27

Production and Applications of Succinic Acid

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27.1 Introduction

The growing global concern over climate change, the greenhouse effect, and dependency on fossil carbon and the increased legislation geared toward environmental protection have urged scientists to develop production methods for the manufacture of industrially important chemicals from renewable resources. Today, several countries all over the world are producing and evaluating the potentials of bio-based chemicals. Among these, succinic acid was identified as one of the most important key chemicals that can be produced biologically [3,22,24,71,87,88,102]. The suitability of this acid as a platform chemical is based on its importance as a key compound in the production of more than 30 commercially important products [6,47,50,121].

Succinic acid, a member of the four-carbon dicarboxylic acid family, is produced as an intermediate of the tricarboxylic acid (TCA) cycle and is one of the important fermentation products of energy metabolism [43,48,53,121]. Succinic acid is also often found in samples of atmospheric aerosol particles [36,101]. Pure succinic acid is solid under atmospheric conditions and its relatively low solubility gives a deliquescence point close to, or above, 100% relative humidity. Once it has deliquesced, it will, however, stay as an aqueous solution down to relative humidity below 60% [90,93]. The physical and chemical properties of this important acid are summarized in Table 27.1.

Traditionally, succinic acid is known as "amber acid." Amber has been used in Europe as a natural antibiotic and general curative for centuries. At that time Europeans had no idea that amber has a very high content of succinic acid, which acts as a curative agent (Fig. 27.1).

In 1546, Georgius Agricola, a mineralist and doctor, purified succinic acid using dry distillation. The dry distillation divided amber into acid, oil, and resin, all of which are exceptionally valuable and useful [100]. Since that time succinic acid has found several applications in the pharmaceutical, agricultural, and food industries [102,121].

Today, succinic acid is used for a wide range of applications and is a critical compound for producing many industrially important products, including surfactants and

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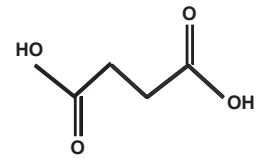
Table 27.1 Physical and Chemical Properties of Succinic Acid

Succinic acid also know as	amber acid; butanedioic acid; dihydrofumaric acid; asuccin; bernsteinsaure; kyselina jantarova
IUPAC name	Butanedioic acid
Molecular formula	$C_4H_6O_4$($HOOCCH_2CH_2COOH$)
Physical state	Colorless, odorless white crystals
Melting point	185–187°C
Boiling point	235°C
Solubility in solvents	Slightly dissolved in ethanol, ether, acetone, and glycerin; not dissolved in benzene, carbon sulfide, carbon tetrachloride, and oil ether
Solubility in water	Soluble
Molar mass	118.09
Specific gravity	1.552
Flash point	206°C
Density	1.56 g/cm^3
Vapor density	3.04
Acidity (pK_a)	$pK_{a1} = 4.2$ $pK_{a2} = 5.6$
Stability	Stable under ordinary conditions
Occurrence	Naturally occurs in plant and animal tissues
Applications	Pharmaceuticals, agriculture, food products, and other industrial uses



Structural Formula of Succinic Acid

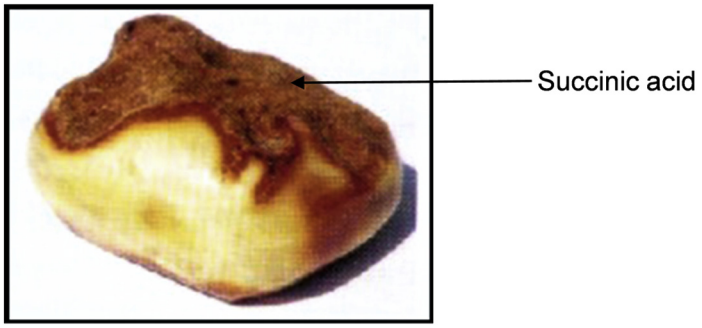


FIGURE 27.1 Amber—the external layer containing succinic acid.

detergents, flavors and fragrances, biodegradable polymers (clothing fibers), food additives, fungicides, and herbicides. It is also used as a starting material for a number of chemicals including adipic acid, *N*-methylpyrrolidinone, 2-pyrrolidinone, succinate salts, 1,4-butanediol, maleic anhydride, tetrahydrofuran, and γ-butyrolactone, which are

used in the pharmaceutical industry. This important organic acid, also known as butanedioic acid, is also used as a plant growth regulator. One of the latest applications of succinic acid is in the production of a new biodegradable plastic, Bionelle, which is an ester of succinic acid and 1,4-butanediol [1,19,64,67,102,121].

Realizing the importance of succinic acid, in this chapter the authors present an exhaustive review of succinic acid production, its market, and its applications. The importance of this review also lies in the fact that it is the first review that also covers the enzymatic regulation of succinic acid production. This review highlights the studies on important microorganisms involved in succinic acid production, its biochemical pathways, and enzyme regulations for the efficient production of this bulk chemical from renewable resources. Its economic importance in correlation with the current market scenario is also covered. This review also summarizes various strategies for the downstream processing and purification of biologically produced succinic acid. In light of current research and worldwide demand, future prospects and outlook are also discussed.

27.2 Market and Companies Involved in Succinic Acid Production

Succinic acid, according to the US Department of Energy (DOE), is one among the top 12 high-value-added bio-based chemicals and is one of the strongest contending biochemicals. Succinic acid, an intermediate in several chemical processes, is a platform chemical for the production of several specialty chemicals. By 2020, it is anticipated that the global market for succinic acid will grow at a compound annual growth rate (CAGR) of 24%. This will be due to its increasing applications and the switch of the chemical industry toward sustainable chemicals sought through biological routes. However, the major challenges include the high cost of processing and huge market competition [107].

The primary applications of bio-based succinic acid over petroleum-based succinic acid are as solvents and lubricants, deicer solutions, cosmetics, food, and pharmaceuticals. Other applications include 1,4-butanediol (BDO), polybutyrate succinate (PBS), plasticizers, and polyester polyols.

With the dismal scenario of fossil fuel availability, the perils of petroleum resource exhaustion, and stringent environmental legislation, there is an increased interest of the government in investments in green chemicals, which will certainly boost the growth of the market in the future. However, the major bottlenecks restraining market growth are the high price of biosuccinic acid and the tedious and cumbersome downstream processes.

Reports indicate that the maximum revenue was generated in the year 2013 with the latest markets of resins, pigments, and coatings. However, it is envisaged that 7 years down the line, BDO may come up as the largest application sector. This is mainly owing to the larger consumption of biosuccinic acid in the production of BDO, in place of maleic anhydride. To replace 1 MT of maleic anhydride, 1.2 MT of biosuccinic acid is desirable [92].

The largest market for succinic acid is in the manufacture of surfactants/detergents or foaming agents. This acid is also used as an ion chelator in electroplating. Succinic acid is used in the food industry mainly as a flavoring agent and as an antimicrobial agent. It is also important in the pharmaceutical industry in the production of antibiotics, amino acids, and vitamins. Depending on its purity, the market price of petrochemically produced succinic acid is about US$5.90–8.80/kg [60,102].

Succinic acid is currently only a niche product, with 30,000 tonnes produced every year, creating a market worth $225 million. The market research firm Frost & Sullivan believes the market will expand sixfold to 180,000 tonnes by 2015, thanks largely to the introduction of biosuccinic acid.

Although the gross economics limits biologically produced succinic acid, the study of the cost of the raw material and the estimation of the potential market volume clearly indicate that the fermentative succinic acid production system will in the near future replace the existing petroleum-based succinic acid production process.

In this direction, the US DOE has funded considerable research since 2005 to develop improved microorganisms and separations technology to reduce the overall cost of bio-based succinic acid. Advances in research have reduced the cost from $2.00 per pound in 1992 to about $0.50 per pound in 2003 for bio-based succinic acid [114], with further reductions in cost being anticipated. Commercialization of new low-cost approaches would significantly increase the market demand for succinic acid and its derivatives.

Europe was the largest market globally for succinic acid in 2013 in terms of value. Germany is the key consumer of succinic acid in Europe. Pharmaceuticals and personal care applications are the fastest growing segment for succinic acid in Europe. The largest manufacturers of bio-based succinic acid in this region are Succinity GmbH (Germany) and Reverdia (The Netherlands). The demand for conventional succinic acid in the region is strong because of high usage in the growing end-use applications. The Asia–Pacific (APAC) region is estimated to grow at a CAGR of 27.4% between 2014 and 2019. However, the North American region is also experiencing a shift from conventional succinic acid to bio-based succinic acid consumption. China is the largest consumer of succinic acid in the APAC region and is expected to grow at a CAGR of 30.5% between 2014 and 2019. The flourishing pharmaceuticals and personal care industry is creating the higher succinic acid demand in the country. The global bio-based succinic acid market is expected to reach a market volume of 710.0 KT by 2020, growing at a CAGR of 45.6% during 2013–20 [35,92].

The global succinic acid market is segmented on the basis of applications. Succinic acid has a wide variety of applications, which include industrial applications (57.1%), pharmaceuticals (15.91%), food and beverages (13.07%), and others (13.92%). All these applications have a huge growth potential, which is ultimately driving the growth of the succinic acid market. Among the industrial applications BDO has the largest market share. The increasing demand for green chemicals is the major driver for the growth in demand of succinic acid in the chemical industry.

As of this writing, no established industrial process for microbial succinic acid production have been reported. But a few companies are claiming to have a process to produce biosuccinic acid on a large scale. Companies like BioAmber (ARD-France/DNP), the association between DSM and Roquette (France), Myriant Technologies LLC (USA), the cooperation between Mitsubishi Chemical Corporation (Japan) and PTT Public Company Limited (Thailand), and BASF and CSM are cooperating in joint ventures for the development of bio-based succinic acid. All these companies are working on the production of succinic acid on a pilot scale with efficient and cost-effective downstream processing. They further claim "We are excited about the market feedback, especially the demand for large quantities. It shows that our customers are eager for succinic acid as an alternative to petroleum-based chemicals."

BioAmber is the first company that has developed a commercially viable technology to produce bio-based succinic acid by fermenting renewable feedstocks such as sugar or cereals. The plant will utilize the US DOE's proprietary *Escherichia coli* bacterium, which is under exclusive license to DNP Green Technology and has been optimized by BioAmber. Through a fermentation process, the microorganisms feed on sugar and carbon dioxide to produce succinic acid. As a result of the microorganisms feeding on carbon dioxide, the technology captures the greenhouse gas rather than emitting it. In addition, the process can use sugars derived from a variety of sources, which makes the technology feedstock flexible.

The other companies BASF and CSM announced joint production development of bio-based succinic acid. The two companies as partners have been working on the development of the industrial fermentation and downstream processing of bio-based succinic acid and plan to start its commercial production by the end of 2016. They claim to demonstrate the economical production of succinic acid on the industrial scale using an innovative pathway on the basis of renewable substrate. Carbon dioxide will be used as a raw material and fixed during the highly efficient fermentation process, contributing further to sustainable development.

The current worldwide use of succinic acid is around 20,000 to 30,000 tonnes per year and this is on the increase by approximately around 10% a year. This is one of the reasons it has attracted another player, with DSM of the Netherlands and Roquette of France announcing that they have combined their efforts to commercialize a fermentation-based process to produce bio-renewable succinic acid from glucose and carbon dioxide using a low-pH yeast. A list of companies involved in the production of biosuccinic acid is presented in Table 27.2.

27.3 Microorganisms Involved in the Production of Succinic Acid

Several anaerobic and facultative anaerobic bacteria that produce succinic acid from carbohydrates have been isolated from rumen and other sources. The rumen is the first division of the stomach of a ruminant animal. More than 200 kinds of bacteria inhabit

Table 27.2 Companies Involved in the Production of Biosuccinic Acid

Name	Organism	Substrate Used	Capacity	Country	Year of Commercialization	Source
BioAmber—DNP Green Technology	*Escherichia coli*	Wheat-derived glucose	2000 tonnes/year	France	2010—11	http://www.bio-amber.com
Mitsubishi Chemical Company—PTT	Bacteria	Biomass	Commercial level	Thailand	2010	http://biopol.free.fr
Royal DSM NV—Roquette	Bacteria/non bacteria, *E. coli*	Glucose/starch	10,000 to 20,000 tonnes/year	France	2011	http://www.chemie.de/news
BASF—CCM (Purac)	*Basfia succiniproducens*	Glycerin or glucose as a feedstock	Commercial quality and volumes	Spain	2010	http://www.foodnavigator.com/Product-Categories
Roquette—Rice University	Genetically engineered *E. coli*	Glucose from corn and wheat	Commercial level	France	2011	http://www.uga.edu/news/artman/publish/081124_household_products.html
Reverdia	*Saccharomyces cerevisiae*	Starch derivatives	10,000 tonnes/year	Italy	2012	http://www.dsm.com/en_US/cWorld/public/media/pages/press-releases/36_succinic acid_plant_2012.jsp
BioEnergy International — Myriant	*E. coli*	Starch	15,000 tonnes	United States	2013	http://biopol.free.fr
Succinity GmbH	*B. succiniciproducens*	No data	10,000 tonnes/year	Spain	2013	http://www.basf.com

the bovine rumen [47]. A number of functionally important rumen bacteria produce succinic acid during fermentation of carbohydrates [45], although succinic acid is seldom detected in measurable amounts because it is rapidly converted to propionic acid [10]. It is a common metabolite of several anaerobic and facultative microorganisms. The fermentative production of succinic acid has been most intensively investigated; the best-known succinic acid producers include *Actinobacillus succinogenes*, *Mannheimia succiniciproducens*, *Ruminococcus flavefaciens*, *Anaerobiospirillum succiniciproducens*, and recombinant *E. coli* [2,27,58,71,102,121].

Propionate-producing bacteria such as *Propionibacterium* species; gastrointestinal bacteria such as *E. coli*, *Pectinatus* sp., and *Bacteroides* sp.; and rumen bacteria such as *Bacteroides amylophilus*, *Prevotella ruminicola*, *Succinimonas amylolytica*, *Succinivibrio dextrinisolvens*, *Wolinella succinogenes*, and *Cytophaga succinicans* are also known good producers of succinic acid [12,22,38,47,85,98,110]. *Anaerobiospirillum succiniciproducens* and *Actinobacillus succinogenes* have been known to be the most efficient succinic acid-producing strains. Both bacteria require four specific enzymes, namely phosphoenolpyruvate (PEP) carboxykinase, malate dehydrogenase, fumarase, and fumarate dehydrogenase, for the production of succinic acid [110]. These strains produce succinic acid at a high yield, using a wide spectrum of carbohydrates as carbon and energy sources, and form fewer byproducts [16,22,78].

Research has also been directed toward the production of succinic acid from fungi. There are many reports in which the fungi *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium viniferum, Byssochlamys nivea*, *Lentinus degener*, and *Paecilomyces varioti* and yeast *Saccharomyces cerevisiae* have been used for the production of succinic acid. These organisms produce succinic acid as a metabolic by-product under aerobic and/or anaerobic conditions [72,94,102,113].

27.4 Synthesis and Enzyme Regulation of Succinic Acid

Succinic acid was originally observed in nature in all plants and animal tissues. It is also found in beer, molasses, meat, eggs, peat, coal, fruit, honey, and urine [109]. It plays a significant role in their intermediary metabolism and the Krebs cycle (Fig. 27.2).

However, scientists could not come up with a way to obtain the succinic acid from the plant and animal tissues in which they observed it. Today, succinic acid can be synthesized in two ways.

27.4.1 Chemical Synthesis

Chemically, succinic acid is manufactured through oxidation of *n*-butane or benzene to maleic anhydride followed by hydration to maleic acid, which is further converted into succinic acid by hydrogenation (Fig. 27.3). During maleic acid hydrogenation, the succinic acid produced is impure and needs further extensive and sometimes complex purification steps to obtain a commercially acceptable product. Several

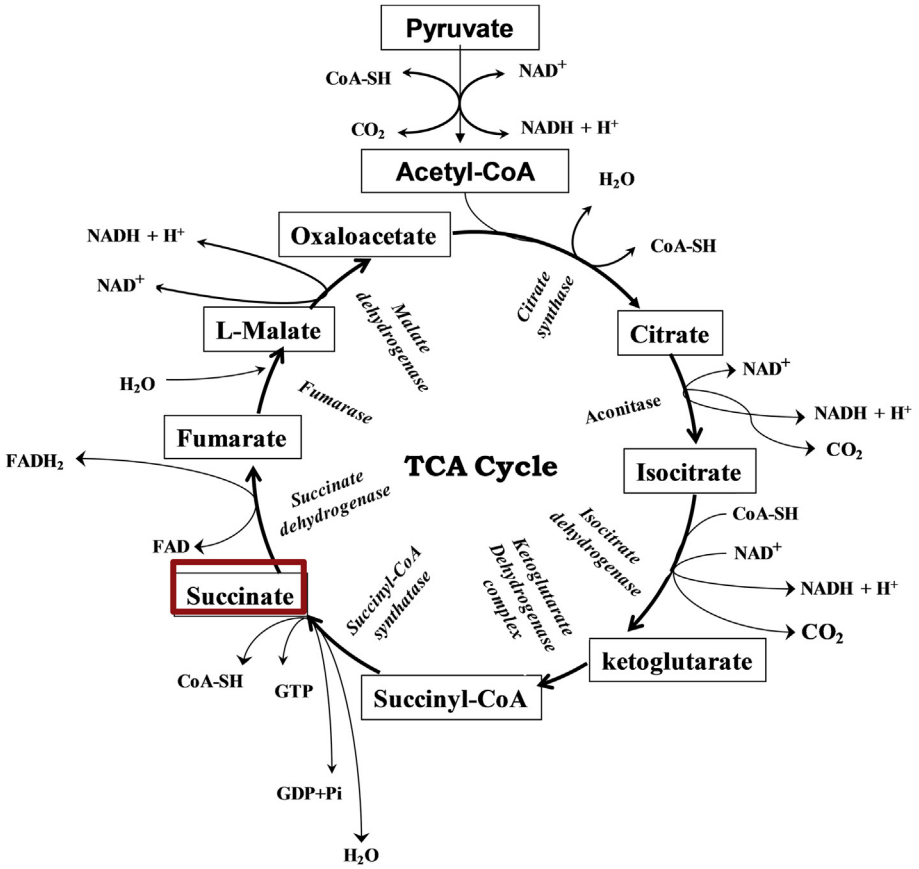


FIGURE 27.2 Chemical synthesis of succinic acid. *TCA*, tricarboxylic acid.

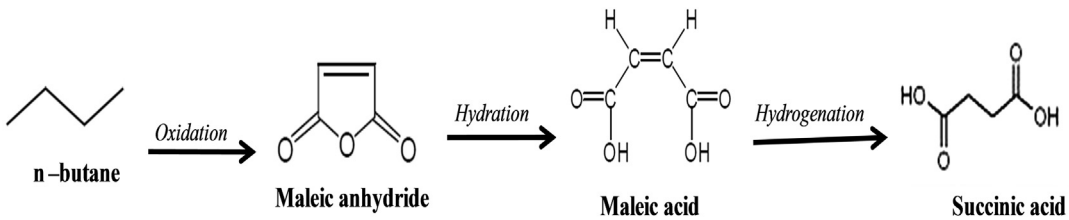


FIGURE 27.3 Biological synthesis of succinic acid.

companies have developed hydrogenation technology for the conversion of maleic anhydride to succinic anhydride [121].

27.4.2 Biological Synthesis

Succinic acid can be produced biologically in the following three pathways: (1) the TCA cycle or Krebs cycle or citric acid cycle, (2) the glyoxalate cycle, and (3) the reductive TCA

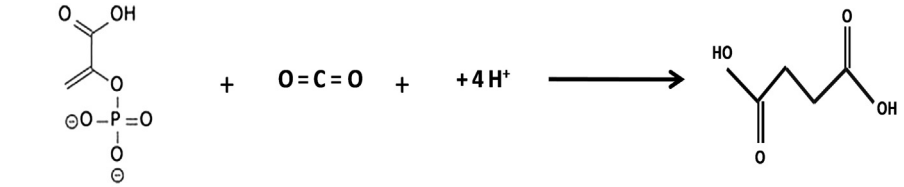


FIGURE 27.4 Reductive arm of the tricarboxylic acid cycle.

cycle. However, during the Krebs cycle and glyoxalate cycle, succinate does not accumulate in cells and further convert into other form; hence these cycles cannot be exploited for succinic acid production.

Anaerobically, succinic acid is produced via the reductive arm of the TCA cycle, in which PEP is converted into oxaloacetate (OAA), resulting in the formation of the end products succinate and propionate (Fig. 27.4). This requires the incorporation of four electrons and 1 mol of CO_2 [17,47].

Metabolism by either the oxidative arm of the TCA cycle or the glyoxylate bypass pathway conserves only four of the six carbons from glucose in the four-carbon succinic acid product. On the other hand, the reductive arm of the TCA cycle produces two four-carbon acids for every glucose molecule metabolized via glycolysis operating in conjunction with pyruvate carboxylase. Thus, anaerobic metabolism is preferred for succinic acid-producing microorganisms [124]. In anaerobes, there is an absence of any enzyme that further converts succinate. Thus, succinate is accumulated in the cell only under anaerobic conditions.

27.4.3 Enzymatic Regulation

Many species of anaerobic and facultative anaerobic bacteria have been studied to understand the enzymatic control regulating succinic acid production, including *Actinobacillus* sp., *A. succiniciproducens*, *R. flavefaciens*, and *E. coli*. In microbes, the production of succinic acid is highly regulated by enzymes that act in coordination. There are four major enzymes involved in the production of succinic acid; they are (1) PEP carboxykinase (PEPCK) or PEP carboxylase (PEPC), (2) malate dehydrogenase, (3) fumarate reductase, and (4) fumarase [97]. Vander Werf et al. [110] studied in detail the mechanisms of enzyme regulation for succinic acid production in *Actinobacillus* sp. and reported that this organism converts PEP to OAA by two enzymes: PEPCK and pyruvate kinase. PEPCK is a CO_2-fixing enzyme that converts PEP to OAA. Succinate is the only fermentation product formed via this pathway [110].

Escherichia coli, one of the best-studied facultative anaerobic bacteria, also produces succinic acid via the reductive arm of the TCA cycle. However, the succinate production pathway involves mainly PEPC as the carboxylating enzyme in place of PEPCK [81]. Under anaerobic conditions, the enzyme functions to direct the conversion of PEP to

succinic acid. PEPC is a regulatory enzyme that replenishes OAA in the TCA cycle of *E. coli* [56]. It is a unique allosteric enzyme in that it has many kinds of effectors such as acetyl-CoA, fructose diphosphate, and GTP [56,118]. Cysteinyl, histidyl, lysyl, and arginyl residues are essential for its catalytic activity. PEPC catalyzes the fixation of CO_2 with PEP to produce OAA and inorganic phosphate. The control of synthesis of this enzyme has already been studied by Teraoka et al. [104]. In *E. coli*, the OAA produced from PEP is converted to malic acid by malate dehydrogenase (*mdh*). The free energy of PEP is dissipated through the conversion of PEP to OAA.

The malic acid thus produced is converted to fumaric acid by the enzyme fumarase. Once fumaric acid is produced in the organism, fumarate reductase catalyzes the reduction of fumaric acid to succinic acid and is the key enzyme under anaerobic conditions when fumaric acid is the terminal electron acceptor [97]. The amino acid sequence of fumarate reductase is similar to that of the TCA cycle enzyme succinate dehydrogenase. These two enzymes have similar functional characteristics (e.g., substrate affinities and reaction rates) and both catalyze the interconversion of fumaric acid and succinic acid [11]. *Escherichia coli* also contains high aspartase and aspartate transaminase activities at levels comparable to those of fumarase and malate dehydrogenase. These two enzymatic activities form an alternative pathway for the formation of fumarate in *E. coli*. This fumarate is subsequently converted into succinate.

Laivenieks [61] reported that PEPCK of *A. succiniciproducens* is more suitable for the production of succinic acid, because it produces ATP and conserves the free energy of PEP. On the other hand, PEPC of *E. coli* dissipates the free energy of PEP. The sequence of *A. succiniciproducens* PEPCK is similar to those of all known ATP/ADP-dependent PEPCKs.

Ruminococcus flavefaciens and other succinate-producing ruminal bacteria use PEPCK rather than PEPC and pyruvate carboxylase as a primary carboxylation enzyme. This has the net effect of conserving energy by substrate-level phosphorylation of GDP. It also provides OAA, which is converted to succinate. This permits the disposal of two pairs of reducing equivalents generated during the conversion of carbohydrates to PEP [99]. Agarwal et al. [1] reported that there is a proportional relationship between enzyme activity and succinic acid production in *Enterococcus flavescens*. They reported maximum activity of PEPCK, which was followed by malate dehydrogenase, fumarase, fumarate reductase, and PEPC when maximum succinic acid was produced under the optimized conditions.

27.5 Estimation of Enzymes Involved in Succinic Acid Production

Teraoka et al. [104], as early as 1970, described the assay of the activity of PEPC in *E. coli*, in a reaction mixture containing cyclohexylammonium PEP, $KH^{14}CO_3$, glutathione, $MgSO_4 \cdot 7H_2O$, NADH, Tris−HCl buffer (pH 8.5), dioxane, malate dehydrogenase, and the

enzyme. They defined 1 unit of the enzyme as the amount that fixes 1 μmol of CO_2 per minute under specific assay conditions. They also monitored the activity of PEPCK by the $^{14}CO_2$ fixation reaction. The assay was devised so as to suppress the action of PEPC, which interferes with the assay of this enzyme. The reaction mixture contained cyclohexylammonium PEP, $KH^{14}CO_3$, ADP, $MnSO_4 \cdot 6H_2O$, L-aspartate, NADH, glutathione, Tris−malate buffer (pH 7.0), malate dehydrogenase, and the enzyme. One unit of enzyme was defined as the amount of the enzyme that fixes 1 μmol of CO_2 per minute under the specific assay conditions. Similarly, Yoshinga et al. [118] described the assay for the enzyme PEPC in a reaction mixture containing PEP, $KHCO_3$, $MgSO_4$, dioxane, $NADH_2$, Tris−H_2SO_4 buffer (pH 8.5), and malate dehydrogenase. They defined 1 unit of activity as the amount of enzyme producing 1 μmol of OAA per minute.

Kameshtha et al. [56] reported that in *E. coli*, the enzyme activity was determined at 30°C by the measurement of NADH oxidation at 340 nm in a coupled reaction system with malate dehydrogenase, using a spectrophotometer equipped with a constant temperature cell housing. The enzyme concentration was determined by measuring the absorbance at 280 nm using a millimolar extinction coefficient of 11.9 cm/mM. Meyer et al. [79] also described the assay of PEPC coupled with malate dehydrogenase. However, they also described the use of a lactate dehydrogenase-coupled system. Similarly, Samuelov et al. [97] monitored the activity of PEPCK in a reaction mixture containing sodium hydrogen maleate buffer (pH 5.5), $MgCl_2$, ADP, $NaHCO_3$, PEP, NADH, cell extract, and malate dehydrogenase. They also estimated the activity of malate dehydrogenase in a reaction mixture containing Tris−HCl buffer (pH 8.1), OAA, NADH, dithiothreitol (DTT), and cell extract. Fumarate reductase activity was also monitored by NADH oxidation. The reaction mixture contained Tris−HCl buffer (pH 8.1), fumarate, NADH, DTT, and cell extract. The enzyme assay was performed under strict anaerobic conditions. The wavelengths and millimolar extinction coefficients for NAD, NADH, NADP, and NADPH were 340 nm and 6.22 cm/mM, respectively.

Chao et al. [13] also reported that PEPCK and PEPC activity in *E. coli* were measured spectrophotometrically by monitoring the appearance of OAA and disappearance of pyruvate. Vander Werf et al. [110] described that PEPCK was monitored in a reaction mixture containing MES buffer (pH 6.6), $MgCl_2$, $MnCl_2$, DTT, ADP, $NaHCO_3$, NADH, malate dehydrogenase, and cell extract. The mixture was incubated at 37°C to activate PEPCK, after which the reaction was started by the addition of PEP. The activity of the enzyme was measured spectrophotometrically under strict anoxic conditions.

Fumarase activity has been determined by Samuelov et al. [97]. They reported that it can be estimated by monitoring the formation of fumarate at 240 nm in a reaction mixture containing Tris−HCl buffer (pH 7.2), L-malate, and cell extract. Similarly, the estimation protocol for determining the activity of fumarate reductase has been described by Vander Werf et al. (1979) [110]. The reaction mixture contained methyl viologen, fumarate, and cell extract. Before the reaction was started, sodium dithionite was added to the cuvettes. The activity was estimated by measuring the change in the absorbance at 578 nm. All the compounds of the reaction mixture except for the cell

extract and the substrate were added to an optical glass cuvette. The cuvette was sealed with a soft rubber stopper and was made anoxic by flushing with N_2 for 5 min. One unit of enzyme activity was the amount of enzyme catalyzing the conversion of 1 μmol of substrate per minute into specific products.

27.6 Enzymatic Regulation of Succinic Acid Production

Amplification of the enzymatic steps involved in the succinate pathway under anaerobic conditions resulted in higher succinate production. For example, overexpression of PEPC (*pepC*) from *E. coli* resulted in 3–5 times more succinic acid [81]. Conversion of fumarate to succinate was improved by overexpressing native fumarate reductase (*frd*) in *E. coli* [31,112]. Certain enzymes not indigenous to *E. coli* can also help increase succinate production, for example, introduction of pyruvate carboxylase (*pyc*) from *Rhizobium etli* into *E. coli* enhanced succinate production [32]. Overexpression of malic enzyme in the presence of inactivated pyruvate formate lyase (*pfl*) and lactate dehydrogenase (*ldh*) significantly increased succinic acid production [41,103]. In this *pfl* and *ldh* mutant, there was a large pyruvate accumulation. Overexpression of malic enzyme in this mutant increased succinate production driven by the high pyruvate pool toward malate formation and subsequently converted to succinate. An inactive glucose phosphotransferase system (*ptsG*) in the same mutant strain (pfl^- and ldh^-) also yielded higher succinate production in *E. coli* [14]. Efforts have been made to develop recombinant *E. coli* strains capable of producing succinic acid with high efficiency. The *Sorghum vulgare ppc* and *Lactococcus lactis pyc* genes were introduced into the *ldh–pta* inactivated and *ldh–pta–ack*-inactivated *E. coli* mutant strains, respectively, to redirect the accumulated pyruvate to OAA [73]. In another approach, a recombinant strain was created by activating the glyoxylate pathway because it requires less NADH in *E. coli*. More recently, a metabolically engineered *E. coli* strain capable of aerobically producing succinic acid through the glyoxylate pathway and the oxidative branch of the TCA cycle was developed by inactivating the succinate dehydrogenase (*sdh*), pyruvate oxidase (*poxB*), *pta–ack*, *aceBAK* operon repressor (*iclR*), and *ptsG* genes. The aerobic fed-batch fermentation of this strain resulted in the production of 58.3 g/L succinic acid in 59 h with a succinic acid yield of 0.85 mol/mol glucose [73,74]. However, production of pyruvic (6.1 g/L) and acetic (3.0 g/L) acids could not be avoided. Further modification of *E. coli*, with advances in measuring intracellular metabolites and carbon flux analysis, may lead to the development of a commercial biocatalytic succinic acid process. Kai et al. [55] and Wang et al. [112] constructed and overexpressed the carbonic anhydrase gene from *Cyanobacterium anabaena* sp. 7120 in *E. coli* to enhance succinic acid synthesis.

The production of succinic acid is highly regulated by enzymes, which act in coordination. Overexpression of the PEPC gene in *E. coli* provided a significant increase in the amount of succinic acid production, from 3.27 to 4.44 g/L [81]. *Escherichia coli* has a malic enzyme, which can interconvert pyruvate and malate. Amplifying the malic enzyme would result in the conversion of pyruvate to malate. The latter can be converted

to succinic acid. Thus succinic acid production could be enhanced by the overexpression of the *sfcA* gene encoding malic enzyme in NZN111 [41,103]. The NZN111 strain was developed to increase succinic acid production, by inactivating the pyruvate:formate lyase (*pfl*) and the lactate dehydrogenase (*ldh*) genes This resulted not only in increased yield of succinic acid but also less formation of ethanol and acetic, formic, and lactic acids under anaerobic conditions. However, the disadvantage associated with the production of a high amount of pyruvic acid was the inhibition of cell growth. This was mainly due to the inability to sufficiently generate NAD [41,103]. Goldberg et al. [31] and Wang et al. [112] reported that the recombinant *E. coli* strain DH5α harboring pGC1002, which contains the *E. coli* fumarate reductase gene *frd ABCD* and the ampicillin-resistance (β-lactamase) gene produced 47.5 g/L succinic acid from 50.8 g/L fumaric acid and 23.7 g/L glucose after 32 h cultivation. The weight yield of succinic acid based on the quantity of fumaric acid consumed was 0.93. A metabolically engineered *E. coli* strain capable of aerobically producing succinic acid through the glyoxylate pathway and oxidative branch of the TCA cycle was developed by inactivating the succinate dehydrogenase (*sdh*), pyruvate oxidase (*poxB*), *pta−ack*, *aceBAK* operon repressor (*iclR*), and *ptsG* genes. The aerobic fed-batch fermentation of this strain resulted in the production of 58.3 g/L succinic acid in 59 h. With the succinic acid yield, the formation of pyruvic acid (6.1 g/L) and acetic acid (3.0 g/L) acids could not be avoided.

27.7 Production and Regulation of Succinic Acid

As of this writing, succinic acid is commercially manufactured by chemical processes. Fermentative production of this important organic acid has many advantages over chemical processes owing to its simplicity and environmentally friendly nature. Succinic acid is reported to be produced and accumulated by a few anaerobic and facultative anaerobic microorganisms as the product of their metabolism. The major constraint in the development of a commercially viable technology for succinic acid production is the lack of cost-effective production methods and downstream processing. Thus, a lot of efforts are still to be made in process development for the production of this organic acid in large amounts in a cost-effective manner. This will facilitate its commercialization and will rightly justify its versatile applications.

The most critical factor affecting succinic acid production is pH. The pH of the medium affects the solubility and availability of CO_2 in both anaerobic and facultative anaerobic microorganisms [23]. Thus by regulating CO_2 availability, the pH regulates the activity of the enzyme responsible for succinic acid production [51,97]. Almost all the studies have reported 37−39°C as the optimal temperature for the growth of rumen microflora and the production of succinic acid. This is because most succinic acid producers are isolated from rumen and the ambient temperature in the rumen of the ruminants and monogastric animals is 39 ± 1°C [96]. Therefore, most of the studies on the growth and end-product formation by the rumen isolates have been carried out at this temperature. Anoxic gases (CO_2, N_2, and H_2) also play a significant role in the cell

growth of anaerobic bacteria. The level of CO_2 in the culture medium affects the metabolic flux and distribution of fermentation products [23,84].

Theoretically, succinic acid can be produced fermentatively from glucose in the presence of CO_2 with the following stoichiometry:

$$\text{Glucose} + 2CO_2 + 4H^+ \rightarrow 2\ \text{Succinic acid} + 2H_2O$$

Therefore, supplies of CO_2 and electron donors are necessary to achieve good succinic acid production.

Similarly, Lee et al. [62] reported that the high level of CO_2 usually achieved by an external supply was found to promote succinic acid production. Subsequently, Lee et al. [67], by changing the headspace from a CO_2 to a N_2 atmosphere, showed a significant effect on both the cell growth and the end-product formation. Under a N_2 atmosphere, cell growth and glucose consumption were poor. In addition to CO_2, H_2 as a potential electron donor can affect cellular metabolism. Succinate is a highly reduced fermentation product using four electrons per molecule formed [17]. Therefore, the effect of the addition of an electron donor, i.e., hydrogen, during the fermentation process is important. Vander Werf et al. [110] also supported that hydrogen increases the succinate/acetate product ratio during glucose fermentation in *A. succinogenes*.

Lee et al. [62], while working with *A. succiniciproducens*, reported that the supply of a mixed gas comprising 5.0% H_2 and 95.0% CO_2 significantly enhanced both cell growth and succinic acid production. Furthermore, the supply of 5% or 10% H_2 shortened the fermentation time, resulting in an increase in succinic acid productivity (1.8 g/L/h for the culture with 5% H_2 supply), which was 1.8 times higher than that obtained in the absence of H_2 and is the highest value reported as of this writing. They further suggested that the decrease in cellular redox potential and the promotion of NADP(H) recycling could be the possible reason for the enhanced effects. Therefore, the external supply of H_2 accelerates the conversion of glucose to succinic acid because of the incorporation of electrons derived from H_2.

Anaerobiospirillum succinicproducens a well-known succinic acid producer, which produced a mixture of succinic acid and acetic acid at a molar ratio of 4:1 from glucose under strictly anaerobic conditions [22]. When *A. succinicproducens* was grown on whey the ratio of succinic acid to acetic acid (g/g) was 5.1−5.8. When *A. succiniciproducens* was cultured on 6.5 g/L glycerol as a carbon source, the yield of succinic acid was 4.9 g/L and 133%, respectively [65]. Lee et al. [68] also used whey as a substrate for the production of succinic acid by *M. succiniciproducens* MBEL55E. O'Herrin and Kenealy [83] reported that *Succinivibrio dextrinisolvens* produces succinate and acetate as the major fermentation products of glucose, and lactate and formate as the minor products. Vander Werf et al. [110] reported that *Actinobacillus* sp. 130Z produced acetate, succinic acid, and formate as major fermentation products of glucose. They further reported that this organism also supported succinic acid production using carbon sources like fructose, mannose, mannitol, sucrose, maltose, lactose, xylose, and cellobiose. To further enhance the production of succinic acid, cofermentation of glucose (4.3 g/L) and

glycerol (3.6 g/L) was carried out, both the carbon sources were completely consumed at the end of fermentation, and the concentration and yield of succinic acid obtained were 8.2 g/L and 104%, respectively. However, the ratio of succinic acid to acetic acid was 9:1, which was lower than that obtained using glycerol alone. Another potential organism, *M. succiniciproducens*, can utilize other carbon sources, like mannitol, arabitol, fructose, xylose, sucrose, maltose, and lactose, as efficiently as glucose [67]. This study reported that it could not, however, utilize xylitol, inositol, sorbitol, glycerol, xylan, or cellulose. Hong et al. [42], while working with *M. succiniciproducens*, used cellulose, a substrate produced during the digestion of feed, to produce succinic acid. Agarwal et al. [1], while working with *E. flavescens*, reported 14.25 g/L succinic acid in 30 h when 3% sucrose was used as the carbon source. To make the process economical Zheng et al. [122] used corn straw hydrolysate as a sugar source for succinic acid production.

Previously, succinic acid production via chemical synthesis from petrochemical or refined sugar has been the focus of interest of most reviewers. However, these expensive substrates have been replaced by alternative sustainable raw materials such as lignocellulosic biomass, which is cheap and abundantly available. Thus, this review focuses on succinic acid production utilizing lignocellulosic material as a potential substrate for solid-state fermentation (SSF) and separate hydrolysis and fermentation (SHF). SSF is an economical single-step process that can be a substitute for SHF—a two-step process in which biomass is hydrolyzed in the first step and fermented in the second step. SSF of lignocellulosic biomass under optimum temperature and pH conditions results in the controlled release of sugar and simultaneous conversion into succinic acid by specific microorganisms, reducing reaction time and costs and increasing productivity [4].

Some nutritional components present in complex nitrogen sources are essential for the growth of cells as well as for succinic acid production [66]. Nutrient supplements such as yeast extract, corn steep liquor, and casein hydrolysate have been reported to improve the nutritional quality of media because they contain growth-promoting compounds in addition to organic nitrogen and carbonaceous compounds [30,44]. The main constituents of yeast extract are purine and pyrimidine bases and vitamins of the B group [44]. Russel [96] and Nghiem et al. [82] reported that *Bacteroides ruminicola*, a versatile bacterium, can have a very high growth yield and it is greatly stimulated by peptone. In another study, biotin-supplemented spent yeast cell hydrolysate was used as an alternative nitrogen source for the efficient production of succinic acid by *A. succinogenes* NJ113, using renewable resources. As a result, when biotin-supplemented spent yeast cell hydrolysate was used with corn fiber hydrolysate, a succinic acid yield of 67.7% was obtained from 70.3 g/L total sugar concentration, with a productivity of 0.63 g/h [15].

Lee et al. [62] examined seven commercial complex organic nitrogen sources (yeast extract, casamino acid, polypeptone, soytone, tryptone, and beef extract) for their effects on the production of succinic acid from *A. succiniciproducens*. They reported that polypeptone enhanced succinic acid production, whereas yeast extract or soytone had no significant effect on cell concentration. However, yeast extract and polypeptone also had

a positive effect on cell growth and succinic acid production [65]. When glycerol was used as the carbon source and polypeptone alone was used as the nitrogen source, it did not support cell growth or succinic acid production. Only when yeast extract was also supplemented, the polypeptone exerted a positive effect on cell growth and succinic acid production. To further confirm the yeast extract-dependent consumption of glycerol, both glycerol and yeast extract were intermittently added. The added glycerol was consumed further, depending on the supplementation with yeast extract. The concentration and yield of succinic acid at the end of fermentation were 219.0 g/L and 160%, respectively.

Corn steep liquor was found to be the best nitrogen source by Lee et al. [68] and Agarwal et al. [1]. They reported that the highest succinic acid concentration was obtained using corn steep liquor while working with *M. succiniciproducens* and *E. coli*. Pretreated molasses is also a good source of succinic acid production. Liu et al. [76] reported a maximum of 50.6 g/L succinic acid in 60 h when cane molasses was used as a sole nitrogen source. They reported that inexpensive cane molasses could be utilized for the economical and efficient production of succinic acid by *A. succinogenes*.

Metal ions are known to play an important role in maintaining cellular metabolism and enzyme activities [91]. Metals like sodium and magnesium have been studied for their effects on succinic acid production by various workers [20,21,62,69,79]. Sodium ions are involved in the formation of transmembrane pH gradient, cell motility, intracellular pH regulation, and nutrient uptake [20,39,59]. Cell growth and succinic acid production in *A. succiniciproducens* were slightly affected by varying NaCl concentration in the medium [62,63]. Similarly, magnesium ions also played an important role in maintaining cellular metabolism and are especially important because they are cofactors for PEPCK/PEPC, the key enzymes in succinic acid production [54,79,91]. A comparison of succinic acid yield and production conditions from various sources is summarized and presented in Table 27.3.

27.8 Recovery Systems for Succinic Acid

The purification and extraction of succinic acid from fermentation broth is one of the major problems scientists are facing. However, there are a few reports on the purification of succinic acid from various microbial sources [20,33,43]. Schematic flow charts showing various methods used for succinic acid recovery are presented in Fig. 27.5. In general, there are no set rules for the purification of succinic acid. Normally, purification is a multistep process comprising several well-defined steps such as neutralization with salt, precipitation, ion exchange, filtration, acidification, electrodialysis, and crystallization [43,58,101].

The major problem regarding purification of succinic acid is that during fermentation many unwanted by-products are formed, like acetic acid, lactic acid, and ethanol. Removing all the impurities increases the cost of downstream processing. Bechthold et al. [6], Baniel and Eyal [5], and Williams [115] reported that more than 60−80% of the

Table 27.3 Comparison of Succinic Acid Yield and Production Conditions From Various Microbial Sources

S. No.	Microorganism Used	Production of Succinic Acid (g/L)	Productivity (g/L/h)	Yield (g/g)	Substrate Used	Mode of Cultivation	Incubation Time (h)	References
1	*Lactobacillus reuteri*	11.3	0.23	–	Cane molasses	Anaerobic batch	48	[57]
2	*Actinobacillus succinogenes* 130Z	66.4	0.79	0.67	Glucose	Anaerobic batch	84	[38]
3	*A. succinogenes* FZ53	105.8	1.34	0.80	Glucose	Anaerobic batch	78	[38]
4	*Anaerobiospirillum succiniciproducens*	33	1.1	0.93	Glucose	Anaerobic batch	30	[82]
5	*A. succiniciproducens*	24	2.1	0.72	Whey	Anaerobic continuous	$D = 0.085$/h	[97]
6	*A. succinogenes*	110	–	–	Glucose	Anaerobic	–	[121]
7	*A. succinogenes*	17.8	–	89%	Corn steep liquor	Anaerobic batch	14	[66]
8	*A. succiniciproducens*	29.6	1.35	0.97	Glucose + glycerol	Anaerobic fed-batch	22	[64–65]
9	*Mannheimia succiniciproducens*	14	1.87	0.70	Glucose	Anaerobic batch	7.5	[67]
10	*Escherichia coli* AFP111	99.2	1.30	1.10	Glucose	Dual phase fed-batch	76	[111]
11	*M. succiniciproducens*	13.5	1.22	0.72	Whey	Batch	11	[68]
12	*A. succinogenes*	33.99	0.88	0.86	Glucose	Repeat batch	38.5	[108]
13	*M. succiniciproducens* MBEL55E	11.73 and 14.3	1.17 and 3.19	56% and 55%	Wood hydrolysate	Anaerobic batch and continuous	24	[58]
14	*E. coli AFP*111	51.0	0.87	70%	Plant hydrolysate	Two-stage batch	120–170 h	[26]
15	*E. coli* HL27659K	43	0.72	0.53	Glucose	Aerobic batch	60	[73]
16	*E. coli*	58.3	1.08	0.62	Glucose	Aerobic fed-batch	54	[74]
17	*M. succiniciproducens*	52	1.8	0.76	Glucose	Anaerobic	30	[67]
18	*Bacteroides fragilis*	12.5	0.416		Tryptone/glucose	Anaerobic	30h	[47]
19	*A. succinogenes*	35.6	1.01	0.82	Wheat	Biorefining strategy	36	[27]
20	*A. succinogenes*	40	–	–	–	Anaerobic	–	[123]
21	*Enterococcus flavescens*	14.25	2.10		Sucrose	Anaerobic	30	[1]
22	*E. coli* AFP184	30–40	1.25	–	Corn steep liquor	Anaerobic	32	[2]
23	*A. succinogenes*	55.2	1.15		Cane molasses	Anaerobic batch/fed-batch	48	[76]
24	*A. succiniciproducens*	83	10.4	0.89	Glucose	Electrodialysis	8	[80]

Continued

Table 27.3 Comparison of Succinic Acid Yield and Production Conditions From Various Microbial Sources—cont'd

S. No.	Microorganism Used	Production of Succinic Acid (g/L)	Productivity (g/L/h)	Yield (g/g)	Substrate Used	Mode of Cultivation	Incubation Time (h)	References
25	*E. coli* NZN111	12–14	1.4	–	Glucogenic carbon source	Anaerobic	10	[116]
26	*A. succinogenes*	53.2	1.21	–	Straw hydrolysate	Anaerobic fermentation	48	[122]
27	*A. succinogenes BE-1*	15.8	0.20	1.23	Crop stalk waste	Anaerobic batch cultivation	72	[71]
28	*E. coli* XZ721	102	0.7	0.80	Minimal medium	Anaerobic fermentation	72	[120]
29	*Yarrowia lipolytica* Y-3314	140	0.86	0.38	Complex medium at lower pH	Anaerobic fermentation	72	[119]
30	*Corynebacterium glutamicum* BOL-3/pAN6-gap	1134	1.67 two-stage process	21 two-stage process	Minimal medium fed-batch in saline solution	Anaerobic fermentation	72	[77]
31	*A. succinogenes* NJ113	60.5	2.16	–	Sucrose	Serum bottle fermentation	48	[49]
32	*Propionibacterium acidipropionici* ATCC 4875	14.8	0.197	0.212	Sorbitol	Anaerobic fermentation	72	[28]

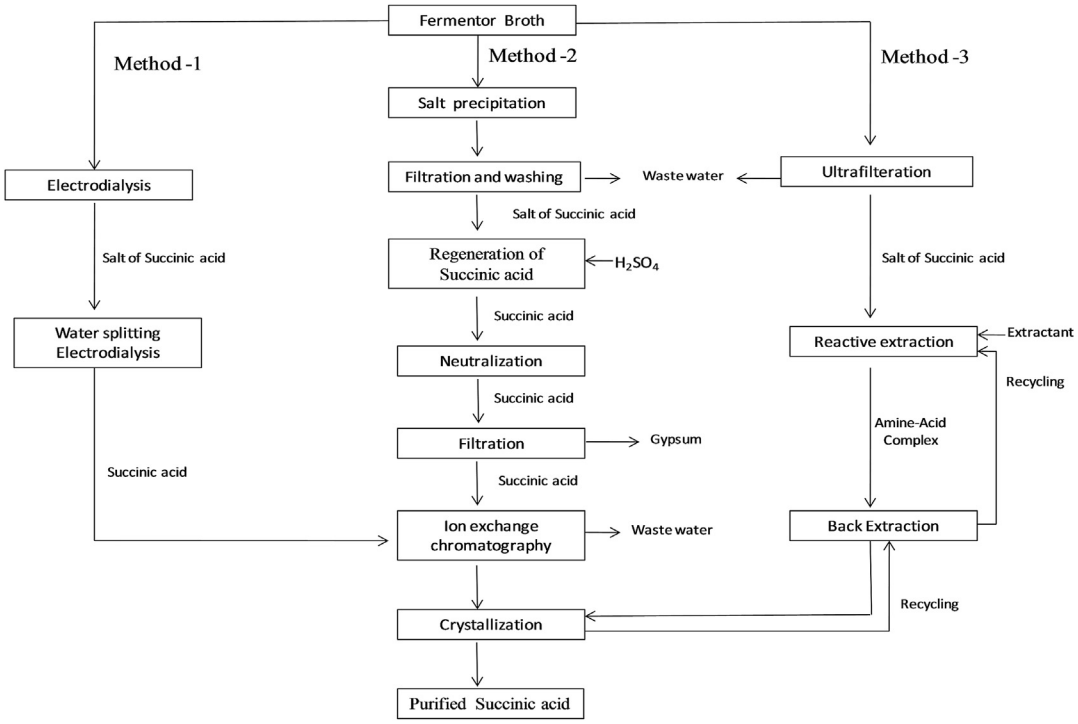


FIGURE 27.5 Recovery procedures for succinic acid.

total production cost is generated by downstream processes. Therefore, a prime concern in the designing of any successful fermentation process is the development of simple, efficient, and economically viable downstream processing for the purification of succinic acid.

The basic technical hurdles faced during purification are to convert salt to acid, to remove cells and protein-like impurities, and to polish the free acid to its required purity [20,21]. There are a few reports on succinic acid recovery that include the following:

1. **Filtration/ultrafiltration**: The isolation of succinic acid from the fermentation broth generally requires ultrafiltration. The fermentation broth is directed through a bypass cross-flow, hollow-fiber ultrafiltration that removes the unwanted proteins and cell debris [95].
2. **Crystallization**: Succinic acid product recovery is based on simultaneous fermentation and crystallization of the calcium salt upon addition of calcium hydroxide to the aqueous fermentation broth [8,20,21]. Furthermore, by a simple filtration process these crystals are recovered and protein and other debris are removed by washing. Addition of concentrated sulfuric acid dissolves the crystal into soluble succinic acid solution and insoluble calcium sulfate molecules. Succinic acid is recovered by filtration and further purified by acid and base ion exchangers [21].

This process generates a large amount of solid waste and slurry, which poses a serious waste management problem for industrial-scale processes [121]. Berglund et al. [8] suggested that succinic acid has a minimum solubility in the presence of bisulfate ions. By adding ammonium bisulfate to diammonium succinate solution, succinic acid can be crystallized from solutions at high purity.

3. **Selective extraction**: This method is also used as an alternative for succinic acid purification. Selective extraction is generally based on acid—base couple extraction and amine-based extraction. The main extraction mechanisms are ion-pair and hydrogen-bond formation [47]. Amine-based extraction is an effective and economic method for separating succinic acid from the fermentation broth because it is operated at normal temperatures and pressures and also proceeds very rapidly [29,105,117]. Amines offer a high affinity for reacting with negatively charged molecules because of their high basicity (electron donor). Therefore, they are suitable for the purification of organic acids like succinic acid [60,89]. Tertiary amines are known to be effective extractants for organic acids. Succinic acid also shows a much more pronounced tendency to aggregate when extracted by amines. This may be because dicarboxylic acids have two potential binding sites, so they can act as a link between other amines and acids to form a large complex or aggregate [9]. Hong and Hong [40] reported the extraction of succinic acid with a solution of mixed tertiary amine triproplamine and trioctylamine as the extraction agent and 1-octanol/*n*-heptane as the diluent.
4. **Ion exchange and sorption**: Ion exchange is a well-reported procedure for the final purification of succinic acid. This technique is used in many studies for the recovery of succinic acid [8,52,70].
5. **Electrodialysis**: This is one of the most suitable processes for the recovery of succinic acid. This is a rapid process for altering the composition and/or concentration of electrolytes in a solution by transferring ions across the semipermeable membranes under the influence of a direct electric current. Zeikus et al. [121] and Glassner and Datta [33] also reported that electrodialysis is an engineering technology that holds higher potential for commercialization and environmentally friendly purification processes for succinic acid. The production of succinic acid by this process is cost-effective and can easily be scaled up for commercial use. The major limitation of this process is that the membranes cannot handle divalent cations; therefore, fermentations neutralized with magnesium or calcium hydroxide cannot be acidified and purified by this process. However, there is still a considerable task to lower the cost of succinic acid recovery technology. Zeikus et al. [121] suggested that decreasing the number of units of operation appears to be a promising way of improving the overall economics. The employment of new purification technologies and integrated process configurations therefore presents exciting possibilities for reducing the number of purification steps and the production costs. Various purification techniques applied for the recovery of succinic acid are presented in Table 27.4.

Table 27.4 Purification and Downstream Techniques Applied for the Recovery of Succinic Acid

Microorganism	Purification Steps	Final Purity %	Final Yield %	References
Anaerobiospirillum succiniciproducens ATCC 53488	Neutralization with calcium hydroxide in the fermentation broth, calcium succinate precipitation, filtration, ion exchange	94.2	—	[21]
A. succiniciproducens ATCC 29305	Ultrafiltration, hollow-fiber cartridge 0.2 μm, electrodialysis, crystallization, liquid—liquid extraction	99	—	[34]
A. succiniciproducens ATCC 29305	Neutralization with ammonium, precipitation, filtration, reflux, reutilization, crystallization	—	90	[8]
Mannheimia succiniciproducens	Reactive extraction, vacuum distillation, crystallization	99.8	73.1	[46]
M. succiniciproducens MBEL55E	Centrifuged 5590X*g*, single reactive extraction, vacuum distillation, crystallization	>99.5	67.05	[101]
Actinobacillus succinogenes CIP 106512	Novel resin-based (cation-exchange resin Amberlite IR 120H) vacuum distillation—crystallization method	99%	89.5%	[75]
A. succinogenes	Aqueous two-phase system (acetone/ammonium sulfate)	94.4%	77.3%	[37]

27.9 Applications and Uses of Succinic Acid

The industrial potential of succinic acid was recognized when it was reported that owing to its linear saturated structure, it could be used as an intermediate for the synthesis of several chemicals of industrial importance. Succinic acid is an exciting building block for the industry because it can be used to make many derivatives that offer benefits in multiple applications in all spheres of life. Succinic acid is a chemical intermediate that has been traditionally used in medicine, in the manufacture of lacquers, and to make perfume esters. It has also been conventionally used in foods as a sequestrant, buffer, and neutralizing agent [18]. Succinic acid is a known plant-growth regulator [25].

Succinates (most often calcium succinate, potassium succinate, and sodium succinate) are very effective after long illnesses and injuries. These are generally used for medicinal purposes as sedatives, antispasmodics, antirheoters, and contraceptive drugs. In addition to this, succinic acid is also used as an inhibitor of potassium ions and an antioxidant. Succinic acid is also a valuable product for people active in sports. Therefore, the acid may be called an "elixir of youth" [18]. Succinic acid has been known as a remedy for alcohol hangovers because of its remarkable effect in supporting the body's natural ability to process acetaldehyde, alcohol's first and most toxic metabolite. These salts make it possible for the patient to regain immunity to diseases as well as intellectual fitness with the ability to concentrate. In addition to this, by the esterification of succinic acid, a solvent dimethyl succinate can be made, which can be marketed as an

environmentally benign solvent [34]. It is also used in vehicle water-cooling systems [121]. Succinic acid compounds also have been patented as salt-substitute flavor-enhancing agents [106]. Succinate salts are also reported to increase propionate production in the rumen. It acts as a glycogenic material and a precursor for protein synthesis [7]. This suggests that the succinate salts can be used as additives to animal feed for ruminants and monogastric animals such as pigs. Hence, the crude succinate salt produced from carbohydrates could find new markets as a product for animal nutrition to reduce the usage of antibiotics (such as monocin and lasalocid) in certain animal feeds [7].

The potential market for succinic acid is estimated at €2.5 billion, with potential use in products such as antifreeze liquids, coolants, solvents, pigments, polyesters, intermediates for the chemical industry (BDO and its derivatives), plasticizers, etc. These products, all of which can be derived from bio-based succinic acid, represent a safe, economical, and environmentally friendly alternative to petrochemicals to accommodate the growing consumer demand.

Succinic acid and its derivates have also been used in the leather industry to improve water repellency and wet strength and in metallurgy to improve the froth floating of various ores [86]. The interest in succinic acid production has considerably increased because of its use as a green feedstock for the manufacture of synthetic resins and biodegradable polymers such as PBS and polyamides [83,114]. It is also used in paints as a coalescing agent for emulsion [62]. The latest application of succinic acid is in the production of a new biodegradable plastic, Bionelle, which is an ester of succinic acid and BDO. This has been manufactured by Showa Highpolymer Co., Ltd. (Tokyo, Japan) [69]. The potential applications of succinic acid based on commodity and specialty chemicals are illustrated in Fig. 27.6 [47].

27.10 Conclusions and Perspectives

Succinic acid has been recognized today by both government organizations and the chemical industry as one of the most promising biochemicals that can be produced from renewable feedstocks to replace a wide range of petroleum-based chemicals. The process to make biosuccinic acid will use simple raw materials as a feedstock, which will consist of glucose (six-carbon unit) and CO_2. The fermentation to produce succinic acid also appears to be much more energy efficient. A life-cycle analysis showed that it would require about 30–40% less energy than a typical chemical production process and so this will save the atmosphere from yet more carbon emissions. So it seems the process will be clean (no waste products), be environmentally friendly (capture CO_2), and use a relatively cheap feedstock for production (glucose) to provide a cheaper source of succinic acid for a growing market.

With the support of companies like BioAmber–DNP Green Technology (USA), BioEnergy International–Myriant (USA), and DSM and Roquette (The Netherlands) and a few others, tremendous efforts and extensive research have been done to make

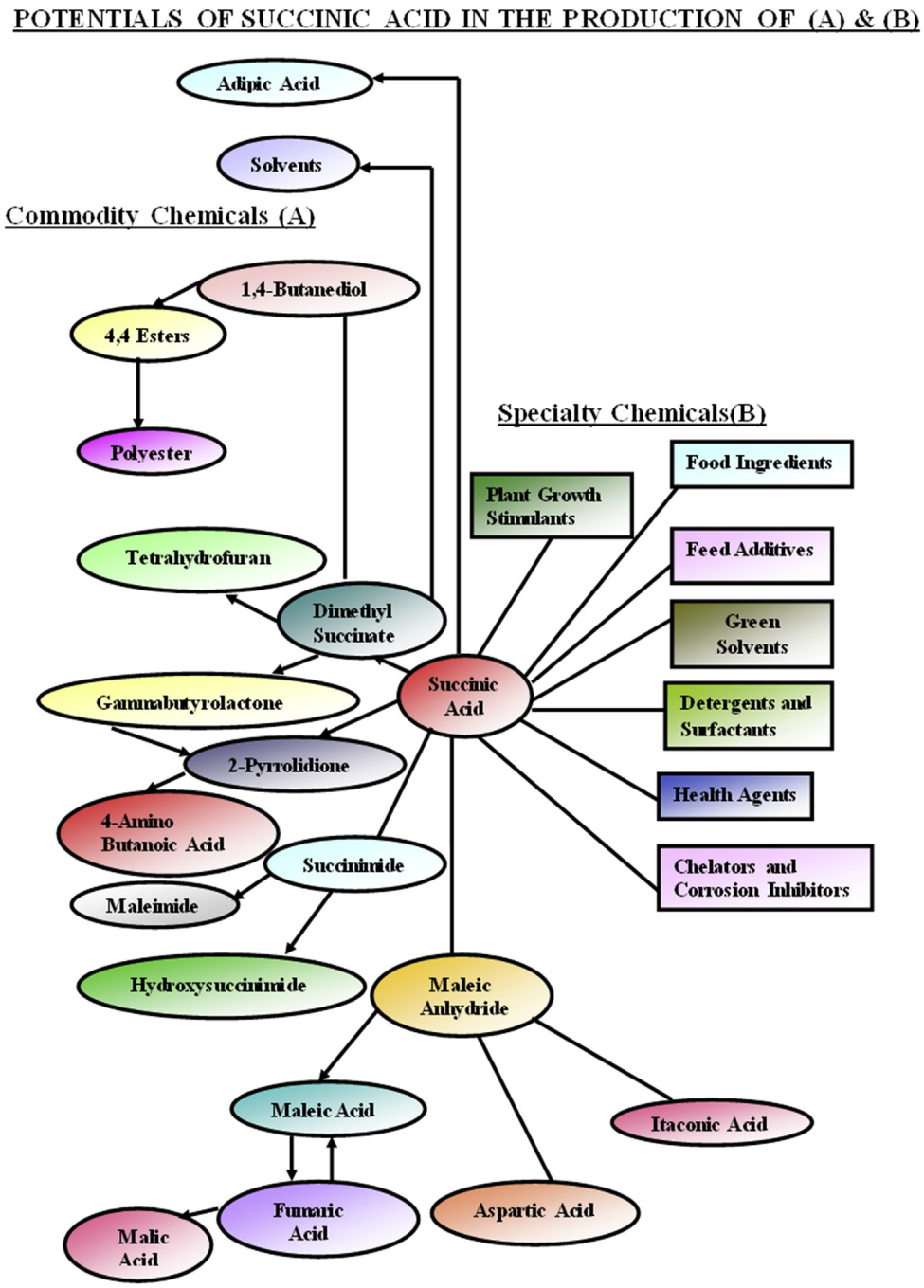


FIGURE 27.6 Potential applications of succinic acid.

biosuccinic acid a reality. While developing the process they all kept their vision on sustainability and the use of "green"/renewable materials (biomass or agricultural feedstocks) for succinic acid production. These players will ensure commercial success by developing less time-consuming and more cost-effective fermentation routes using the existing production infrastructure with available and next-generation feedstocks. This global industrial revolution will definitely change the world forever.

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