenic strains use has started to gain a foothold in the market, since they allow significant ~~citric acid~~CA synthesis improvements. Currently, the inventions are equally divided between primary and waste substrates, but the trend of patents with residue use is destinated to grow in a circular economy perspective.

~~5. Other fungi~~

~~The technological innovation literature reports the use of other fungi for the citric acid production, as an alternative to the *A. niger*. The related patents are mainly concentrated in the 70s, nevertheless, the interest has waned for the achieved low efficiencies. In this regard, the following paragraphs focus on: *Candida* sp., *Penicillium* sp. and other strain of *Aspergillus,* considered as the most representative for the technological innovation (Figure 6).~~

~~*5.1 Candida sp.*~~

~~The required conditions for the citric acid production by *Candida* sp. fermentation, reported in patents DE4407441, US4178211, GB1464334, include: a carbon source, mainly glucose, with a concentration higher than 200 g/L, a nitrogen concentration between 50 and 150 mM and an atmospheric oxygen saturation in the fermenter of 20%, pH around 5.0, at 30°C [131]–[133]. The glucose substitution is proposed in patent GB1418561, where the *C. guilliermondii* strain produces citric acid metabolizing cane or beet molasses after the addition of essential nutrients. The process~~

~~needs a pH value between 2.5 and 4.5 (controlled by ammonia addition), at 22-35°C, at aerated conditions obtained by sparging air. In order to maintain the high citric acid production rate, the medium is recovered and replaced with fresh solution [134]. On the other hand, the possibility to use hydrocarbon fraction is verified in the further inventions. In this regard, alpha-olefins and n-paraffin, both with 8-20 C atoms, or a mix of them, are employed for the metabolism of *C. tropicalis*, *C. lipolytica*, *C. intermedia* and *C. bruptii*, in patents US4424274 and JPS5779890. The treatments require the concentration of dissolved oxygen in a range of 10-30 ppm with a final acid concentration greater than 150 g/L [135], [136]. The invention GB1428440 uses *Candida* sp. in a two-fermentation phases; in the first step the fungal is cultured in an aerated tank containing at least one n-paraffin, for its adaptation, thereafter, it is cultured in a second fermenter where, further inorganic nitrogen is added at acid pH [137]. N-paraffin (9-20 C atoms) is employed also in patent GB1418511, for the *C. lipolytica* and *C. oleophila* metabolisms in a medium with a high fluoroacetate concentration. A specific agent (pentachlorophenol, 2,4-dinitrophenol, 4,5,6-7-tetrachloro-2(trifluoromethyl)benzimidazole, mesoxalonitrile[p(trifluoromethoxy) phenol]-hydrazone or dicoumarin) is added to the medium to decouple the substrate, in order to increase its bioavailability [138]. N-paraffin (10-18 C atoms, with the concentration of 3-20% v/v), sugar, glycerin, ethanol, acetic acid, butyric acid or animal or vegetable fats or oil are suitable for the *C. lipolytica* and *C. tropicalis* metabolisms, as confirmed by the inventions GB1380938, GB1297243, GB1204635. In these processes, the medium is enriched by inorganic nitrogen and other nutrient salts, at pH 1.5-3.5, under aerobic conditions, at 20-35°C [139]-[141].~~

~~*5.2 Penicillium sp.*~~

~~The patents US3652396, GB878151 include the fungal strains *P. adameizi* and *P. restrictum* with hydrocarbon, sucrose or cane and beet molasses, as carbon sources. In both cases, pretreatments are not necessary and inorganic nutrient (e.g. nitrogen, phosphorous and magnesium) are added. The fermentation is carried out at pH around 7, at 28°C [142], [143].~~

~~5.3 *Aspergillus sp.*~~

~~Patent GB581389 mentions *A. wentii*, as an alternative to *Aspergillus* strain, which metabolizes cane sugar enriched with nutrient salts (e.g. ammonium nitrate, peptone, magnesium sulphate, dipotassium hydrogen phosphate, potassium chloride, zinc sulphate, ferric chloride and calcium chloride). When the fungal has reached the maximum growth rate, the residual culture medium is recovered and replaced by a fresh fermentation liquor containing 15-20% of carbohydrates (e.g. sucrose, maltose, lactose, glucose, dextrose, levulose). The treatment is carried out at pH 3.0, maintained by calcium carbonate or an alkali metal hydroxide addition [144]. Alternatively, patent GB797390 suggests molasses as carbon source. Before the fermentation, the broth medium is treated by ion exchange resins, $Ca(OH)_2$, alkaline ferrocyanides or complex-forming agents, for the metal removal, mainly iron. The broth pH is maintained around 2.5 at the 32°C [145].~~

~~6.~~5. Discussion and perspectives

Figure 3~~7~~ shows a whole ~~citric acid~~CA production process which~~With the aim to~~ summarizes the treatments described in the present review, involving *A. niger*. As reported in the supporting materials (Tables S1), the technological innovation literature describes the further use of other fungi [131], [132], [141]–[145], [133]–[140]. Nevertheless, the related patents are mainly concentrated in the 70s and the interest has waned for the achieved low efficiencies; for this reason, these techniques has not been discussed in the present review. ~~(reported in the Tables S1), Figure 7 shows a general citric acid production process.~~

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-The first identified variable in the scheme (Figure 3A) is the ~~possibility of~~possible ~~a~~ pre-treatment, if food or agriculture waste ~~are~~ is used as substrate for the fungal growth. This step ~~is possibly~~could be required to remove metals and/or to make carbon source bio-available for the fungal metabolism. Currently, the industrial production mainly uses sugars from hydrolysis of plant starch. The following step includes the fermentation, ~~I~~irrespective of the selected conditions ~~and the substrate kind (glucose or sucrose), the following step includes the fermentation~~. The average

conditions which promote the fungal metabolism, with ~~a consequent~~ high sugar conversion rate (around 95%) and a high acid concentration (100-150 g/L) ~~are~~include: sugar concentration 10~~%~~-15% (w/v), pH 1.5-4.5, stirring speed 120-400 rpm, temperature 27°-35°C, air supply and nutrient salts addition (mainly 0.2% w/v of $(NH_4)_2SO_4$~~, 0.2%~~ and~~w/v~~ of KH_2PO_4 and 0.1% of $MgSO_4$) and/or metal addition (Mn, Fe and Zn). A final phase of recovery and purification closes the treatment~~ schema.~~.

As concern the technological evolution overview, the sharp increase ~~in~~ of the number of patents ~~related to the citric acid bioproduction,~~ in the last 20 years, confirms the strong interest for ~~this topic~~the acid bioproduction, especially in China. As showed in Figure 3B~~8~~, there is an evident change in the invention origin, from European and US to Chinese, starting from 2000. The reason can be found in a modification ~~a~~ of the trade routes, ~~modification~~ which currently identify China as one of the most important export-oriented country. In this regard, 824,000 tons were exported from China, principally to India (9%), Turkey (5.2%), Japan (5%), Mexico (5%) and Indonesia (3.9%), in 2015. In the same year, 1,007 tons of ~~citric acid~~CA was imported to China mainly from Germany (30%), Canada (18%), Japan (17%) and Austria (12%), with a whole cost of USD 5 million [146].

Furthermore, considering the ~~further~~ increase of the ~~citric acid~~CA demand, China aimed at the production process improvement with an increase of its economic exportation load of about 10%, from 2015 to 2017. In this regard, Figure 4A~~9a~~ attributes to China the 75% of the whole trade value connected with the ~~citric acid~~CA exportation, followed by Netherland, USA and Germany (each one with a contribution around 3%). On the other hand, USA (12%), Germany (8%), Mexico (5%) and India (5%) represent the main importers in the world (Figure 4~~9b~~B). Whereas, the Chinese production independence is confirmed by an importation trade lower than 1% [147].

Additional information about the main critical aspects addressed in the patents can be deduced from the trends in Figure 3B~~8~~. In this regard, the current technological innovation, has focused on

the identification of innovative substrates, mainly agriculture waste, in agreement with the scientific literature which tends to fulfill the circular economy rudiments [148].

6. Conclusions

The present review proves the necessity of a technological innovation study to understand the real state-of-art in a specific field. A patented invention is designed for the implementation on a real scale and it should be commercially available. Furthermore, for the grant of a patent it is necessary to prove the actual novelty level, ensuring the innovation level of the review. Compared to the traditional literature, the invention overview helps with the market previsions thanks to the additional information related to the origin and the state of development [27]. This focus on the patents about the ~~citric acid~~CA production, over a long period of time, highlighted the growing interest for this topic and a technology progress, in agreement with the market demand. In this regard, a relevant increase of the waste substrate proves the development of the circular economy model. Further studies, with a similar approach, could deep the aspect of the ~~Furthermore, there is a change in the~~ fermentation design change. The scientific literature confirms as the research ~~which ha~~has moved towards the conversion of the most consolidated submerged process to the innovative solid-state fermentation (*koji* process) [4], [149]–[151]. This simple and eco-sustainable technique combines the possibility of an agro-industrial waste use with a low energy demand and a minimum wastewater ~~productio~~production [27]. The choice of a limited in-depth of this topic is due to the low number of the related patents, probably connected with the current scale-up difficulties. This technique cannot be implemented in the bioreactors for submerged process and many criticalities are still due to the lack of standardized processes. ~~n.~~

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