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Bioplastic Compounds of Succinic Acid from Agriculture Waste; Date Palm Syrup And Date Palm Fronds

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Abstract

Excessive use of petrochemical-based plastics lead to severe plastic pollution by accumulation in soil, drainage system blockage, also each year trillions of plastic residues released into the ocean. In some cases, burning plastic waste releases toxic gases and may cause acid rain. Bioplastics, biodegradable, are eco-friendly alternatives to petroleum-based plastics, reduce environmental impacts and utilize non-edible waste products for decomposition by microorganisms, making them a significant topic in the twenty-first century. Succinic acid has wide medical and industrial application, in addition to being a solution to the problem of plastic pollution for use in the manufacture of bioplastics. One of the most important biomass wastes in the Iraqi environment that are candidates for biorefinery is palm waste due to its environmental friendliness and high sugar content. In this study, succinic acid was investigated in the fermentation product of poor-quality date palms Zahid and palm fronds. The residues of Zahid dates with palm fronds and the addition of soybean were used for the production of succinic through the process of batch fermentation and solid-state fermentation using a mixture of yeasts *Saccharomyces cerevisiae* and *P. stipitis* ATCC 58785 and fungi *Trichoderma reesei* and *Aspergillus niger* after pretreatment. The succinic acid was estimated using GC mass, and the quantities of acid produced from date juice from Zuhdi dates syrup were 20.6g/L, while the optimal ratio of acid produced from palm fronds with soybeans was estimated at 2.28g/L and production improved to 3.51 g/L by fed batch fermentation. Biotonic acid, oxalic acid malic acid were detected in significant amount as 10.82,23.68 and 70.40 g/L respectively from date syrup. This research opens the horizons for the use of the biomass of the dates palm in the production of bioplastics. Consolidated bioprocessing of lignocellulose to succinic acid through a microbial cocultivation system consolidated bioprocessing.

Keywords: Date palm, Palm front, Bioplastic, GC

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Introduction

Succinic acid is a four-1,4 dicarboxylic acid as a precursor chemical for various industrially important polymers and are included among the top 12 possible bio-based compounds by the U.S. Department of Energy, along with fumaric and malic acids [1]. The annual market for succinic acid polymers is projected to reach approximately 25 million tonnes [2]. The food additive, pharmaceutical, surfactant, and biodegradable plastic sectors have all made extensive use of succinic acid. Growing interest has been paid to its manufacturing as an alternative for chemical raw materials due to fossil fuel depletion and environmental issues [1,3].

Polybutylene succinate (PBS), butylene succinate-co-butylene adipate (PBSA), polyethylene succinate (PESu), polybutylene succinate-co-butylene-fumarates (PBSFs), polybutylene succinate-co-L-lactate (PBSL), polybutylene succinate-co-diethylene succinates (PBDEGs), polybutylene succinate-co-ethylene succinates (PBESs)and polybutylene succinate-co-butylene terephthalates (PBST) are a few examples of commercially available bioplastics [4]. Additionally, because of its distinct qualities namely biodegradability, and biocompatibility, the global bioplastic industry is projected to attain a valuation of $25.93 billion by 2029AD. [5].

Succinic acid is a traditional fossil fuel produced by partially hydrogenating maleic anhydride and hydrating or directly hydrogenating maleic acid using heavy metal catalysts in organic solvents at high temperature and pressure. The process, involving high temperatures and pressure, is expensive, hazardous, emits Green House Gas (GHG), and depletes fossil fuels, making it potentially harmful to the environment. By 2035AD, the International Energy Agency (IEA) projects a considerable 75% decrease in fossil fuel use [4,5].

The chemical industry and governments have shifted to biosynthesis for a sustainable global energy future because to the depletion of fossil fuel resources and the high consumer demand for environmentally

advantageous energy [6]. There have been attempts of effectively commercialise the synthesis of succinic acid via the sustainable route of microbial fermentation. A bio-based production process accounted for 38 kilotons, or 49%, of the succinic acid created in 2013 [7].

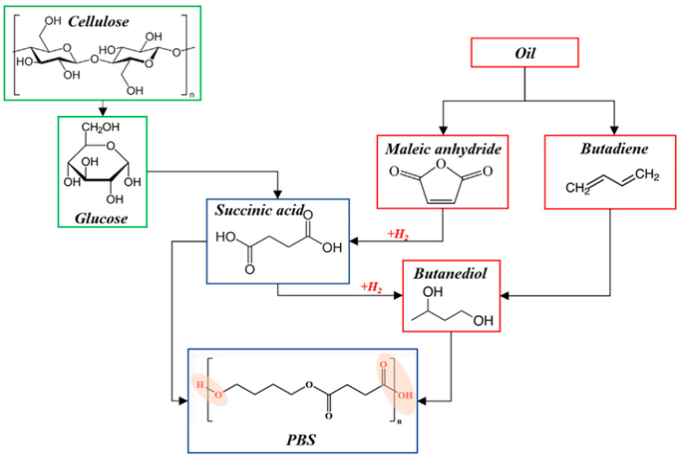


Figure 1. The flowchart illustrates the process of synthesizing polybutylene succinate (PBS) from either fossil or biobased sources [8].

Microorganisms can produce succinic acid at room temperature and pressure utilizing renewable resources. Recent reports of optimised fermentation processes have shown bio-succinic acid produces of up to 1.78 mol for each mol of glucose at concentrations of up to 146 g/L [9,10]. However, the large-scale production of biosuccinic acid has been challenged by poor yields and high costs. While manufactured from traditional petrochemical procedures only costs USD 1.05 –1.29/kg, bio-succinic acid manufacture costs USD 1.66–2.2/kg. Its demand for culture during the fermentation and downstream processing may be the cause of the expensive manufacture. The downstream processing, which is the direct outcome of many purification and recovery procedures carried out to obtain high purity (>98%) for effective PBS manufacturing, makes for 60% of the entire processing cost of bio-succinic acid synthesis [11].

Due to the rapid depletion of fossil resources and the need for natural energy sources, the biosynthesis of Succinic acid from renewable energy sources is attracting more interest due to its environmental sustainability [2]. One of the most important environmental wastes that are candidates for bioremediation in the Iraqi environment is palm waste. In a biorefinery conception and modelling of the circular economy, the utilisation of lignocellulosic biomass waste can also enable the synthesis of bio-based building blocks such as succinic acid, lactic acid, and propionic acid. Iraq is among the top nations with the highest number of date palm trees, which contributes to a large volume of garbage that is not consumed. To determine the date palm tree's possible energy availability, various components were examined. The date palm fronds as a cheap, plentiful, and non-food biomass that refers to a renewable and suitable source of cellulosic fibres appropriate for the synthesis of biopolymer [12]. Date palm frond compositions established through dry weigh, The cellulose, hemicellulose and lignin were Palm leaf cellulose 25.01%, hemicellulose 32.89% and lignin 42.10% [13,14].

It is essential pretreatment of biomass through chemical or physical techniques in order to effectively production succinic acid from lignocellulos [2]. Three basic techniques are used in the bioconversion of lignocellulose: simultaneous hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and simultaneous saccharification and co-fermentation (SSCF). One of their major shortcomings, though, is that during the fermentation route, a significant number of hydrolases must be added. This raises the cost of production significantly and makes large-scale manufacturing impractical [1]. As an alternative, scientists have worked hard and developed the idea of consolidated bioprocessing (CBP), which mixes microbial fermentation, lignocellulose hydrolysis, and hydrolase synthesis all in one step. But few wild-type strains exist in nature that can use CB to directly synthesise succinic acid from biomass [15]. *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes*, *Pichia kudriavzevii*, *S. cerevisiae*, *Basfia succiniciproducens*, *Rrecombinant Escherichia coli* and *Mannheimia succiniciproducens* are among the bacterial strains that have been assessed and commercialised for this purpose. Another modified genetically bacterial strain that has shown improved succinic acid production efficiency is *Corynebacterium glutamicum*. [4].

The aims of this research to investigate succinic acid production from pretreated-lignocellulosic biomass from date syrup and date palm frond using fungal co-culture by different fermentation technology. The concept is that through the solid-state stage, *A. niger* and *T. reesei* combined cellulolytic activity liberated sugars from the biomass mixture and slurry fermentation stage *P. stipitis* ATCC 58785 and *S. cerevisiae* were transformed into a mixed organic acid product, with the primary product getting succinic acid, simultaneously.

Material and methods

Preparation of raw material

Collect damaged Zahdi dates in date factories and those that have fallen on the farm land, remove dust from them, separate the pits from the dates, collect them in bags, and store them in the laboratory at room temperatures until

use. The dates palm fronds collected from Baghdad Garden in Iraq were washed, peeled, and crushed until a puree was obtained.

Hot extraction method of dates

Zahdi dates is a medium-sized semi-dry dates, which are very sweet was used due to their local availability in large quantities, to prepare date juice by the hot extraction method. The pitted dates were mixed with tap water in a ratio of 1:1 in a heated saucepan at a temperature of 80 °C for half an hour with continuous rotation and left for the second day when it was filtered through gauze. The remaining dates were added to it as much as its volume and mixed with an electric mixer and filtered in the same way. The resulting juice is diluted with water to obtain the required concentrations, taking into account the determination of the pH value at 4.5.

Acid hydrolysis of biomass dates palm fronds

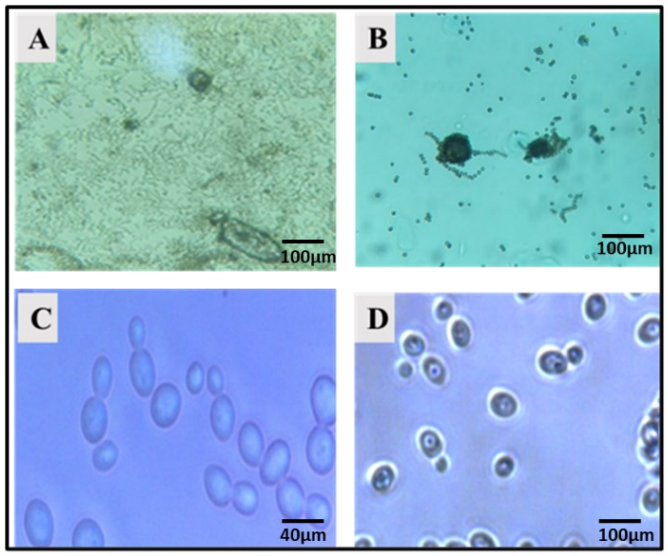
Pretreatment of dates palm fronds by acid hydrolysis was carried out [16]. The acid concentration was 5.0% of sulfuric acid as 50 mL of dilute acid for each 10 g of raw material of palm fronds and the contact time 15 min while heating in autoclave. After cooling, the mixture was filtered through a filter paper.

Detoxification

The detoxification process was carried out by the addition of 2.5% (m/v) of active carbon, the mixture was subjected to stirring at 200 rpm, at 30° C, for 1 h. The mixture was then centrifuged again and filtered. Concentrated hydrolysate with glucose concentration was obtained by evaporation. The pH of hydrolysate was adjusted to 6.0 by 5M NaOH before fermentation.

Microorganism

Strains were used in fermentation experiments (Figure 2), *T. reesei* and *A. niger* were supplied by the agriculture director biotechnology center Iraq. The yeasts *S. cerevisiae* and *P. stipitis* ATCC 58785, *P. stipitis* ATCC 58785 was bought from the American Type Culture Collection (ATCC) [17]. Fungal inoculum was prepared from pores of seven-day old potato dextrose agar (PDA) slant stock cultures. This culture was spread out on PDA plates, suspended in sterile deionized water, and cultured for seven days at 30 °C. The spores formed on the plates were washed with sterile modified Mandel's medium containing 2 g glucose, 2.0 g KH_2PO_4, 0.3 g $MgSO_4 \cdot 7H_2O$, 0.4 g $CaCl_2 \cdot 2H_2O$, 0.3 g NH_2CONH_2, 1.4 g $(NH4)_2SO_4$, 1.0 g Proteose peptone No. 2, 0.2 mL Tween-80, 5 mg $FeSO_4 \cdot 7H2O$, 1.4 mg $ZnSO_4 \cdot 7H_2O$, 1.6 mg $MnSO_4 \cdot H2O$, 2.0 mg $CoCl_2$ per L of deionized water [18]. To generate the fungal inocula, spore suspensions containing 1 × 10^6 to 1 × 10^7 spores/mL were transferred to 50-mL centrifuge tubes and incubated in an incubator shaker at 30 ◦C, 150 rpm for two days. The strains were used in fermentation experiments. The Yeast inoculum was prepared by taking a complete transfer (Loopful) from the mother culture growing on the yeast extract agar and then cultivating it in a glass flask with a capacity of 250 mL containing 40 mL of liquid yeast medium (YEL). The flasks were incubated in a shaking incubator at 30°C for 48 h at a 150 rpm to obtain a culture containing about (1.5 x 10^6 cells/mL). Use this medium to inoculate fermentation media.



Figer 2. Microorganism used in study. A; *T. reesei*, B; *A. niger*, C; *S. cerevisiae* and D; *P. stipitis* ATCC 58785.

Fermentation experiments

The batch culture fermentation of dates palm syrup

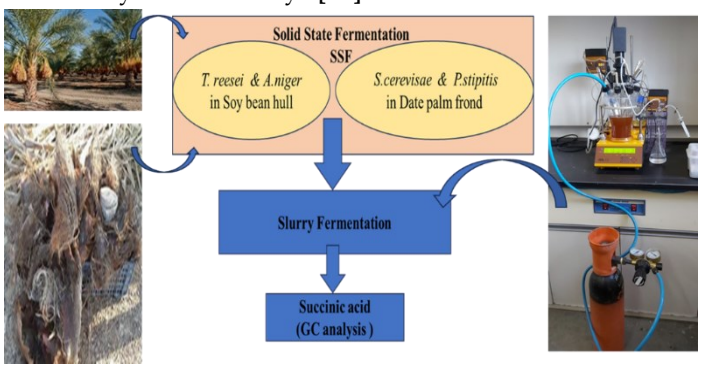
The batch fermentation was carried out in 1-L LAMBDA MINIFOR stirred tank bioreactor fermenter. For co-culture fermentation 5% (v/v) of inoculum size of *S. cerevisiae* was transferred into the fermentation media of total sugar as 60 g/L. Fermentation experiments was performed in bioreactor with a total working volume of 0.75 L at 30°C for 72 h. For C6 sugar fermentation, *S. cerevisiae* was subjected to anaerobic incubation at 30°C for 24 h with agitation at 150 rpm on first phase of fermentation time. Subsequently, 5% (v/v) of inoculum size of *P. stipitis* ATCC 58785 was transferred into the fermentation culture in the bioreactor with the help of a syringe. On second phase of fermentation time C5 sugar fermentation with agitation at 300 rpm, 30°C and aeration conditions at 0.25 vvm [19]. To monitor the fermentation process, the fermenter was vented using a syringe attached to an air-tight tube connected to a water column filled with 70% ethanol. If the pH dropped to less than 6.0, ammonium bicarbonate was used to re-neutralize the

medium. After fermentation, 3 ml sample of the fermented biomass was collected for later analysis.

The fermentation of dates found.

Solid-State Fermentation experiments

The fungi were grown on milled biomass substrates under static conditions. The moisture content of both substrates was adjusted to 70% using modified Mandel's medium. These were then sterilized using a laboratory autoclave at 121 ◦C for 15 min. Solid-state pre-cultures containing mixed *T. reesei* and *A. niger* in soybean hulls were prepared by adding 10% (v/w) of two-day-old inoculum cultures of T. reesei in a 250-mL Erlenmeyer flask containing 7.5 g sterilized soybean hull. *A. niger* was inoculated into the same flask one day after *T. reesei* addition to ensure adequate growth of *T. reesei* before *A. niger* addition and thus balanced cellulase and β-glucosidase activities. Two-day-old *S. cerevisiae* and *P. stipitis* ATCC 58785 inoculate (10% w/v) were grown separately in 500-mL flasks containing 22.5 g pretreatment dates palm fronds. All flasks were incubated in a humidified incubator at 30 ◦C, 95% relative humidity for seven days [20].



Figer 3. Schematic diagrams of two-stage fermentation for succinic acid production combining Solid state fermentation (SSF) and Slurry fermentation

Slurry Fermentation Stage

Following the solid-state pre-cultivation phase, 50.0 mL of 0.05 M sodium acetate buffer (pH 4.8) was used to suspend the static co-cultures of T. reesei and A. niger in soybean hulls. After a full 20 minutes of mixing, the liquid was transferred to a 500 mL baffled flask containing the yeast pre-culture in dates palm fronds. Following that, the liquid was stirred to suspend all of the solids and the fungus mycelia. The mixed cultures dry solids content reached 20% (w/w), with a mass ratio of 3:1 between dates palm frond and soybean husk mass. After that, the flasks with the mixed pre-culture slurry were incubated for four days at 350 rpm and 35 °C in an incubator shaker. Samples were taken out of the liquid phase and centrifuged for ten minutes at 12,000 rpm.

Analyses

The percentage of extractives, moisture, ash, lignin, cellulose, holocellulose, and hemicellulose content were investigated according to Sluiter et. al. [21]. Sugars (cellobiose, glucose, and xylose) and were analysed using a HPLC (sykam -German 2013AD) [21]. Organic acids such succinic acid, biotonic acid, oxalic acid and malic acid were analysis used Dani Master GC Fast Chromatograph System D0305AFS in Environment and Water Department. Equations was used to compute the concentration in the sample [22].

Concentration in sample(µg/mL) = (Area sample/ Average area standard) ×0.50mg/mL.

Statistical analysis

Data obtained were analysed by one way ANOVA using SPSS statistics.

Results

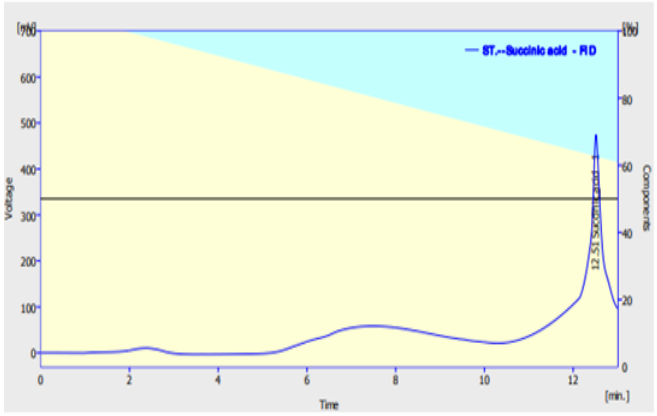
Date palm chemical composition

The chemical composition of the Zahdi date syrup showed that the total sugar 73,23 reducing sugar 54.30 with glucose 29.7 and fructose 28.6, these results agree with Mahdi et.al. [23]. The compositions of date palm fronds were showed that the cellulose, hemicellulose and lignin content of the samples Zahdi date fronds about 42.1,23.5 and 30.6 % respectively. These results support the findings of earlier studies [24, 13]. The samples' lignin and hemicellulose content, which demonstrated a high proportion in comparison with a previous work by Jassem et.al. [12], hemicellulose and lignin were 38% and 33% respectively but less in cellulose content 30%. The different parts had different compositions as well as differs in type and country. In this study, the combined treatment using fungi and chemical treatment was chosen to improve biomass digestibility [25]. The use of cheap raw materials, as biomass, might be a way to lower production expenses within the biorefinery concept and circular economy of the manufacture of organic acid as succinic acid, propionic acid and lactic acid [3].

Succinic acid production

Batch culture fermentation dates syrup

Using cocultures of *S. cerevisiae* and *P. stipitis* ATCC 58785, the maximum succinic acid concentration resulted in the batch culture fermentation dates syrup under the present study was 20.17 g/L (Figure 4&5) after 48 h of fermentation, yield 0.34g/g and productivity 0.42g/L h. Concentrations of succinic acid began to rise at 24 hours and continued to do so for 48 hours. Thereafter, they were constant for 60 hours before declining to 12.4 g/L at 72 hours.

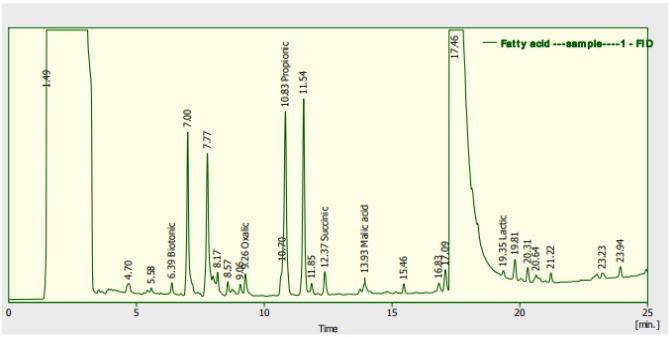


Result Table (Uncal - ST.--Succinic acid - FID)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	12.512	802.419	150.170	100.0	100.0	0.10	Succinic acid
	Total	802.419	150.170	100.0	100.0		

Figure 4. Analysis of standard succinic acid result showed retention time 12.512 min.

Sample : Sample ---1 ISTD Amount : 0
Inj. Volume [mL] : 0 Dilution : 1



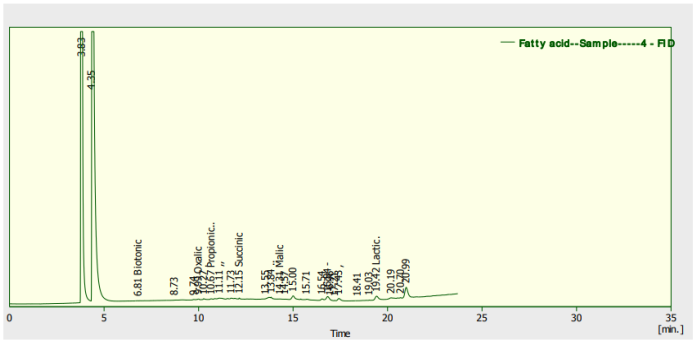
Result Table (Uncal - Fatty acid ---sample----1 - FID)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.487	103178.126	986.980	85.5	27.5	1.79	
2	4.697	276.256	31.824	0.2	0.9	0.15	
3	5.583	237.676	21.299	0.2	0.6	0.09	
4	6.387	154.652	38.295	0.1	1.1	0.07	Biotonic
5	7.003	2531.094	560.510	2.1	15.6	0.07	
6	7.773	1853.737	407.412	1.5	11.4	0.07	
7	8.173	51.818	22.860	0.0	0.6	0.04	
8	8.573	68.003	22.699	0.1	0.6	0.05	
9	9.063	94.587	25.100	0.1	0.7	0.07	
10	9.263	334.050	68.302	0.3	1.9	0.08	Oxalic
11	10.703	2.286	0.577	0.0	0.0	0.01	
12	10.827	410.646	178.448	0.3	5.0	0.04	Propionic
13	11.540	3670.232	698.310	3.0	19.5	0.08	
14	11.853	107.213	27.946	0.1	0.8	0.07	
15	12.370	323.667	69.946	0.3	2.0	0.08	Succinic
16	13.933	348.008	52.799	0.3	1.5	0.07	Malic acid
17	15.457	173.205	35.514	0.1	1.0	0.08	
18	16.833	142.077	26.507	0.1	0.7	0.09	
19	17.087	306.058	63.103	0.3	1.8	0.08	
20	17.460	5115.416	6.046	4.2	0.2	0.33	
21	19.350	101.266	19.506	0.1	0.5	0.08	Lactic
22	19.810	296.503	62.277	0.2	1.7	0.08	
23	20.307	271.859	52.640	0.2	1.5	0.08	
24	20.640	72.665	15.005	0.1	0.4	0.08	
25	21.217	174.204	34.698	0.1	1.0	0.08	
26	23.227	139.777	18.338	0.1	0.5	0.13	
27	23.937	211.813	38.760	0.2	1.1	0.09	
	Total	120646.893	3585.700	100.0	100.0		

Figure 5. Sample succinic acid from date palm syrup.

The succinic acid production occurred at the same time as the glucose consumption. Because *S. cerevisiae* tolerates low pH levels well, it is the best option for producing succinic acid [26]. In current study, succinic concentration result was compatible with result in the combined production of the bioethanol and succinic acid ascertained by SSCF from rice straw that has been treated employing genetically modified *S. cerevisiae* SHY07-1 were 91.9 g/L and 29.3 g/L for bioethanol and succinic acid respectively [27In the same way, the concentrations of bioethanol and succinic acid reached 120.77 and 34.84 g/L, respectively, when processed sugarcane bagasse was utilized [28]. Examined is the production of succinic acid by S. cerevisiae as the TCA cycle pathway's intermediate. S. cerevisiae does not, however, naturally accrue succinic acid at large concentrations; the highest concentration measured by the natural isolate Z28 was 1.13 g/L [29]. Succinic acid synthesis may be significantly enhanced by metabolically engineering S. cerevisiae along three main pathways: the glyoxylate route, the oxidative TCA cycle, and the reductive TCA cycle [30]. Promising development in fermentation process for succinic acid synthesis by yeast hosts, two yeasts, *P. stipitis* and *S. cerevisiae* were used to ferment the hydrolysate soybean hull, according to estimates, *P. stipitis* could produce 116.2 kg of bioethanol and 20.4 kg of the organic acid from 1 tonne of soybean hull, whereas S. cerevisiae could produce 124.0 kg of bioethanol and 36.5 kg of succinic acid [31]. Toward economical production of succinic acid by *Actinobacillus succinogenes* [32], instead of using yeast extract and glucose, biomass hydrolysate and waste yeast hydrolysate were effectively utilized as sources of nitrogen and carbon.

Inj. Volume [mL] : 0.001 Dilution : 1



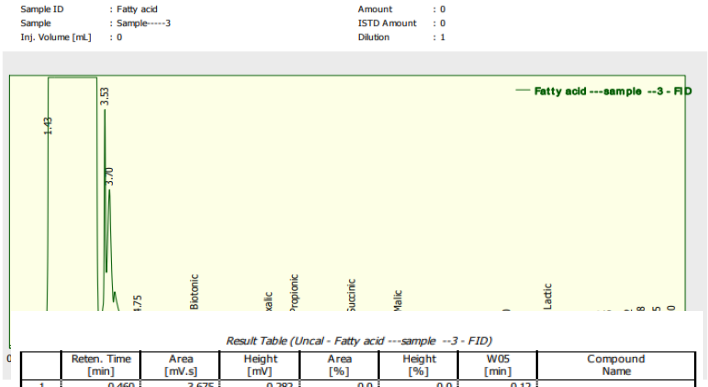
Result Table (Uncal - Fatty acid--Sample-----4 - FID)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.827	771.134	58.516	12.6	5.7	0.05	
2	4.347	3698.392	827.444	60.2	80.7	0.13	
3	6.813	1.173	0.100	0.0	0.0	0.03	Biotonic
4	8.727	0.070	0.054	0.0	0.0	0.01	
5	9.743	11.597	1.659	0.2	0.2	0.13	
6	9.990	23.653	2.855	0.4	0.3	0.11	Oxalic
7	10.273	26.476	3.421	0.4	0.3	0.10	.
8	10.673	21.825	2.369	0.4	0.2	0.15	Propionic..
9	11.107	94.241	4.097	1.5	0.4	0.32	,,
10	11.727	24.232	2.953	0.4	0.3	0.16	
11	12.153	32.590	3.619	0.5	0.4	0.05	Succinic
12	13.550	25.658	2.456	0.4	0.2	0.23	
13	13.840	170.780	8.586	2.8	0.8	0.30	..
14	14.307	47.887	2.522	0.8	0.2	0.36	Malic
15	14.573	30.164	2.191	0.5	0.2	0.25	
16	15.003	225.138	14.613	3.7	1.4	0.17	
17	15.713	65.500	3.419	1.1	0.3	0.29	
18	16.537	53.505	5.692	0.9	0.6	0.18	
19	16.837	163.339	14.592	2.7	1.4	0.19	-
20	16.963	18.144	4.421	0.3	0.4	0.05	
21	17.207	3.462	1.205	0.1	0.1	0.05	
22	17.430	87.481	8.323	1.4	0.8	0.16	,
23	18.407	0.883	0.099	0.0	0.0	0.03	
24	19.030	2.317	0.919	0.0	0.1	0.05	
25	19.420	33.255	5.640	0.5	0.5	0.11	Lactic.
26	20.187	59.277	4.189	1.0	0.4	0.28	
27	20.697	27.847	2.134	0.5	0.2	0.08	
28	20.987	422.268	37.523	6.9	3.7	0.17	
	Total	6142.288	1025.611	100.0	100.0		

Figure 6. Sample succinic acid from date palm found.

The fermentation dates found

Succinic acid with trace concentration around 0.18 g/L was produced via the solid-state pre-fermentation stage before the slurry fermentation stage began. There was no discernible rise in succinic acid levels in the first 24 h. Following the achievement of maximal glucose production was achieved, succinic acid concentrations began to rise for 24 to 48 hours, then stabilised up to 60 h and then reached its highest level (2.28 g/L) at 72 h. Concurrent with the ingestion of glucose, succinic acid has been produced. As there was no longer any available glucose after 72 hours, succinate levels significantly decreased (**Figure 6**).



Result Table (Uncal - Fatty acid ---sample --3 - FID)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.460	3.675	0.282	0.0	0.0	0.12	
2	1.267	33.800	7.353	0.0	0.3	0.08	
3	1.433	109592.664	992.573	86.4	36.4	1.83	
4	3.530	3803.132	870.332	3.0	31.9	0.06	
5	3.697	10012.531	573.168	7.9	21.0	0.18	
6	4.753	1721.227	97.267	1.4	3.6	0.27	
7	5.160	207.909	17.122	0.2	0.6	0.19	
8	6.037	202.601	29.531	0.2	1.1	0.08	
9	6.853	115.547	20.436	0.1	0.7	0.11	Biotonic
10	8.540	131.579	16.327	0.1	0.6	0.11	
11	9.177	21.961	4.072	0.0	0.1	0.08	
12	9.407	16.307	2.928	0.0	0.1	0.09	
13	9.590	60.372	3.813	0.0	0.1	0.08	Oxalic
14	10.537	55.051	3.551	0.0	0.1	0.13	Propionic
15	11.387	47.020	5.980	0.0	0.2	0.11	
16	12.057	24.866	2.968	0.0	0.1	0.12	
17	12.660	56.337	3.734	0.0	0.1	0.11	Succinic
18	13.773	65.179	10.111	0.1	0.4	0.10	
19	14.380	80.762	4.140	0.1	0.2	0.17	Malic
20	15.433	36.050	6.007	0.0	0.2	0.09	
21	15.913	17.857	2.503	0.0	0.1	0.11	
22	16.143	14.388	2.439	0.0	0.1	0.10	
23	16.373	21.095	3.276	0.0	0.1	0.10	
24	18.080	12.141	1.886	0.0	0.1	0.08	
25	18.400	173.004	18.038	0.1	0.7	0.15	
26	18.663	50.100	6.222	0.0	0.2	0.13	
27	19.927	70.568	3.314	0.1	0.1	0.11	Lactic
28	21.017	15.810	1.280	0.0	0.0	0.39	

Figure 7. Sample succinic acid from date palm syrup by fed batch and SSF.

In the solid state fermentation used soybean hulls, which are high in protein and are excellent substrates for stimulating the development of fungal cellulase enzyme, were successively infected with T. reesei and A. niger It is required to combine C rich biomass, such as date palm frond, with N-rich biomass substrates, such as soybean hulls, to create an adequately high C:N ratio, which is supposed to be led to stimulating fungal growing and hydrolytic enzyme actions in order to encourage overproduction of organic acids during the slurry fermentation stage [20]. To promote slower-growing T. reesei, avoid overgrowth and domination by faster growth *A. niger*, *A. niger* was inoculum one day after *T. reesei* inorder to create balanced and sustained enzyme cellulase and -glucosidase creation [33], so as to increase production, a fed batch of fermentation medium was added to reach 3.51g/L as recommended by Li et.al.[34]. The study found that fibrous bed bioreactor technique and fed batch fermentation effectively improved succinic acid production, achieving a productivity of 0.69 g/L h and a concentration of 140.6 g/L (**Figure 7**).

Other organic acid, citric and oxalic acids were generated at 0.89 and 0.52 g/L, respectively, during the slurry fermentation stage, whereas malic acid was detected at 0.56 g/L.

Nevertheless, the ability to directly produce succinic acid from lignocellulose biomass is limited to a few numbers of wild-type strains in nature. Actinobacillus succinogenes, a versatile wild-type bacterial host capable of metabolizing various carbon sources, is advantageous for optimal bio-conversion of C5 and C6 sugars into succinic acid [35]. succinic acid produce by Actinobacillus succinogenes BE-1 were generated 15.8 and 17.8 g/L after enzymatic hydrolysis of cotton stalk and corn stalk respectively by anaerobic batch fermentation technology [36].

The way to improve productivity by using specialized isolates, concurring to its natural habitat. In a 32-hour batch fermentation, one research used Anaerobiospirillum succiniciproducens to produce 23.8 g/L of succinic acid from wood hydrolysate generated via oak wood treated with a steam explosion, supplemented with corn steep liquor [37]. Another study Actinobacillus succinogenes was utilized in a 24-hour batch fermentation to produce 22.5 g/L succinic acid from hemicellulose hydrolysate obtained from sugarcane bagasse pretreatment [38].

By using specific strain were achievement, one of the greatest amounts of succinic acid bioproduction (0.92g/g) from food waste (potato waste) fermentation observed to date, by Actinobacillus succinogenes, 32.2 g/L of succinic acid were achieved with a productivity of 0.64 g/L • h [39]. Another strategy proposed in the future to develop productivity is to use immobilizing cell technology to avoid cell flocculation and facilitate separation of the product from the cells, making it reusable, and this is what Cao et.al. [40] were found. On the immobilizing fermentation technology, the same strain was cultivated in five rounds of repeated batch culture in loofah sponge matrices with sodium hydroxide to produce succinic acid from cane molasses, high succinic acid content, yield, and productivity were

Fermentation modes	Production (g/ L)	Yield(g/g)	Productivity (g/L h)
Batch fermentation Dates syrup	20.17±0.250[a]	0.340±0.020[a]	0.420±0.030[a]
SSF (dates palm fronds)	2.28±0.250[b]	0.228±0.011[b]	0.031±0.005[b]
SSF +fed batch (dates palm fronds)	3.51±1.850[b]	0.351±0.019[a]	0.048±0.004[b]

Note: Mean values with the same letter in the same column do not differ significantly according to the Tukey's test ($p > 0.05$).

demonstrated by the findings, which were 45.6 g/L, 0.76 g/g, and 1.9 g/L.h, respectively.

Biomass that is commonly utilised in large-scale fermentative succinic acid synthesis. These feedstocks have the potential to be typically less expensive, which is a benefit. A limitation is that the sugars in this form of plant material are challenging to obtain; thus, pretreatment and hydrolysis are needed to separate the sugars from the lignin, hemicellulose, and cellulose that make up the biomass. As a result, a number of contaminants and products of sugar breakdown are created, including soluble lignin compounds that can function as inhibitors, furfural, 5 hydroxymethyl furfural, acetate, and formate. It takes a lot of work and consequently extra expenses in the downstream processing section to limit the influence of these components, particularly if high purity succinic acid is to be generated. Increasing the furfural tolerance of producing strains is one method of overcoming the inhibitory effect of these contaminants and sugar breakdown product.

Table 1; Summary of production from date syrup and Dates palm fronds by different fermentation modes

Conclusions

Concerns about the environment the and the rapid depletion of non-renewable fossil fuels have created interest in researching the biotechnological method of producing succinic acid. The problem of plastic pollution has emphasized the necessity of synthesizing bioplastics like polybutylene succinate (PBS), derived from succinic acid. Because of availability and high lignocellulosic content dates palm waste biomass for the production of succinic acid and its related bioplastics, it is may be a potential replacement for the more expensive pure carbon source. consolidated bioprocessing (CBP) method is the final configuration for cheap hydrolysis and cellulosic biomass fermentation. In addition, an effective microbial strain should also generate the desired products with high titer, yield, and productivity. Thus, the two primary methods for direct succinic acid synthesis from lignocellulose through consolidated bioprocessing (CBP), are metabolic engineering and establishing microbial co-cultivation systems.

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Conflicts of Interest:

The authors declare no conflict of interest.

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