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MINI-REVIEW

Recent advances in production of succinic acid from lignocellulosic biomass

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Abstract Production of succinic acid via separate enzymatic hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) are alternatives and are environmentally friendly processes. These processes have attained considerable positions in the industry with their own share of challenges and problems. The high-value succinic acid is extensively used in chemical, food, pharmaceutical, leather and textile industries and can be efficiently produced via several methods. 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 recently 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 SSF and SHF. SSF is an economical single-step process which can be a substitute for SHF — a two-step process where 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. In addition, main process parameters which influence SHF and SSF processes such as batch and fed-batch fermentation conditions using different microbial strains are discussed in detail.

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Introduction

Succinic acid ($C_4H_6O_4$), a dicarboxylic acid, was isolated for the first time from microbial fermentation in 1546. Its traditional name is amber acid, but it is also known as butanedioic acid. It is formed by plants, animals and microorganisms, but its maximum production is obtained through anaerobic fermentation by microbes. Succinic acid originates from carbohydrate fermentation and is extensively used in chemical markets and industries which are producing food, green solvents and biodegradable plastics and ingredients used for the stimulation of plant growth (Zeikus et al. 1999). Succinate is the feedstock for several industrial products including tetrahydrofuran, adipic acid, 1,4-butanediol, and aliphatic esters (Willke and Vorlop 2004). Green technologies are highly required today in chemical industries because huge amounts of wastes and chemicals generated from these petrochemical industries are affecting the environment. Production of succinic acid via the fermentation route is a novel and green technology because CO_2 is consumed during the process (Song and Lee 2006; Zeikus et al. 1999).

Succinic acid plays a significant role in the biological metabolism as an important organic acid that occurs in animals, plants, humans and microorganisms. The industrial potential of succinic acid was recognized by Zeikus (1980) for the first time. Succinic acid has four existing markets. In the largest of these markets, succinic acid is mostly used as a surfactant, additive, foaming agent and detergent. Second, it is also used as an ion chelator which prevents corrosion and

pitting in the metal industry. The third application is the food market where it is used as an acidulate, pH regulator, antimicrobial and flavoring agent. The fourth market is the pharmaceutical industry where it is used as an additive for the production of vitamins, antibiotics and amino acids (McKinlay et al. 2007). Song and Lee (2006) also reported on succinic acid production and the various industrial products that can be formed from it. Succinic acid is an important commercial product which is also a precursor to industrially important compounds as shown in Fig. 1.

SSF of lignocellulosic biomass is a novel technique which can substitute for SHF, which is a two-step process where enzymatic hydrolysis and fermentation occur separately. SSF of lignocellulosic biomass by using microorganisms has several advantages over the two-step hydrolysis because energy consumption and cost of the pretreatment for glucose conversion to the end products are reduced. SSF under optimum temperature and pH conditions results in the controlled release of sugar, and the introduction of thermotolerant organisms can enhance the efficiency of the SSF process, bringing operating temperature closer to the optimum temperature of the cellulase enzyme (John et al. 2009). An economically feasible organic acid production requires low-cost lignocellulosic raw materials which can be supplied continuously in large quantities as a feedstock in polymer and other industries. Hence, several attempts have been made to find cheap lignocellulosic raw material for the economically feasible production of organic acids such as

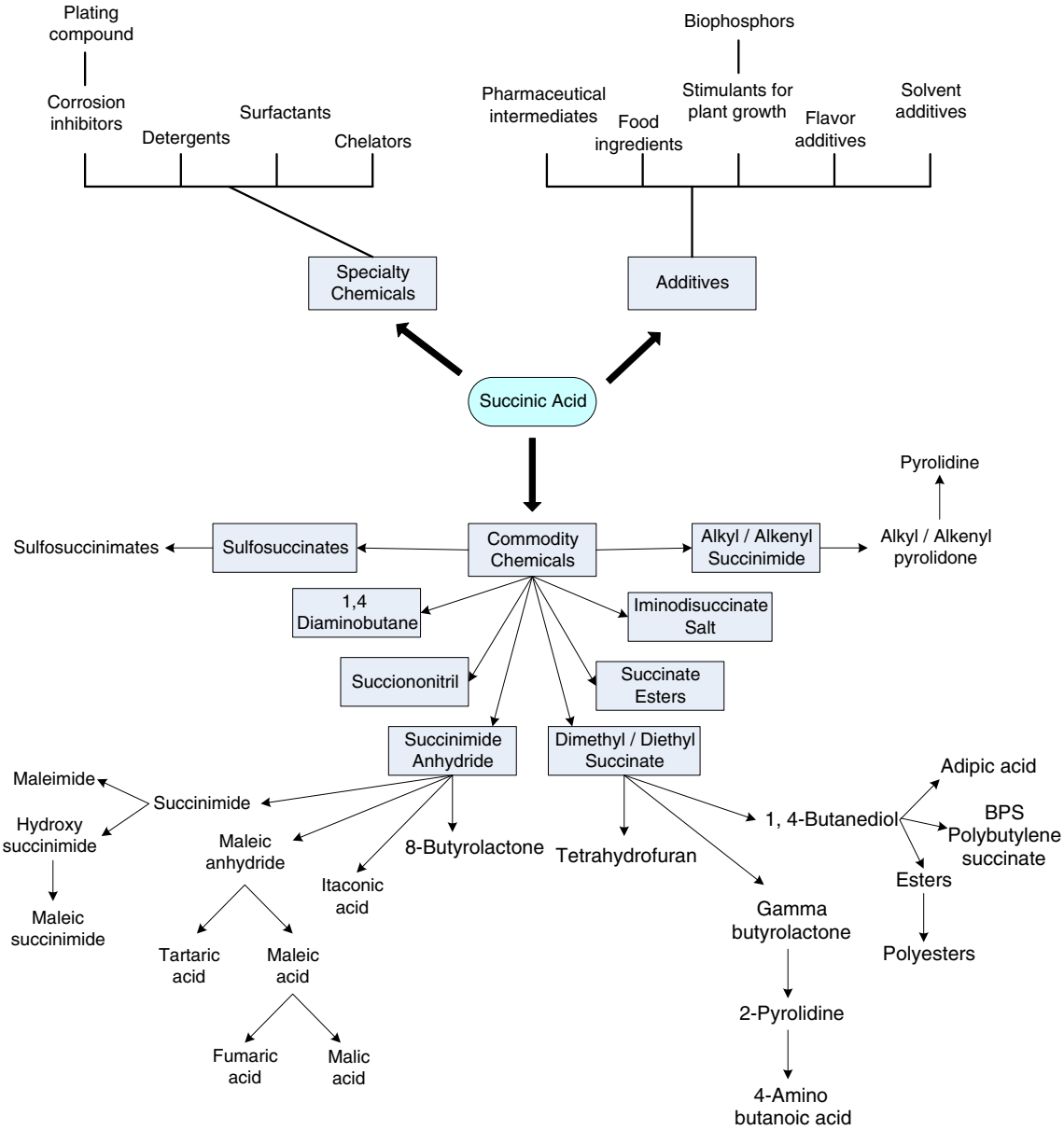

Fig. 1 Map of routes to various products formed from succinic acid

lactic acid (Garde et al. 2002; Hamzah et al. 2009; Miura et al. 2004; Park et al. 2004).

Bio-based succinic production by microorganisms will soon replace the currently used petroleum-based succinic acid due to the high price of raw materials. Depending on product purity and the increase in maleic anhydride price, its cost ranges from \$5.9 to 9.0 kg^{-1}. The cost of succinic acid production is also affected by its productivity, raw material costs, yield and product recovery system. Thus, the cost of maleic anhydride is directly affected by the cost of succinic acid (Song and Lee 2006). Succinic acid is considered as one of the high-value added products because its annual worldwide demand reaches to 30 million tons. Succinic acid production has reached 294, 819 tons/year (Luo et al. 2010). The selling price of succinic acid was calculated as \$2.26/kg; however, when lignocellulosic biomass sugar cane is used as a feedstock the price of succinic acid reduced to \$1/kg. Using efficient downstream process and optimization, succinic acid price may even dip below \$0.36/kg (Efe et al. 2013). The Research Institute of Innovative Technology is producing 50,000 tons of succinate per year using a genetically modified strain of *Corynebacterium glutamicum* on an industrial basis. Such developments will help promote the bio-based production of succinate to penetrate the market and be commercialized. Hence, there is a need for cheaper bio-based production of succinic acid from renewable sources that will not only replace the chemical method (Beauprez et al. 2010) but will also help reduce the price of biosuccinic acid to more competitive values.

This review describes the recent advances in succinic acid production from lignocellulosic biomass which has been of great interest, because it is cheap and abundantly available all year round. Previously, succinic acid and other organic acids were produced from petrochemical based products and glucose, which is more expensive than lignocellulosic biomass. Lignocellulosic biomass are mostly obtained from plant wood, grass, and forestry, municipal and agricultural wastes and is available in abundance all year round. The use of lignocellulosic biomass recently in SSF process has gained popularity due to its simplicity and specificity (Hamzah et al. 2009). Thus, this review encompasses insights into the recent and advanced techniques of SSF (a one-step conversion of lignocellulosic biomass directly to succinic acid) and SHF (a two-step process involving enzymatic hydrolysis and fermentation using microorganisms). To the best of our knowledge, no review has been done on succinic acid production from cheap and renewable lignocellulosic biomass via SSF.

Potential of lignocellulosic material for succinic acid production

Industrially important compounds are produced from fermentable sugars obtained through pretreatment of lignocellulosic biomass. Different types of microorganisms consisting of bacteria and fungi are used to degrade lignocellulosic biomass into glucose monomers (Kumar et al. 2008). The annual production of lignocellulosic feedstocks is about 180 million tons obtained from agricultural, industrial and forests. The worldwide production of wood from terrestrial plants is 1.3×10^{10} metric tons/year, of which coal accounts for 7×10^{9} metric tons energy, enough to fulfill two-thirds of the world energy requirements. These billion tons of biomass produced per year are converted into biofuels, organic acids and other high value added products (Demain et al. 2005).

Lignocellulosic wastes are quite abundant, and they are obtained from agricultural and industrial resources. The estimated biomass waste generated worldwide from cereal crops was 2.9×10^{3} million tons, plantation crops was 5.4×10^{2} million tons and that of oil seed crops was 1.4×10 million tons (Rajaram and Verma 1990). Among all of them, only 10 % is utilized for palm oil and kerosene oil as end products, while the other 90 % (empty fruit bunches [EFB], trunks, fronds, fibers, kernels and palm oil effluent) were just wasted. Lignocellulosic biomass such EFB, oil palm trunks (OPT), oil palm fronds (OPF), rice straw, wheat straw, corn stem, corn husk, corn cobs, palm, etc., contain a high amount of cellulose components. It has been calculated that cellulose production from biomass was around 1.5 trillion tons/year. High value added and environmentally friendly products such as succinic acid can be produced from these low-cost and inexhaustible source of lignocellulosic raw materials (Kim et al. 2006). In order for the raw material to be considered, a good substrate for the production of organic acids, it must have the following characteristics: available abundantly throughout the year, renewable, cheap, produces less amounts or no by-products formation, stereo-specific, high production rate, low level of contaminants and no competing food value (John et al. 2006).

Pretreatment of the lignocellulosic material

Lignocellulosic biomass consists of cellulose, hemicellulose and lignin which form a complex structure as depicted in Fig. 2. Lignocelluosic biomass structures are quite tough and require enzymatic hydrolysis as a pretreatment for conversion into fermentable sugars with an appropriate duration that can be accepted for industrial time frame. Pretreatment of lignocellulosic biomass changes its structure and makes it more accessible for the enzymes. The main purpose of pretreatment is to disrupt the structure of lignin, break its seal and crystalline structure of cellulose biomass, thus making it more easily available for enzymes (Mosier et al. 2005). Simultaneously, the accessibility of surface area is improved. The pretreatment also helps to remove the protection of lignin and cellulose trapped by hemicelluloses, which provides resistance to enzymatic hydrolysis. These factors greatly

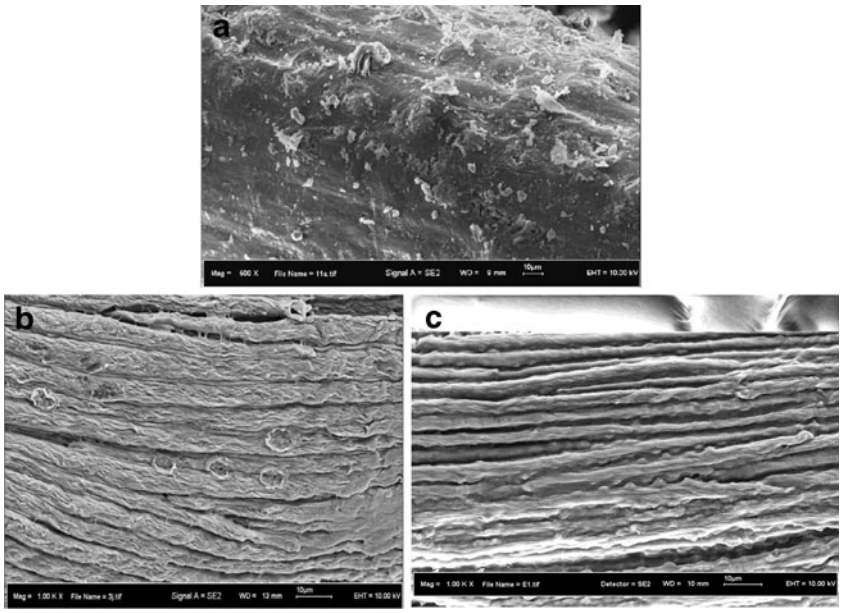

Fig. 2 SEM images of lignocellulose fibre from empty fruit bunch (**a**) untreated lignocellulose fibre (**b**) pretreated lignocellulose fibre using conventional alkali method (**c**) pretreated lignocellulose fibre using microwave alkali method. (Hamzah et al. 2011)

affect the rate of cellulose hydrolysis, resulting in low production of fermentable sugar. The lignocelluosic seal breaks down by pretreatment as shown in Fig. 2b and c.

Lignocellulosic schematic pretreatment revealed that 90 % removal of lignin from lignocelluloses enhances sugar concentration by four times as compared with untreated wood. During hydrolysis, cellulose availability of the enzyme was further increased and fermentation was enhanced when the concentration of lignin was decreased in the lignocellulosic biomass. Another critical point is the crystalline structure of cellulose where the cellulose chains are tightly bonded with each other by hydrogen bonding. Hydrogen bonding occurs in between subunits of sugars in cellulose chains which formed a 3D lattice-like structure (Rose and Bennett 1999). Due to this crystalline nature, cellulose is water-insoluble and its hydrolysis are also restricted for the enzymes in aqueous solution. During pretreatment the hydrogen bonding is deformed, and the cellulose structure becomes amorphously disordered. Such amorphous structure is easily available for enzymatic degradation as compared to crystalline structure, which is more restricted.

The pretreatment of lignocellulosic biomass can be made more effective if the pretreatment process has low energy and capital cost and is environmental friendly. In addition, its recovery must be high, the lignocellulosic biomass size should be small, lignin content must be reduced and sugar concentration must be very low because they slow down the fermentative activity of an enzyme (Chandra et al. 2007). Research on the pretreatment of lignocellulosic biomass for organic acids and valuable products is still ongoing. A pretreatment method should be developed such that it can be used for all types of biomass and achieve an efficiency close to 100 %. In this regard, non-food/non-star chic/cellulosic materials are great choices and their optimization and pretreatments methods are under investigation (Agbor et al. 2011).

Physical treatment methods include steam explosion, reduction of biomass size, hydrothermolysis, where acid, base and cellulose solvents are the most frequently chemical additives used in most chemical methods (Kamm et al. 2006). Pretreatment of lignocellulosic materials on the basis of pH can be divided into acidic, basic and neutral pretreatments, and the processes are most frequently used for pretreatment of lignocellulosic biomass (Galbe and Zacchi 2007). A combination of microwave-alkali (MW-A) pretreatment of lignocelluloses material has been performed as an alternative method over conventional heating. Application of such pretreatment of lignocellulosic material has been reported as on rice straw (Zhu et al. 2005), wheat straw (Zhu et al. 2006), switch grass (Hu et al. 2008) and empty fruit bunches fiber (Hamzah and Idris 2008). In principle, microwave irradiation produces heat by direct interaction between a heated object and an applied electromagnetic field which makes it different from super facial heat transfer. Thus, the heating is volumetric and rapid. Due to this radiation the polar parts of lignocellulosic material become hotter and produce hot *spots* on its surface with the combination of heterogeneous material which improves the disruption of the recalcitrant structure of lignocelluloses. The MW-A pretreatment improved the cellulose composition by about 60–80 %, whereas lignin and hemicelluloses were effectively dissolved in the aqueous solution of alkali (Hamzah and Idris 2008; Lai and Idris 2013; Zhu et al. 2005, 2006) as shown in Fig. 2c.

Non-thermal effect is produced because of the electromagnetic field in the microwave which causes the

destruction of crystalline cellulose structure (de la Hoz et al. 2005). Silicon on the surface of EFB lignocelluloses was removed by the microwave pretreatment irradiation (Hamzah and Idris 2008). Softwood lignocellulosic biomass contains about 10 % of silicon, which is toxic for SSF and enzyme systems. The changes which take place on the surface of lignocelluloses biomass surface are observed by scanning electron microscope (SEM) as shown in Fig. 2c.

Recently, a novel pretreatment method for empty fruit bunch fiber (EFBF) was developed using the sequential pretreatment with alkali and dilute acid (Kim et al. 2012). The EFB was initially treated with dilute sulfuric acid to remove 90 % hemicellulose and 32 % of lignin, and then subjected to sodium hydroxide where 70 % of lignin has removed. As a result of this pretreatment method, 82 % cellulose, 30 % lignin and less than 1 % hemicellulose were obtained. The efficiency of enzyme has increased because the morphology of pretreated biomass obtained were porous, rough and irregularly ordered which are desirable for enzyme digestibility. In another study, when an empty fruit bunch (EFB) was pretreated by aqueous ammonia, a glucose yield of 41.1 % and productivity of 0.11 g l^{-1} h^{-1} were obtained (Jung et al. 2011).

Although there are many pretreatment methods available, only some of them can be industrialized based on environmental and economic considerations. These methods require a high temperature which is normally achieved by either conventional heating (Hu et al. 2008) or microwave irradiation (Hamzah and Idris 2008). Thus, for industrialization, it is important to develop an efficient, cheap and environment-friendly method for pretreatment of lignocellulosic biomass.

Succinate production by SHF from lignocelluosic biomass and refined sugars

SHF has been widely used in the production of succinic acid from lignocellulosic materials such as corncobs, corn stover, rapeseed, rice straw, wheat straw, switch grass, bagasse, and empty fruit bunches. Biotransformation of lignocellulosic materials and refined sugars and its complete hydrolysis has been performed by various enzymes that act in synergy. This synergistic study is of great importance because different factors, such as enzyme concentration, substrate concentration, the presence of inhibitors, surfactants and adsorption rates, influence complete degradation of lignocellulosic materials. A combination of different enzymes results in higher productivity with the least amount of time that exhibits greater efficiency compared to the sum of individual concentration of each enzyme (Van Dyk and Pletschke 2012).

The production of succinic acid from refined sugars such as glucose, fructose, galactose, lactose, xylose and sucrose as substrates by *Escherichia coli* has been well studied while *Actinobacillus succinogenes* and *Anaerobiospirillum succinoproducens* were less commonly used for such substrates. The use of *E. coli* on glucose in a batch and dual phase aeration fermentation system results in a high succinate concentration of 101 g l^{-1} (Hodge et al. 2009). Also, high yield and productivity (1.2 g g^{-1} and 1.31 g l^{-1} h^{-1}) were obtained when using two-stage batch and batch dual phase aeration fermentation system, respectively (Chatterjee et al. 2001; Ma et al. 2011).

Succinic acid can also be produced from lignocellulosic biomass such as corn stalk, cane molasses, plant hydrolysates and soft wood hydrolysates using *E. coli.* Using corn stalk enzymatic hydrolysate as a substrate, succinic acid concentration of 57.8 g l^{-1} was obtained by *E. coli.* This process was carried out in a stage aeration fermentor for the first 12 h and was performed in anaerobic culture. However, when softwood hydrolysates were used as a substrate, succinic acid concentration decreased to 42.2 g l^{-1} using *E. coli*. In this process, dual-phase aeration process occurred in a batch fermentation process (Hodge et al. 2009). But when cane molasses were used as a substrate using the same fermentation process concentration, the productivity further decreased to 26 g l^{-1} and 0.52 g l^{-1} h^{-1}, respectively, by recombinant strain of *E. coli*. Thus, there is no increase in concentration and productivity, and only yield has increased to 0.87 g g^{-1} (Agarwal et al. 2007).

The disadvantages of SHF process when utilizing *E. coli* on glucose, sucrose and galactose are low yield and productivity, high cost of substrates, and difficultly in handling *E. coli* due its pathogenic nature. The production concentration, yield and productivity of succinic acid obtained from refined sugars by SHF are detailed in Table 1.

Glucose, galactose/lactose medium can be used to produce succinic acid using *A. succiniciproducens.* When glucose and galactose are used in a fermentation process, galactose efficiency is not affected. Production of succinic acid is increased and cost is also lowered by co-fermentation of galactose lactose. Different studies have suggested that a high amount of succinic acid concentration 83 g l^{-1} and productivity of 10.4 g l^{-1} h^{-1} were obtained. All the experiments were strictly anaerobic under high concentration of CO_2. This result clearly indicated that succinate high productivity was obtained under a continuous culture of fermentation condition while high yield was obtained under anaerobic and batch fermentation conditions. Succinate production using glycerol as a substrate was higher as compared to glucose, because succinate yield was increased and acetate production decreased (Glassner and Datta 1992; Lee et al. 2003b; 1999; McKinlay and Vieille 2008; Nghiem 1997).

Currently, genetically engineered strains *A. succinogenes*, *A. succinoproducenes* and *Mannheimia succinoproducens* are used for a high amount succinic acid production from lignocellulosic biomass. Lignocelluosic hydrolysate as a

Table 1 Succinic acid concentration, productivity and yield obtained from glucose/glycerol, galactose as a substrate via SHF

Substrate	Method	Microbe	Succinic acid			Reference
			Conc. ($g\ l^{-1}$)	Prod. ($g\ l^{-1}\ h^{-1}$)	Yield ($g\ g^{-1}$)	
Glucose	Anaerobic, batch	*A. succinogenes*	105.8	1.36	0.83	(Guettler et al. 1996)
			60.2	1.3	0.75	Liu et al. 2008a)
Glucose	Anaerobic, continuous culture	*A. succiniciproducens*	83	10.4	0.88	(McKinlay and Vieille 2008)
			34.4	1.8	0.86	Lee et al. 1999)
	Anaerobic, batch		32.2	1.2	0.99	(Nghiem 1997)
			50.3	2.1	0.9	(Glassner and Datta 1992)
Glycerol Glucose/ Glycerol Galactose Glucose			19	0.15	1.6	(Lee 2001)
			29.6	1.35	0.97	(Lee 2001)
			15.3	1.46	0.87	(Lee et al. 2003b)
Glucose	Anaerobic, batch	*M. succiniciproducens*	14	1.87	0.70	(Lee et al. 2002a)
			10.5	1.75	0.59	(Song et al. 2007)
	Anaerobic, continuous		8.2	3.19	0.55	(Kim et al. 2004)
	Anaerobic, fed-batch Batch		52.4	1.8	1.66	(Lee et al. 2006)
			13.4		0.97	
	Anaerobic, batch	*E. coli*	12.8	0.29	0.64	(Stols and Donnelly 1997)
	Anaerobic, batch,		73.4	0.61	1.06	(Jantama et al. 2008a)
	Aerobic, batch		86.5	0.9	0.93	
	Aerobic, (fed-batch)		58.3	0.72	0.62	(Lin et al. 2005
			8.3	0.16	0.72	
	Anaerobic, fed-batch		40	0.42	1.06	(Sánchez et al. 2005)
	Dual phase aeration, fed-batch		77	0.71	0.75	(Andersson et al. 2009)
	Dual phase aeration, batch		51	0.52	0.54	Gokarn et al. 1998)
	Dual phase aeration, batch		101	1.18	0.78	(Hodge et al. 2009)
	Dual phase aeration, batch		99.2	1.31	1.1	(Ma et al. 2011)
	Dual phase aeration, batch		38	1.27	0.8	(Vemuri et al. 2002)
Fructose Xylose	Dual phase aeration, batch		30	1.01	0.7	
Sucrose	Dual phase aeration, batch		24	0.81	1.2	(Chatterjee et al. 2001)
Glucose	Micro-aerobic, fed-batch	*C. glutamicum*	23	3.83	0.19	(Okino et al. 2005)
	with membrane for cell recycling	*C. glutamicum (metabolically engineered)*	146	3.17	0.92	(Okino et al. 2008)
	Aerobic, batch	*S. cerevisiae*	3.62	0.022	0.072	(Raab et al. 2010)

This table summarizes some of the recent studies using glucose/glycerol, sucrose, galactose as a substrate with the performed respective succinic acid yield, concentration and productivity using SHF

carbon source and nitrogen source, and a mixture of hydrolysate of waste yeast and alkali utilizing *A. succinogenes* as the microbe were utilized for succinate production. The process was reported to be economical because for the first time the high price $MgCO_3$ was replaced with cheap pH regulating alkalis ($Mg(OH)_2$ and NaOH), which reduced fermentation cost by 55.9 % and increased productivity to 56.4 g l^{-1} with high yields of succinic acid up to 0.73 g g^{-1} (Li et al. 2011). Similarly, when cane molasses were used a substrate by bacterium *A. succinogenes* CGMCC1593, succinic acid concentration of (50.6 g l^{-1}) with a yield of 0.8 g g^{-1} after 60 h in a batch fermentation was obtained. Li et al. (2010b) produced succinic acid from the wastes of crop stalk (cotton stalk and corn stalk) using a batch fermentation under the same condition in a stirred bioreactor. The yield and productivity of succinic acid were improved using fed-batch process fermentation process.

Lignocellulosic biomass such wood hydrolysate, whey and raw whey were used as a substrate for succinate production using *A. succiniciproducens.* When whey was used as a substrate, a high concentration of succinic acid 34.7 g l^{-1} and yield of 0.91 g g^{-1} was obtained under anaerobic and fed batch continuous process; however, high productivity was noted up to 3.3 g l^{-1} h^{-1} using continuous fermentation conditions (Lee et al. 2008; 2003a; Samuelov et al. 1999).

Succinic acid was produced from low-cost substrate whey and corn steep liquor (CSL) by *M. succiniciproducens* MBEL55E. Lee et al. (2003a) reported that when whey-

based culture and CSL were fermented under aerobic and batch conditions, the yield of succinic acid obtained was up to 71 % and productivity was 1.18 g l^{-1} h^{-1}. These results were quite similar when whey-based medium having yeast extract as substrate were introduced — succinate productivity of 1.21 g l^{-1} h^{-1} was obtained. However, under anaerobic conditions, productivity increased to 3.90 g l^{-1} h^{-1}, and the process was effective as compared to aerobic fermentation conditions.

Recently, the sugar obtained from lignocellulosic biomass using SHF has been mostly used for succinic acid fermentation. The lignocellulosic wastes are cheaper, which makes the process more economical. However, these lignocellulosic feedstocks also contain inhibitors that sometimes affect the efficiency and yield of succinic acid formation. These inhibitors include furans, phenolic compounds, and weak acid, and these can be partially removed by activated carbon adsorption. Many pretreatment methods are used for lignocellulosic biomass which can effectively produce cellulose and also remove these inhibitors. Much of the work has been performed using lignocellulosic substrate by SHF are described in detail and shown in Table 2.

Succinic acid production by simultaneous saccharification and fermentation (SSF)

The production of succinic acid from lignocellulosic biomass can be made more effective by combining the two steps: enzymatic hydrolysis of the substrate and its fermentation into a single step known as SSF. Many studies were performed to degrade the lignocellulosic material into organic acids by using different enzymes and microbes.

However, data on SSF using lignocellulosic biomass for succinic acid are rather limited. When corn fiber was used as a substrate and the efficient SSF was introduced, a higher amount of succinic acid was produced using *A. Succinogenes*. In this process, alkaline pretreatment was used on the corn fiber before the SSF and a good amount of succinic acid yield was obtained. Succinic acid yield increases when cellulase are added in addition to cellobiose. Maximum concentration of succinic acid obtained was 47.4 g l^{-1}, yield 0.72 g g^{-1} with a productivity of 0.99 g l^{-1} h^{-1} in a 5-l stirred bioreactor. This process produced high yields of succinic acid and has the potential to be industrialized (Zheng et al. 2010). SSF with the rapeseed meal (RSM) as the substrate using *A. succinogenes* produced a succinate concentration 15.5 g l^{-1}, and a yield of 12.4 g g^{-1} dry matter at pH of 6.4. However, the yield and productivity obtained in this process were lower as compared when corn fiber was used as a substrate (Chen et al. 2011a,b). Table 3 illustrates some of the recent work done in the past decade in the use of various lignocellulosic materials for the production of succinic acid using SSF.

Bioreactor systems: batch and fed-batch

In SSF, the production cost can be lowered by increasing the substrate concentration which reduces the use of energy and water. However, it is quite difficult in batch experiments to operate at high substrate concentration due to the high viscosity of the medium. Thus, the problem was solved by using a fed-batch process where high concentration of substrate was initially mixed at the beginning of the fermentation process. Generally, in SSF, all the media (enzymatic hydrolysis and cultivation medium) and treated lignocelluloses were mixed together in a single vessel (Marques et al. 2008). Then the broth was set to the required pH that can tolerate both processes before autoclaving at 121 °C for 15 min. A simple kinetic model was developed in this study for the optimal succinic acid production from glucose in batch fermentation process by *M. Succiniciproducens*. Succinate production follows the Luedeking–Piret model and the parameters for growth and non-growth values were 1.619 and 0.355 h^{-1}, respectively. Under optimal conditions and high CO_2 atmosphere, the yield improved considerably. This model suggests that the parameters used will be quite useful for succinic acid production from lignocellulosic biomass (Song et al. 2008).

Both batch and fed-batch fermentation processes were used for succinic acid production from corn straw hydrolysates using *A. succinogenes*. The parameters used for both processes were similar and the reaction was performed in a 5-l stirred bioreactor. During batch fermentation succinic acid concentration of 45.5 g l^{-1} in 48 h with a rate of 0.81 g l^{-1} h^{-1} were obtained. With fed batch fermentation succinic acid concentration of 53.2 g l^{-1} was obtained in 44 h at a rate of 1.21 g l^{-1} h^{-1}. The results revealed that succinic acid concentration, yield and rate were much higher in fed batch than batch fermentation process. The fermentation time for the batch fermentation was 48 h, while in fed batch it was reduced to 44 h. The succinic acid productivity in batch fermentation with SSF (0.99 g l^{-1} h^{-1}) was superior than SHF (0.95 g l^{-1} h^{-1}) (Zheng et al. 2009). In a batch SSF process, addition of sterile $CaCO_3$ solutions or powder to control the pH reduction in the medium can be intermittently done after 24 h of cultivation (Park et al. 2004) or added during the earlier part of cultivation. However, in most of the studies, $CaCO_3$was added to the medium during the earlier part of cultivation. Meanwhile, in fed batch reactor, the addition of the succinic acid neutralizer was controlled by a pH controller (Marques et al. 2008).

Succinic acid was produced from wood hydrolysate a lignocellulosic biomass by *M. succiniciproducens* MBEL55E. For the first time, zylose and glucose were used as a carbon source. Batch and continuous fermentation process were used and succinic acid concentration of 11.73 g l^{-1} and yield of 1.17 g l^{-1} h^{-1} were obtained. High yield of succinic acid from the inexpensive wood hydrolysate was obtained by

Table 2 Succinic acid concentration, productivity and yield obtained from different lignocellulosic substrate, microbe and fermentation systems via SHF

Substrate	Method	Microbe	Succinic acid			Reference
			Conc. ($g\ l^{-1}$)	Prod. ($g\ l^{-1}\ h^{-1}$)	Yield ($g\ g^{-1}$)	
Corn fiber hydrolyzate	Anaerobic batch	*A. succinogenes*	70.3	0.63	0.96	(Chen et al. 2011b)
			70.6	0.7	0.88	(Guettler et al. 1996)
			35.4	0.98	0.73	(Chen et al. 2010)
Corn stalk and cotton stalk			15.8	0.6	1.23	(Li et al. 2010b)
Corn stover			56.4		0.73	(Li et al. 2011)
Sugarcane bagasse hydrolyzate			22.5	1.01	0.43	(Borges and Pereira 2011)
Corn straw			45.5	0.95	0.81	
	Anaerobic, Fed-batch		53.2	1.21	0.83	(Zheng et al. 2009)
Cane molasses	Anaerobic Batch		46.4	0.97	0.8	
	Fed-batch		55.2	1.15	0.81	(Liu et al. 2008b)
Whey	Anaerobic, fed-batch	*A. succiniciproducens*	34.7	1.02	0.91	(Samuelov et al. 1999)
Whey	Anaerobic, continuous Culture		19.8	3	0.64	
Whey	Anaerobic, continuous Culture		14.3	3.3	0.71	(Lee et al. 2008)
Wood hydrolyzate	Anaerobic, batch		24	0.74	0.88	(Lee et al. 2003b)
Wood hydrolysate	Anaerobic, batch	*M. succiniciproducens*	11.7	1.17	0.56	(Kim et al. 2004)
Whey	Anaerobic, batch		13.4	1.18	0.71	(Lee et al. 2003a)
Whey	Anaerobic, continuous		6.4	3.9	0.69	
Plant hydrolysates (Glucose, xylose)	Dual Phase aeration Batch	*E. coli*	51.0	0.52	0.54	(Gokarn et al. 1998)
Softwood dilute acid Hydrolyzate			42.2	0.78	0.72	(Hodge et al. 2009)
Cane molasses			26	0.87	0.52	(Agarwal et al. 2007)
Corn stalk enzymatic Hydrolyzate			57.8	0.8	0.87	(Wang et al. 2011)
Wheat straw		*F. succinogenes*	1.55	0.022	0.05	(Li et al. 2010a)
Orange peel			1.75	2.025	0.044	(Li et al. 2010a)

Note: summarizes some of the recent studies using lignocellulosic biomass with the performed respective succinic acid yield, concentration and productivity using SHF

this novel bacteria, and this process can easily be industrialized. The productivity was increased from 1.17 to 3.19 $g\ l^{-1}\ h^{-1}$ by using a continuous process of fermentation (Kim et al. 2004).

Similarly, metabolically engineered process was developed to enhance succinic acid production without the production of any side products. There are three CO_2-fixing reaction catalyzed by three enzymes: PEP phophoenolpyruvate, PEP carboxylase and PEP carboxykinase. Among them, PEP carboxykinase was considered to be a better agent for succinic acid production and anaerobic growth of *M. Succinoproducens*. This metabolically engineered strain produced succinic acid concentration of 13.4 $g^{-1}\ l^{-1}$ from a glucose concentration of 20 $g^{-1}\ l^{-1}$. Yields of 0.97 mol succinic acid per mol of glucose were achieved without the formation of any side products. However, when a fed-batch process was introduced, succinic acid concentration 52.4 $g^{-1}\ l^{-1}$, yield 1.16 $g\ g^{-1}$ and a productivity of 1.8 52.4 $g^{-1}\ l^{-1}\ h^{-1}$ was achieved. Hence, in fed batch process both the concentration and productivity as well as succinate yield was improved as compared to batch fermentation process. Therefore, fed batch fermentation was considered as a more efficient process for succinate production (Lee et al. 2006). SSF process was introduced and rapeseed meal was utilized as a carbon source, and a high concentration 52.4 $g\ l^{-1}$ and yield 12.4 $g\ g^{-1}$ of succinic acid were achieved using *A. succinogenes* (Chen et al. 2011a).

Table 3 Succinic acid and Lactic Acid production via SSF and its concentration, productivity and yield using different substrate, microbe and fermentation systems

Substrate	Microbe	Conc. (gl-1)	Prod. (gl-1h-1)	Yield (gg-1)	Reference
Succinic Acid					
Corn stover	*A. succinogenes*	47.4	0.99	0.72	(Zheng et al. 2010)
Rapeseed	*A. succinogenes*	23.4	0.33	0.12	(Chen et al. 2011a)
Lactic Acid					
Cellulose	*L. bulgaricus*				
	NRRL B-548	57	0.45	0.80	(Venkatesh 1997)
Corncob	*Rhizopus sp.* *MK-96-1196*	24	0.33	0.90	(Miura et al. 2004)
Barley	*L. casei* *NRRL B-441*	162	3.37	0.87 to 0.98	(Linko and Javanainen 1996)
Wheat bran					
	L. casei and L. delbrueckii	123	2.3	0.95	(John et al. 2006)
Wheat starch	*L.lactis ssp. lactis* *ATCC 19435*	92	3.2	0.77 to 1	(Hofvendahl et al. 1999)
Pretreated wood	*L. delbrueckii*	48–62	4.3	0.57	(Moldes et al. 2000)
Cellulose	*L.coryniformis ssp.* *torquens ATCC 25600*	4.5	0.5	0.89	(Yáñez et al. 2003)
Potato starch	*R. oryzae, R. arrhizus*	20	0.52	0.85 to 0.92	(Huang et al. 2005)

The advantage of SSF process is that it eliminates additional steps in the hydrolysis process. Similarly, glucose and zylose concentration remain low because it was quickly utilized by *A. succinogenes*, which decreases the chances of substrate and product inhibition in fermentation process (Zheng et al. 2010). In SSF sugar concentration was kept low under controlled conditions. In order to make the SSF process more efficient, further study on the process should be carried out under controlled conditions of sugar utilization and enzymatic hydrolysis.

Aerobic and anaerobic

Cultivation can be done either anaerobic or aerobic depending on the metabolic pathway of the selected strain. Succinic acid was obtained as a major end product in anaerobic conditions mostly using rumen bacteria such as *A. succinogenes*, *M. succiniciproducens*, *A. succiniciproducens* and *E. coli* from lignocelluolosic biomass. On the other hand, succinic acid production using these bacteria is still limited, because these are partially pathogenic and operates at anaerobic condition with slow growth in acidic condition, high glucose levels and osmotic stress (Song and Lee 2006). For the concentration of lactic acid production, most of the lactobacillus was run under strictly anaerobic condition (Garde et al. 2002). Similarly, economic production of succinic acid was obtained in anaerobic conditions from corn fiber hydrolysates as the carbon source using a rumen bacteria *A. succcinogenes* NJ113. The highest yield of succinic acid of more than 72.0 % was obtained in a 7.5-l anaerobic fermentor culture. A compound such as furfural that inhibits the fermentation growth can be removed by the addition of $CaCO_3$ to enhance fermentability of corn fiber hydrolysates for enhanced succinic acid production (Chen et al. 2010).

Difference between SSF and SHF Process

As mentioned earlier, lignocelluloses' conversion into cellulose fraction and the production of succinic acid can be done by two methods: SHF or SSF. SHF refers to the physical separation process, in which lignocellulosic bioconversion occurs in two steps in two different reactors, while SSF is a one-step process that occurs within the same bioreactor (Xu et al. 2009). Several studies (McKinlay and Vieille 2008) revealed that production of succinic acid from cellulose can be done more effectively by combining two steps — enzymatic hydrolysis and microbial fermentation — into a single step known as SSF. SSF is very effective, efficient and a time-saving process. Figure 3 clearly shows the detailed process for succinic acid production through SSF and the conventional two-step fermentation processes (SHF).

In SSF, cellulose has to be hydrolyzed to produce glucose and the glucose formed is simultaneously converted to organic acid. The process eliminates saccharification of fermentable sugars step before conversion. Hence, it is capable of substantially decreasing the utilization of the enzyme. From the industrial view point this is important, as a faster saccharification rate can be achieved in a reduced reactor volume. Moreover, the SSF process also eliminates the use of different reaction vessels for both processes. Venkatesh

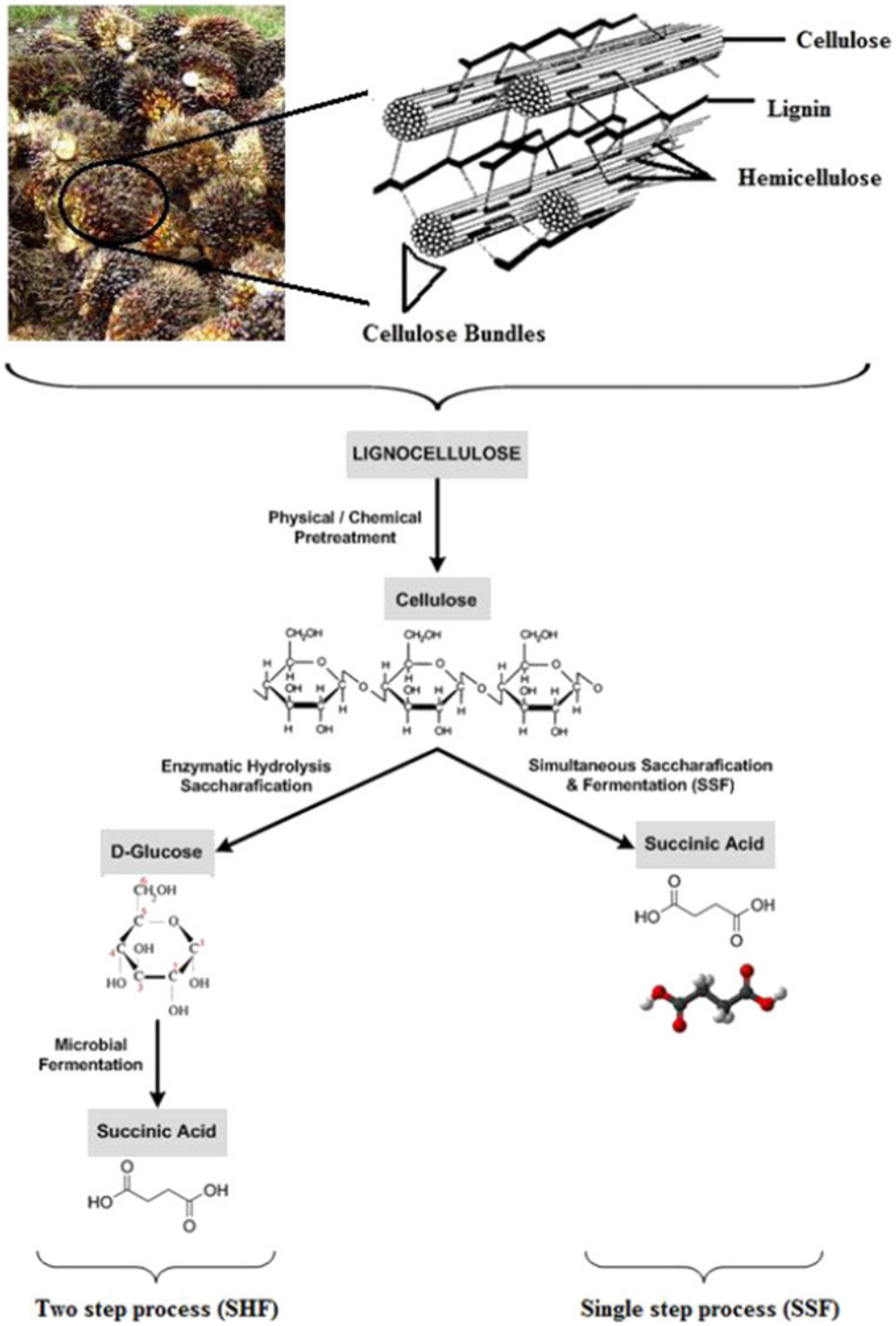

Fig. 3 Schematic diagram for succinic acid production from lignocellulose via SHF and SSF

(1997) had previously attempted to produce lactic acid from pure cellulose through SSF. In SHF, the glucose produced was capable of competitively inhibiting the *cellulase* and resulted in a low hydrolysis rate. In order to overcome this problem, large amount of enzyme is required which results in an increased production cost.

However, it is also observed that some time in SSF process the concentration and productivity achieved tend to be lower than SHF. The product concentration attained was as high as 70.3 g l^{-1} when corn fiber was used as the substrate and *A. succinogenes* as microbes via SHF, while concentration of 47.4 g l^{-1} was obtained when the corn stover was used as the substrate and *A. succinogenes* via SSF. However, the metabolic engineering of the strains and fermentation conditions can solve this problem.

SSF of lignocellulosic materials has several advantages and can replace the two-step fermentation process SHF, because it can reduce costs by replacing high amount of biomass consumption and also achieve high productivity by controlling the release of sugar (John et al. 2009). Ideally, in the SSF process, the in situ hydrolysis and cultivation eliminate the inhibition of the enzyme either by mono- or disaccharide accumulation (Han and Chen 2008). By combining the enzymatic hydrolysis and cultivation, the glucose produced from the saccharification process is directly used by succinic acid microbes in the cultivation process. Moreover, more cellulose is converted to glucose in the SSF as compared to SHF. Findings of studies revealed that the saccharification rate in SSF is faster than the single saccharification process. Consequently, the higher saccharification rate increases productivity and reduces reactor volume and capital cost (Zhang et al. 2007). Hence, the SSF process is seen to be a more comprehensive yet a simple process for utilization of lignocellulosic material.

Metabolic evolution and new insights in succinic acid production

New techniques in metabolic engineering have resulted in the creation of new metabolic pathways or modification in the current pathways for optimal production of succinic acid which are not achievable by native metabolic pathways. Therefore, metabolic engineering techniques are employed in which different innovative technologies and tools are used to make efficient synthetic pathways to produce high amounts of the desired product in an effective way (Lee et al. 2012).

A metabolically engineered, advanced, cost-effective and efficient method was recently used for the production of succinic acid from *M. succiniciproducens*. In this process, glucose was introduced as a carbon source. A high yield of succinic acid 67.05 % with productivity 1.75 g l^{-1} h^{-1} was obtained under anaerobic and continuous fermentation conditions. A cost-effective process LPK 7 strain was used which resulted in succinate purity greater than 99.7 % (Song et al. 2007).

In due course of time, the field of synthetic microbiology will likely be developed by designing biosynthetic pathways using different computational tools. Advances in genome-scale metabolic pathways and computationally predicted pathways identified necessary parts of the variants which can speed up the developing field of synthetic pathway engineering. System biology will potentially bring breakthroughs and will be one of the major tools in the near future only when computational and experimental successes go hand in hand in an integrated manner by utilizing applied synthetic biology approaches (Medema et al. 2012).Metabolic evolution in cellular level in *E. coli* bring significant changes which improved succinate production. The energy required for succinate production becomes more efficient by conserving carboxykinase (pck) phosphoenolpyruvate (PEP) evolved which resulted in a major pathway for succinate production. Gene expression and PCK enzyme activity level significantly increased in two stages due to several mutations occurring during the metabolic evolution process (Zhang et al. 2009).

Succinic acid production yield was also enhanced by using simple and low-cost salt fermentation media. Gene deletion technique (genetic engineering) was introduced in which anaerobic genes (Doha, earth, ackA) were deleted, and the pathway of succinate production remained the same for the regeneration of NAD^+ which increased growth of dicarboxylic acids. After deletion of the genes, the best succinate biocatalyst strains (KJ060(ldhA, adhE, ackA, focA, pflB) and KJ073(ldhA, adhE, ackA, focA, pflB, mgsA, poxB)) increased succinate production which reached up to 622–733 mM with a yield of 1.2–1.6 per mol of metabolized glucose (Jantama et al. 2008a). In *E. coli*, further engineering techniques were applied on genetic level to achieve high yields of succinic acid. The strains KJ091 (DldhA, DadhE, DackA, DfocA-pflB, DmgsA, DpoxB) was further improved by using genetic techniques. Deletion of 2-ketobutyrate formate-lyase (tdcE; pyruvate formate- lyase homologue) and threonine decarboxylase (tdcD; acetate kinase homologue) reduced acetate level by 50 % and increased succinic acid production by 10 %, as succinic acid titer reach to (700 mM), yield (1.5 mol mol^{-1} glucose) and productivity (0.9 g l^{-1} h^{-1}). Thus, stains KJ134 and KJ 122 using anaerobic, batch fermentation and salt medium can be used as a bioacalyst for commercial succinate production (Jantama et al. 2008b).

In addition, system metabolic engineering techniques were introduced in *E. coli* to enhance succinic acid production from 5.8 to 86.5 g l^{-1}. PEP carboxykinase has evolved which is a major carboxylating enzyme responsible for succinic acid production in *E. coli*. Similarly, glucokinase and galactose permease were induced, because glucose-specific PEP-dependent PTS were inactivated. These metabolic engineering approach and evolved characteristics are similar to those of the native succinate producing host rumen bacteria in *E. coli* (Lee et al. 2012).

The metabolic engineering techniques in *E. coli* and *M. succinoproducenes* were to some extent successful in increasing succinic acid productivity and yield due to the deletion of genes which slow down the succinic acid fermentation process or the genes which are responsible for producing side products. Similar studies need to be performed in the future in *A. succinoneges* and *A. succinoproducenes* which will increase succinate yield and productivity. Therefore, the prospect of using these metabolic engineered

strains, low-cost lignocellulosic biomass and one-step saccharification and fermentation process (SSF) instead of two-step process (SHF) will result in low price and industrially feasible succinic acid production.

Conclusion

Bio-based succinic acid has become an important green intermediate for deriving both commodity and specialty products plus additives. The production of such biological succinate via both SHF and SSF has the potential to reduce the use of non-sustainable resources and carbon footprint. The rapid development of knowledge in genetic engineering has resulted in promising strains suitable for industrial bio-based production of succinate production mostly via the SHF. Parameters that influence the viability of the bioprocess are yield, concentration and productivity. The yield which is very much related to the variable cost of the substrate will be significantly attractive due to the use of lignocelluloses materials which are abundantly available and cheap. Thus, biological succinate production is currently seen as economically competitive compared to petrochemical-derived succinate because of the cheap lignocelluloses feedstock, reasonable product concentration, productivity and yields.

Hence, the prospect of producing succinic acid via SSF has a promising future due to the one-step process which combines enzymatic hydrolysis and microbial fermentation in a single bioreactor. The success of SSF in producing lactic acid from lignocelluloses has spurred interest in using SSF for succinic acid production. Although lignocelluloses can be used as substrates, they need to be pretreated accordingly so as to allow structural changes to occur which will assist the subsequent enzymatic hydrolysis and fermentation process. Obstacles in the recovery and purification of succinate are currently being solved using downstream processes such as vacuum distillation, single reactive extraction and crystallization where purity as high as 99.5 % is achievable. The possibility of an integrated biorefinery is within reach with a proper approach which is precisely optimized. Considering the advances in efficient downstream process, strain development, reduced feedstock cost, reduced greenhouse gas emission and large potential market size have raised hopes for higher earnings and profits in succinic acid production in the future.

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