



BIO-SUCCINIC ACID: AN ENVIRONMENT-FRIENDLY PLATFORM CHEMICAL

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Abstract

Global research for biomass-based products is swelling gradually due to depletion of fossil-based raw material as well as their negative effects on the environment. The hazardous chemical processes used for production of valuable industrial chemicals are directly responsible for environmental pollution. Therefore, use of alternative renewable resources as feedstock to produce such chemicals is gaining popularity. Succinic acid (SA) is platform chemical which can be used to produce bulk industrial chemicals with huge global demand such as adipic acid, 1,4-butanediol, maleic anhydride. Non-hazardous biological fermentation process can be used to produce succinic acid, which can be further converted to these chemicals. The bio-based approach uses CO₂ as supplement during the process and it replaces fossil-based raw materials; therefore, it is environment friendly. Mostly, pure sugars or sugars derived from crop residues or lignocellulosic materials are used to produce succinic acid. However, utilization of agricultural waste aromatic compounds to produce succinic acid has not been investigated in detail and not being implemented anywhere. Lignin waste management is a problem for the cellulosic bio-refineries and finding the way for its biological utilization is still in the stage of research and development. Unlike sugar metabolism, bioconversion of aromatic compounds is relatively complicated. Interestingly, bioconversion of agricultural waste aromatic compounds could be targeted towards production of succinic acid. The purpose of the present review is to highlight this possibility.

Keywords

Succinic acid, Platform chemical, Market potential, Lignin, Aromatic substrates.

1. Introduction

Succinic Acid (SA) is a biochemical compound produced in almost all organisms, aerobic as well as anaerobic either as an intermediate or end product of their metabolic pathway. Knowing the positive impact of succinic acid on human health, Nobel Prize winner, Robert Koch popularized succinate by means of an industrial organic compound in food industries and generated interest intended for its high scale industrial production. Due to its impressive utilization to produce various industrial chemicals like 1, 4-butanediol, 2-pyrrolidinone, tetrahydrofuran, and various other conventional products like pigments, additives, detergents, pharmaceuticals intermediate, etc., succinic acid becomes a valuable chemical [1,2]. The production of SA was initiated

by Agricola in 1546. Initially, it was extracted from Amber through dry distillation which releases acidic anhydride. At that time, the succinic acid used to recognize by odor only. Later, numerous chemical approaches of succinic acid extraction from Amber were developed such as extraction by solvents like petroleum ether, methanol, and ammonium carbonate. At the end of 18th-century, chemical-based industries started using fossils fuels (petroleum, coal and natural gases) or catalytic hydrogenation of maleic anhydride to produce succinic acid. However, due to the concern of their limited natural feedstock, and negative environmental impacts, biotechnology is now playing a vital role to develop environment friendly processes to produce succinic acid [3].

Bio-based succinic acid has various benefits over its chemically synthesized counterpart as it utilizes CO₂ during the process, renewable raw materials are used and produce fewer toxic byproducts. Moreover, succinic acid fermentation not only utilizes CO₂ but also produces three important products together i.e. ethanol, succinic acid, and diethyl succinate. Michigan biotechnology institute (USA) had isolated the first commercial micro-organism “*Anaerobiospirillum succiniciproducens*” as a natural succinic acid producer. Some other micro-organisms isolated for this purpose are *Actinobacillus succinogenes*, *Mannheimia succiniciproducens* and *Basfia succiniciproducens* [4]. Low product yield and low productivity are the major limitations of these natural producers. Metabolic engineering can resolve these problems by modifying the biosynthetic pathway as desired. Metabolic engineering can be applied to natural producers and or any other microorganism whose metabolic pathways are well studied such as *Escherichia coli*, *Corynebacterium glutamicum*, and *Saccharomyces cerevisiae*. However, *E. coli* is usually selected as the model organism for metabolic engineering to produce succinic acid as its genome, proteome, metabolome and culture conditions are well established. Thus, significant interest has been observed to develop metabolically engineered strain for succinic acid production and various technologies to transform succinic acid into industrially important derivatives [5, 6].

As the applications of succinic acid are very vast, so its demand for at commercial level is high. In the 1990s, total succinic acid was manufactured around 18,000 MT (metric tons) and estimated to increase with 27.4% CAGR to 768 million MT by the year 2025. Major markets spread over for succinic acid use are surfactants/detergents, anti-corrosion agents, flavoring agents or antimicrobial agents in foods, health industry including vitamins, antibiotics, etc. In 2009, *Bioamber* and *Biohub* are two major French corporations have begun pilot-plant for succinic acid production. They

used genetically engineered *E. coli* strains for this. Later in 2010, Myriant and MBI International started pilot plant production using *Escherichia coli* and *Actinobacillus succinogenes*, respectively in the United States. As the value of bio-based succinic acid production increases, at the end of 2010, German-based BASF and Dutch CSM firms also started bio-succinic acid production in Spain. BASF isolated their own novel rumen bacteria *Basfia succiniciproducens* for succinic acid production. Later on, Japanese agencies (Mitsubishi and Ajinomoto) came up with scaling-up of succinic acid production by using the *Corynebacterium* strain [7, 8]. All of these companies use direct sugar sources like corn starch, sugarcane molasses even though there are other low-cost and abundant cellulosic biomass such as wood hydrolysate [9] also available for succinic acid production. Although these materials need to be pretreated to release carbohydrate molecules available, still they are less expensive than refined carbohydrates. Apart from cellulosic source, aromatic compounds source (lignin) have the potential for succinic acid production. This review signifies the susceptibility of the succinic acid as an important building block chemical.

2 Succinic acid: Production approaches

Succinic acid production methods through biological routes are well studied along with genetic level modifications. Bio-based succinic acid production is non-hazardous and environmentally friendly while the petroleum-based method uses harmful chemicals for succinic acid and their derivatives development. Vaswani and Sudeep [10] defined both the chemical and biological processes for succinic acid synthesis. The chemical method starts with the conversion of maleic anhydride to succinic anhydride by hydrogenation with the aid of catalyst like Ni/Si alloy. After that, hydrolysis converts it into succinic acid. But hydrogenation causes toxicity and increases the cost of the whole process. However, the non-toxic biochemical approach uses glucose and CO₂ as substrates to produce succinic acid (Figure 1).

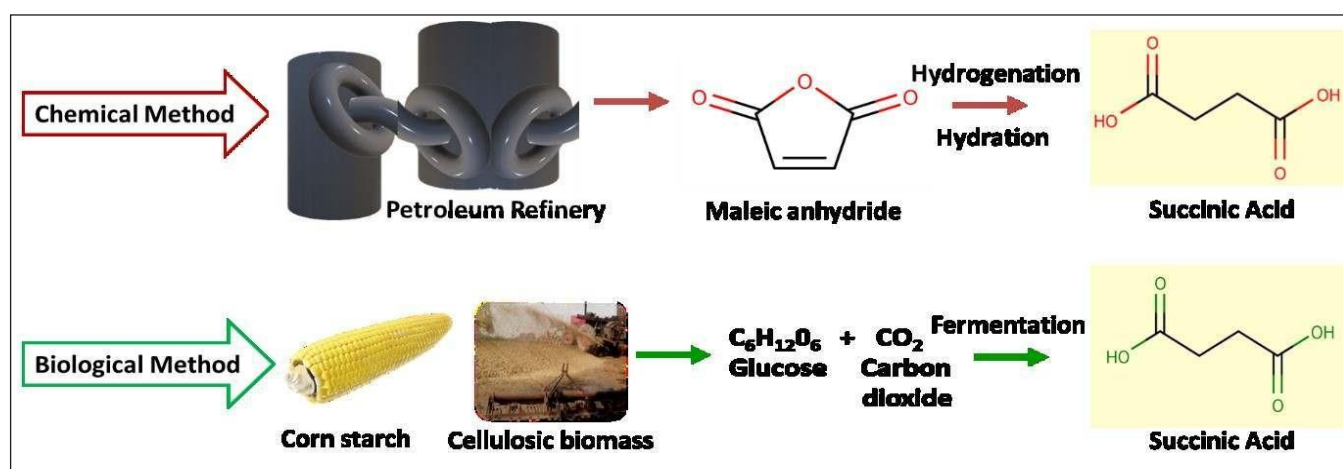


Figure 1: Succinic acid production via a chemical and biological method.

There are various applications of succinic acid in the field of food and chemical industry. It is used to produce adipic acid, 1,4 butanediol, tetrahydrofuran, succinonitrile, succinimide, polyesters, resins, acidulants, etc. Figure 2 describes various applications of succinic acid. Moreover, it is utilized in detergent and surfactant formations, as chain extender, ion-

chelator, and foaming agent preparations. It is also used for making resins, inks, coating materials, plasticizers, liquors, plant growth stimulants, electrolyte bath additives, biodegradable plastics, anti-microbial agents, pH modifiers and flavoring agents.

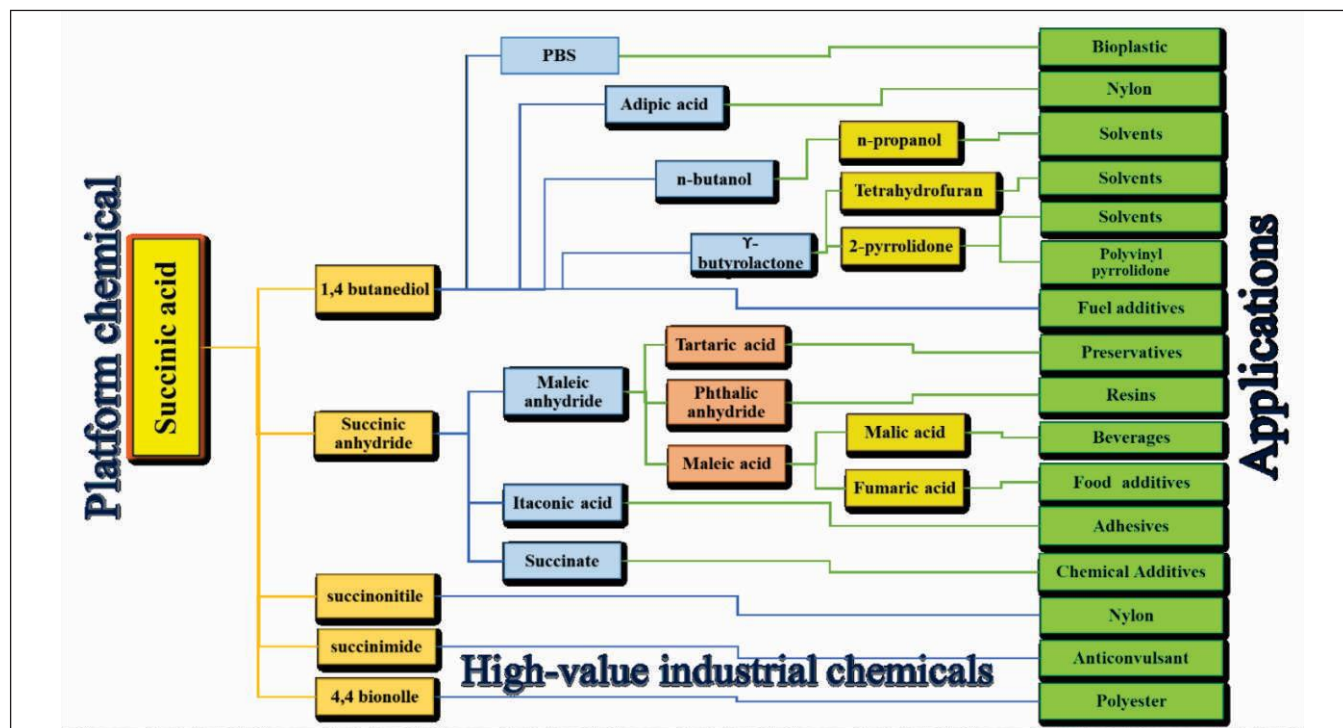


Figure 2: Applications of succinic acid as a platform chemical.

3 Global market potential of succinic acid

The global market of succinic acid is expected to reach at around 701 million USD by 2021. The global demand for succinic acid is around 40,000 tons/year-45,000 tons/year. A comparative analysis of the global market potential of succinic acid against other valuable industrial chemicals are presented in Figure 3. The major succinic acid manufacturers using petrochemical process are Kawasaki Kasei Chemicals, Linyi Lixing Chemical, Anhui Sunsing Chemicals, Mitsubishi Chemical, Nippon Shokubai, Gadiv Petrochemical, Astatech, R-Biopharm, Evonik, Thyssenkrupp [11]. On the other hand, major bio-based succinic acid manufacturers are BioAmber, Reverdia, and Succinity (Table 1). In India, Thirumalai Chemicals Ltd., A. B. Enterprises, Chemicals Chamber and others (shown in Table 2) are chemical-based manufacturers of succinic acid [12,13].

As per the authors’ knowledge, till now there is no bio-based succinic acid producing company in India.

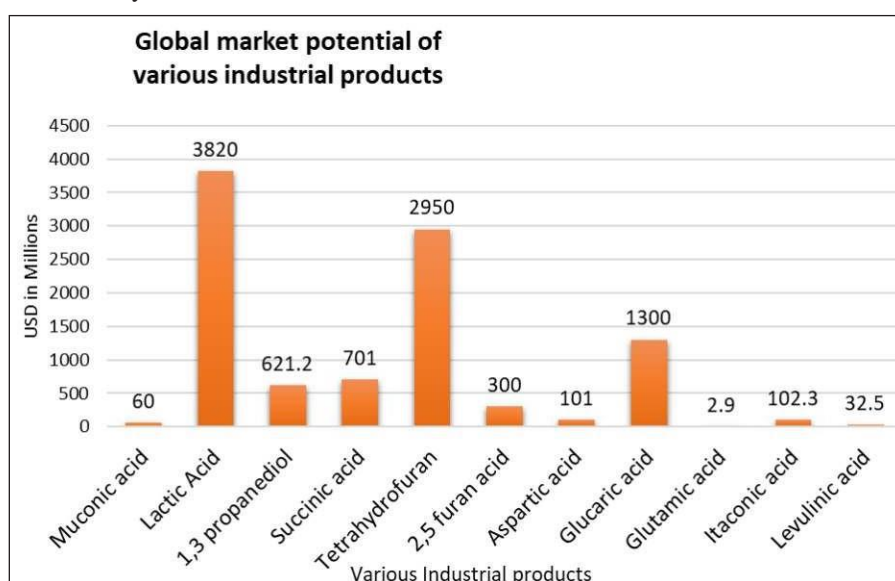


Figure 3: The global market potential of various industrial products.

Table 1: Succinic acid key player in world.

Company Name	Location
Shanghai Runkey Biotech Co., Ltd	China
Linyi Lixing Chemical Co., Ltd.	China
HeBei Oukai Biotechnology Co.,Ltd	China
A & Z Food Additives Co., Ltd.	China
ZSIKI KFT	Hungary
BioAmber	United States
Myriant	United States
Succinity	Germany
Reverdia	Italy
Gadiv Petrochemical Industries Ltd.	Israel
Nippon Shokubai	Japan
Mitsubishi Chemical Holdings	Japan
Kawasaki Kasei Chemicals Ltd.,	Japan
BASF	Germany
DSM	Netherlands

Table 2: Succinic acid key player in India.

Company Name	Location
B Joshi Agrochem Pharma	Mumbai
Thirumalai Chemicals Ltd.	Mumbai
Alpha Chemika	Mumbai
A. B. Enterprises	Mumbai
Axiom Chemicals Pvt. Ltd.	Vadodra
Meru Chem Pvt. Ltd.	Mumbai
Antares Chem Private Limited	Mumbai
Labdhi Chemicals	Mumbai
Arnish Laborates Private Limited.	Mumbai
Alpha Chem	Ambala
Natural Organics India	Delhi
Kapchem International	Mumbai
Chemicals Chamber	Delhi

31 Economic Value of Succinic Acid

The current bio-based production of compounds and polymers (excluding-fuel) is estimated to be around 50 million tons [14]. But still, chemical production from fossil-fuels was significantly higher, which was accounted to be 330 MT in 2009 [15]. The market of petrol-based production of succinic acid was estimated to decline by 2019 because of its negative influence on the environment. Due to this, bio-based

production processes are preferred for use in the pharmaceutical and cosmetics industries. Its non-toxicity and high purity help in taking care of the personal health of consumers [16]. Global market of succinic acid can be divided into different sectors such as, pharmaceuticals (15%), industrial (57.1%), food (13.07%) and others (13.92%).

Among various applications of succinic acid, the largest application is BDO (1, 4-butanediol) that boosted the international bio-succinic acid marketplace by about 17% in the year 2013. BDO is used as a solvent in many industries. It can be further converted to γ -butyrolactone (GBL) and tetrahydrofuran (THF). So, OMR industry analyst and Grand view research estimated the demand for succinic acid increases with CAGR of 35% till 2020.

On an industrial scale, butyrolactone is mainly manufactured from maleic anhydride. So, the replacement of maleic anhydride (MAN) by bio-succinic acid will take over this market, which is less expensive and environment-friendly. Polyurethane, a thermosetting polymer is another example that shows increases in the market possibility of bio-succinic acid. A new polyurethane class Non-isocyanate based polyethenes (NIPUs) has recently been developed, which will also increase the demand for bio-succinic acid [17]. About 25,000 to 30,000 MT (metric tons) succinic acid produces annually and expected to rise 10% every year. The addressable market of succinic acid will replace mainly the maleic anhydride, adipic acid, phthalic anhydride market. Because it can be used as a substitute for these chemicals. The succinic acid price in the market relies upon its purity, productivity, yield, recovery process, and raw material cost.

The succinic acid produced from the organic process is usually sold at a price of USD 5.9 to 9 per kg.

32 Expansion of bio-based succinic acid productions

There are various companies that are using bio-based production routes succinic commercial production of acid. Major companies producing bio-succinic acid are BASF, BioAmber, Mitsubishi, Myriant, DSM, Mitsui & Co, Succinity, Roquette Freres S.A, Purac, and Reverdia. Most of them are joint ventures (JV) except Myriant. The main bio-based succinic acid production market was covered by BioAmber, Succinity, and Reverdia with the production capacity of 350 MT, 500 MT, and 300 MT respectively [18]. BioAmber, Reverdia, and Succinity were able to produce 3,800 MT in the year 2011. Further, the Myriant corporation also enhanced the production volume with a dramatic increase in their production units. The world's first largest bio-based succinic acid industry BioAmber is located in France and now able to produce succinic acid nearly 3,000 MT/year. All the bio-based succinic acid-producing companies are using sugars extracted from different crops or biomasses. Table 3 shows the foremost bio-based succinic acid-producing player in the succinic acid market along with the details of major production capacity, kind of substrates, and class of microbes used in production [19].

Table 3: Bio-Based succinic acid-producing companies

Company name	Location	Substrate	Microbe	Commercial Operation	Production (tons/year)
Bioamber (in JV with Mitsui)	Sarnia, Ontario, Canada	Corn, wheat, cassava, rice, sugarcane, sugar beets	Bacteria	August 2015	30,000
Myriant	Lake Providence, LA, USA	Sorghum grain, Ligno cellulosic sugar from corn stover	Bacteria	June 2013	14,000
Reverdia (Roquette-DSM JV)	Cassano Spinola, Italy	starch from corn	Yeast	December 2012	10,000
Succinity (BASF-Corbion Purac JV)	Barcelona, Spain	sucrose, glucose	Bacteria	March 2014	10,000

4 Substrate and micro-organisms for succinic acid: Natural and Engineered

Corn waste, sugarcane molasses, wheat grains, whey, lignocellulosic biomass or pure sugars are commonly used substrates for fermentation. Glucose is the main carbon source used to study the metabolic rate of microbes. Natural succinic acid-producing microbes such as *Actinobacillus carinogens*, *Anaerobicspirillum succiniproducens*,

Mannheimia succinicproducens are restricted to glucose only, and cannot utilize a variety of substrates [20]. Genetically engineered micro-organisms such as *Corynebacterium glutamicum*, *E. coli*, and a new specific isolate *Basfia succinoproducens* were also investigated for their role in succinic acid production [21]. *Basfia succinoproducens* isolated from the rumen gut is a natural succinic acid-producing microbe. Different kinds of bacteria,

natural and genetically engineered, were being used for getting a higher titer of succinic acid. Lignocellulosic sugar and direct pure sugar derivatives are the mostly used substrates to produce succinic acid. Some researchers suggested that corn cob, cane molasses, starch, and lignocellulosic biomass are the substrates useful for succinic acid production [22-41].

A comparative analysis of succinic acid yield and titer on different carbon sources and hydrolysates is depicted in Figures 4 and 5.

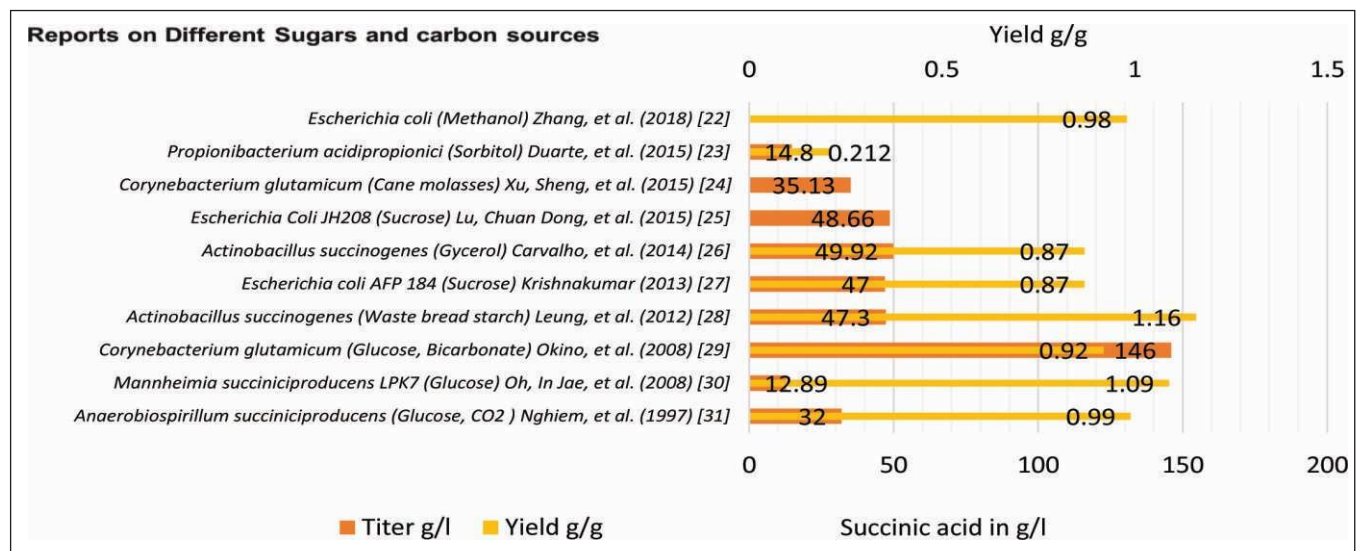


Figure 4: Comparative study of the bacterial fermentation of different sugar derivatives and other carbon sources for succinic acid production.

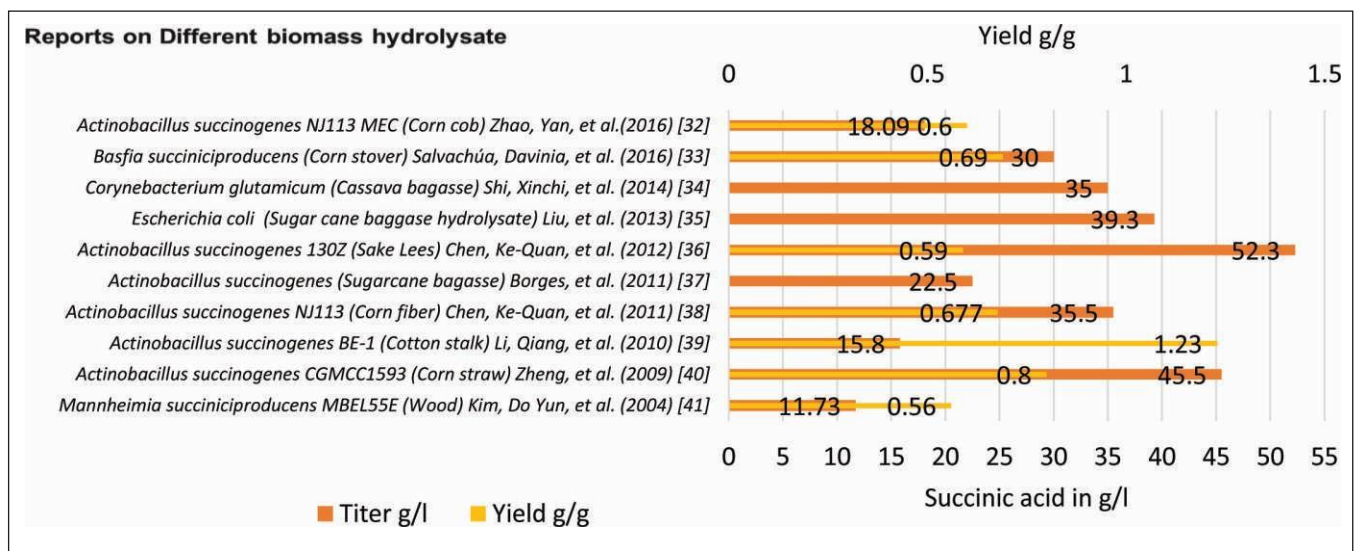


Figure 5: Comparative study of the bacterial fermentation of different biomass hydrolysates for succinic acid production.

5. Metabolic engineering strategies for succinic acid production

Glucose is the starting substrate for biosynthesis of succinic acid in microbial cells. Initially, glycolysis occurs till phosphoenolpyruvate (PEP) and pyruvate formation. When

CO₂ enters the system under anaerobic condition, it activates enzymes like phosphoenolpyruvate carboxylase (ppc), phosphoenolpyruvate carboxylase kinase (ppck), and pyruvate carboxylase (pyc). Their carboxylases catalyze PEP

and pyruvate to produce oxaloacetate. At this stage, the citric acid cycle appears as a reverse second half citric acid cycle to produce succinic acid (Figure 6). Other byproducts such as acetate, ethanol, lactate formate also start producing from pyruvate. Apart from that, keto-glutarate and malate form amino acid such as aspartate and glutamate. Figures 6 (A and

B) described all probable products formed along with succinic acid-producing under anaerobic conditions. The theoretical yield measured for the succinic acid from glucose is 0.66g/g. One mole of CO₂ is used per mole of succinic acid obtained.

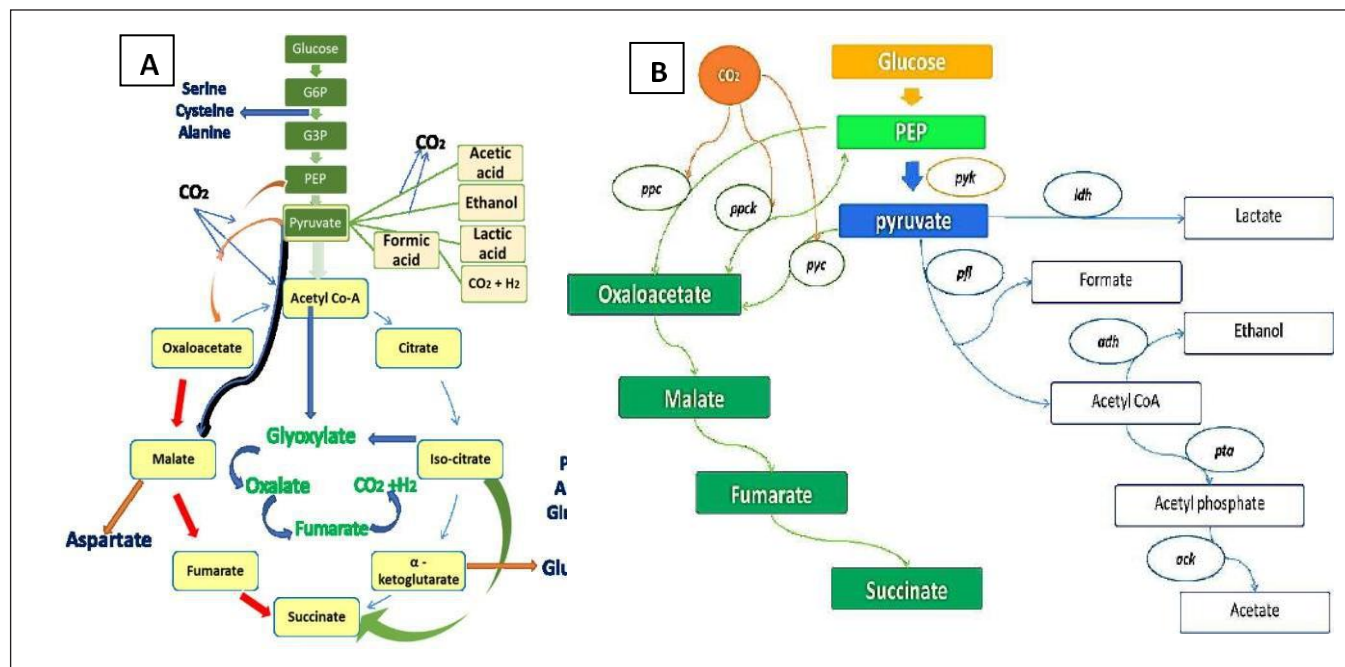


Figure 6: (A) Major metabolic routes to succinic acid production, (B) Bio-synthetic pathway of the succinic acid from glucose: Green branch of the pathway is representing succinic acid and white is for the by-product.

6. Potential to use aromatic substrates

Mostly, sugar derivatives were used to produce succinic acid, but aromatic compounds and phenol derivatives have not been studied to produce succinic acid. However, the phenolic derivatives were studied for other industrial chemicals such as adipic acid, muconic acid, but not for succinic acid. The phenol degradation potential by different micro-organisms is shown in Figure 7 [42-50]. A higher degradation rate for aromatics like catechol, phenol, benzoic acid, p-coumarate, 4-hydroxy benzoate was achieved by the implementation of a genetic engineering approach. Researchers have studied the degradation of the aromatic into products like adipic acid, pyruvate, lactate, muconic acid by *Pseudomonas putida*. Vanillin, pyridine and di-carboxylic acid were produced from *Rhodococcus josti* [51-57]. This has been done by deleting gene of *ldhA*, *pflB*, *pta* and *ackA* and overexpression of *pyc* in *E. coli* and in other naturally succinic acid-producing micro-organisms. Table 4 clearly differentiates the strategies used for the metabolic change in micro-organism for utilizing

diverse categories of phenolics. For muconic acid production, *PcaHG*, *CatBC* and *vdh* genes suppression and overexpression of the *AroY*, *AsbF*, *CatA* genes were accomplished in *P. putida*. So, there are possibilities for enhancing the succinic acid production from phenol derivatives by appropriate metabolic engineering strategies.

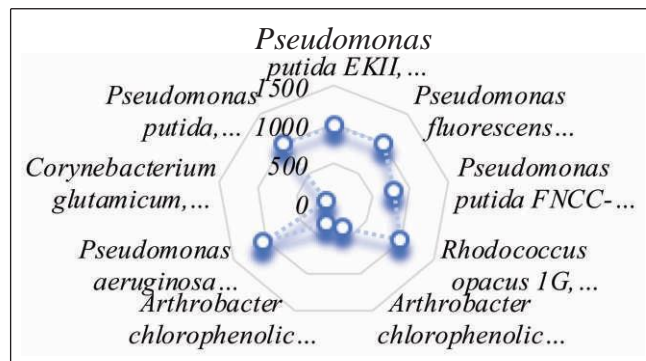


Figure 7: Comparative study of phenol degradation (mg/l) in bacteria for succinic acid production.

Table 4: Phenol derivatives used to produce various valued products.

Sr. No.	Substrate	Micro-organism	Bio-Product	Gene deletion	Gene addition	Approach	Product/yield	Refs
1.	Catechol, phenol, and benzoic acid using glucose as additional	<i>Pseudomonas putida</i> Kt2440	Muconic acid adipic acid, Nylon	catB	benAB CD, catA	Homologous recombination and plasmid insertion	13 g/L	[56]
2.	Catechol, phenol, and benzoic acid using glucose as additional	<i>Corynebacterium glutamicum</i> MA-2	Muconic acid	muconate cyclo isomerase (catB)	Catechol-1,2-dioxygenase (catA)	Homologous recombination and plasmid insertion	85 g/L	[54]
3.	Benzoate, p-coumarate, phenol, 4-hydroxy benzoate,	<i>Pseudomonas putida</i> Kt2440	Muconic acid, Adipic acid	Pca HG	AroY, Dmp KLMONP	Homologous recombination	13.5 g/L	[52]
4.	Benzoate, p-coumarate	<i>Pseudomonas putida</i> Kt2440	Pyruvate Lactate	aceEF	catBCA	Homologous recombination and plasmid insertion	41.17 g/g	[51]
5.	Wheat straw	<i>Rhodococcus jostii</i> RHA1	Vanillin	vdh	-	Homologous recombination	96 mg/L	[57]

7. Downstream processing techniques for succinic acid purification

The succinic acid comes in the market as a white crystalline powder form. When succinic acid is coming from the microbial broth, it should be clear and without any impurity especially, from hydrolysates which are having color, minerals and metal impurities in excess amount. Therefore, purification of the compound is very crucial for any kind of product that comes in the market. There are various methods established for succinic acid purification, some of them are patented processes developed by succinic acid-producing companies and some are research-based methods. Table 5 describes the purification process developed and currently used by bio-based succinic acid manufacturing companies.

Cost-effective downstream processes and clarification methods are always desirable to cut the purification cost. Three major categories of the purification are electro dialysis,

salt precipitation and ammonia-based neutralization (Figure 8). These are mostly used due to their recyclability and low cost. Electrodialysis is done by ion-exchange membranes with electrolytic conductivity to transfer ions faster for separation of product. It can be reused several times and very effective in getting high yield [58]. In the salt precipitation process, calcium hydroxide is used for neutralization and precipitate formation. Then, the solid is dissolved by adding sulfuric acid and finally passed through ion-exchangers to get clarified product [59]. Third, ammonia neutralization is used to produce salts of succinic acid as di-ammonium succinate after the addition of liquor ammonia. It can be transformed into mono form through heating at 135°C with pressure 50 psi. Final crystals of acid are dissolved in water and purified again with ion exchangers [60]. In all the three processes, ion exchangers are used for extracting the final highly purified product. So that only the purified product will come without any other salt or metal ion impurities.

Table 5: Succinic acid purification process developed by bio-succinic acid companies.

Company	Purification Process
BASF	Succinic acid is salted out first as a precipitate, then, passed through an ion-change column with a mild temperature near 50 to 60°C.
BioAmber	Ammonia treatment to form di-ammonium succinate salt then heated to convert it into mono-ammonium succinate. Further dissolving in reverse osmosis water system.
Mitsubishi	Precipitation of succinic acid by reducing the overhead pressure below the atm. pressure.
Myriant	Succinic acid is purified from the vacuum dried concentrate of the broth, then acidic treatment separated it from ammonium sulfate.

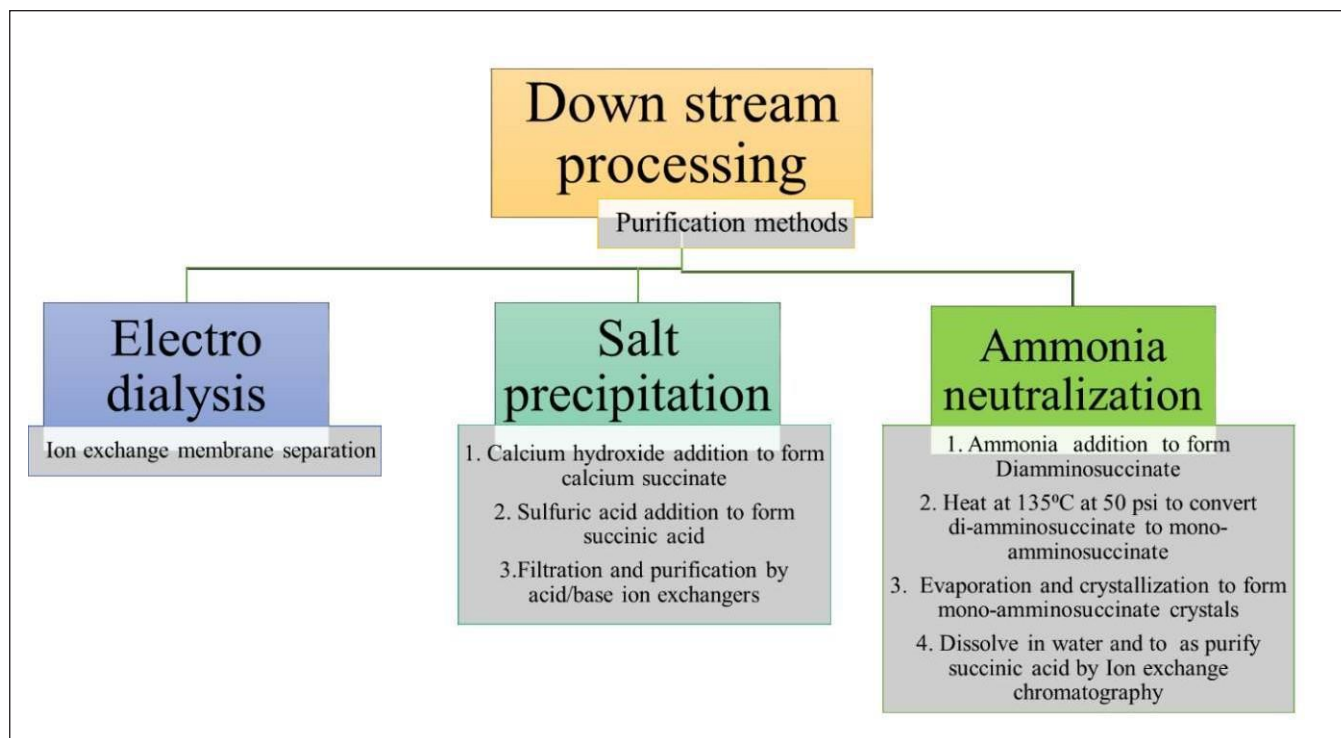


Figure 8: Various downstream processing methods for succinic acid.

8 Conclusion

Succinic acid is well studied for its potential as a platform chemical, which has vast applications such as the formation of polymers. The process development for enhancement of yield and titer has been done by metabolic engineering. Due to the depletion of petroleum-based resources, alternate bio-based approaches are being explored. Easy sugar-based process for succinic acid production is developed and marketed; however, aromatic substrates are not evaluated yet for succinic acid production. Therefore, to compete with the increasing demand for polymers, this basic platform chemical needs to be produced in bulk quantity and preferably from biomass. Thus, succinic acid production from low-cost agro-industrial wastes has become a major research area.

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