

Harnessing yeasts for sustainable succinic acid production: advances in metabolic engineering and biorefinery integration

Vasiliki Korka^{1,2}, Apostolos Petropoulos², Sofia Maria Ioannidou², Carol Sze Ki Lin³, Apostolis Koutinas², Patrick Fickers^{1,*}

¹Microbial Processes and Interactions, TERRA Teaching and Research Centre, Gembloux Agro-Bio Tech, University of Liège, Av. de la Faculté, 2B, 5030 Gembloux, Belgium

²Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece

³School of Energy and Environment, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China

*Corresponding author. Microbial Processes and Interactions, TERRA Teaching and Research Centre, Gembloux Agro-Bio Tech, University of Liège, Av. de la Faculté, 2B, 5030 Gembloux, Belgium. E-mail: pfickers@uliege.be

Editor: Ewelina Celińska

Abstract

This review highlights the potential of *Yarrowia lipolytica* and other yeasts as sustainable producers of bio-based succinic acid (SA), a key platform chemical with applications in bioplastics, solvents, and pharmaceuticals. Recent advances in metabolic engineering have substantially improved SA titers, yields, and productivities in yeasts. These improvements were achieved by reconstructing biosynthetic pathways, disrupting gene involved in side-metabolism and/or expressing heterologous genes involved in critical metabolic functions. The use of renewable feedstocks, including crude glycerol, agricultural residues, food waste hydrolysates, and industrial by-products, has shown promise in reducing both production costs and environmental impacts. Innovative downstream separation techniques, such as *in situ* extraction, membrane filtration, and crystallization, further contribute to process sustainability. Integrating yeast-based SA production into circular biorefineries and adopting continuous production systems are promising strategies for enhancing economic feasibility and minimizing ecological footprints. Although challenges related to scale-up and process integration persist, ongoing advancements in genetic engineering and bioprocessing technologies position yeast-based processes as a viable route for sustainable, large-scale bio-based SA production within a circular bioeconomy framework.

Keywords: yeast; succinic acid; metabolic engineering; biorefineries; life cycle assessment; technoeconomic evaluation

Introduction

Succinic acid (SA) is a four-carbon dicarboxylic acid of considerable industrial significance, classified as a C4 platform chemical. Global production of SA currently reaches 50,000 tonnes annually, with approximately 20% derived from bio-based sources (Spekreijse et al. 2021). The market is projected to grow to \$515.8 million by 2030. Since 2004, SA has been classified as one of the top 12 bio-based platform chemicals by the U.S. Department of Energy due to its strategic importance in the future chemical industry (Werpy and Petersen 2004). SA versatility lies in its role as a chemical building block for the synthesis of value-added compounds including adipic acid, 1,4-butanediol, tetrahydrofuran, N-methylpyrrolidone, 2-pyrrolidone, and γ -butyrolactone (Yang et al. 2024). Additionally, SA is used in the manufacture of plasticizers, lubricants, food additives, green solvents, pharmaceuticals, and bioplastics (Spekreijse et al. 2021, Gyan et al. 2024). One of the most prominent applications is the synthesis of poly(butylene succinate) (PBS), a biodegradable polymer produced via the copolymerization of SA with 1,4-butanediol (Barletta et al. 2022).

SA can be produced either through the chemical conversion of maleic anhydride (chemical-based route) or by microbial processes (bio-based route) (Saxena et al. 2017). The chemical process relies heavily on fossil-based feedstocks and contributes significantly to environmental impacts. Although bio-based SA can offset climate change by saving 4.5 to 5 tonnes of carbon dioxide per tonne of SA compared to the chemical-based route, it currently competes with food production, as its synthesis relies on corn-based glucose syrup and requires extensive land and water use (Song et al. 2014, Mancini et al. 2022). Furthermore, the cost of bio-based SA remains higher than its chemical-based counterpart (€2.61/kg vs. €2.25/kg; Spekreijse et al. 2021), mainly due to the expensive downstream separation and purification processes (DSP) (Kurzbek and Weuster-Botz 2010). To improve economic feasibility and sustainability, alternative production strategies have been explored. These include the use of low-cost renewable carbon sources or agro-industrial side streams, and optimization of both microbial cell factories and process parameters (Kumar et al. 2024, Lin et al. 2024). For instance, the use of crude hydrolysates from agricultural residues can reduce

Received 31 May 2025; revised 25 July 2025; accepted 10 September 2025

© The Author(s) 2025. Published by Oxford University Press on behalf of FEMS. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<https://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site-for further information please contact journals.permissions@oup.com

production costs to \$1.6–1.9/kg (Dickson et al. 2021). Additional cost reductions may be achieved by developing integrated biorefineries that co-produce SA alongside other high-value compounds (e.g. phenolic extract) (Ioannidou et al. 2022).

At present, microbial production of SA is mainly based on bacteria or yeast species. Numerous wild-type and engineered bacterial strains have been considered for SA production, with some *Escherichia coli* engineered strains achieving among the highest SA production titers (Vemuri et al. 2002; Wang et al. 2018; Zhu et al. 2020). Bacteria mainly produce SA by CO₂ fixation under anaerobic conditions, with high titer, yield and productivity (Li et al. 2013, Kumar et al. 2024, Lin et al. 2024). However, the production of SA using bacterial strains faces significant challenges, as most require a neutral pH for optimal growth. Under these conditions, the primary product is succinate, necessitating a costly and complex purification process to convert it into SA. Compared to bacteria, yeasts exhibit greater resistance to acidity, permitting the generation of SA under low pH conditions (below 4.2), greatly simplifying downstream processing, minimizes the need for pH control and the contamination risks, key benefits for industrial-scale applications (Ma et al. 2020, Li et al. 2021). Yeasts also have limitations, including predominantly lack of CO₂ fixation, and lower productivities compared to bacterial systems. However, advances in genetic engineering and metabolic pathway optimization have allowed yeasts, such as *Yarrowia lipolytica*, to achieve SA titers similar to those of bacterial strains (Table 1). Moreover, some studies using *Saccharomyces cerevisiae* have efficiently produced SA under fermentation where air inlet is enriched with CO₂ (Yan et al. 2014, Malubhoy et al. 2022).

In addition, yeasts are capable of catabolizing a broad range of substrates, with *Y. lipolytica* particularly adept at utilizing both hydrophilic and hydrophobic carbon sources, including sugars, lignocellulosic hydrolysates, fatty acids, lipids, waste oils, crude glycerol, and acetate, low-cost, renewable feedstocks, the use of which enhance the economic feasibility and sustainability of SA production (Ma et al. 2020).

In recent years, several reviews have been published on various aspects of SA production, including general reviews (Li et al. 2021, Kumar et al. 2024) as well as more specific ones that focus on *Y. lipolytica* as a SA producer (Sun et al. 2025a), the utilisation of various renewable carbon sources and metabolic engineering approaches (Narisetty et al. 2022a, Jiang et al. 2017, Dai et al. 2020, Mitrea et al. 2024), downstream separation schemes (Dai et al. 2020, Kumar et al. 2020a, Kumar et al. 2024) and methods for enhanced CO₂ fixation (Lin et al. 2024). The present review provides a comprehensive and up-to-date overview that integrates all key areas of SA production research using yeast strains, from strain metabolic engineering advances to techno-economic analysis, and life cycle assessment. We focus on *Y. lipolytica* as a promising cell factory for SA production. We compare metabolic engineering strategies, SA titer, productivity, and yield achieved in *Y. lipolytica* with those reported for other yeast strains (*S. cerevisiae* and *Issatchenkia orientalis*) and for *E. coli*. We also discuss the ability of yeasts to utilize crude hydrolysates, advances in downstream processing, and the integration of SA production into biorefineries. Finally, we present an environmental and economic sustainability assessment of yeast-based SA production.

Succinic acid and metabolic pathways

In cells, SA predominantly exists in its dissociated form, succinate, due to the nearly neutral pH of the mitochondrial matrix (Porcelli et al. 2005) and its pK_a values (4.16 and 5.61). Therefore,

in this review, the term succinate will be used when considering biochemical pathways, while SA will be used in all other contexts (i.e. in DSP) for consistency. Depending on the microorganism and culture conditions, succinate can be synthesized through different metabolic pathways. It is an intermediate of the oxidative tricarboxylic acid (oTCA) cycle, which takes place in the mitochondrial matrix of eukaryotes and the cytoplasm of prokaryotes. The oTCA cycle operates primarily under aerobic conditions and in the presence of carbon sources such as glucose or glycerol, with the main function of generating NADH, FADH₂, and GTP from acetyl-CoA (Figure 1). In this cycle, succinate is formed from succinyl-CoA by succinyl-CoA synthetase (Scs) and is subsequently oxidized to fumarate by the succinate dehydrogenase (SDH) complex, composed of five subunits (Sdh1–5), which transfers electrons to ubiquinone (coenzyme Q) in the electron transport chain. Although Sdh5 is not essential for the assembly of the Sdh1–Sdh4 subunits, it plays a crucial role in the flavination (i.e. covalent FAD attachment) of the Sdh1 (Hao et al. 2009, Gao et al. 2016).

Succinate can also be generated from isocitrate via the glyoxylate cycle, which bypasses the two decarboxylation steps of the oTCA cycle. This pathway involves two specific enzymes: isocitrate lyase (Icl), which cleaves isocitrate into glyoxylate and succinate, and malate synthase (Mls), which condenses glyoxylate with acetyl-CoA to form malate (Figure 1). In yeasts, the glyoxylate pathway occurs in the peroxisome and is active during growth on non-fermentable carbon sources such as acetate or fatty acids, facilitating gluconeogenesis.

The third pathway for succinate production is the reductive TCA (rTCA) cycle, which operates in reverse to the oTCA cycle. This CO₂-fixing pathway enables the regeneration of NAD⁺ and FAD cofactors under anaerobic conditions (Hugler et al. 2005). CO₂ is fixed with α -ketoglutarate to form isocitrate, which is then converted into succinate through several intermediates (Figure 1). The rTCA cycle can also be supplied with oxaloacetate generated from pyruvate or phosphoenolpyruvate (PEP) via pyruvate carboxylase (Pyc) and PEP carboxykinase (Pck), respectively. Additionally, malate can be synthesized from pyruvate via malic enzyme and subsequently enters the rTCA cycle. As a carbon fixation route, the rTCA cycle offers the highest theoretical yield of succinate; however, its efficiency is often limited by intracellular NADH depletion under anaerobic conditions. In *E. coli*, succinate is synthesized under anaerobic conditions as an end-product through the rTCA cycle (Zhu et al. 2014, Zheng et al. 2021). Under these conditions, succinate is derived from oxaloacetate but cannot be further converted into succinyl-CoA since the reaction is thermodynamically unfavorable (Birney et al. 1996, Thakker et al. 2012). Although yeasts can also synthesize succinate via the rTCA pathway under anaerobic conditions, the efficiency is low due to the thermodynamic constraints of converting oxaloacetate into succinate and because the relevant enzymes are subject to glucose repression (Raab and Lang 2011). Additionally, redox imbalance further limits succinate biosynthesis from glucose under anaerobic conditions (Cui et al. 2023).

Efficient succinate production via the rTCA cycle has been achieved in engineered *E. coli* strains such as AFP111/pTrc99A-pyc, using a two-phase fermentation process comprising an aerobic growth stage followed by an anaerobic production phase (Vemuri et al. 2002, Table 1). In yeasts, implementing similar strategies is more complex due to the difficulty of balancing oxidative and reductive metabolism (Franco-Duarte et al. 2017). Nevertheless, several studies have reported successful engineering of the rTCA cycle in yeast for succinate production (Xiao et al. 2014, Yan et al. 2014, Cui et al. 2023, see below for details).

Table 1. Genetically modified strains for SA production (upper case letter, gene overexpression; lowercase letter, gene disruption; ALE, adaptative laboratory evolution; F, shake flask; B, batch bioreactor, FB, fed-batch bioreactor).

Strain	Genotype	Culture mode	Carbon Source	pH control	SA titer (g/L)	Yield (g/g)	Productivity (g/L.h)	Reference
Y-3314	<i>sdh2Δ</i>	F	Glycerol	6	45.5	N.R.	0.27	(Yuzbashev et al. 2010)
Y-4215	Chemical mutagenesis & ALE of Y-3314	FB	Glucose	2.7 ^b	50.2	0.43	0.93	(Yuzbashev et al. 2016)
H222-A22	Replacing the promoter of <i>SDH2</i>	FB	Glycerol	5	25.1	0.26	0.15	(Jost et al. 2015)
PGC01003	<i>sdh5Δ</i>	FB ^f	Crude Glycerol ^a	6	160.2	0.40	0.40	(Gao et al. 2016), (Li et al. 2018b)
PSA02004	ALE of PGC01003 in glucose	B	Glucose	6	209.7	N.R.	0.65	(Yang et al. 2017)
		FB	FVW ^a	6	65.8	0.50	0.69	(Li et al. 2018d)
		FB	OFMSW ^a	5.5–6	140.6	0.47	0.44	(Stylianou et al. 2023)
		B	SCB ^a	6	66.7	0.51	0.78	(Ong et al. 2019)
PGC52	<i>sdh5Δ, ach1Δ</i>	F	Glycerol	2.5–3.5 ^b	32.2	0.58	0.33	(Cui et al. 2017)
PGC62	ScPCK	F	Glycerol	2.5–3.5 ^b	12.05	0.29	0.13	(Cui et al. 2017)
					30.15	0.50	0.31	(Cui et al. 2017)
PGC91	<i>sdh5Δ, ach1Δ</i>	F	Glycerol	2.5–3.5 ^b	26.16	0.41	0.27	(Cui et al. 2017)
PGC202	YIPYC in PGC52	FB	Glycerol	3.4 ^b	110.7	0.53	0.80	(Cui et al. 2017)
	Y1SCS2 in PGC62	B	Glucose	5	53.6	0.61	0.52	(Yu et al. 2018)
		FB ^f	MFV ^a	2.8 ^b	71.6	N.R.	0.40	(Li et al. 2019a)
		B	FVW ^a	3.5 ^b	32.6	0.61	0.60	(Li et al. 2019a)
		B	AW ^a	3.5 ^b	40	0.56	0.50	(Li et al. 2019a)
PSA3	ALE PSA02004 at low pH	B ^f	Biochar-textile ^a	4.5 ^b	28.8	0.61	0.33	(Li et al. 2019b)
ST8578	AsPCK, ICL, MLS, MDH, KGDH, SCS2, SpMAE1, <i>ach1Δ</i> , Downregulation of <i>SDH1</i> , ALE	FB	Glucose	3	76.8	0.20	0.24	(Li et al. 2018a)
		FB	Glucose	5	35.3	0.26	0.61	(Babaei et al. 2019)
PSA02004PP	XR, XDH and XK in PSA02004	FB	Xylose	4 ^b	22.3	0.15	0.14	(Prabhu et al. 2020)
RIY420	GUT1 in PGC01003	B	SCB ^a	4.5 ^b	5.6	0.14	0.09	(Ong et al. 2020)
PGC62-SYF	TbFRD, Y1SCS2, Y1ICL, Y1MLS and Y1YHM2 in PGC62 strain	FB	Glycerol	6	178	0.46	0.44	(Jiang et al. 2021)
PGC62-SYF-Mae	SpMAE in PGC62-SYF	F	Glucose	N.R. ^b	21.6	0.61	0.30	(Jiang et al. 2021)
PSA02004PP-ACS 5.0	ALE and ACS in PSA02004PP	FB	Glucose	5.5	101.4	0.37	0.70	(Jiang et al. 2021)
Y1-005	AOX, CAT, DAS, DAK, MgXR, MgXDH, MgXK, FBA2, TAL2, FBP1, HSP70	B	Acetate	6.8	5.1	0.23	0.05	(Narisetty et al. 2022b)
		F	Methanol	N.R.	0.92	N.R.	N.R.	(Zhang et al. 2023)
Hi-SA2	<i>sdh5Δ</i> EcFUM, SpMAE1, Y1MDH2, TbFRD, Y1MDH1 in PGC91 ALE	FB	Glucose	2.5 ^b	111.9	0.79	1.79	(Cui et al. 2023)

Table 1. Continued

Strain	Genotype	Culture mode	Carbon Source	pH control	SA titer (g/L)	Yield (g/g)	Productivity (g/L.h)	Reference
Hi-SA2YIGsh2	YIGSH2 in Hi-SA2	FB	Corncob ^a	3.3 ^b	45.3	0.71	1.42	(Zhong et al. 2024b)
E501	ALE Hi-SA2 in SA 50 g/L	FB	Glucose	2.5–3 ^b	89.62	0.61	1.87	(Zhong et al. 2024a)
E501XF	YIHXX1 and YIPFK1 in E501	FB	Glucose	3.5	112.54	0.67	2.08	(Zhong et al. 2024a)
BDic5	YIXK, SsXR, SsXDH, ScPCK, YISCS2, YIDIC	FB	Glucose-Xylose	6.5	102.44	N.R.	0.39	(Ge et al. 2025)
	sdh5Δ, achΔ		Corn stovel ^a		105.4	0.35	0.53	
ASDH5-A11	YAL10_A07997g	FB	Glycerol	5.5	88.5	0.37	1.23	(Sun et al. 2025b)
	sdh5Δ							
UBR2 _{CBS} -DHA-SA-AnDCT-02	PYC2 to UBR2 _{CBS} -DHA-SA-AnDCT-02	F	Glycerol	5–6	35	0.60	0.36	(Malubhoy et al. 2022)
(2)-PYC2oe								
IoAura3 + SA	PYC, MDH, FUM, FRD	F	Glucose	5	11.63	0.12	0.11	(Xiao et al. 2014)
g3473Δ/PaGDH-DAK/g3837Δ	SpMAE1, PaGDH, DAK, g3473Δ, g3837Δ, GpdΔ, PdcΔ in IoAura3 + SA	FB	Glucose, Glycerol	3	109.5	0.65	0.54	(Tran et al. 2023)
g3473Δ/PaGDH-DAK/ScSUC2	ScSUC2 in g3473Δ/PaGDH-DAK	FB	Sugarcane juice ^a	3	104.6	0.63	1.25	(Tran et al. 2023)
AFP1111/pTtc99A—pyc	PYC	FB ^c	Glucose	7	99.2	1.14 ^d	1.3	(Vemuri et al. 2002)
	pflΔ, ldhΔΔ							
KMG111	mutation in PTSG ^e	FB ^c	Corn stalk ^a	7	129.2	0.86 ^d	2.15 ^d	(Wang et al. 2014)
NZN111	pflΔ, ldhΔ, ptsgΔ, MGTA ^e	FB ^c	Cassava starch ^a	7	100.6	0.86	2.32 ^d	(Chen et al. 2014)
E. coli	pflBΔ, ldhΔΔ	FB ^c	Glycerol	7	72.7	1.13 ^d	0.70 ^d	(Li et al. 2018c)
MLB/pTtc99a-pck	ldhΔΔ, pflBΔ, PCK	FB ^c	Crude glycerol ^a	7	66.8	1.24 ^d	0.70 ^d	
JW1021	pflΔ, ldhΔ, ptsgΔ	FB ^c	Corn stalk ^a	7	113.95	0.91 ^d	3.25 ^d	(Wang et al. 2018)

^aCarbon source refers to hydrolysates from: FVW, Fruits and Vegetables waste; OFMSW, Organic Fraction of Municipal Solid Waste; SCB, Sugarcane bagasse; MFW, Mix Food Waste; AW, Agricultural waste.

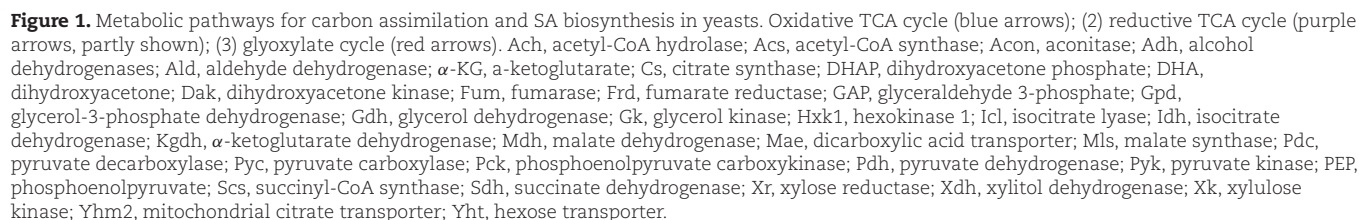
^bCultures have been carried out without pH control. The value indicated the pH at the end of the fermentation.

^cDual phase fermentation, aerobic growth and anaerobic production of SA.

^dThe calculation is based on glucose consumed and SA produced during the anaerobic phase.

^ePFL, pyruvate formate lyase; LDHA, lactate dehydrogenase; PTSG, enzyme of the phosphotransferase system; MGTA, magnesium transporter.

^f*n*-situ fibrous bed bioreactor.



In native yeast metabolism, succinate is generally a transient intermediate that does not accumulate. To achieve significant succinate accumulation, metabolic engineering is required to redirect carbon fluxes and partially impairs the oTCA cycle. Common engineering targets include genes encoding succinate dehydrogenase (Sdh) in the oTCA cycle; isocitrate lyase (Icl) and malate synthase (Mls) in the glyoxylate cycle; and pyruvate carboxylase (Pyc), malate dehydrogenase (Mdh), fumarase (Fum), and fumarate reductase (Frd) in the rTCA pathway (Figure 1). To this end, several metabolic engineering strategies have been developed in yeasts such as *Y. lipolytica*, *S. cerevisiae*, and *I. orientalis*; the first of which being the most promising one. This section will focus in depth on the metabolic engineering strategies developed in *Y. lipolytica* to increase SA titer, productivity, and/or yield and compare its performances to that obtained for *S. cerevisiae*, *I. orientalis* and even *E. coli* (refer to Table 1 for a detailed comparison).

Y. lipolytica is a non-conventional yeast commonly found in environments rich in lipids and proteins (Nicaud 2012, Mamaev and Zvyagil'skaya 2021). As an oleaginous yeast, part of its metabolism

The disruption or downregulation of genes encoding Sdh subunits has primarily been considered for SA accumulation. The mutant strains Y-3374 and Y-3314 were generated by disrupting *SDH1* (YALI0D11374g) and *SDH2* (YALI0D23397g), respectively, in the Po1f laboratory strain (Yuzbashev et al. 2010). While both mutants showed markedly reduced growth on glucose, strain Y-3314 was able to produce SA at a titer of 45.5 g/L from glycerol in buffered media (pH 6), with a productivity of 0.27 g/(L·h). Without medium buffering, the SA titer and productivity dropped to 17.4 g/L and 0.10 g/(L·h), respectively. Strain Y-3314 (*sdh2::URA3*) was further subjected to chemical mutagenesis using N-methyl-N'-nitro-N-nitrosoguanidine. The resulting mutant, Y-3753, produced SA with a titer and yield of 42.1 g/L and 0.39 g/g glucose, respectively, in a fed-batch bioreactor without pH control (Yuzbashev et al. 2016).

In a similar approach, Jost et al. replaced the native promoter of *SDH2* (YAL10D23397g) in strain H222-AZ2 with the regulated weak PO1T promoter. This led to a 64% decrease in SDH activity and resulted in an SA titer of 25.1 g/L with a productivity of 0.15 g/(L·h) in a 1 L bioreactor using glycerol medium under oxygen-limited conditions (Jost et al. 2015).

In another study, disruption of the *SDH5* gene (YAL10F11957g) enabled strain PGC01003 to produce 5.5 g/L SA from glycerol, a 13-fold increase compared to the parental strain Po1g. However, PGC01003 exhibited impaired growth on glucose and accumulated acetic acid. Upon optimization of culture conditions, PGC01003 produced 160.2 g/L SA with a yield of 0.40 g/g and a productivity of 0.40 g/(L·h) from crude glycerol (Gao et al. 2016).

Various strategies were also employed to eliminate side-products formation and to redirect pyruvate toward SA production. These strategies include deletion of genes involved in side-product formation or overexpression of genes encoding key enzymes of the oTCA cycle, reductive carboxylation, and the glyoxylate cycle. The disruption of the *ACH1* gene (YAL10_E30965g) in PGC01003, encoding acetyl-CoA hydrolase, resulted in strain PGC52, improved growth on glycerol, reduced acetate accumulation from 7.5 g/L to 0.2 g/L, and increased the SA titer by 27% in shake-flask cultures (Cui et al. 2017). However, the reduction in acetate formation led to an accumulation of acetyl-CoA, which inhibited pyruvate dehydrogenase (Pdh), causing excess intracellular pyruvate. To redirect the excess pyruvate towards succinate, overexpression of *Pck* and/or *Pyc* (YAL10_C24101g) in PGC52 yielded varying outcomes, with the best improvement seen when expressing the *Pck* gene from *S. cerevisiae*, resulting in strain PGC62. This strain was further modified by overexpressing the gene for the β -subunit of succinyl-CoA synthetase (*Scs2*), generating strain PGC202, which produced 110.7 g/L SA with a yield of 0.53 g/g glycerol after 138 hours of fed-batch culture without pH control (Cui et al. 2017). On glucose, PGC202 produced 53.6 g/L SA with a yield and productivity of 0.61 g/g glucose and 0.52 g/(L·h), respectively (Yu et al. 2018).

Another strategy involved further engineering of PGC62 by overexpressing the *Frđ* gene from *Trypanosoma brucei* and endogenous genes encoding *Scs2* (YAL10D04741g), *Icl* (YAL1C24124g), *Mls* (YAL10E15708g, *Mls1*; YAL10D19140g, *Mls2*), and *Yhm2* (YAL10B10736g) (a mitochondrial citrate transporter). The resulting strain, PGC62-SYF, also expressed *SpMAE* (a C4-dicarboxylic acid transporter from *Schizosaccharomyces pombe*). In a glucose-fed batch bioreactor operated at pH 5.5, strain PGC62-SYF-*SpMAE* produced 101.4 g/L SA, with a yield and productivity of 0.37 g/g glucose and 0.70 g/(L·h), respectively (Jiang et al. 2021).

In *sdhΔ* mutants, the oTCA cycle is disrupted, preventing the conversion of succinate to fumarate and subsequently to oxaloacetate. As a result, less FADH₂ and NADH are generated, reducing ATP production. To address this, various strategies were used to restore glucose metabolism. Yuzbashev et al. (2016) evolve the strain Y-3753 in a glucose-limited chemostat for 840 hours, yielding strain Y-4215, which produced 50.2 g/L SA, 19% increase in SA titer compared with strain Y-3753, within 54 hours in a glucose fed-batch culture, with a yield of 0.43 g/g glucose. Yang et al. used adaptive laboratory evolution (ALE) on PGC01003, leading to strain PSA02004 with enhanced glucose uptake. This strain achieved a titer of 65.8 g/L SA with a productivity of 0.69 g/(L·h) from glucose in a 2 L bioreactor, with acetate as the sole by-product (Yang et al. 2017). ALE of PSA02004 under low pH conditions yielded strain PSA3, which produced 76.8 g/L SA from glucose at pH 3 over 324 hours in repeated fed-batch culture, with a yield of 0.20 g/g glucose (Li et al. 2018a).

Babaei et al. combined most of the previous mentioned strategies to construct a strain that efficiently produce SA. First, they reduced SDH activity by 77% by truncating the *SDH1* promoter, maintaining glucose-dependent growth. Disruption of *ACH1* and overexpression of *SpMAE1* allowed strain ST8507 to produce 1.2 g/L SA in four days culture in deep-well plate using glycerol, while the parental strain produced any SA. However, the specific growth rate of ST8507 decreased from 0.49 to 0.33 h⁻¹. Further overexpression of the *Pck* gene from *Actinobacillus succinogenes* generated strain ST8510. Overexpressing endogenous genes encoding *Icl* and *Mls* and *Mdh*, in ST8507 improved the SA titer by 58% (~2 g/L). Additionally, enhancing oTCA flux by overexpressing *Kgdh* and *Scs2* encoding genes led to strain ST8578, which produced 3.3 g/L SA (a 280% increase over ST8507). ALE of ST8578 yielded improved glucose utilisation, with a final titer of 35.3 g/L SA at pH 5, and a yield and productivity of 0.26 g/g and 0.61 g/(L·h), respectively in a fed-batch bioreactor culture (Babaei et al. 2019).

Efforts to enhance glycerol metabolism and broaden carbon source (i.e. xylose, acetate, and methanol) utilization in *Y. lipolytica* have also been considered. To further optimize glycerol metabolism in strain PGC01003, strain RIY420 was constructed by overexpressing *GUT1* (YAL10F00484g) encoding glycerol kinase. In fed-batch culture, RIY420 produced 178 g/L SA with a yield of 0.46 g/g glycerol and a productivity of 0.44 g/(L·h), representing a 10% productivity improvement over PGC01003 (Ong et al. 2020). Prabhu et al. (2020) engineered strain PSA02004 by overexpressing endogenous *XR* (xulose reductase, YAL10D07634g), *XDH* (xylitol dehydrogenase, YAL10E12463g), and *XK* (xylulose kinase, YAL10F10923g) genes (Fig 1). In bioreactor batch culture on pure xylose, strain PSA02004PP produced 11.2 g/L SA, with a yield and productivity of 0.19 g/g xylose and 0.13 g/(L·h), respectively. Fed-batch culture yielded 22.3 g/L SA, with a yield and productivity of 0.15 g/g and 0.14 g/(L·h). Strain BDic5, engineered for xylose catabolism (*YlXK*, *SsXR*, *SsXDH*) and SA anabolism (*sdh5Δ*, *ach1Δ*, *ScPCK*, *YlSCS2*, *YlDIC*), produced 102.4 g/L SA with a productivity of 0.39 g/(L·h) from glucose and xylose in shake flasks under fed-batch conditions (Ge et al. 2025). Narisetty et al. (2022b) expressed *E. coli* acetyl-CoA synthase (*Acs*) in PSA02004PP and used ALE to enhance acetate tolerance. The resulting strain, ACS 5.0, produced 5.1 g/L SA in acetate medium over 96 hours.

Zhang et al. (2023) reconstructed a methanol catabolic pathway in *Y. lipolytica* by expressing *Pichia pastoris* genes for alcohol oxidase (*Aox*, PAS_chr4_0821), catalase (*Cat*, PAS_chr2-2_0131), dihydroxyacetone synthase (*Das*, PAS_chr3_0832), and dihydroxyacetone kinase (*Dak*, PAS_chr3_0841). However, the engineered strain could not grow on methanol, likely due to insufficient precursor regeneration. To resolve this, xylose catabolism genes (*XR*, *XDH*, *XK*) from *Meyerozyma guilliermondii* and *P. pastoris* genes coding fructose 1,6-bisphosphate aldolase (*Fba2*, PAS_chr1-1_0319), transaldolase (*Tal2*, PAS_chr2-2_0338) and fructose-1,6-bisphosphatase (*Fbp1*, PAS_chr3_0868) were expressed and the corresponding protein targeted to the peroxisome to allow xylulose 5-phosphate (*Xu5p*) regeneration. Overexpression of *Hsp70* and disruption of *SDH5* enabled SA production. The final strain, Y1-005, produced 0.92 g/L SA using methanol as the sole carbon source.

Another efficient strategy for enhancing SA production in engineered yeast strains involves leveraging the rTCA pathway. Cui et al. reconstructed part of the rTCA cycle in strain PGC91 by expressing *Frđ* from *Trypanosoma brucei*, *Fum* from *E. coli*, and endogenous *Mdh1* (YAL10D16753g) yielding in strain PGC91-rT with a 30.8% higher SA yield but 52.5% lower biomass. ALE restored growth on glucose, and mitochondrial targeting of *TbFrđ*, *YlPyc*, and *YlFum* improved SA synthesis. The final strain, Hi-SA2, which

also overexpresses *SpMAE1* and *Mdh2* (YALIOE14190g), produced 111.9 g/L SA in 62 hours from glucose with a yield of 0.79 g/g and productivity of 1.79 g/(L·h) at pH 2.5 (Cui et al. 2023). ALE on Hi-SA2 for SA tolerance produced strain E501, which achieved 89.6 g/L SA at 1.87 g/(L·h). Further improvement of glucose uptake and glycolysis by overexpression of gene from endogenous hexose transporters *Yht1* (YALIOC06424g), *Yht3* (YALIOF19184g) and *Yht4* (YALIOE23287g), as well as the key enzymes from the glycolytic pathway, namely hexokinase (*Hxk1*, YALIOB22308g), phosphofructokinase (*Pfk1*, YALIOD16357g) and pyruvate kinase (*Pyk1*, YALIOF09185g) led to strain E501XF, which produced 112.5 g/L SA at pH 3.5 with a yield and productivity of 0.67 g/g and 2.08 g/(L·h), respectively (Zhong et al. 2024a).

Finally, Sun et al. identified YALIOA07997g, a gene associated with SA tolerance under stress (40 g/L SA, pH 3). Its overexpression in an *sdh5Δ* strain led to an SA titer of 88.5 g/L with a productivity of 1.23 g/(L·h), representing a 66% increase over the parental strain (Sun et al. 2025b).

Other yeast strains

A significant amount of research has focused on the metabolic engineering of *S. cerevisiae* to enhance SA production. Similar to *Y. lipolytica*, these strategies involve the disruption of SDH-encoding genes and/or the overexpression of genes encoding key enzymes in the rTCA pathway, mainly *Pyc*, *Mdh*, *Fum*, and *Frd*. Several studies have demonstrated the ability of engineered *S. cerevisiae* strains to produce SA under anaerobic or microaerobic conditions with CO₂ fixation, which offers significant advantages from an industrial viewpoint (Agren et al. 2013, Yan et al. 2014, Malubhoy et al. 2022). The strain UBR2_{CBS}-DHA-SA-AnDCT-02(2)-PYC2oe achieved an SA titer of 35 g/L with a yield of 0.60 g/g glycerol and a productivity of 0.36 g/(L·h) in shake flask culture with the addition of CaCO₃; currently the highest values reported for *S. cerevisiae* in the literature (Malubhoy et al. 2022). In this strain, the rTCA pathway was partially reconstructed in the cytosol by overexpressing genes encoding the endogenous *Mdh3*, the heterologous fumarase from *Rhizopus oryzae* (RoFumR), and the peroxisomal fumarate reductase from *Trypanosoma brucei* (TbFrd), along with the overexpression of genes encoding the dicarboxylic acid transporter *Dic02* from *Aspergillus niger* (AnDct-02) and *Pyc2* (Xiberras et al. 2020, Malubhoy et al. 2022). These genetic modifications were made into *S. cerevisiae* strain UBR2CBS-DHA previously engineered to convert its native FAD-dependent glycerol catabolic pathway into an NAD-dependent one (Klein et al. 2016). Compared to *Y. lipolytica*, the efficiency of SA production using *S. cerevisiae* strains remains relatively low. However, there is significant potential for further improvement in both titers and yields. Notably, Reverdia, a leading company in this field, reported in a patent a genetically engineered *S. cerevisiae* strain capable of producing up to 100 g/L of SA at pH (Choi et al. 2015). However, the details of the strain construction are not fully disclosed.

I. orientalis (also known as *Candida krusei* or *Pichia kudriavzevii*) is a non-model yeast that exhibits exceptional natural tolerance to low pH (down to 2) and high osmotic pressure (Tan et al. 2025), and shows promise for efficient SA production. Part of the rTCA cycle was reconstructed in the cytoplasm of strain SD108 by overexpressing the genes encoding *Pyc*, *Mdh*, *Fum*, and *Frd* (Xiao et al. 2014). The resulting strain, IoΔura3 + SA, was further engineered by overexpressing the *SpMae1* transporter and disrupting genes encoding glycerol-3-phosphate dehydrogenase (*Gpd*) and pyruvate decarboxylase (*Pdc*) to block glycerol and ethanol production, respectively, as well as the dicarboxylic acid importer

G3473. Additional modifications included the overexpression of a glycerol dehydrogenase (*Gdh*) gene from *Pichia angusta* and the endogenous *Dak* gene. Finally, to relieve glucose-induced catabolite repression on glycerol consumption, the G3837 hexokinase gene was deleted, resulting in strain g3473Δ/PaGDH-DAK/g3837. This strain produced 109.5 g/L SA with a yield of 0.63 g/g glucose equivalent and a productivity of 0.54 g/(L·h) in a fed-batch culture using minimal medium with glucose and glycerol, maintaining the pH at 3 (Tran et al. 2023).

Renewable feedstocks for succinic acid synthesis

Glucose is currently the most used substrate in bioprocesses for SA production, but its cost remains a major barrier to the process economic viability (Morales et al. 2016). As a result, researchers have explored a range of alternative substrates for yeast strains to support more sustainable and cost-effective SA production. In the literature, most studies on the use of renewable feedstocks for SA production by yeast are related to *Y. lipolytica* based on its remarkable flexibility in utilizing both hydrophilic and hydrophobic carbon sources, including sugars, lignocellulosic hydrolysates, fatty acids, lipids, waste oils, crude glycerol, and acetate (Ma et al. 2020). Additionally, efforts to widen *Y. lipolytica* substrate range have included the introduction of new metabolic pathways and improvements to the strain tolerance toward specific feedstocks (Zhong et al. 2024b, Zhang et al. 2023).

Crude glycerol

Compared to glucose, glycerol has a higher degree of reduction and represents a promising carbon source for SA synthesis. Crude glycerol is a byproduct of biodiesel production, with approximately 1 kg of crude glycerol generated for every 10 kg of biodiesel produced (Koutinas et al. 2014). According to the EU Biofuels Annual Report (Flach et al. 2019), biodiesel production in the EU was estimated at 11.2 million tonnes (Mt) in 2023, suggesting an annual availability of approximately 1.12 Mt of crude glycerol in the EU-28. Crude glycerol has been used as a substrate for SA production by *Y. lipolytica* strain PGC01003 in fed-batch cultures, achieving an SA titer of 160.2 g/L, a yield of 0.4 g/g, and a productivity of 0.4 g/(L·h) (Gao et al. 2016). Using an *in-situ* fibrous bed bioreactor (isFBB), in which cells were immobilized on sugarcane bagasse, SA production reached 209.7 g/L with a productivity of 0.65 g/(L·h) in fed-batch culture of strain PGC01003 (Li et al. 2018b). Engineered yeast strains have demonstrated SA production efficiencies comparable to, or even exceeding, those of engineered *E. coli* when using crude glycerol. For example, *E. coli* MLB/pTrc99a-pck produced only 5.9 g/L SA when cultivated in untreated crude glycerol. However, when the same strain was cultivated in crude glycerol pretreated with activated carbon, it produced 66.8 g/L SA with a yield of 1.24 g/g and a productivity of 0.70 g/(L·h) during the anaerobic phase (Li et al. 2018c).

Food supply chain wastes

Food wastes are generated at multiple stages across value chains, including industrial production, distribution, consumption, and waste management. According to Eurostat, total food waste in the EU was approximately 59 Mt million tonnes (Mt) in 2022 (Eurostat 2022). Major components of food waste include the organic fraction of municipal solid waste (OFMSW), fruit and vegetable residues, and waste from catering services. It is estimated that by 2050, the volume of OFMSW could reach 3.4 billion tonnes

(Bandini et al. 2022). Currently, organic waste is predominantly managed through landfilling or incineration that are processes associated with significant environmental burdens and limited economic return. A fed-batch culture in isFBB using *Y. lipolytica* strain PGC202 and a glucose-based hydrolysate derived from mixed food waste achieved 71.6 g/L SA after 177 hours, values similar with cultures using commercial glucose-based media (53.6 g/L SA after 104 h) (Li et al. 2019a, Yu et al. 2018). In the same study, the authors employed a fruit and vegetable waste hydrolysate (i.e. glucose-based) in a batch bioreactor culture with strain PGC202, without pH control, resulting in 32.6 g/L SA with a productivity of 0.6 g/(L·h) and a yield of 0.61 g/g glucose (Li et al. 2019a). Using the same hydrolysate and supplementing with 4% corn steep liquor (CSL), strain PSA02004 produced 140.6 g/L SA in fed-batch mode, with a productivity of 0.44 g/(L·h) (Li et al. 2018d). OFMSW hydrolysate was also used as a carbon source in cultures with *Y. lipolytica* strain PSA02004, applying a two-stage pH regulation strategy that gradually reduced the pH from 6.0 to 5.5 over 30 hours. This strategy yielded 54.4 g/L SA, with a productivity of 0.82 g/(L·h) and a yield of 0.44 g/g (Stylianou et al. 2021). The same strain was employed in combined SA production and *in-situ* extraction using an electrochemical membrane bioreactor (EMB), which increased the final SA titer to 66.7 g/L, with a yield of 0.51 g/g and a productivity of 0.78 g/(L·h) (Stylianou et al. 2023). In contrast, few studies have explored SA production by engineered *E. coli* strains using food waste hydrolysates. Huang et al. (2019) used a hydrolysate derived from sweet potato waste with *E. coli* HD134, achieving 18.6 g/L SA with a yield of 0.94 g/g after 48 hours in shake flask culture.

Agricultural residues

Agricultural activities generate various residues that could serve as valuable feedstocks for SA production. Between 2006 and 2015, the production capacity of agricultural residues (e.g. dry biomass from leaves and stems) in the EU-28 was estimated at approximately 442 Mt. In the EU, most of these residues originate from cereals (328.52 Mt, 74.25%), followed by oil-bearing crops (73.1 Mt, 16.52%) and permanent crops (21.86 Mt, 4.94%) (Camia et al. 2018). Residues with limited competitive uses, such as corn stover, which is not essential for soil conservation, animal bedding, or bioenergy production, are promising candidates for use as industrial feedstocks.

Li et al. (2019a) performed batch fermentations with *Y. lipolytica* strain PGC202 using hydrolysates derived from agricultural residues, including sugarcane bagasse, wheat straw, and corn stalks. These cultures yielded 40 g/L SA with a yield of 0.56 g/g and a productivity of 0.50 g/(L·h). Ong et al. (2019) demonstrated that *Y. lipolytica* strain PSA02004 can simultaneously metabolize glucose and xylose from sugarcane bagasse hydrolysates for SA production (32.2 g/L SA with a yield of 0.58 g/g and a productivity of 0.33 g/(L·h)). In another study, sugarcane bagasse hydrolysate was used in batch bioreactor cultures with the strain PSA02004PP, producing 5.6 g/L SA with a yield of 0.14 g/g (Prabhu et al. 2020). Corn stover hydrolysate was also used in fed-batch cultures with *Y. lipolytica* strain BDic5, achieving 105.4 g/L SA with a yield of 0.35 g/g and a productivity of 0.53 g/(L·h) (Ge et al. 2025). Mancini et al. (2022) reported the use of corn stover hydrolysate in *S. cerevisiae* cultures maintained at a low constant pH of 3.1, yielding 43 g/L SA with a yield of 0.69 g/g and a productivity of 0.45 g/(L·h).

Lignocellulosic hydrolysates often contain inhibitory compounds such as furfural, which can hinder microbial growth and SA production. The strain Hi-SA2-YIGsh2, engineered to overexpress the GSH2 gene (YALI0C17831g) encoding glutathione syn-

thetase from *Y. lipolytica*, exhibits elevated intracellular levels of reduced glutathione. This enhances resistance to reactive oxygen species and improves tolerance to toxic compounds in hydrolysates. Using undetoxified corn cob hydrolysate containing mainly glucose and xylose, this strain produced 45.3 g/L SA with a yield of 0.71 g/g and a productivity of 1.42 g/(L·h) (Zhong et al. 2024b).

Food industry products and coproducts

The main food industry coproducts that could serve as substrates for SA production include corn-derived glucose syrups, sucrose-rich juices (e.g. sugarcane juice), and molasses. (Tran et al. (2023) overexpressed the SUC2 gene from *S. cerevisiae* in an *I. orientalis* g3473Δ/PaGDH-DAK strain to enable sucrose assimilation. Using sucrose-rich sugarcane juice in fed-batch culture at pH 3, the engineered strain produced 104.6 g/L SA, with a yield of 0.63 g/g glucose and a productivity of 1.25 g/(L·h). Rapeseed oil was also evaluated as a carbon source for SA production using *Y. lipolytica* VKM Y-2412 in fed-batch culture, resulting in 69.0 g/L SA (Kamzolova et al. 2014).

Other feedstocks

SA production by engineered yeasts has been evaluated using textile waste hydrolysates. In 2015, global textile waste generation was approximately 92 Mt, and it is projected to reach 148 Mt by 2030 (Li et al. 2019b). This waste stream represents a promising raw material for platform chemical production, as 35–40% of textile waste consists of cellulose (Hu et al. 2018). Biochar-treated textile waste hydrolysate, rich in glucose, was used as fermentation feedstock with the *Y. lipolytica* strains PGC202 and PSA02004. Strain PGC202 efficiently produced 28.8 g/L SA with a yield of 0.61 g/g and a productivity of 0.33 g/(L·h) in isFBB. In contrast, strain PSA02004 was unable to fully consume the available glucose, resulting in a significantly lower SA titer of 3.6 g/L (Li et al. 2019b).

These aforementioned researches underscore the capability of yeast, most notably *Y. lipolytica*, to efficiently metabolize a broad spectrum of alternative carbon sources obtained from renewable feedstocks, including crude glycerol, lignocellulosic hydrolysates, and various industrial and agricultural waste streams.

Downstream separation and purification of succinic acid

Downstream processes contribution to the total cost of the final product are comprised between the 30 and 40% (Straathof 2011). In the case of bacterial strains, the requirement for neutral pH during the culture phase in bioreactor significantly impacts SA production costs, with DSP contributing up to 60% of the total production expenditure (Kurczrock and Weuster-Botz 2010). As already stated, at neutral pH, succinate exists in salt form in the culture medium at neutral pH, which must be acidified during the DSP stage. In addition to medium neutralization, the DSP process must include unit operations for separating biomass, metabolic by-products (e.g. acetate, formate), and impurities (e.g. anions, cations, proteins, pigments), as well as evaporation and crystallization of SA.

Various DSP processes for SA purification (Figure 2) have been developed, exhibiting different SA recovery efficiencies and purities. SA purification strategies can be classified into *in-situ* processes, which integrate bioreactor operation with SA extraction, and sequential processes, where SA is produced first in the bioreactor and subsequently purified from the culture broth.

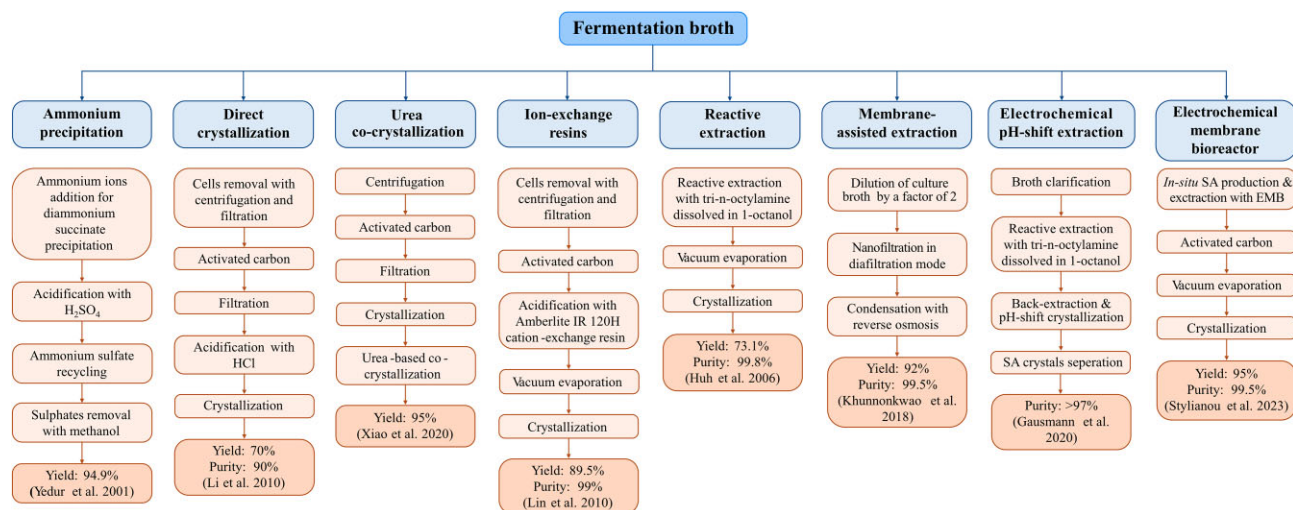


Figure 2. Strategies for succinic acid separation and purification.

Precipitation has been widely used for recovering organic acids from culture broths (Kurzrock and Weuster-Botz 2010). This method typically involves the addition of a base to precipitate succinate salts, followed by filtration and acidification with H_2SO_4 to solubilize SA and remove salts as a by-product stream. A calcium hydroxide-based process achieved SA crystal purity of 94.2% (Datta et al. 1992). (Alexandri et al. 2019) also investigate the calcium precipitation in culture broths derived from spent sulphite liquor (SSL), resulting in 81% purity and a low recovery yield of 13%. Similar results were observed using a wheat-based fermentation broth, with 13% recovery yield and 30% SA purity (Luque et al. 2009). Major drawbacks of this method include the generation of solid calcium sulfate as a by-product and low recovery yields (Lee and Kim 2011). Alternatively, a diammonium succinate process using ammonia as the neutralizing agent demonstrated a SA recovery yield of up to 94.9%. Unlike the process with calcium hydroxide, the use of H_2SO_4 results in ammonium sulfate, which can be recycled and reused in the culture stage (Yedur et al. 2001).

Direct crystallization has also been explored. Luque et al. (2009) acidified the culture broth to pH 2 to convert SA to its undissociated form. The process includes vacuum evaporation to remove volatile acids and concentrate SA, followed by crystallization at 4°C. SA crystal purities of 90–97% and recovery yields of 61–75% were reported using synthetic (artificial) media. One-step crystallization via HCl acidification of glucose-based broth yielded 90% purity and 70% recovery (Li et al. 2010).

Cooling crystallization with an extra crystallization step using urea increased SA recovery to 95%, producing SA-urea co-crystals suitable for succinimide production (Xiao et al. 2020). Other direct crystallization methods include acidification and ion-exchange resin-based approaches. The latter resulted in 89.5% recovery and 99% purity (Lin et al. 2010). A cation-exchange resin acidification method yielded 79% recovery and 96% purity (Alexandri et al. 2019). Due to impurities in culture broths, crystallization is generally the final DSP step, following preliminary purification operations such as activated carbon treatment and filtration (Mancini et al. 2020).

Reactive extraction uses amines in organic solvents to form amine-acid complexes. Amines are effective for recovering organic acids because they serve as electron donors and interact with negatively charged species (Kurzrock and Weuster-Botz 2010). Different types of amines (primary, secondary, tertiary) have

been tested in various solvents. Extraction must occur at pH values below the acid pKa, as amines react with the undissociated form (Jun et al. 2007). Quaternary amines, which interact with both dissociated and undissociated acids, are difficult to regenerate by back-extraction (Lee et al. 2008). Tertiary amines such as trioctylamine in 1-octanol have achieved recovery yields of 67–73.1% and purities higher than 99% (Huh et al. 2006, Song et al. 2007). Kurzrock and Weuster-Botz, (2011) also reported high recovery yields using reaction systems combining different amines and organic solvents, with trihexylamine in 1-octanol as the most effective combination. Alexandri et al. (2019) reported a 73% recovery yield and 97.2% purity using trioctylamine in 1-hexanol with pH swing back-extraction.

Membrane-assisted techniques, including ultrafiltration, nanofiltration, reverse osmosis, emulsion liquid membranes, and electrodialysis, have also been investigated. These methods offer economic and energy efficiency due to selective compound separation, reduced chemical consumption, and fewer DSP unit operations (Sadare et al. 2021). Pressure-driven filtration and osmosis are typically used as initial steps to clarify broths (Jansen and Van Gulik 2014), though industrial application is limited by membrane fouling (Prochaska et al. 2018).

A two-stage membrane-based process was conducted combining nanofiltration in diafiltration mode followed by concentration of the retentate by reverse osmosis, leading to a SA recover yield of 92% and a SA purity of 99.5% (Khunnonkwao et al. 2018). Emulsion liquid membrane separation, a form of advanced liquid-liquid extraction, has also been applied. Othman et al. (2018) developed an optimized method using this approach, achieving 98% recovery and nearly 100% purity of SA crystals.

Electrodialysis utilizes ion-exchange membranes and electrical potential to transport ionic species. This process allows the selective recovery of dissociated carboxylic acids at pH values above their pKa (Jones et al. 2021). The first report of electrodialysis for SA recovery achieved 60% yield and 80% purity (Glassner and Datta 1992). (Fu et al. 2014) converted sodium succinate to SA using a bipolar membrane electrodialysis (EDBM) system, achieving 96.8% recovery. When combined with ultrafiltration and reactive extraction, EDBM yielded over 90% recovery (Prochaska et al. 2018). Gausmann et al. (2020) demonstrated a sustainable strategy using electrochemical pH-shift DSP combined with back-extraction and crystallization to recover SA crystals with more

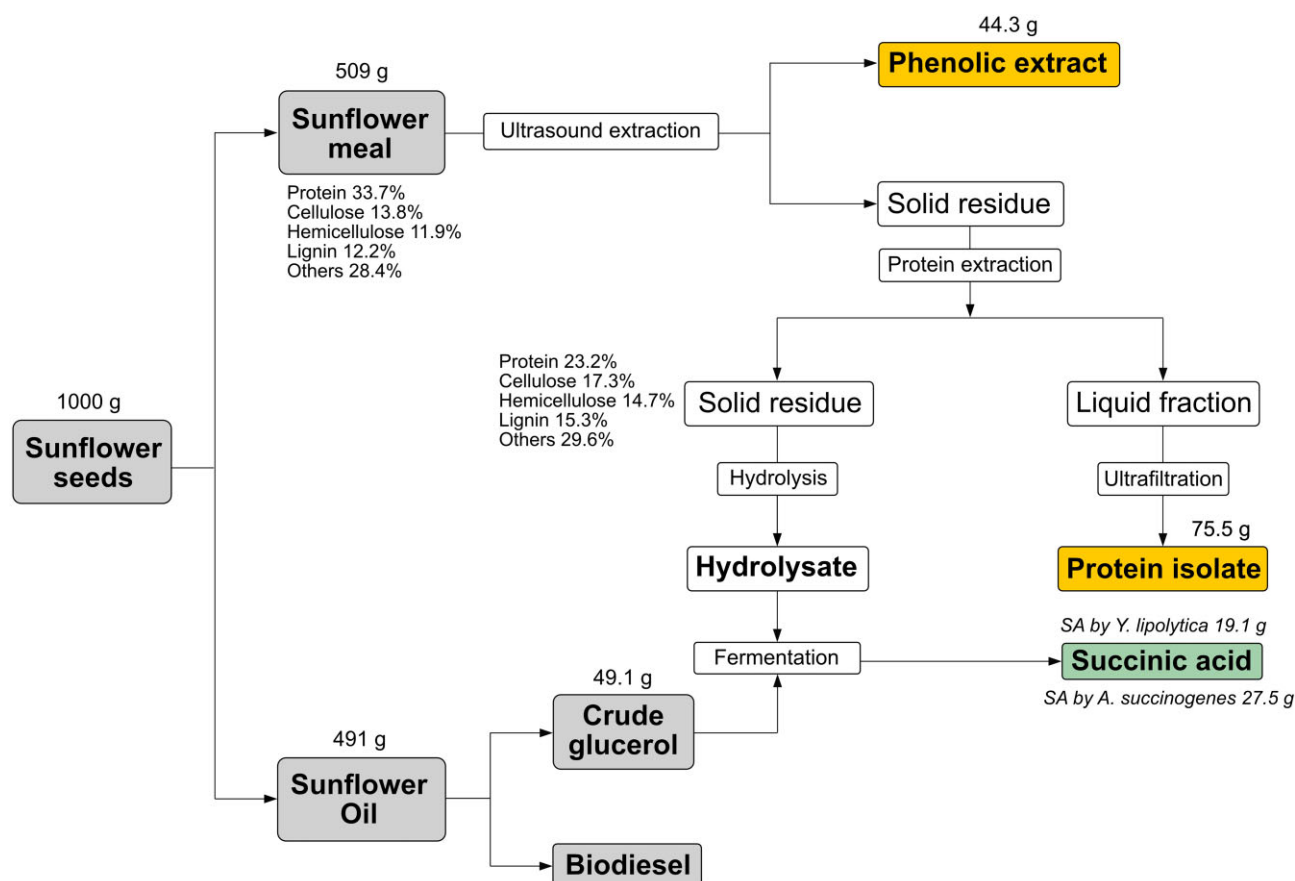


Figure 3. Biorefinery concept for SA production (Modified from Efthymiou et al. 2021).

than 97% purity. Industrial implementation depends heavily on optimizing current efficiency and energy consumption (Fu et al. 2014).

An integrated in-situ electrochemical membrane bioreactor (EMB) process was developed by Pateraki et al. (2019) using *Bacillus succiniciproducens* cultivated on SSL. In-situ succinate extraction reduced product inhibition for the producing microorganism, increasing yield by 15% and productivity by 32%. Additionally, OH^- generated at the cathode reduced NaOH requirements for pH control by 19.3%. Stylianou et al. (2023) applied a similar EMB-based process using engineered *Y. lipolytica* strains and municipal biowaste hydrolysates. This approach improved SA production efficiency, minimized base consumption, and allowed operation at a reduced pH (from 6 to 5.5), achieving a 95% recovery yield and 99.95% purity. Combining yeast fermentation with an EMB-based process presents a promising approach by omitting cells centrifugation and acidification steps. The low pH of the broth enhances coulombic efficiency, as one carboxyl group of succinic acid remains undissociated near its pKa, reducing the electrons needed for ion transportation. These findings underscore the importance of low-pH fermentation for efficient in-situ SA extraction.

Integrated biorefineries for SA production

The higher market price of bio-based SA compared to its fossil-derived counterpart indicates that conventional bioprocesses must be restructured and integrated into circular biorefineries using crude renewable resources. This approach can alleviate

key process bottlenecks, including the replacement of commercial carbon sources and nutrients with low-cost hydrolysates, and enhance economic viability through the coproduction of multiple end-products targeting diverse applications and markets. The comprehensive exploitation of all biomass components can enable the generation of both commodity (e.g. bio-based SA) and specialty products (e.g. bioactive compounds).

To date, only a limited number of biorefinery concepts incorporate SA production by engineered yeasts. One such example involves the valorization of biodiesel industry by-products, such as sunflower meal (SFM) and crude glycerol, for SA production using *A. succinogenes* and *Y. lipolytica* PSA2004 within an integrated biorefinery framework (Figure 3) (Efthymiou et al. 2021). In this process, a protein concentrate and phenolic-rich compounds were first extracted from SFM. The residual solids were subjected to thermochemical and enzymatic treatments to generate a nutrient-rich hydrolysate, which supported SA production by *A. succinogenes* in fed-batch culture, yielding 34 g/L SA with a yield of 0.6 g/g and productivity of 0.71 g/(L·h). Crude glycerol, combined with SFM hydrolysate, served as a carbon source for SA production by *Y. lipolytica* PSA2004 in fed-batch cultures, achieving 69.1 g/L SA, a productivity of 1.26 g/(L·h), and a yield of 0.39 g/g.

In the PERCAL project, a novel biorefinery concept (Figure 4) was developed based on OFMSW for the production of SA for incorporation in polyurethane dispersions, and biosurfactants (Ioannidou et al. 2023a). The carbohydrate-rich fraction of OFMSW was hydrolyzed to generate a sugar-rich substrate for SA production by *Y. lipolytica* (Stylianou et al. 2023), while the oil and protein fractions were utilized for biosurfactant synthesis. In that

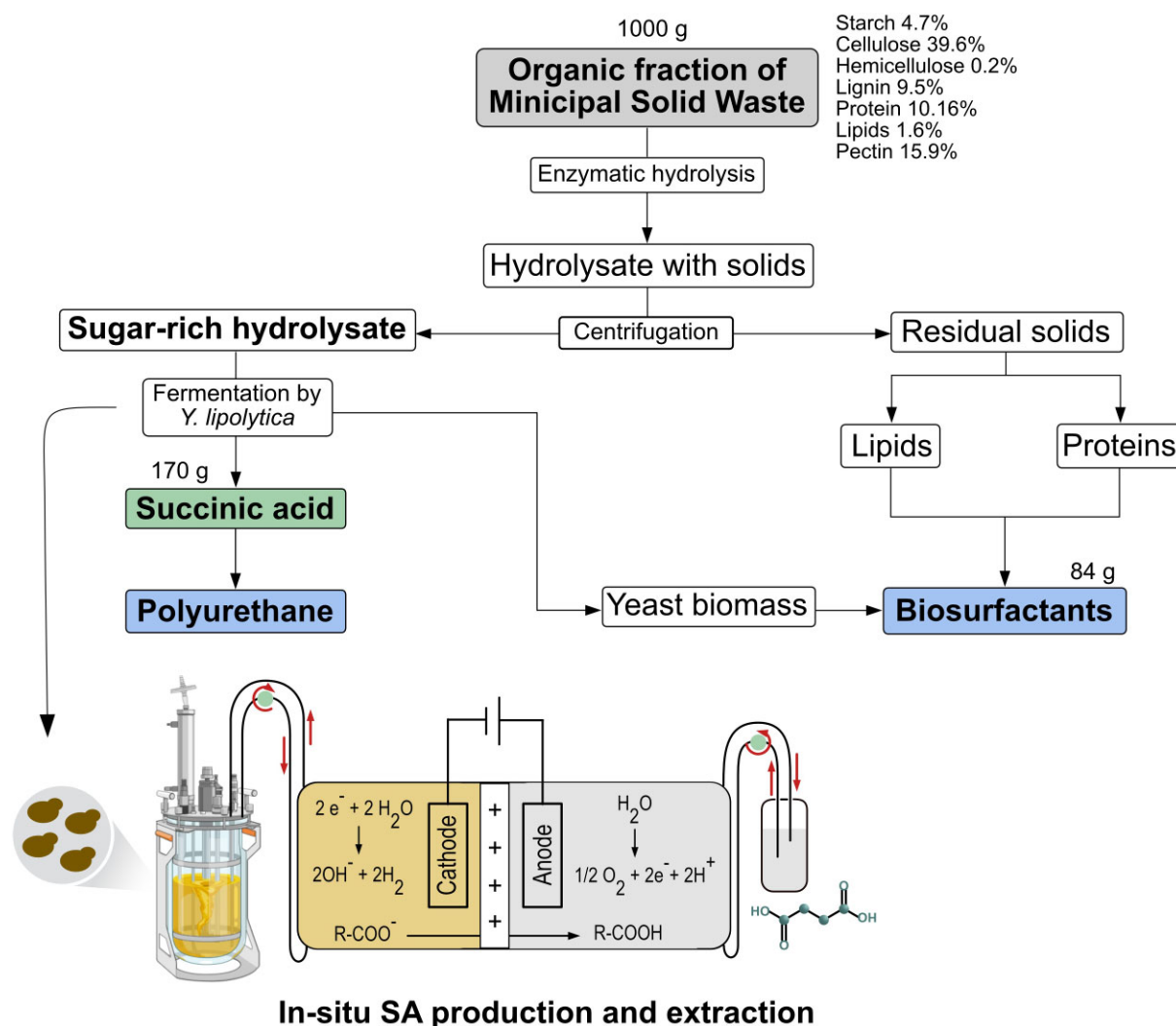


Figure 4. Biorefinery approach for in-situ production and extraction of SA by *Y. lipolytica* (Modified from Ioannidou et al. 2023a, Stylianou et al. 2023).

biorefinery, an EMB was employed for in-situ SA production and extraction, enhancing process integration and efficiency.

Most biorefineries reported in the literature utilize bacterial strains for bio-based SA production. For instance, Filippi et al. (2022) developed a biorefinery concept using winery residues (grape pomace, stalks, and wine lees). Bacterial cellulose was derived from grape pomace, while grapeseed oil and polyphenols were extracted from the same stream. Wine lees were valorized to produce ethanol, antioxidants, tartaric acid, and nutrient-rich hydrolysates, which were subsequently used in fed-batch culture of *A. succinogenes* for SA production. Patsalou et al. (2020) designed a biorefinery process for the valorization of citrus peel waste into SA and co-products. Essential oils (0.43%) and pectin (30.53%) were first extracted, and the residual biomass was hydrolyzed to yield fermentable sugars.

Sustainability assessment

In the last decade, the SA market price considering both fossil and bio-based origin ranges between \$1.46–4.06/kg (E4tech, RE-CORD, WUR 2015; CredenceResearch 2024; Imarc 2024). The wide market price fluctuation underscores the volatile SA market, which as most bio-based platform chemicals are influenced by various fac-

tors, such as market demand, flexibility in final applications, production cost associated with the available technologies and crude renewable feedstocks, competition with established fossil-based counterparts, and co-product development. The case of BioAmber, the commercial-scale SA production facility in Canada, has been presented by Li and Mupondwa 2021. Despite the high capital investment of \$147 million and the annual production capacity of 30,000 t, where economies of scale can be achieved, the plant failed to achieve profitability, as the production cost of \$2.23/kg_{SA} was significantly higher than BioAmber's projected targets. Key issues included underperformance in market penetration, inability to reach full production capacity and overestimation of co-product value. A sensitivity analysis showed that even with improved conversion routes and the inclusion of co-products, profitability remained difficult to attain at the assumed market prices.

The current economic performance of conventional bioprocesses for SA production could be improved via integration within novel biorefinery concepts producing multiple bio-based products. Literature-cited techno-economic indicators of bio-based SA production processes, including Fixed Capital Investment (\$2.88–16.75/kg_{SA}), Cost of Manufacture (COM) (\$0.88–2.32/kg_{SA}) and Minimum Selling Price (MSP) (\$0.99–2.26/kg_{SA}), vary significantly

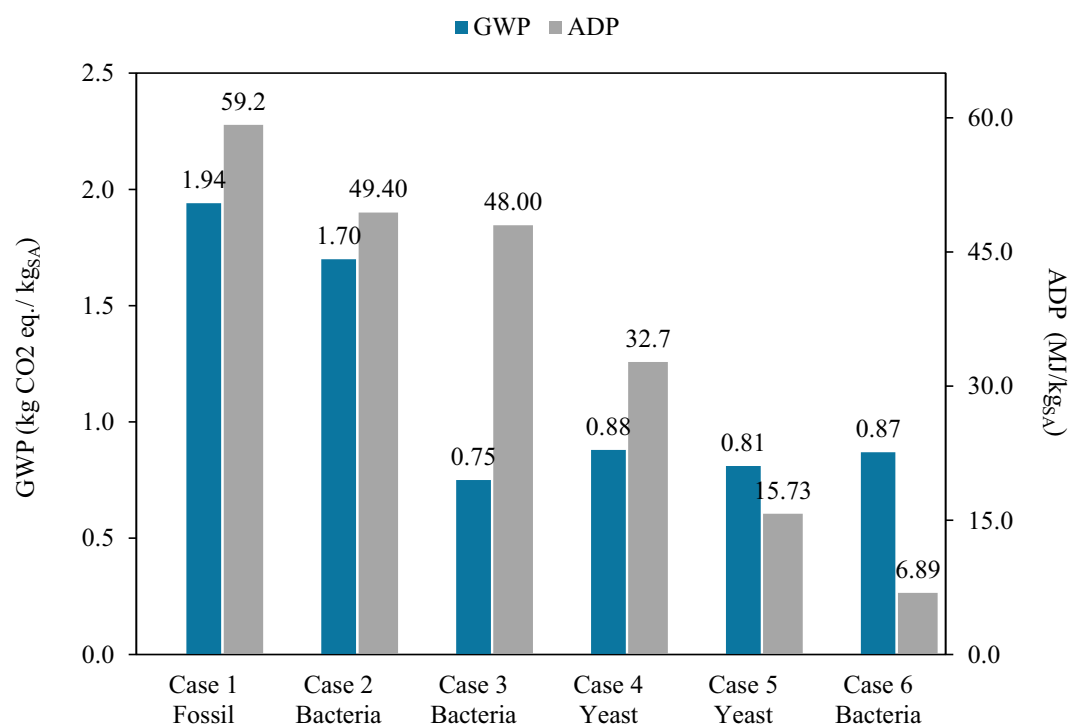


Figure 5. Global warming potential (GWP) and abiotic depletion (ADP) values for different SA production processes using fossil and renewable resources. Case 1. Fossil-based SA (Cok et al. 2014); Case 2. Anaerobic bacterial culture to succinate salt at pH 7 with SA purification via an electrodialysis-based DSP process (EU electricity production grid) (Cok et al. 2014); Case 3. Anaerobic bacterial culture to succinate salt at pH 7 with SA purification via an electrodialysis-based DSP process (France electricity production grid) (Cok et al. 2014); Case 4. Low pH yeast fermentation with direct crystallization-based SA purification (France electricity production grid) (Cok et al. 2014); Case 5. Yeast fermentation at pH 6 with in-situ production and extraction using a electrochemical membrane bioreactor (100% renewable electricity) (Ioannidou et al. 2023b); Case 6. Bacterial fermentation with NH₃ and salt separation with continuous ion exchange columns. Ammonium phosphate is produced as a co-product fertilizer (Moussa et al. 2016).

depending on the feedstock used and the respective co-products developed, the production capacity, and the selected technologies employed (Ioannidou et al. 2020). Ladakis et al. (2022) developed a biorefinery utilising an OFMSW stream to produce bio-based SA via fermentation of the carbohydrate fraction and to extract lipids/fats and proteins for the production of various co-products. The MSP of SA (\$1.1–2.39/kg_{SA}), estimated for an annual production capacity of 60,000 t, was influenced by co-product market prices and OFMSW management fees (Ladakis et al. 2022). Ioannidou et al. (2023b) reported a MSP of \$2.7/kg_{SA} when SA production was achieved with the EMB technology using the engineered *Y. lipolytica* PSA02004 strain cultivated on crude hydrolysates derived from an OFMSW stream, for which a conservative management fee (\$35/t) was considered. The OFMSW management fees in EU countries (\$35–118/t) vary depending on the specific country and region. The OFMSW constitute major renewable feedstocks for the industrial production of bioeconomy products because they are currently available at significant quantities in all EU countries, while their current management involves either low value practices (e.g. composting) or environmentally unfavorable practices (e.g. landfilling).

Melitos et al. (2025) reported that the MSP of SA production using an engineered *E. coli* strain cultivated on crude glycerol (\$3.81/kg_{SA}) was 20.5% lower than the respective MSP estimated when the same strain was cultivated on pure glycerol (\$4.79/kg_{SA}). Crude glycerol is currently produced in EU countries mainly from biodiesel production plants and therefore its future availability depends on the biodiesel market. Furthermore, the crude glycerol production capacity is limited in order to sustain economies

of scale for the production of fermentation products in all EU countries.

The global warming potential (GWP) (1.94 kg CO₂-eq/kg_{SA}) and the abiotic depletion fossil (ADP) (59.2 MJ/kg SA) for petro-based SA production using maleic anhydride (Cok et al. 2014) can be reduced if it is substituted by bio-based SA production (Figure 5). The environmental performance of SA production is mainly affected by utilities (e.g. steam, electricity), bases, acids and chemical requirements. The exploitation of renewable electricity-driven technologies at different bioprocessing stages may reduce the overall environmental impact. Cok et al. (2014) reported low GWP (0.88 kg CO₂-eq/kg_{SA}) and ADP (32.7 MJ/kg_{SA}) values for an SA production bioprocess using corn-derived glucose via low-pH yeast fermentation with direct crystallization-based SA purification, considering a European electricity production mix. When an EMB system is employed for SA production, low GWP (0.81 kg CO₂-eq/kg_{SA}) and ADP (15.73 MJ/kg_{SA}) values can be achieved (Figure 5) when only renewable electricity (photovoltaics) is employed (Ioannidou et al. 2023b). Moussa et al. (2016) presented the environmental performance of SA production from sorghum using the process developed by Myriant Corporation that is based on *E. coli* cultures using NH₃ as a neutralising agent followed by salt separation with continuous ion exchange columns, evaporation and crystallisation. Ammonium phosphate is produced as a co-product fertilizer. The GWP (0.87 kg CO₂-eq/kg_{SA}) and the non-ren Fossil CED (6.89 MJ/kg_{SA}) values are among the lowest reported in the literature (Moussa et al. 2016). This is mainly attributed to the negative values of GWP and non-ren Fossil CED of ammonium sulphate production as co-product, considering a system

expansion approach to avoid the production of petroleum-derived ammonium sulphate. This study highlights the importance of biorefinery development towards improved environmental sustainability.

Morales et al. (2016) presented a multicriteria sustainability assessment of SA production from sugar beet and lignocellulosic biomass based on *in-silico* SA production by engineered *E. coli* strains, considering thermodynamic-based flux analysis. Six alternative bio-based SA production routes were assessed using different combinations of fermentation conditions (i.e. acidic, neutral and high sugar concentration) and DSP technologies (i.e. reactive extraction, electrodialysis and ion exchange). SA production by low pH fermentation coupled with reactive extraction for SA purification led to the lowest environmental impact, while fermentations carried out at high sugar concentrations coupled with reactive extraction led to cost-competitive SA production. However, process hazards issues should be addressed for both processes in order to ensure process safety with minimal environmental loads and worker exposure.

The development of a biorefinery within a conventional sugarcane bioethanol facility in Brazil for the simultaneous production of bioethanol from sucrose and SA from hemicellulose hydrolysates derived from sugarcane bagasse using the bacterial strain *A. succinogenes* resulted in a production cost of \$2.32/kg_{SA} (Klein et al. 2017). The sugarcane bagasse-based bioprocess included pretreatment with dilute acid, detoxification and fermentation of C5 hydrolysates, followed by a DSP process based on acidification with H₂SO₄, evaporation, adsorption using a zeolite bed, evaporation of SA solution in a multiple-effect evaporator, crystallization and drying of the crystals.

Challenges and prospects

SA is recognized as a valuable platform chemical due to its diverse industrial applications. However, sustainability concerns highlight the need to shift away from processes that rely on commercial sugars toward those utilizing crude renewable feedstocks. To address this challenge, a broader range of renewable resources must be evaluated for their potential in SA production using engineered yeast strains. This shift necessitates the genetic modification of yeast to enable the assimilation of multiple carbon sources. For example, an alginate catabolic pathway has been introduced into *E. coli* and *S. cerevisiae*, enabling these organisms to utilize alginate and mannitol derived from macroalgae for ethanol production via fermentation (Wargacki et al. 2012, Enquist-Newman et al. 2014).

Integrating bio-based SA production within biorefineries and incorporating electricity-driven technologies can significantly enhance process profitability and reduce environmental impacts. For instance, conventional pretreatment of lignocellulosic biomass such as liquid hot water, alkaline treatment, dilute acid, and steam explosion, can account for up to 40% of the total bioprocess cost (Kumar et al. 2020b, Haldar and Purkait 2021). These technologies are also associated with high energy demands and environmental concerns. In contrast, deep eutectic solvents (DESs) offer promising advantages, including lower cost, non-toxicity, and biodegradability Filippi et al. (2023). Additionally, non-thermal plasma technologies provide a sustainable alternative for biomass pretreatment, operating under ambient temperature and air pressure conditions (Vanneste et al. 2017). Argeiti et al. (2024) demonstrated the effectiveness of a non-thermal air plasma bubble reactor for pretreating brewers spent grains, enabling efficient

utilization of all available sugars (e.g. dextrans, cellulose, hemicellulose) in this by-product. Consequently, developing sustainable pretreatment strategies is crucial for extracting fermentable sugars from crude renewable resources and enhancing the overall sustainability of bio-based SA production.

Traditional SA production processes predominantly rely on batch or fed-batch microbial cultures, which, despite their effectiveness, result in elevated production costs. In contrast, continuous bioprocesses offer several advantages, including extended operation duration, reduced machine downtime (e.g. fewer cleaning and sterilization cycles), and increased volumetric productivity over the total cycle time due to minimized turnaround. These features contribute to lowering the overall cost of production. Furthermore, continuous operations facilitate steady-state conditions, resulting in consistent product quality and enabling seamless integration between upstream and downstream processing (Outram et al. 2025, Schroedter et al. 2025).

In-situ production and extraction strategies are particularly beneficial in continuous systems. For chemicals such as SA, such integration minimizes the accumulation of inhibitory by-products in the culture broth, facilitates pH regulation, improve microbial metabolic efficiency through the supply of reductive energy into the metabolic pathways, and reduces both environmental impact and production costs (Stylianou et al. 2023).

Regarding strain development, advanced genetic engineering tools can support the construction of robust yeast strains capable of producing SA at higher yields and productivities while simultaneously utilizing multiple carbon sources, including hexoses and pentoses, without triggering carbon catabolite repression. Ultimately, a major hurdle to the commercial viability of bio-based products remains the effective scale-up of laboratory development to industrial production. Future work should focus on developing scalable process engineering solutions that meet industrial standards and economic benchmarks.

Conclusion

Yeasts represent a promising and versatile platform for the sustainable production of SA. Their tolerance to low-pH environments and ease of genetic modification make them attractive candidates compared to bacterial hosts. Moreover, recent advances in metabolic engineering and synthetic biology have enhanced the ability of various yeast strains to efficiently convert renewable feedstocks into SA and improve its final titer and/or yield. These traits, combined with their GRAS status, position yeasts as a valuable alternative for developing economically viable and environmentally friendly bioprocesses for SA production.

Author contributions

Vasiliki Korka: Conceptualization, Data curation, Writing original draft, Visualization

Apostolos Petropoulos: Data curation, Writing original draft

Sofia-Maria Ioannidou: Data curation, Writing original draft

Carol Sze Ki Lin: Review & editing

Apostolis Koutinas: Conceptualization, Review & editing

Patrick Fickers: Conceptualization, Writing original draft, Validation, Review & editing

Conflict of interest: The authors have declared no competing interests (financial or nonfinancial) that are relevant to the content.

Funding

Vasiliki Korka was supported by the Onassis Foundation Scholarship [Scholarship ID: F ZU 037-1/2024-2025].

References

- Agren R, Otero JM, Nielsen J. Genome-scale modeling enables metabolic engineering of *Saccharomyces cerevisiae* for succinic acid production. *J Ind Microbiol Biotechnol* 2013;**40**:735–47. <https://doi.org/10.1007/s10295-013-1269-3>.
- Alexandri M, Vlysidis A, Papapostolou H et al. Downstream separation and purification of succinic acid from fermentation broths using spent sulphite liquor as feedstock. *Sep Purif Technol* 2019;**209**:666–75. <https://doi.org/10.1016/j.seppur.2018.08.061>.
- Argeiti C, Psaki O, Filippi K et al. Biorefinery electrification of brewers' spent grains using plasma bubbles for sustainable production of poly(3-hydroxybutyrate). *Chem Eng J* 2024;**496**:153548. <https://doi.org/10.1016/j.cej.2024.153548>.
- Babaei M, Rueksomtawin Kildegaard K, Niaei A et al. Engineering oleaginous yeast as the host for fermentative succinic Acid production from glucose. *Front Bioeng Biotechnol* 2019;**7**:361. <https://doi.org/10.3389/fbioe.2019.00361>.
- Bandini F, Taskin E, Bellotti G et al. The treatment of the organic fraction of municipal solid waste (OFMSW) as a possible source of micro- and nano-plastics and bioplastics in agroecosystems: a review. *Chem Biol Technol Agric* 2022;**9**:4. <https://doi.org/10.1186/s40538-021-00269-w>.
- Barletta M, Aversa C, Ayyoob M et al. Poly(butylene succinate) (PBS): materials, processing, and industrial applications. *Prog Polym Sci* 2022;**132**:101579. <https://doi.org/10.1016/j.progpolymsci.2022.101579>.
- Birney M, Um H-D, Klein C. Novel mechanisms of *Escherichia coli* succinyl-coenzyme A synthetase regulation. *J Bacteriol* 1996;**178**:2883–9. <https://doi.org/10.1128/jb.178.10.2883-2889.1996>.
- Camia A, Robert N, Jonsson R et al. Biomass production, supply, uses and flows in the European Union. First results from an integrated assessment. EUR 28993 EN, Luxembourg: Publications Office of the European Union, 2018, JRC109869. ISBN 978-92-79-77237-5, <https://doi.org/10.2760/539520>.
- Chen C, Ding S, Wang D et al. Simultaneous saccharification and fermentation of cassava to succinic acid by *Escherichia coli* NZN111. *Bioresour Technol* 2014;**163**:100–5. <https://doi.org/10.1016/j.biortech.2014.04.020>.
- Choi S, Song CW, Shin JH et al. Biorefineries for the production of top building block chemicals and their derivatives. *Metab Eng* 2015;**28**:223–39. <https://doi.org/10.1016/j.ymben.2014.12.007>.
- CredenceResearch. Succinic Acid Price Analysis 2024: Charts, Price, Trends, News, Forecast and Historical Data Read. 2024. <https://www.credenceresearch.com/alchemica-insights/succinic-acid-prices?utm> (May 2025, date last accessed).
- Cok B, Tsiropoulos I, Roes AL et al. Succinic acid production derived from carbohydrates: an energy and greenhouse gas assessment of a platform chemical toward a bio-based economy. *Biofuels, Bioprod Bioref* 2014;**8**:16–29. <https://doi.org/10.1002/bbb.1427>.
- Cui Z, Gao C, Li J et al. Engineering of unconventional yeast *Yarrowia lipolytica* for efficient succinic acid production from glycerol at low pH. *Metab Eng* 2017;**42**:126–33. <https://doi.org/10.1016/j.ymben.2017.06.007>.
- Cui Z, Zhong Y, Sun Z et al. Reconfiguration of the reductive TCA cycle enables high-level succinic acid production by *Yarrowia lipolytica*. *Nat Commun* 2023;**14**:8480. <https://doi.org/10.1038/s41467-023-4245-4>.
- Dai Z, Guo F, Zhang S et al. Bio-based succinic acid: an overview of strain development, substrate utilization, and downstream purification. *Biofuels, Bioprod Bioref* 2020;**14**:965–85. <https://doi.org/10.1002/bbb.2063>.
- Datta R, Glassner DA, Jain MK et al. Fermentation and purification process for succinic acid. *US Patent* 1992;**5**,168,055.
- Dickson R, Mancini E, Garg N et al. Sustainable bio-succinic acid production: superstructure optimization, techno-economic, and lifecycle assessment. *Energy Environ Sci* 2021;**14**:3542–58. <https://doi.org/10.1039/D0EE03545A>.
- Eftymiou M-N, Pateraki C, Papapostolou H et al. Restructuring the sunflower-based biodiesel industry into a circular bio-economy business model converting sunflower meal and crude glycerol into succinic acid and value-added co-products. *Biomass Bioenergy* 2021;**155**:106265. <https://doi.org/10.1016/j.biombioe.2021.106265>.
- Enquist-Newman M, Faust AME, Bravo DD et al. Efficient ethanol production from brown macroalgae sugars by a synthetic yeast platform. *Nature* 2014;**505**:239–43. <https://doi.org/10.1038/nature12771>.
- Eurostat. 2022. Eurostat Report 2022. <https://ec.europa.eu/eurostat/web/products-eurostat-news/w/ddn-20240927-2>. (May 2025, date last accessed).
- E4tech, RE-CORD, WUR. From the Sugar Platform to biofuels and biochemicals. 2015. Contract No. ENER/C2/423-2012/SI2.673791.
- Fickers P, Benetti P, Wache Y et al. Hydrophobic substrate utilisation by the yeast *Yarrowia lipolytica*, and its potential applications. *FEMS Yeast Res* 2005;**5**:527–43. <https://doi.org/10.1016/j.femsyr.2004.09.004>.
- Fickers P, Cheng H, Sze K et al. Sugar alcohols and organic acids synthesis in *Yarrowia lipolytica*: where are we? *Microorganisms* 2020;**8**:574. <https://doi.org/10.3390/microorganisms8040574>.
- Filippi K, Papapostolou H, Alexandri M et al. Integrated biorefinery development using winery waste streams for the production of bacterial cellulose, succinic acid and value-added fractions. *Bioresour Technol* 2022;**343**:125989. <https://doi.org/10.1016/j.biortech.2021.125989>.
- Filippi K, Stylianou E, Pateraki C et al. Pretreatment of grape pomaces and stalks using deep eutectic solvents for succinic acid production integrated in a biorefinery concept. *Waste Biomass Valor* 2023;**14**:2857–72. <https://doi.org/10.1007/s12649-023-02047-1>.
- Flach B, Lieberz S, Bolla S. US Department of Agriculture (USDA) Foreign Agricultural Service. EU Biofuels Annual 2019, GAIN Report Number NL9022. Washington, DC, USA: Global Agricultural Information Network (GAIN) Report, 2019.
- Franco-Duarte R, Bessa D, Gonçalves F et al. Genomic and transcriptomic analysis of *Saccharomyces cerevisiae* isolates with focus in succinic acid production. *FEMS Yeast Res* 2017;**17**:6. <https://doi.org/10.1093/femsyr/fox057>.
- Fu L, Gao X, Yang Y et al. Preparation of succinic acid using bipolar membrane electrodialysis. *Sep Purif Technol* 2014;**127**:212–8. <https://doi.org/10.1016/j.seppur.2014.02.028>.
- Gao C, Yang X, Wang H et al. Robust succinic acid production from crude glycerol using engineered *Yarrowia lipolytica*. *Biotechnol Biofuels* 2016;**9**:179. <https://doi.org/10.1186/s13068-016-0597-8>.
- Gausmann M, Kocks C, Doeker M et al. Recovery of succinic acid by integrated multi-phase electrochemical pH-shift extraction and crystallization. *Sep Purif Technol* 2020;**240**:116489. <https://doi.org/10.1016/j.seppur.2019.116489>.
- Ge M, Sha Y, Lu M et al. Construction and optimization of efficient glucose-xylose co-fermenting yeast *Yarrowia lipolytica* for green

- and sustainable succinic acid production from lignocellulosic biomass. *Green Chem* 2025;**27**:450–63. <https://doi.org/10.1039/D4GC04189E>.
- Glassner DA, Datta R. Process for the production and purification of succinic acid. US Patent 1992;**5**,834.
- Gyan K, Afedzi AEK, Tanypramphan P et al. A review of the advances in detoxification strategies of lignocellulosic hydrolysate for bio-based succinic acid production. *Biocatal Agric Biotechnol* 2024;**60**:103323. <https://doi.org/10.1016/j.bcab.2024.103323>.
- Haldar D, Purkait MK. A review on the environment-friendly emerging techniques for pretreatment of lignocellulosic biomass: mechanistic insight and advancements. *Chemosphere* 2021;**264**:128523. <https://doi.org/10.1016/j.chemosphere.2020.128523>.
- Hao H-X, Khalimonchuk O, Schraders M et al. SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. *Science* 2009;**325**:5944. <https://doi.org/10.1126/science.1175689>.
- Hu Y, Du C, Pensupa N et al. Optimisation of fungal cellulase production from textile waste using experimental design. *Process Saf Environ Prot* 2018;**118**:133–42. <https://doi.org/10.1016/j.psep.2018.06.009>.
- Huang M, Cheng J, Chen P et al. Efficient production of succinic acid in engineered *Escherichia coli* strains controlled by anaerobically-induced nirB promoter using sweet potato waste hydrolysate. *J Environ Manage* 2019;**237**:147–54. <https://doi.org/10.1016/j.jenvman.2019.02.041>.
- Hugler M, Wirsen CO, Fuchs G et al. Evidence for autotrophic CO₂ fixation via the reductive tricarboxylic acid cycle by members of the ϵ subdivision of Proteobacteria. *J Bacteriol* 2005;**187**:3020–7. <https://doi.org/10.1128/JB.187.9.3020-3027.2005>.
- Huh YS, Jun Y-S, Hong YK et al. Effective purification of succinic acid from fermentation broth produced by *Mannheimia succiniciproducens*. *Process Biochem* 2006;**41**:1461–5. <https://doi.org/10.1016/j.procbio.2006.01.020>.
- Imarc. Succinic acid prices, trend, chart, demand, market analysis, news, historical and forecast data report, 2025 Edition. Report ID: SR112025A22649. 2024. <https://www.imarcgroup.com/succinic-acid-pricing-report>.
- Ioannidou SM, Filippi K, Kookos IK et al. Techno-economic evaluation and life cycle assessment of a biorefinery using winery waste streams for the production of succinic acid and value-added co-products. *Bioresour Technol* 2022;**348**:126295. <https://doi.org/10.1016/j.biortech.2021.126295>.
- Ioannidou S-M, López-Gómez JP, Venus J et al. Techno-economic evaluation and life cycle assessment for sustainable alternative biorefinery concepts using the organic fraction of municipal solid waste. *Green Chem* 2023a;**25**:4482–500. <https://doi.org/10.1039/D3GC00244F>.
- Ioannidou SM, Stylianou E, Pateraki C et al. Techno-economic and environmental sustainability assessment of succinic acid production from municipal biowaste using an electrochemical membrane bioreactor. *Chem Eng J* 2023b;**473**:145070. <https://doi.org/10.1016/j.cej.2023.145070>.
- Ioannidou SM, Pateraki C, Ladakis D et al. Sustainable production of bio-based chemicals and polymers via integrated biomass refining and bioprocessing in a circular bioeconomy context. *Bioresour Technol* 2020;**307**:150594. <https://doi.org/10.1016/j.biortech.2020.123093>.
- Jansen ML, Van Gulik WM. Towards large scale fermentative production of succinic acid. *Curr Opin Biotechnol* 2014;**30**:190–7. <https://doi.org/10.1016/j.copbio.2014.07.003>.
- Jiang M, Ma J, Wu M et al. Progress of succinic acid production from renewable resources: metabolic and fermentative strategies. *Bioreour Technol* 2017;**245**:1710–7. <https://doi.org/10.1016/j.biortech.2017.05.209>.
- Jiang Z, Cui Z, Zhu Z et al. Engineering of *Yarrowia lipolytica* transporters for high-efficient production of biobased succinic acid from glucose. *Biotechnol Biofuels* 2021;**14**:145. <https://doi.org/10.1186/s13068-021-01996-w>.
- Jones RJ, Massanet-Nicolau J, Guwy AJ. A review of carboxylate production and recovery from organic wastes. *Bioresour Technol* 2021;**16**:100826. <https://doi.org/10.1016/j.biteb.2021.100826>.
- Jost B, Holz M, Aurich A et al. The influence of oxygen limitation for the production of succinic acid with recombinant strains of *Yarrowia lipolytica*. *Appl Microbiol Biotechnol* 2015;**99**:1675–86. <https://doi.org/10.1007/s00253-014-6252-z>.
- Jun Y-S, Huh YS, Park HS et al. Adsorption of pyruvic and succinic acid by amine-functionalized SBA-15 for the purification of succinic acid from fermentation broth. *J Phys Chem C* 2007;**111**:13076–86. <https://doi.org/10.1021/jp072606g>.
- Kamzolova SV, Vinokurova NG, Dedyukhina EG et al. The peculiarities of succinic acid production from rapeseed oil by *Yarrowia lipolytica* yeast. *Appl Microbiol Biotechnol* 2014;**98**:4149–57. <https://doi.org/10.1007/s00253-014-5585-y>.
- Khunnonkwao P, Jantama K, Kanchanatawee S et al. A two steps membrane process for the recovery of succinic acid from fermentation broth. *Sep Purif Technol* 2018;**207**:451–60. <https://doi.org/10.1016/j.seppur.2018.06.056>.
- Klein BC, Silva JFL, Junqueira TL et al. Process development and techno-economic analysis of bio-based succinic acid derived from pentoses integrated to a sugarcane biorefinery. *Biofuels Bioprod Bioref* 2017;**11**:1051–64. <https://doi.org/10.1002/bbb.1813>.
- Klein M, Carrillo M, Xiberras J et al. Towards the exploitation of glycerol's high reducing power in *Saccharomyces cerevisiae*-based bioprocesses. *Metab Eng* 2016;**38**:464–72. <https://doi.org/10.1016/j.jymben.2016.10.008>.
- Koutinas AA, Vlysidis A, Pleissner D et al. Valorization of industrial waste and by-product streams via fermentation for the production of chemicals and biopolymers. *Chem Soc Rev* 2014;**43**:2587–627. <https://doi.org/10.1039/c3cs60293a>.
- Kumar B, Bhardwaj N, Agrawal K et al. Current perspective on pre-treatment technologies using lignocellulosic biomass: an emerging biorefinery concept. *Fuel Process Technol* 2020a;**199**:106244. <https://doi.org/10.1016/j.fuproc.2019.106244>.
- Kumar R, Basak B, Jeon B-H. Sustainable production and purification of succinic acid: a review of membrane-integrated green approach. *J Cleaner Prod* 2020b;**277**:123954. <https://doi.org/10.1016/j.jclepro.2020.123954>.
- Kumar V, Kumar P, Maity SK et al. Recent advances in bio-based production of top platform chemical, succinic acid: an alternative to conventional chemistry. *Biotechnol Biofuels* 2024;**17**:72. <https://doi.org/10.1186/s13068-024-02508-2>.
- Kurzrock T, Weuster-Botz D. Recovery of succinic acid from fermentation broth. *Biotechnol Lett* 2010;**32**:331–9. <https://doi.org/10.1007/s10529-009-0163-6>.
- Kurzrock T, Weuster-Botz D. New reactive extraction systems for separation of bio-succinic acid. *Bioprocess Biosyst Eng* 2011;**34**:779–87. <https://doi.org/10.1007/s00449-011-0526-y>.
- Ladakis D, Stylianou E, Ioannidou S-M et al. Biorefinery development, techno-economic evaluation and environmental impact analysis for the conversion of the organic fraction of municipal solid waste into succinic acid and value-added fractions. *Bioresour Technol* 2022;**354**:127172. <https://doi.org/10.1016/j.biortech.2022.127172>.

- Lee EZ, Huh YS, Jun Y-S et al. Effect of operating variables on back-extraction characteristics of succinic acid from organic phase. *Biotechnol Bioproc E* 2008;**13**:342–6. <https://doi.org/10.1007/s12257-007-0184-8>.
- Lee SC, Kim HC. Batch and continuous separation of acetic acid from succinic acid in a feed solution with high concentrations of carboxylic acids by emulsion liquid membranes. *J Membr Sci* 2011;**367**:190–6. <https://doi.org/10.1016/j.memsci.2010.10.057>.
- Li C, Gao S, Li X et al. Efficient metabolic evolution of engineered *Yarrowia lipolytica* for succinic acid production using a glucose-based medium in an in situ fibrous bioreactor under low-pH condition. *Biotechnol Biofuels* 2018a;**11**:236. <https://doi.org/10.1186/s13068-018-1233-6>.
- Li C, Gao S, Yang X et al. Green and sustainable succinic acid production from crude glycerol by engineered *Yarrowia lipolytica* via agricultural residue based in situ fibrous bed bioreactor. *Bioreour Technol* 2018b;**249**:612–9. <https://doi.org/10.1016/j.biortech.2017.10.011>.
- Li C, Ong KL, Cui Z et al. Promising advancement in fermentative succinic acid production by yeast hosts. *J Hazard Mater* 2021;**401**:123414. <https://doi.org/10.1016/j.jhazmat.2020.123414>.
- Li C, Ong KL, Yang X et al. Bio-refinery of waste streams for green and efficient succinic acid production by engineered *Yarrowia lipolytica* without pH control. *Chem Eng J* 2019a;**371**:804–12. <https://doi.org/10.1016/j.cej.2019.04.092>.
- Li C, Yang X, Gao S et al. Hydrolysis of fruit and vegetable waste for efficient succinic acid production with engineered *Yarrowia lipolytica*. *J Cleaner Prod* 2018d;**179**:151–9. <https://doi.org/10.1016/j.jclepro.2018.01.081>.
- Li C, Huang B, He Q et al. Production of succinate from simply purified crude glycerol by engineered *Escherichia coli* using two-stage fermentation. *Bioresour Bioprocess* 2018c;**5**:41. <https://doi.org/10.1186/s40643-018-0227-3>.
- Li Q, Wang D, Wu Y et al. One step recovery of succinic acid from fermentation broths by crystallization. *Sep Purif Technol* 2010;**72**:294–300. <https://doi.org/10.1016/j.seppur.2010.02.021>.
- Li X, Mupondwa E. Empirical analysis of large-scale bio-succinic acid commercialization from a technoeconomic and innovation value chain perspective: bioAmber biorefinery case study in Canada. *Renew Sustain Energy Rev* 2021;**137**:110587. <https://doi.org/10.1016/j.rser.2020.110587>.
- Li X, Zhang M, Luo J et al. Efficient succinic acid production using a biochar-treated textile waste hydrolysate in an in situ fibrous bed bioreactor. *Biochem Eng J* 2019b;**149**:107249. <https://doi.org/10.1016/j.bej.2019.107249>.
- Li Y, Li M, Zhang X et al. A novel whole-phase succinate fermentation strategy with high volumetric productivity in engineered *Escherichia coli*. *Bioresour Technol* 2013;**149**:333–40. <https://doi.org/10.1016/j.biortech.2013.09.077>.
- Lin F, Li W, Wang D et al. Advances in succinic acid production: the enhancement of CO₂ fixation for the carbon sequestration benefits. *Front Bioeng Biotechnol* 2024;**12**:1392414. <https://doi.org/10.3389/fbioe.2024.1392414>.
- Lin SKC, Du C, Blaga AC et al. Novel resin-based vacuum distillation-crystallisation method for recovery of succinic acid crystals from fermentation broths. *Green Chem* 2010;**12**:666–71. <https://doi.org/10.1039/b913021g>.
- Liu H-H, Ji X-J, Huang H. Biotechnological applications of *Yarrowia lipolytica*: past, present and future. *Biotechnol Adv* 2015;**33**:1522–46. <https://doi.org/10.1016/j.biotechadv.2015.07.010>.
- Luque R, Lin CSK, Du C et al. Chemical transformations of succinic acid recovered from fermentation broths by a novel direct vacuum distillation-crystallisation method. *Green Chem* 2009;**11**:193–200. <https://doi.org/10.1039/B813409J>.
- Ma J, Gu Y, Marsafari M et al. Synthetic biology, systems biology, and metabolic engineering of *Yarrowia lipolytica* toward a sustainable biorefinery platform. *J Ind Microbiol Biotechnol* 2020;**47**:845–62. <https://doi.org/10.1007/s10295-020-02290-8>.
- Malubhoy Z, Bahia FM, de Valk SC et al. Carbon dioxide fixation via production of succinic acid from glycerol in engineered *Saccharomyces cerevisiae*. *Microb Cell Fact* 2022;**21**:102. <https://doi.org/10.1186/s12934-022-01817-1>.
- Mamaev D, Zvyagilskaya R. *Yarrowia lipolytica* : a multitolerant yeast species of ecological significance. *FEMS Yeast Res* 2021;**21**:2. <https://doi.org/10.1093/femsyr/foab008>.
- Mancini E, Dickson R, Fabbri S et al. Economic and environmental analysis of bio-succinic acid production: from established processes to a new continuous fermentation approach with in-situ electrolytic extraction. *Chem Eng Res Des* 2022;**179**:401–14. <https://doi.org/10.1016/j.cherd.2022.01.040>.
- Mancini E, Mansouri SS, Gernaey KV et al. From second generation feed-stocks to innovative fermentation and downstream techniques for succinic acid production. *Crit Rev Environ Sci Technol* 2020;**50**:1829–73. <https://doi.org/10.1080/10643389.2019.1670530>.
- Melitos G, Misailidis N, Petrides D et al. Process synthesis, design and techno-economic assessment of bio-succinic acid production. *Food Bioprod Process* 2025;**149**:224–37. <https://doi.org/10.1016/j.fbp.2024.11.020>.
- Mitrea L, Teleky B-E, Nemes S-A et al. Succinic acid—A run-through of the latest perspectives of production from renewable biomass. *Heliyon* 2024;**10**:e25551. <https://doi.org/10.1016/j.heliyon.2024.e25551>.
- Morales M, Ataman M, Badr S et al. Sustainability assessment of succinic acid production technologies from biomass using metabolic engineering. *Energy Environ Sci* 2016;**9**:2794–805. <https://doi.org/10.1039/C6EE00634E>.
- Moussa HI, Elkamel A, Young SB. Assessing energy performance of bio-based succinic acid production using LCA. *J Cleaner Prod* 2016;**139**:761–9. <https://doi.org/10.1016/j.jclepro.2016.08.104>.
- Narisetty V, Okibe MC, Amulya K et al. Technological advancements in valorization of second generation (2G) feedstocks for bio-based succinic acid production. *Bioresour Technol* 2022a;**360**:c127513. <https://doi.org/10.1016/j.biortech.2022.127513>.
- Narisetty V, Prabhu AA, Bommarreddy RR et al. Development of hypertolerant strain of *Yarrowia lipolytica* accumulating succinic acid using high levels of acetate. *ACS Sustain Chem Eng* 2022b;**10**:10858–69. <https://doi.org/10.1021/acssuschemeng.2c02408>.
- Nicaud J-M. *Yarrowia lipolytica*. *Yeast* 2012;**29**:409–18. <https://doi.org/10.1002/yea.2921>.
- Ong KL, Fickers P, Lin CSK. Enhancing succinic acid productivity in the yeast *Yarrowia lipolytica* with improved glycerol uptake rate. *Sci Total Environ* 2020;**702**:134911. <https://doi.org/10.1016/j.scitotenv.2019.134911>.
- Ong KL, Li C, Li X et al. Co-fermentation of glucose and xylose from sugarcane bagasse into succinic acid by *Yarrowia lipolytica*. *Biochem Eng J* 2019;**148**:108–15. <https://doi.org/10.1016/j.bej.2019.05.004>.
- Othman N, Jusoh N, Mohar MS et al. Extraction of succinic acid from real fermentation broth by using emulsion liquid membrane process. *Malaysian J Anal Sci* 2018;**22**:1090–101. <https://doi.org/10.17576/mjas-2018-2206-20>.
- Outram V, Yiakoumetti A, Green C et al. Enhancing the stability of continuous fermentations for platform chemical

- production. *iScience* 2025;**28**:111786. <https://doi.org/10.1016/j.isci.2025.111786>.
- Pateraki C, Andersen SJ, Ladakis D et al. Direct electrochemical extraction increases microbial succinic acid production from spent sulphite liquor. *Green Chem* 2019;**21**:2401–11. <https://doi.org/10.1039/C9GC00361D>.
- Patsalou M, Chrysargyris A, Tzortzakis N et al. A biorefinery for conversion of citrus peel waste into essential oils, pectin, fertilizer and succinic acid via different fermentation strategies. *Waste Manage (Oxford)* 2020;**113**:469–77. <https://doi.org/10.1016/j.wasman.2020.06.020>.
- Porcelli AM, Ghelli A, Zanna C et al. pH difference across the outer mitochondrial membrane measured with a green fluorescent protein mutant. *Biochem Biophys Res Commun* 2005;**326**:799–804. <https://doi.org/10.1016/j.bbrc.2004.11.105>.
- Prabhu AA, Ledesma-Amaro R, Lin CSK et al. Bioproduction of succinic acid from xylose by engineered *Yarrowia lipolytica* without pH control. *Biotechnol Biofuels* 2020;**13**:113. <https://doi.org/10.1186/s13068-020-01747-3>.
- Prochaska K, Antczak J, Regel-Rosocka M et al. Removal of succinic acid from fermentation broth by multistage process (membrane separation and reactive extraction). *Sep Purif Technol* 2018;**192**:360–8. <https://doi.org/10.1016/j.seppur.2017.10.043>.
- Raab AM, Lang C. Oxidative versus reductive succinic acid production in the yeast *Saccharomyces cerevisiae*. *Bioengineered Bugs* 2011;**2**:120–3. <https://doi.org/10.4161/bbug.2.2.14549>.
- Sadare OO, Ejekwu O, Moshokoa MF et al. Membrane purification techniques for recovery of succinic acid obtained from fermentation broth during bioconversion of lignocellulosic biomass: current advances and future perspectives. *Sustainability* 2021;**13**:6794. <https://doi.org/10.3390/su13126794>.
- Saxena RK, Saran S, Isar J et al. Production and applications of succinic acid. *Current developments in biotechnology and bioengineering*, Elsevier, 2017, 601–30. <https://doi.org/10.1016/B978-0-444-63662-1.00027-0>.
- Schroedter L, Schneider R, Venus J. Transforming waste wood into pure L-(+)-lactic acid: efficient use of mixed sugar media through cell-recycled continuous fermentation. *Bioresour Technol* 2025;**419**:132010. <https://doi.org/10.1016/j.biortech.2024.132010>.
- Straathof AJJ. The proportion of downstream costs in fermentative production processes. In: Moo-Young M. (ed.), *Comprehensive Biotechnology*, Second Edition. Elsevier, 2011, 811–4.
- Song H, Dotzauer E, Thorin E et al. Techno-economic analysis of a straw-based biorefinery system for power, heat, pellet and bioethanol production. *Int J Energy Res* 2014;**38**:551–63. <https://doi.org/10.1002/er.3039>.
- Song H, Huh YS, Lee SY et al. Recovery of succinic acid produced by fermentation of a metabolically engineered *Mannheimia succiniciproducens* strain. *J Biotechnol* 2007;**132**:445–52. <https://doi.org/10.1016/j.jbiotec.2007.07.496>.
- Spekreijse J, Vikla K, Vis M et al. Bio-based value chains for chemicals, plastics and pharmaceuticals: a comparison of bio based and fossil based value chains. Luxembourg: Publications Office of the European Union, 2021.
- Stylianou E, Carvajal-Arroyo JM, Ladakis D et al. Development of an electrochemical membrane bioreactor for succinic acid production and in situ separation with engineered *Yarrowia lipolytica* cultivated on municipal biowaste hydrolysate. *Chem Eng J* 2023;**466**:142877. <https://doi.org/10.1016/j.cej.2023.142877>.
- Stylianou E, Pateraki C, Ladakis D et al. Bioprocess development using organic biowaste and sustainability assessment of succinic acid production with engineered *Yarrowia lipolytica* strain. *Biochem Eng J* 2021;**174**:108099. <https://doi.org/10.1016/j.bej.2021.108099>.
- Sun M-L, Fang D, Zeng D et al. Leveraging stress tolerance property to enhance succinic acid production and cell metabolic activity of *Yarrowia lipolytica*. *ACS Sustain Chem Eng* 2025a;**13**:5210–9. <https://doi.org/10.1021/acssuschemeng.4c10404>.
- Sun T, Sun M-L, Lin L et al. Advancing succinic acid biomanufacturing using the nonconventional yeast *Yarrowia lipolytica*. *J Agric Food Chem* 2025b;**73**:100–9. <https://doi.org/10.1021/acs.jafc.4c09990>.
- Tan S-I, Liu Z, Tran VG et al. *Issatchenkia orientalis* as a platform organism for cost-effective production of organic acids. *Metab Eng* 2025;**89**:12–21. <https://doi.org/10.1016/j.ymben.2025.02.003>.
- Thakker C, Martínez I, San K-Y et al. Succinate production in *Escherichia coli*. *Biotechnol J* 2012;**7**:213–24. <https://doi.org/10.1002/biot.201100061>.
- Tran VG, Mishra S, Bhagwat SS et al. An end-to-end pipeline for succinic acid production at an industrially relevant scale using *Issatchenkia orientalis*. *Nat Commun* 2023;**14**:6152. <https://doi.org/10.1038/s41467-023-41616-9>.
- Vanneste J, Ennaert T, Vanhulsel A et al. Unconventional pretreatment of lignocellulose with low-temperature plasma. *ChemSusChem* 2017;**10**:14–31. <https://doi.org/10.1002/cssc.201601381>.
- Vemuri GN, Eiteman MA, Altman E. Succinate production in dual-phase *Escherichia coli* fermentations depends on the time of transition from aerobic to anaerobic conditions. *J Ind Microbiol Biotechnol* 2002;**28**:325–32. <https://doi.org/10.1038/sj.jim.7000250>.
- Wang J, Wang H, Yang L et al. A novel riboregulator switch system of gene expression for enhanced microbial production of succinic acid. *J Ind Microbiol Biotechnol* 2018;**45**:253–69. <https://doi.org/10.1007/s10295-018-2019-3>.
- Wang J, Zhang B, Zhang J et al. Enhanced succinic acid production and magnesium utilization by overexpression of magnesium transporter mgtA in *Escherichia coli* mutant. *Bioresour Technol* 2014;**170**:125–31. <https://doi.org/10.1016/j.biortech.2014.07.081>.
- Wargacki AJ, Leonard E, Win MN et al. An engineered microbial platform for direct biofuel production from brown macroalgae. *Science* 2012;**335**:308–13. <https://doi.org/10.1126/science.1214547>.
- Werpy T, Petersen G. Top value added chemicals from biomass: volume I- results of screening for potential candidates from sugars and synthesis gas. 2004. <https://doi.org/10.2172/15008859>.
- Xiao H, Shao Z, Jiang Y et al. Exploiting *Issatchenkia orientalis* SD108 for succinic acid production. *Microb Cell Fact* 2014;**13**:121. <https://doi.org/10.1186/s12934-014-0121-4>.
- Xiao Y, Zhang Z, Wang Y et al. Two-stage crystallization combining direct succinimide synthesis for the recovery of succinic acid from fermentation broth. *Front Bioeng Biotechnol* 2020;**7**:471. <https://doi.org/10.3389/fbioe.2019.00471>.
- Xiberras J, Klein M, de Hulster E et al. Engineering *Saccharomyces cerevisiae* for succinic acid production from glycerol and carbon dioxide. *Front Bioeng Biotechnol* 2020;**8**:566. <https://doi.org/10.3389/fbioe.2020.00566>.
- Yan D, Wang C, Zhou J et al. Construction of reductive pathway in *Saccharomyces cerevisiae* for effective succinic acid fermentation at low pH value. *Bioresour Technol* 2014;**156**:232–9. <https://doi.org/10.1016/j.biortech.2014.01.053>.
- Yang X, Wang H, Li C et al. Restoring of glucose metabolism of engineered *Yarrowia lipolytica* for succinic acid production via a simple and efficient adaptive evolution strategy. *J Agric Food Chem* 2017;**65**:4133–9. <https://doi.org/10.1021/acs.jafc.7b00519>.
- Yang Y, Zhao J, Zhou Y et al. Progress on production of succinic acid by *Actinobacillus succinogenes*: new opportunities for cheap biomass and waste gas utilization. *J Cleaner Prod* 2024;**434**:140005. <https://doi.org/10.1016/j.jclepro.2023.140005>.
- Yedur S, Berglung KS, Dunuwila DD. Succinic acid production and purification. 2001, US patent 6,265,190.

- Yu Q, Cui Z, Zheng Y et al. Exploring succinic acid production by engineered *Yarrowia lipolytica* strains using glucose at low pH. *Biochem Eng J* 2018;**139**:51–6. <https://doi.org/10.1016/j.bej.2018.08.001>.
- Yuzbashev TV, Bondarenko PYu, Sobolevskaya TI et al. Metabolic evolution and ^{13}C flux analysis of a succinate dehydrogenase deficient strain of *Yarrowia lipolytica*. *Biotech Bioeng* 2016;**113**:2425–32. <https://doi.org/10.1002/bit.26007>.
- Yuzbashev TV, Yuzbasheva EY, Sobolevskaya TI et al. Production of succinic acid at low pH by a recombinant strain of the aerobic yeast *Yarrowia lipolytica*. *Biotech Bioeng* 2010;**107**:673–82. <https://doi.org/10.1002/bit.22859>.
- Zhang S, Guo F, Yang Q et al. Improving methanol assimilation in *Yarrowia lipolytica* via systematic metabolic engineering combined with compartmentalization. *Green Chem* 2023;**25**:183–95. <https://doi.org/10.1039/D2GC02783F>.
- Zheng T, Xu B, Ji Y et al. Microbial fuel cell-assisted utilization of glycerol for succinate production by mutant of *Actinobacillus succinogenes*. *Biotechnol Biofuels* 2021;**14**:23. <https://doi.org/10.1186/s13068-021-01882-5>.
- Zhong Y, Gu J, Shang C et al. Sustainable succinic acid production from lignocellulosic hydrolysates by engineered strains of *Yarrowia lipolytica* at low pH. *Bioresour Technol* 2024b;**408**:131166. <https://doi.org/10.1016/j.biortech.2024.131166>.
- Zhong Y, Shang C, Tao H et al. Boosting succinic acid production of *Yarrowia lipolytica* at low pH through enhancing product tolerance and glucose metabolism. *Microb Cell Fact* 2024a;**23**:291. <https://doi.org/10.1186/s12934-024-02565-0>.
- Zhu F, Wang C, San K-Y et al. Metabolic engineering of *Escherichia coli* to produce succinate from woody hydrolysate under anaerobic conditions. *J Ind Microbiol Biotechnol* 2020;**47**:223–32. <https://doi.org/10.1007/s10295-020-02259-7>.
- Zhu X, Tan Z, Xu H et al. Metabolic evolution of two reducing equivalent-conserving pathways for high-yield succinate production in *Escherichia coli*. *Metab Eng* 2014;**24**:87–96. <https://doi.org/10.1016/j.ymben.2014.05.003>.