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Biomanufacturing of food-grade citric acid and comprehensive utilization of its production wastewater

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Abstract

Citric acid is an important and multi-functional organic acid, which is widely used in food, medicine, chemical industry and other fields. It is the largest edible organic acid in the world. *Aspergillus niger* is an important strain for biosynthesis of citric acid, which is mainly produced by simultaneous saccharification and fermentation with starch raw materials. The reuse of citric acid wastewater is the development direction of comprehensive utilization of citric acid wastewater. In the future, with the combination of novel technology strategies such as gene editing technology and synthetic biology technology, the establishment of fermentation strategies with high yield, high yield, high production intensity and clean production is the development direction to realize efficient green manufacturing in citric acid industry.

Keywords: citric acid; *Aspergillus niger*; synchronous saccharification fermentation; wastewater utilization.

Practical Application: This paper provides a reference for the efficient green bio-manufacturing of food grade citric acid.

1 Application of citric acid in food industry

1.1 Citric acid

Citric acid is a natural ingredient in animals and plants and an intermediate product of physiological metabolism, which is one of the most widely used organic acids in food, medicine, chemical industry and other fields (Barretti et al., 2022). Citric acid has a pleasant sour taste, refreshing entrance, no after-sour taste, safe and non-toxic; it has high solubility in water and can be directly absorbed and metabolized by organisms. Based on this excellent characteristic, citric acid is widely used in the food industry (Vázquez-Rodríguez & Escalante, 2022).

Citric acid is the largest edible organic acid in production and consumption in the world. It is widely used in food, medicine and chemical industry (Chavez-Esquivel et al., 2022). Citric acid is the second largest fermented product in the world, and its output is second only to alcohol production. In recent years, with the application of citric acid in emerging industrial fields, such as nanomedicine, drug transport and tissue engineering (Yan et al., 2022), the global market demand is increasing, and now it is growing at a rate of 5% every year. As citric acid is one of the most productive fermentation products in the world, the increase of citric acid production, even if the increase is small, will produce huge social and economic benefits. In order to meet the growing market demand, the significance of developing an efficient method for synthesizing citric acid has become increasingly prominent.

1.2 Application of citric acid in food industry

Citric acid is multifunctional and non-toxic (GRAS, generally recognized as safe), which is recognized as a safe food additive by the expert committee of FAO/WHO and is called the first edible sour agent. In the beverage industry and brewing wine, it can not only endow the product with fruit flavor, but also has the functions of solubilization, buffering and antioxidation, so as to coordinate the components such as pigment, aroma and sugar, and form a harmonious taste and aroma. At the same time, it can enhance the anti-microbial antiseptic effects (Plesoianu et al., 2022). The role of citric acid in jam and jelly is mainly to give the product sour taste and adjust pH to the narrowest range suitable for pectin gel (Soares et al., 2022). In frozen food, fat and oil, it has the characteristics of chelating and regulating pH, strengthening the effect of antioxidants and enzyme inactivation, and improving the stability of frozen food (Eshun et al., 2022). In the pharmaceutical industry, such as foaming agents, citric acid and sodium carbonate aqueous solution react together to produce a large amount of CO_2, which can quickly dissolve the active ingredients and improve the taste ability.

2 Research status of biological manufacture of food-grade citric acid

2.1 Strain

Citric acid is a kind of natural organic acid, which is mainly synthesized by chemical synthesis and biological fermentation.

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Compared with chemical synthesis, biosynthesis has the advantages of low energy consumption and renewable resources. More than 99% of citric acid in the world is synthesized by biological fermentation. Microbial fermentation is the most important method of industrial production, and about 99% of the products in the world are produced by fermentation (Jin et al., 2021).

Microbial fermentation strain can be divided into yeast fermentation and *Aspergillus niger* fermentation. *Yarrowia lipolytica* can use different kinds of carbon sources such as alkanes and glucose to accumulate a large amount of citric acid, which has been used in production. However, it also accumulates a large amount of by-product isocitrate (5%-10%) during yeast fermentation, which makes it difficult to separate and purify citric acid and limits its large-scale application (Rywińska & Rymowicz, 2010). Förster used recombinant *Y. lipolytica* H222-S4 (p671CL1) to increase the expression of sucrase under the condition of inducible XPR2 promoter and multi-copy ICL1. The yield of citric acid was 0.33 $g{\cdot}L^{-1}{\cdot}h^{-1}$, and the ratio of by-product was reduced with less than 5% (Förster et al., 2007). By knocking out ATP- citrate lyase (ACL) gene and overexpressing isocitrate lyase (ICL), Liu et al. (2013) decreased the yield of isocitrate by-product. The application of genetic engineering in strain transformation reduces the accumulation of by-products to a certain extent, and the stability of recombinant engineering strain and the safety of citric acid need to be further investigated.

A. niger is a food-grade safe filamentous fungus, which is widely used in the production of organic acids and industrial enzyme preparations (Yin et al., 2017). As a cell factory for protein production, *A. niger* can secrete protein efficiently and is a high-producing strain of natural polysaccharide-degrading enzymes such as glucoamylase, amylase, xylanase and pectinase. *A. niger* has the advantages of rich enzyme system, high fermentation efficiency and few by-products, which can well regulate the flux of glycolysis and the secretion of citric acid from mitochondria and cytoplasm, which is still an important direction for the development of citric acid fermentation. The modern industrial production of *A. niger* seeds mainly adopts the secondary culture method (as shown in Figure 1). The mature *A. niger* seeds should be cultured first, and then transferred to fermentation culture. The process of *A. niger* seed culture requires the preparation of mature spores, and then the spores are inoculated to form mature mycelium pellets. The spores are prepared by solid-state culture, and a batch of mature spores need to be screened by plate, and the culture process is expanded step by step, such as plate culture, eggplant bottle culture, bran-koji bucket and so on. The preparation process is tedious and long (the preparation period is more than 30 days). The secondary seed culture cycle is also long, only spore germination takes more than 12 h, consuming a lot of auxiliary time and production cost (Wang et al., 2017a). Therefore, how to improve the seed culture process of *A. niger*, shorten the growth cycle and reduce the production cost is an important problem to be solved in citric acid production.

2.2 Fermentation materials

Microbial fermentation is the most important way to produce citric acid, and more than 80% of the products are obtained by deep fermentation of *A. niger* containing glucose or sugar processing by-products and agricultural products processing waste (Yu et al., 2018). With the increasing global demand for citric acid and the increasing pressure of market competition, the application of cheap raw materials has gradually become the focus of citric acid research. Industrial and agricultural processing wastes are used to produce citric acid (Papadaki & Mantzouridou, 2019). The application of low-cost and waste raw materials broadens the range of citric acid raw materials, reduces production costs and alleviates environmental pressure. However, the composition of these raw materials is often complex, and complex pre-treatment is

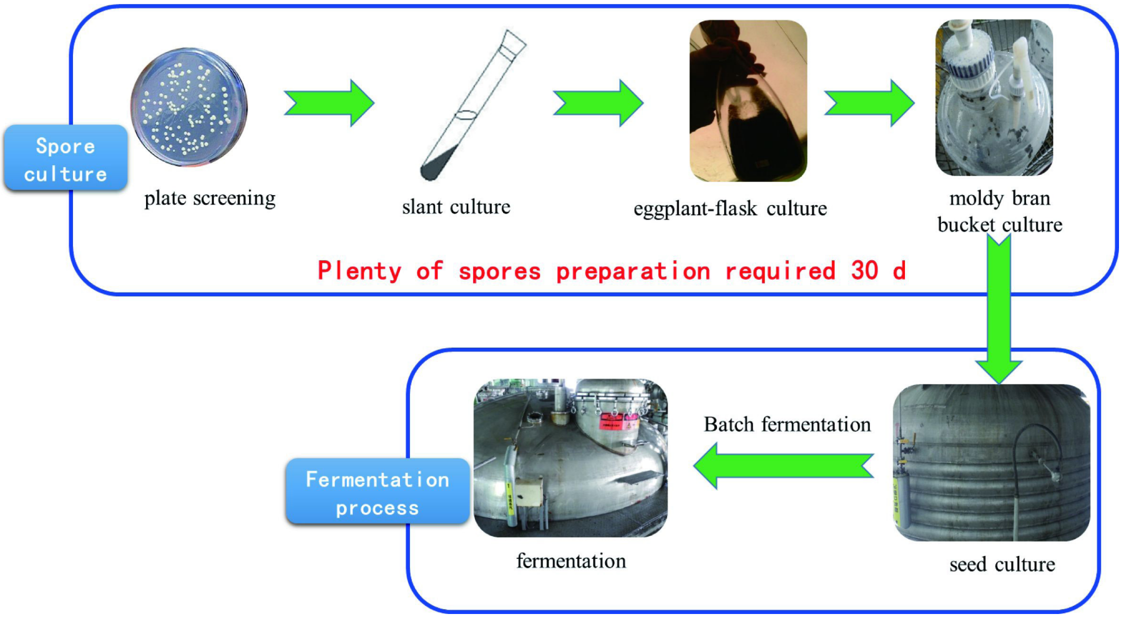

Figure 1. Seed preparation of *Aspergillus niger* in the industrial production of citric acid.

needed to fully release nutrients, resulting in the difficulty of product extraction in the later stage. Throughout the whole production process of citric acid, the production cost increases instead of decreasing. In addition, it is necessary to change the rheological properties of raw materials to improve the ability of oxygen and mass transfer. Therefore, the application of by-product raw materials in large-scale production of agricultural products needs to be further studied, and starch raw materials are still an important choice for the industrial production of citric acid.

In the production of citric acid by microbial fermentation, the use of high purity sugars such as glucose and sucrose can obtain higher yield but higher industrial production cost (John et al., 2009) . In comparison, it is more economical and competitive for coarse starch raw materials to replace refined sugar (Huang et al., 2014). However, starchy materials cannot be directly used as carbon sources. They need to go through gelatinization, liquefaction and saccharification in order to obtain fermentable sugars (Lv et al., 2016; Wang et al., 2016). The filamentous fungus *A. niger*, with the advantages of easy operation, extensive substrate, high yield and few by-products, is an excellent cell factory for synthesizing citric acid. *A. niger* secretes a variety of hydrolytic enzymes such as α-amylase and glucoamylase during fermentation, which is helpful for starch raw materials to be used to synthesize citric acid (Wang et al., 2016).

In order to reduce the production cost, the simultaneous saccharification fermentation (SSF) can be integrated with the saccharification process and fermentation process. In addition, the synchronous saccharification fermentation mode can not only reduce the production cost, but also solve the problem of high sugar inhibiting cell growth. This fermentation method has been successfully applied to alcohol fermentation (Szymanowska-Powałowska et al., 2014) and production of organic acids such as fumaric acid (Saha et al., 2015) , itaconic acid (Huang et al., 2014) and lactic acid (Lv et al., 2016). *Aspergillus niger* itself can secrete a large amount of glucoamylase (up to 10 g L^{-1}), and the saccharification step can be almost reduced or even eliminated. Citric acid is widely produced by *A. niger* synchronous fermentation. However, in the process of citric acid fermentation, with the accumulation of citric acid, the fermentation pH decreased significantly, especially when the pH decreased below 2.0, the glucoamylase activity was significantly destroyed. The loss of glucoamylase activity would affect the glucose supply, and then reduce the citric acid synthesis rate. Further, it would lead to a high content of residual total sugar at the end of fermentation (Wang et al., 2017b). The method of regulating pH in fermentation broth and improving glucoamylase activity by adding calcium salt cannot meet the actual needs of industrialization, because the resulting calcium citrate cannot be effectively separated from mycelium. The problems of high fermentation residual sugar significantly reduces the conversion ratio between substrate and product, and further makes the process of product separation and purification difficult (Lv et al., 2016). Moreover, a large number of complex equipment, such as membrane separation and column chromatography, are needed to solve the separation and extraction problems caused by high fermentation residual sugar.

3 Biological production of citric acid fermentation

According to the culture method, the production of citric acid by fermentation can be divided into three modes: surface fermentation, solid state fermentation and submerged fermentation (Angumeenal & Venkappayya, 2013). Submerged fermentation has the advantages of low labor intensity, high production efficiency, small space occupation and high degree of automation, which is the most important way of industrial production of citric acid. More than 80% of citric acid products are obtained by this fermentation method.

Batch fermentation is the main fermentation method of citric acid, but it has some defects, such as low energy efficiency, long auxiliary time, low utilization rate of equipment and so on. In comparison, continuous fermentation mode (semi-continuous fermentation) has more advantages. Citric acid synthesis belongs to partial growth coupling type, which is not conducive to the formation of continuous process, but researchers have never stopped exploring this fermentation mode. Rywińska *et al*. used *Y. lipolytica* Wratislavia AWG7 fermentation to produce citric acid, developed a novel fermentation method, and cultured to a certain stage, discharged a certain amount of fermentation liquid and added fresh medium to continue fermentation, repeated the above operation. When 40% fresh medium was replaced, yeast cells could maintain cell vitality for a long time (Rywińska and Rymowicz, 2010). Based on the on-line control of biosensor, Moeller *et al*. fermented glucose to produce citric acid by *Y. lipolytica*. The fermentation methods such as batch fermentation, fed-batch fermentation, repeated fed-batch fermentation and repeated fed-batch fermentation were investigated respectively. The yield of citric acid reached 100 g/L (3 d), but a large amount of by-product isocitrate was accumulated in the fermentation process, which limited its large-scale application (Moeller et al., 2010).

The study of citric acid in semi-continuous or continuous fermentation is mainly focused on yeast, but there are few reports on continuous culture of *A. niger.* The special mycelium structure of *A. niger* will restrict the transport of dissolved oxygen in the process of continuous culture, further lead to abnormal cell metabolism and citric acid synthesis. Based on the above problems, it is a great challenge to develop a safe and reliable method for continuous culture of *A. niger*. In spite of this, some researchers have proposed to immobilize *A. niger* cells by immobilization to control the size of mycelial pellets. However, there are side reactions in the immobilization reaction system, and more importantly, the rate-limiting step of product synthesis (mass transfer rate) is limited by the immobilization system. Under the condition of gradual cell aging and cell failure to update, it is difficult to maintain the high cell activity of mycelium pellets in the culture medium, which limits the further application of this method. Therefore, how to effectively control the shape of mycelium pellet in the process of continuous culture and maintain cell vitality is an important topic to realize the continuous fermentation of *A. niger*.

4 Extraction of citric acid and its wastewater treatment

4.1 Extraction of citric acid

At present, citric acid is mainly produced by *A. niger* in submerged fermentation. Citric acid extraction methods mainly include traditional calcium salt extraction method and chromatographic extraction method. Calcium salt extraction method refers to the solid-liquid separation and removal of mycelium residue and other solids in citric acid fermentation broth. The, the fermentation broth was clarified by re-filtration, and after heating, calcium carbonate and other calcium-containing compounds were used to neutralize acid and base to form calcium citrate crystals. Through solid-liquid separation and collection, it enters the lower stage of re-decomposition and continues purification, from which the solution separated by solid-liquid separation is citric acid extraction wastewater (waste sugar water) as shown in Figure 2A. The chromatographic extraction method means that the citric acid fermentation broth removes the mycelium residue by solid-liquid separation, clarifies the fermentation broth through membrane filtration, enters the chromatographic separation system for separation, and obtains the citric acid extraction solution. The waste liquid produced in this separation process is the chromatographic residue, as shown in Figure 2B. The chemical oxygen demand (COD) in the waste water produced by citric acid reaches 350 kg/t per ton and the concentration is as high as 10000 ~15000 ppm. The components of chromatographic residue and waste-sugar broth are similar, which mainly contain fiber, mycelium, organic acid, sugar, protein colloid, pigment, mineral and other metabolites, which have a great impact on the COD of wastewater. Therefore, the resource utilization of chromatographic residue and waste-sugar broth has extremely important practical significance for environmental protection, saving resources and realizing clean production and recycling of resources.

4.2 Comprehensive utilization of citric acid extraction wastewater

Citric acid is the second-generation fermentation product in the world. however, a large amount of citric acid wastewater is produced because each ton of citric acid wastewater can produce

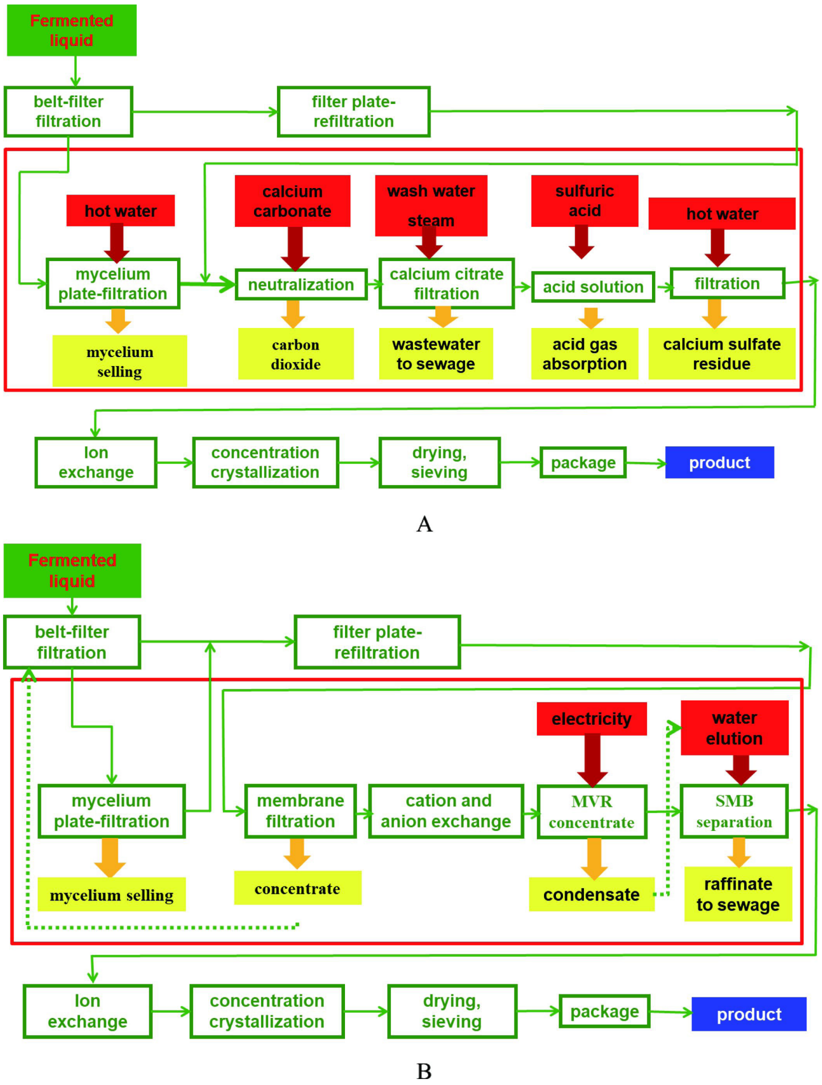

Figure 2. Two typical extraction processes of citric acid products (A. Calcium salt extraction method; B. Chromatographic extraction method).

about 50~60 tons of wastewater. In 2017, China produced more than 1.2 million tons of CA and more than 60 million tons of citric acid wastewater, which contains high levels of COD. Such a huge output of citric acid wastewater has brought great pressure on environmental protection, and wastewater treatment has seriously limited the development of CA industry.

Citric acid wastewater is mainly treated by biological treatment, Fenton reagent, photosynthetic bacteria, emulsified liquid membrane and so on, among which biological treatment is the most widely used. Traditionally, wastewater is treated first by anaerobic digestion, then by aerobic digestion, rather than by energy cycle (Nasr et al., 2012). Ding *et al*. used Fenton reagent to treat citric acid wastewater to improve its dewatering performance while maintaining the fertilizer performance of citric acid wastewater sludge (Ding et al., 2018). However, physical and chemical treatment methods such as Fenton reagent method and microwave radiation method are not suitable for industrial scale application because of their high treatment efficiency, complex operation, high reagent consumption and the risk of secondary pollution (Tekin et al., 2006).

In the industrial production, the current treatment methods of citric acid fermentation wastewater mainly include the terminal treatment method with anaerobic-aerobic digestion as the core, and the clean production method characterized by resource utilization of citric acid wastewater. Among them, anaerobic-aerobic digestion process is the most mature and widely used technical method in citric acid fermentation industry to treat wastewater. The biggest problems faced by this method are that the equipment occupies a large area with high investment and operation cost. Moreover, the treatment is not thorough, the effluent cannot meet the national industrial wastewater discharge standard, and a lot of water resources will be wasted. Based on the concept of fermentation ecological engineering, Xu et al. (2015, 2016) constructed a citric acid-biogas double fermentation coupling system, during which the raw materials such as cellulose, hemicellulose, lignin and pectin, which cannot be used by *A. niger*, as well as the metabolic by-products of *A. niger*, are converted into economically valuable by-products biogas (energy substances) through medium-temperature anaerobic digestion. After water resource treatment, the effluent from anaerobic digestion is reused in the next batch of citric acid fermentation, so as to eliminate the discharge of citric acid fermentation wastewater and reduce the consumption of fresh water. The citric acid-biogas double fermentation coupling system removes the aerobic digestion treatment unit with high investment and operation cost in the traditional wastewater treatment process, and greatly reduces the treatment cost of citric acid fermentation wastewater. However, the process stability for industrial production and application needs to be further optimized.

5 Prospect

Because of its excellent functional properties, the application field of citric acid in food industry will be further expanded in the future. *A. niger* is still an important food-grade citric acid production strain, combined with gene editing technology and synthetic biology technology will further improve the citric acid production technology. A large amount of organic wastewater will be produced in the process of citric acid production, which poses a great burden on the environment. Based on the concept of fermentation ecology, the efficient utilization of citric acid wastewater is still the main direction of wastewater treatment in the future.

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References

Angumeenal, A. R., & Venkappayya, D. (2013). An overview of citric acid production. *Lebensmittel-Wissenschaft + Technologie*, 50(2), 367-370. http://dx.doi.org/10.1016/j.lwt.2012.05.016.

Barretti, B. R. V., Almeida, V. S., Ito, V. C., Silva, B. M., Carvalho Filho, M. A. S., Sydney, E. B., Demiate, I. M., & Lacerda, L. G. (2022). Combination of organic acids and heat-moisture treatment on the normal and waxy corn starch: thermal, structural, pasting properties, and digestibility investigation. *Food Science and Technology (Campinas)*, 42, e33120. http://dx.doi.org/10.1590/fst.33120.

Chavez-Esquivel, G., Cervantes-Cuevas, H., & Vera-Ramírez, M. A. (2022). Effect of dual modification with citric acid combined with ultrasonication on hydrolysis kinetics, morphology and structure of corn starch dispersions. *International Journal of Biological Macromolecules*, 222(Pt B), 1688-1699. http://dx.doi.org/10.1016/j.ijbiomac.2022.09.218. PMid:36179871.

Ding, N., Peng, C., Ren, Y., Liu, Y., Wang, P., Dong, L., Liu, H., & Wang, D. (2018). Improving the dewaterability of citric acid wastewater sludge by Fenton treatment. *Journal of Cleaner Production*, 196, 739-746. http://dx.doi.org/10.1016/j.jclepro.2018.06.139.

Eshun, N. A., Dorleku, W.-P., Saalia, F. K., Alvarez, M. N., & Aryee, A. N. A. (2022). Effect of organic acid pre-treatment on polyphenol oxidase induced browning and sensory quality of frozen yam chips. *Applied Food Research*, 2(2), 100165. http://dx.doi.org/10.1016/j.afres.2022.100165.

Förster, A., Aurich, A., Mauersberger, S., & Barth, G. (2007). Citric acid production from sucrose using a recombinant strain of the yeast *Yarrowia lipolytica. Applied Microbiology and Biotechnology*, 75(6), 1409-1417. http://dx.doi.org/10.1007/s00253-007-0958-0. PMid:17447058.

Huang, X., Chen, M., Lu, X., Li, Y., Li, X., & Li, J.-J. (2014). Direct production of itaconic acid from liquefied corn starch by genetically engineered *Aspergillus terreus. Microbial Cell Factories*, 13(1), 108. http://dx.doi.org/10.1186/s12934-014-0108-1. PMid:25162619.

Jin, S., Sun, F., Hu, Z., Liu, L., Li, J., Du, G., Li, Y., Shi, G., & Chen, J. (2021). Improving *Aspergillus niger* seed preparation and citric acid production by morphology controlling-based semicontinuous cultivation. *Biochemical Engineering Journal*, 174, 108102. http://dx.doi.org/10.1016/j.bej.2021.108102.

John, R. P., Anisha, G. S., Nampoothiri, K. M., & Pandey, A. (2009). Direct lactic acid fermentation: focus on simultaneous saccharification and lactic acid production. *Biotechnology Advances*, 27(2), 145-152. http://dx.doi.org/10.1016/j.biotechadv.2008.10.004. PMid:19013227.

Liu, X. Y., Chi, Z., Liu, G. L., Madzak, C., & Chi, Z. M. (2013). Both decrease in ACL1 gene expression and increase in ICL1 gene expression in marine-derived yeast *Yarrowia lipolytica* expressing INU1 gene enhance citric acid production from inulin. *Marine Biotechnology (New York, N.Y.)*, 15(1), 26-36. http://dx.doi.org/10.1007/s10126-012-9452-5. PMid:22562483.

Lv, X., Yu, B., Tian, X., Chen, Y., Wang, Z., Zhuang, Y., & Wang, Y. (2016). Effect of pH, glucoamylase, pullulanase and invertase addition on the degradation of residual sugar in L-lactic acid fermentation by *Bacillus coagulans* HL-5 with corn flour hydrolysate. *Journal of the Taiwan Institute of Chemical Engineers*, 61, 124-131. http://dx.doi.org/10.1016/j.jtice.2016.01.007.

Moeller, L., Grünberg, M., Zehnsdorf, A., Strehlitz, B., & Bley, T. (2010). Biosensor online control of citric acid production from glucose by *Yarrowia lipolytica* using semicontinuous fermentation. *Engineering in Life Sciences*, 10(4), 311-320. http://dx.doi.org/10.1002/elsc.200900073.

Nasr, N., Elbeshbishy, E., Hafez, H., Nakhla, G., & El Naggar, M. H. (2012). Comparative assessment of single-stage and two-stage anaerobic digestion for the treatment of thin stillage. *Bioresource Technology*, 111, 122-126. http://dx.doi.org/10.1016/j.biortech.2012.02.019. PMid:22366605.

Papadaki, E., & Mantzouridou, F. T. (2019). Citric acid production from the integration of Spanish-style green olive processing wastewaters with white grape pomace by *Aspergillus niger. Bioresource Technology*, 280, 59-69. http://dx.doi.org/10.1016/j.biortech.2019.01.139. PMid:30754006.

Plesoianu, A. M., Nour, V., Tutulescu, F., & Ionica, M. E. (2022). Quality of fresh-cut apples as affected by dip wash treatments with organic acids and acidic electrolyzed water. *Food Science and Technology (Campinas)*, 42, e62620. http://dx.doi.org/10.1590/fst.62620.

Rywińska, A., & Rymowicz, W. (2010). High-yield production of citric acid by *Yarrowia lipolytica* on glycerol in repeated-batch bioreactors. *Journal of Industrial Microbiology & Biotechnology*, 37(5), 431-435. http://dx.doi.org/10.1007/s10295-009-0687-8. PMid:20047064.

Saha, B. C., Nichols, N. N., Qureshi, N., Kennedy, G. J., Iten, L. B., & Cotta, M. A. (2015). Pilot scale conversion of wheat straw to ethanol via simultaneous saccharification and fermentation. *Bioresource Technology*, 175, 17-22. http://dx.doi.org/10.1016/j.biortech.2014.10.060. PMid:25459799.

Soares, C. M. S., Morais, R. A., Silva, R. R., Freitas, B. C. B., Melo, A. A. Fo., & Martins, G. A. S. (2022). Development and optimization of the jam production process of P*outeria cf. gardneriana* Radlk (guapeva). *Food Science and Technology (Campinas)*, 42, e00821. http://dx.doi.org/10.1590/fst.00821.

Szymanowska-Powałowska, D., Lewandowicz, G., Kubiak, P., & Błaszczak, W. (2014). Stability of the process of simultaneous saccharification and fermentation of corn flour. The effect of structural changes of starch by stillage recycling and scaling up of the process. *Fuel*, 119, 328-334. http://dx.doi.org/10.1016/j.fuel.2013.11.034.

Tekin, H., Bilkay, O., Ataberk, S. S., Balta, T. H., Ceribasi, I. H., Sanin, F. D., Dilek, F. B., & Yetis, U. (2006). Use of Fenton oxidation to improve the biodegrability of a pharmaceutical wastewater. *Journal of Hazardous Materials*, 136(2), 258-265. http://dx.doi.org/10.1016/j.jhazmat.2005.12.012. PMID: 16423452.

Vázquez-Rodríguez, J. A., & Escalante, F. M. E. (2022). Analysis of the stability of phycocyanin when trehalose and citric acid are used as protectants in nutraceutical gelatin candies under in vitro digestion assays'. *Food Science and Technology (Campinas)*, 42, e40621. http://dx.doi.org/10.1590/fst.40621.

Wang, B., Chen, J., Li, H., Sun, F., Li, Y., & Shi, G. (2017a). Pellet-dispersion strategy to simplify the seed cultivation of *Aspergillus niger* and optimize citric acid production. *Bioprocess and Biosystems Engineering*, 40(1), 45-53. http://dx.doi.org/10.1007/s00449-016-1673-y. PMid:27573803.

Wang, B., Li, H., Zhu, L., Tan, F., Li, Y., Zhang, L., Ding, Z., & Shi, G. (2017b). High-efficient production of citric acid by *Aspergillus niger* from high concentration of substrate based on the staged-addition glucoamylase strategy. *Bioprocess and Biosystems Engineering*, 40(6), 891-899. http://dx.doi.org/10.1007/s00449-017-1753-7. PMid:28389849.

Wang, L., Cao, Z., Hou, L., Yin, L., Wang, D., Gao, Q., Wu, Z., & Wang, D. (2016). The opposite roles of agdA and glaA on citric acid production in Aspergillus niger. *Applied Microbiology and Biotechnology*, 100(13), 5791-5803. http://dx.doi.org/10.1007/s00253-016-7324-z. PMid:26837219.

Xu, J., Bao, J. W., Su, X. F., Zhang, H. J., Zeng, X., Tang, L., Wang, K., Zhang, J. H., Chen, X. S., & Mao, Z. G. (2016). Effect of propionic acid on citric acid fermentation in an integrated citric acid-methane fermentation process. *Bioprocess and Biosystems Engineering*, 39(3), 391-400. http://dx.doi.org/10.1007/s00449-015-1522-4. PMid:26658985.

Xu, J., Su, X.-F., Bao, J.-W., Chen, Y.-Q., Zhang, H.-J., Tang, L., Wang, K., Zhang, J.-H., Chen, X.-S., & Mao, Z.-G. (2015). Cleaner production of citric acid by recycling its extraction wastewater treated with anaerobic digestion and electrodialysis in an integrated citric acid–methane production process. *Bioresource Technology*, 189, 186-194. http://dx.doi.org/10.1016/j.biortech.2015.04.016. PMid:25898079.

Yan, Y., Wang, L., Si, Z., Zhang, X., & Yuan, W. (2022). A novel cocrystal of metformin hydrochloride with citric acid: Systematic synthesis and computational simulation. *European Journal of Pharmaceutics and Biopharmaceutics*, 179, 37-46. http://dx.doi.org/10.1016/j.ejpb.2022.08.013. PMid:36041596.

Yin, X., Shin, H. D., Li, J., Du, G., Liu, L., & Chen, J. (2017). P*gas*, a Low-pH-induced promoter, as a tool for dynamic control of gene expression for metabolic engineering of Aspergillus niger. *Applied and Environmental Microbiology*, 83(6), e03222-16. http://dx.doi.org/10.1128/AEM.03222-16. PMid:28087530.

Yu, B., Zhang, X., Sun, W., Xi, X., Zhao, N., Huang, Z., Ying, Z., Liu, L., Liu, D., Niu, H., Wu, J., Zhuang, W., Zhu, C., Chen, Y., & Ying, H. (2018). Continuous citric acid production in repeated-fed batch fermentation by *Aspergillus niger* immobilized on a new porous foam. *Journal of Biotechnology*, 276-277, 1-9. http://dx.doi.org/10.1016/j.jbiotec.2018.03.015. PMid:29588182.