

A Review on the Valorization of Lignocellulosic Biomass for Succinic Acid Production: Strengths and Weaknesses

Patrizia Casella^a, Raffaele Loffredo^{a,b}, Maria A. Rao^b, Roberto Balducchi^c, Antonio Molino^{a,*}

^aItalian National Agency for New Technologies, Energy and Sustainable Economic Development – sustainability, biotechnology and agroindustry division, Bioprocess and Bioproducts Laboratory (ENEA-SSPT-BIOAG-PROBIO), Piazz. le Enrico Fermi 1, 80055 Portici (NA), Italy

^bDepartment of Agricultural Sciences, University of Naples, Federico II, Via Università 100, 80055 Portici (NA), Italy

^cItalian National Agency for New Technologies, Energy and Sustainable Economic Development Energy and Sustainable Economic Development – sustainability, biotechnology and agroindustry division, Bioprocess and Bioproducts Laboratory (ENEA-SSPT-BIOAG-PROBIO), Strada Statale Jonica km 419 + 500, 75026 Rotondella (MT), Italy
antonio.molino@enea.it

Bio-based succinic acid production holds great promise for the process sustainability through the use of renewable resources such as lignocellulosic biomass and the use of microorganisms that can utilize CO₂ in fermentation processes. A critical review of the latest findings on succinic acid production from lignocellulosic biomass was discussed, by highlighting all the key aspects of optimization processes at different levels of the process from pre-treatment to succinic acid production. Chemical-physical pretreatments seemed to be the most appropriate for the pretreatment of lignocellulosic biomass, but many strategies are still needed to solve the problem of variability in initial composition, the presence of post-treatment inhibitory compounds and the use of green solvent. In addition, the saccharization-fermentation process seemed to be the most suitable for the fermentative process of hydrolysates obtained from pretreatment to produce bio-based succinic acid. Certainly new studies and strategies are still needed to overcome the weaknesses of the whole process of succinic acid production from lignocellulosic biomass.

1. Introduction

The succinic acid is one of the most valuable bulk chemicals for many applications in food, chemical and pharmaceutical sectors. Nowadays, it is still synthesized chemically through the hydrogenation of petroleum-based maleic acid or maleic anhydride. This route has been no longer sustainable for the use of fossil source and the environmental problems, and for the energy-intensive consumption. So, a sustainable bio-based production is necessary to substitute this highly impacting conventional process.

The Bio-based production of succinic acid can be performed as intermediate in the tricarboxylic acid cycle (TCA) through fermentation process. Natural succinic acid producer, like the bacteria *Actinobacillus succinogenes* and *Mannheimia succiniciproducens*, and genetically engineered strain as *Escherichia coli* and *Saccharomyces cerevisiae* are able to use CO₂ through fermentation for the production of succinic acid by reducing the carbon footprint of the process (Song & Lee, 2006).

Although fermentation processes can be an effective alternative to chemical synthesis processes, efficient strategy are necessary since many difficulties needed to be overcome such as cost-effectiveness, yield and productivity of succinic acid (Gao et al., 2023). The valorization of lignocellulosic biomass can be a strategy to increase the sustainability of bio-based succinic production and to reduce waste production for new green products and materials but new insights are necessary to overcome the major weaknesses.

The process of converting lignocellulosic biomass into succinic acid mainly consisted of several steps: pretreatments, hydrolysis, fermentation, and downstreaming process. The pretreatment and the enzymatic hydrolysis are fundamental preliminary steps for the availability of fermentative sugars for succinic acid producers strain but optimized operative conditions were necessary to convert cellulose and hemicellulose to

glucose and xylose and by reducing potential unwanted compounds (Stefanidis *et al.*, 2014). Also the performance of fermentation needed to be assessed and optimized to enhance succinic acid concentration, productivity, yield and to avoid the production of by-products. Furthermore, the downstreaming process is one of the most critical steps since it contributed about 50 % of the final cost of succinic acid.

In this review the production of succinic acid from different lignocellulosic biomass were analyzed in the most recent works. A critical assessment was carried out on operative conditions and succinic acid production. Furthermore, the main challenge faced in pretreatments and processes have been discussed by highlighting news perspectives.

2. Lignocellulosic biomass

The employment of lignocellulosic biomass to produce fuels and chemicals was gaining interest over the last decades. These biomasses derived principally from residues/waste of various production chains in agro-industrial sectors that were quite abundant with a production of 2×10^{11} tons per year worldwide (Kumar *et al.*, 2020). The most plentiful were rice, wheat, maize, and sugarcane, due to the intensive use in agriculture sector for food and livestock (Vivek *et al.*, 2017).

Since lignocellulosic biomass was potentially rich in fermentable sugars, these could be used as substrates for the biotransformation processes. Although lignocellulosic biomass was considered an ideal feedstock for the production of bio-based succinic acid, some critical issues limited their use. The principal critical issues concerned the variability in the composition of cellulose, hemicellulose and lignin and the consequent optimization of pretreatment processes.

Before the fermentation process, a series of pre-treatments were needed to free cellulose, and hemicellulose, that were bounded in a complex matrix, together with lignin. Furthermore, after the pre-treatments carbohydrates (cellulose and hemicellulose) were broken down into their monomers, such as glucose and xylose, that could be used by microorganisms for the fermentation process.

The harsh conditions of the pre-treatments, like high temperatures, and non-specificity of the process led to the formation of many by-products, such as organic acids, furans and phenolic compounds, identified as potential inhibitors of the growth of microorganism and consequently of the productivity of the fermentative processes.

3. Pretreatments

Lignocellulosic biomasses were a complex matrix in which the compounds contained in greatest quantities were cellulose (40-60% dw), hemicellulose (10-40% dw) and lignin (15-30% dw) (Lu *et al.*, 2021). Based on the different feedstocks, the percentage composition of these three components may varied significantly as it possible to see in Table 1.

Table 1: composition of lignocellulosic biomass from different sources (dw %).

Lignocellulosic biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reference
Corn stalks	50	20	30	Christopher <i>et al.</i> (2017)
Corn stover	38	23	20	Wan & Li (2010)
Sugarcane bagasse	35	36	16	Sasaki <i>et al.</i> (2003)
Rice straw	36	21	24	Imman <i>et al.</i> (2015)
Wheat straw	40	21	23	Khan <i>et al.</i> (2012)
<i>Mischantus</i> straw	42	25	23	Dąbkowska <i>et al.</i> (2019)

It was possible to note that the composition of lignocellulosic biomass strongly depended on the source. The corn stalks had the major percentage of cellulose (50% dw) and lignin (30% dw), but the hemicellulose percentage (20% dw) was the lowest among the analyzed biomasses (Christopher *et al.*, 2017). The highest content of hemicellulose was found in sugarcane bagasse (36% dw) (Sasaki *et al.*, 2003). While, straw (wheat, rice and *Mischantus*) were the biomass that contained the highest percentage of lignin (> 23% dw.).

Due to the complexity of lignocellulosic matrix, pretreatment processes were a crucial first step to make sugars available for the microorganisms involved in fermentation process. The pretreatment altered the lignocellulose structure, removed lignin, and made cellulose more accessible to enzymatic hydrolysis that converted the carbohydrates polymers into fermentable sugars, such as glucose and xylose. This is a multistep step through the synergistic action of cellulolytic and hemicellulolytic enzymes (Yang *et al.*, 2011). There were developed numerous pretreatment techniques, that could be grouped into 3 different categories: physical, chemical, and chemical-physical.

Physical methods included mechanical processes, such as grinding and ultrasound treatment that required high energy and high operational costs. Although another limitation to the use of physical methods was the low yields, Putri et al., 2023 tested peracetic and alkaline peroxide under assisted ultrasound pre-treatment on rice straw and bagasse by obtaining an increase of cellulose content of 107% and 68% respect to initial feedstock.

Mineral acids (such as HCl, H₂SO₄, H₂PO₄, oxalic acid) and bases (calcium and NaOH and aqueous ammonia) and/or organic green solvent (organosolv methods) were mainly used in chemical pretreatments. Jampatesh et al. (2019) compared acid pretreatment on rice straw with hydrochloric, phosphoric, and sulfuric acid at 1M at different hydrolysis times (15, 30, 45, and 60 min) and obtained the best concentration of sugars (10 g/l glucose) and (18 g/L xylose) by using HCl for 60 min. Lo et al. 2020 optimized the acid pretreatment of sweet sorghum bagasse at 50 °C, 1 hour to obtain 29.2 cellulosic glucose after enzymatic hydrolysis. Chemical methods were widely used for the pretreatment of lignocellulosic biomass, the most important strength is the high obtained yield while the disposal of acids and bases can be a considered a weaknesses.

The steam explosion was the most used chemical-physical pretreatment where lignocellulosic biomass is exposed at high temperature (160-260°C) and a pressure (5-50 atm) to a saturated steam for short times (Lee et al., 2003). Kuglarz et al. 2018 tested steam explosion on rapeseed straw by using H₂SO₄ (1% solution) in a batch reactor at 180 °C for 10 min to test several feedstock ratios (10, 15, 20, 25% w/v) and to test several enzymatic dosage (Cellulase activity). The authors obtained the best glucan recovery (98.7%) by using 20% w/v of feedstock and the best glucose concentration equal to 23.3 g/L and xylose concentration equal to 4.5 g/L after the enzymatic hydrolysis (13% dosage).

The steam explosion can be considered economically advantageous and environmental sustainable if compared to the techniques previously mentioned. However, despite these benefits, the harsh conditions (high temperature and pressure) during steam explosion generated several unwanted compounds, such organic acids, furans (furfural and HMF (Hydroxymethylfurfural)), and phenolic compounds that could negatively affect the following enzymatic hydrolysis and the succinic acid production during fermentation process. In fact, these compounds are known as inhibitors for the microbial growth during fermentation process.

In Table 2 there were reported the major inhibitor compounds generated after the steam explosion and critical concentration that can compromise the succinic acid production.

Table 2: Inhibitor compounds found in pre-treated lignocellulosic biomass and their critical concentration.

Class	Compound	Microorganism employed	Critical concentration (g/L)	Reference
Organic acids	Acetic acid	<i>A. succinogenes</i>	33.7	Lin et al. (2008)
	Piruvic acid		59.2	
	Formic acid		10.8	
Furan derivates	Hydroxymethylfurfural	<i>A. succinogenes</i>	10	Dessie et al. (2019)
	Furfural		6	
Phenolic compounds	Vanillin	<i>Corynebacterium glutamicum</i>	0-2	Xu et al. (2015)
	Syringaldehyde		0-2	

Among the inhibitors, the phenolic compounds had a strong effect at low concentration (till to 2 g/l) as observed by Xu et al. (2015) for vanillin and syringaldehyde that totally inhibited *C. glutamicum* activity. These inhibitors can downregulated some genes for the expression of pyruvate carboxylase and phosphofructokinase, that had crucial role in pathways for sugar uptake, CO₂ fixation and reducing power supply (Xu et al., 2022). Organic acids seemed most tolerated at high concentrations till to 59.2 g/l for pyruvic acid and 33.7 g/l for acetic acid (Lin et al. 2008) than furan derivates as furfural and HMF.

4. Succinic acid production starting from different lignocellulosic biomass.

In table 3 some recent works were reported on the production of succinic acid by using lignocellulosic biomass as sugars feedstocks. Vallecilla-Yepez et al (2021) obtained the best SA concentration equal to 27.8 g/L at a yield of 0.61 g/g by using corn stover hydrolysate after liquid hot water and enzymatic hydrolysis treatment. The authors highlighted that the highest SA concentration was reached under the effect of sodium acetate buffer added in control and in corn stover hydrolysate medium.

Table 3: lignocellulosic biomass used for the production of succinic acid through fermentation process. Pretreatments before fermentation and microorganisms employed for fermentation, sugar concentrations after hydrolysis (g/L), succinic acid concentration (g/L) and yield (g/g^{biomass/sugars}) were reported. SA = succinic acid; NR = not reported

Biomass	Pretreatment	Microorganism	Sugars concentration (g/L)	SA concentration (g/L)	SA yield (g/g)	Reference
Oil palm trunk	Carboxylic acid (formic, oxalic, citric acids) and	<i>A. succinogenes</i>	33.6 (total sugars)	2.5	0.13	Bukhari et al. (2020)
Oil palm trunk	Sulphuric acid	<i>A. succinogenes</i>	44.38 (total sugars)	10.6	0.41	Bukhari et al. (2020)
Sweet sorghum bagasse	Phosphoric acid (5–99°C)	<i>A. succinogenes</i>	32.75 (g x 100 g of biomass) (glucose)	17.8	0.56	Lo et al. (2020)
Corn fiber	Liquid hot water	<i>A. succinogenes</i>	NR	27.8	0.61	Vallecilla-Yepez et al. (2021)
Rice straw	Peracetic acid (20ml/g biomass, 3 h 35°C),	<i>Proteus vulgaris</i>	20 (glucose)	3.6	0.18	Putri et al. (2023)
Sugarcane bagasse	alkaline peroxide (20ml/g biomass 10 h 35°C) assisted by ultrasound	<i>Proteus vulgaris</i>	20 (glucose)	5.1	0.25	Putri et al. (2023)
Rice straw	Sodium hydroxide (0.1–2.0 M), 121°C 5 min	<i>Escherichia coli</i>	100 (glucose)	32	0.32	Sawisit et al. (2018)
Rice straw	Acid pretreatment (4 h room temp.) and autoclaving	<i>E. coli</i>	100 (glucose)	14	0.14	Jampatesh et al. (2019)
Sugarcane bagasse	Sodium hydroxide	<i>A. succinogenes</i>	130 (glucose)	41	0.32	Chen et al. (2021)
Mischantus straw	(glycerol:water mixture and H ₂ SO ₄) Organosolv	<i>A. succinogenes</i>	44.2 (total sugars)	23.5	NR	Dąbkowska et al. (2019)
Rapeseed straw	H ₂ SO ₄ (1% solution) Steam explosion	<i>S cerevisiae</i>	23.3 g/l (glucose)	9.3	0.6	Kuglarz et al. 2018
Wheat straw	No pretreatments	<i>Fibrobacter succinogenes</i>	NR	1.5	0.05	Li et al. (2010)

Dąbkowska et al. (2019) reached the concentration of 23.5 g/L of succinic acid after the optimization of the ratio of *Mischantus* straw hydrolysate at 75:25 v/v hydrolysate to medium mixing ratio. Sawisit et al. (2018) investigated the simultaneous saccharification and fermentation (SSF) at batch and fed-batch by using alkaline pre-treated and hydrolysed rice straw treated by using cellulose and xylanase enzymes. The authors achieved the highest succinic acid production under fed-batch SSF than batch SSF by using the same hydrolysate feedstock and by using the engineered *E. coli* KJ122. A novel *in-situ* semi- simultaneous saccharification and co-fermentation (SSSCF) was developed by Chen et al. 2021 that reached the SA concentration of 41 g/L by using undried alkali-pretreated sugarcane bagasse residuals that was enzymatically hydrolyzed and progressively in the same bottles, SA fermentation was started by adding growth medium and *A. succinogenes* inoculum (10% v/v). Lo et al. 2020 after the optimization of the acid pretreatment of sweet sorghum bagasse (at 50 °C, 1 hour) and starting from 29.2 g/L cellulosic glucose after enzymatic hydrolysis, reached the concentration of 17.8 g/L of succinic acid under strictly anaerobic condition by supplying 2.5 M Na₂CO₃ and CO₂ (0.5 vvm) in a 3–L reactor. Jampatesh et al. (2019) obtained the best result on SA concentration 14 g/L at the yield of 0.14 g/g by using 70% of hydrolysate as feedstock for the fermentation process. Bukhari et al. (2020) tested different organic acid (oxalic, citric and formic acids) to pretreat oil palm trunk biomass. The authors observed that the pre-treatment by using oxalic acid was the best to reach the

highest recovery of glucose >60% but the best results on succinic acid production was obtained when citric acid was used to treat the biomass.

These recent findings on the production of succinic acid from lignocellulosic biomass have shown how process optimization started from characterization of the biomass composition to the optimization of the pretreatment process, which almost always required double pre-treatment (chemical or physical/chemical and enzymatic hydrolysis) except when the saccharification and co-fermentation approach was experimented. On the fermentation process, the aspects that seemed to have the greatest influence on the performance of succinic acid production were: the appropriate amount of hydrolysate as feedstock, the use of CO₂ or indirect sources of CO₂, the use of pH control buffers, and the use of engineered strains such as *S. cerevisiae* and *E. coli* that could optimize the process compared with the use of natural SA producer strains such as *A. succinogenes*. Furthermore, among fermentation methodologies, simultaneous saccharification-fermentation appeared to be the most promising for succinic acid production from lignocellulosic biomass. Another critical aspect that requires further studies is the optimization of biomass pretreatment processes to reduce the production of inhibitory compounds for a better valorization of lignocellulosic biomasses through the optimization of the pretreatment process or by developing detoxification technologies of the pretreated.

5. Conclusions

The evaluations on the most recent findings highlighted how several optimizations and new strategies are still necessary on the production of succinic acid from lignocellulosic biomass, starting from the pre-treatment of the biomass due to the variable initial composition of the matrix till to the fermentation process.

Acknowledgments

This work was supported by The National Recovery and Resilience Plan (NRRP) - Next Generation EU – Mission 4, Component 2, Investment 1.4, under the “National Research Centre for Agricultural Technologies”, Agritech, [grant number I63C22000350007].

Reference

- Bukhari, N. A., Jahim, J. M., Loh, S. K., Nasrin, A. B., Harun, S., Abdul, P. M., 2020. Organic acid pretreatment of oil palm trunk biomass for succinic acid production. *Waste and Biomass Valorization*, 11, 5549–5559.
- Chen, J., Yang, S., Alam, M. A., Wang, Z., Zhang, J., Huang, S., ... Xu, J., 2021. Novel biorefining method for succinic acid processed from sugarcane bagasse. *Bioresource Technology*, 324, 124615.
- Christopher, M., Mathew, A.K., Kiran Kumar, M., Pandey, A., Sukumaran, R.K., 2017. A biorefinery-based approach for the production of ethanol from enzymatically hydrolysed cotton stalks. *Bioresource Technology*, 242, 178–183.
- Dąbkowska, K., Alvarado-Morales, M., Kuglarz, M., Angelidaki, I., 2019. Miscanthus straw as substrate for biosuccinic acid production: Focusing on pretreatment and downstream processing. *Bioresource technology*, 278, 82–91.
- Dessie, W., Xin, F., Zhang, W., Zhou, J., Wu, H., Ma, J., Jiang, M., 2019. Inhibitory effects of lignocellulose pretreatment degradation products (hydroxymethylfurfural and furfural) on succinic acid producing *Actinobacillus succinogenes*. *Biochemical Engineering Journal*, 150, 107263.
- Gao, X., Tong, X., Zhang, Y., Xue, S., 2023. The sustainable production of succinic anhydride from renewable biomass. *iScience*, 26(7), 107203.
- Imman, S., Arnthong, J., Burapatana, V., Champreda, V., Laosiripojana, N., 2015. Fractionation of rice straw by a single-step solvothermal process: Effects of solvents, acid promoters, and microwave treatment. *Renewable Energy* 83, 663–673.
- Jampatesh, S., Sawisit, A., Wong, N., Jantama, S. S., Jantama, K., 2019. Evaluation of inhibitory effect and feasible utilization of dilute acid-pretreated rice straws on succinate production by metabolically engineered *Escherichia coli* AS1600a. *Bioresource technology*, 273, 93–102.
- Khan, T. S., Mubeen, U., 2012. Wheat straw: A pragmatic overview. *Current research journal of biological sciences*, 4(6), 673–675.
- Kuglarz, M., Alvarado-Morales, M., Dąbkowska, K., Angelidaki, I., 2018. Integrated production of cellulosic bioethanol and succinic acid from rapeseed straw after dilute-acid pretreatment. *Bioresource Technology*, 265, 191–199.
- Kumar, A., Rapoport, A., Kunze, G., Kumar, S., Singh, D., Singh, B., 2020. Multifarious pretreatment strategies for the lignocellulosic substrates for the generation of renewable and sustainable biofuels: A review. *Renewable Energy*, 160, 1228–1252.

- Lee, P. C., Lee, S., Hong, S. H., Chang, H. N., Park, S. C., 2003. Biological conversion of wood hydrolysate to succinic acid by *Anaerobiospirillum succiniciproducens*. *Biotechnology letters*, 25, 111–114.
- Li, Q., Siles, J. A., Thompson, I. P., 2010. Succinic acid production from orange peel and wheat straw by batch fermentations of *Fibrobacter succinogenes* S85. *Applied microbiology and biotechnology*, 88, 671–678.
- Lin, S. K. C., Du, C., Koutinas, A., Wang, R., Webb, C., 2008. Substrate and product inhibition kinetics in succinic acid production by *Actinobacillus succinogenes*. *Biochemical Engineering Journal*, 41(2), 128–135.
- Lo, E., Brabo-Catala, L., Dogaris, I., Ammar, E. M., Philippidis, G. P., 2020. Biochemical conversion of sweet sorghum bagasse to succinic acid. *Journal of bioscience and bioengineering*, 129(1), 104–109.
- Lu, J., Li, J., Gao, H., Zhou, D., Xu, H., Cong, Y., ... Jiang, M., 2021. Recent progress on bio-succinic acid production from lignocellulosic biomass. *World Journal of Microbiology and Biotechnology*, 37, 1–8.
- Putri, D. N., Pratiwi, S. F., Perdani, M. S., Rosarina, D., Utami, T. S., Sahlan, M., Hermansyah, H., 2023. Utilizing rice straw and sugarcane bagasse as low-cost feedstocks towards sustainable production of succinic acid. *Science of The Total Environment*, 862, 160719.
- Sasaki, M., Adschiri, T., Arai, K., 2003. Fractionation of sugarcane bagasse by hydrothermal treatment. *Bioresource Technology* 86, 301–304.
- Sawisit, A., Jampatesh, S., Jantama, S. S., Jantama, K., 2018. Optimization of sodium hydroxide pretreatment and enzyme loading for efficient hydrolysis of rice straw to improve succinate production by metabolically engineered *Escherichia coli* KJ122 under simultaneous saccharification and fermentation. *Bioresource technology*, 260, 348–356.
- Song, H., Lee, S. Y., 2006. Production of succinic acid by bacterial fermentation. *Enzyme and microbial technology*, 39(3), 352–361.
- Stefanidis, S. D., Kalogiannis, K. G., Iliopoulou, E. F., Michailof, C. M., Pilavachi, P. A., Lappas, A. A., 2014. A study of lignocellulosic biomass pyrolysis via the pyrolysis of cellulose, hemicellulose and lignin. *Journal of analytical and applied pyrolysis*, 105, 143–150.
- Vallecilla-Yepe, L., Ramchandran, D., Long, D., Saha, R., Wilkins, M. R., 2021. Corn fiber as a biomass feedstock for production of succinic acid. *Bioresource Technology Reports*, 16, 100868.
- Vivek, N., Sindhu, R., Madhavan, A., Anju, A. J., Castro, E., Faraco, V., ... Binod, P., 2017. Recent advances in the production of value added chemicals and lipids utilizing biodiesel industry generated crude glycerol as a substrate—metabolic aspects, challenges and possibilities: an overview. *Bioresource technology*, 239, 507–517.
- Wan, C., Li, Y., 2010. Microbial pretreatment of corn stover with *Ceriporiopsis subvermiformis* for enzymatic hydrolysis and ethanol production. *Bioresource Technology*, 101, 6398–6403.
- Xu, C., Alam, M. A., Zhang, J., Wang, Z., Chen, H., Xie, C., ... Xu, J., 2022. Identification of lignocellulosic derivatives inhibiting succinic acid fermentation and molecular mechanism investigation. *Industrial Crops and Products*, 187, 115466.
- Xu, H. T., Wang, C., Zhou, Z. H., Chen, Z. J., Cai, H., 2015. Effects of lignocellulose-derived inhibitors on growth and succinic acid accumulation by *Corynebacterium glutamicum*. *Biotechnology and bioprocess engineering*, 20, 744–752.
- Yang, B., Dai, Z., Ding, S. Y., Wyman, C. E., 2011. Enzymatic hydrolysis of cellulosic biomass. *Biofuels*, 2(4), 421–449.