Production of Succinic Acid from Arundo Donax Hydrolysate for Bio-based Polymers Synthesis

R. Turco*, M. Di Serio*, M. Mazzocca*, V. Russo*, R. Tesser*, R. Vitiello*, D. Cimini**, C. Schiraldi**

*University of Naples Federico II, Department of Chemical Sciences, Complesso Universitario di Monte Sant'Angelo, Naples, Italy, **University of Campania Luigi Vanvitelli, Department of Experimental Medicine, Naples, Italy

Abstract

This work aims to provide an integrated process based on the biotechnological production of succinic acid (SA) using a lignocellulose biomass (Arundo donax) as raw material for the synthesis of a biodegradable plastic, i.e. poly(butylene succinate) (PBS).

Introduction

The US Department of Energy (DOE) included succinic acid (SA) in the "Top 10" list of biomass-derived compounds, considering its high potential as building block in the field of the industrial chemistry. In fact, this dicarboxylic acid with four carbon atoms (1,4-butanedioic acid) represents an important commercial product as well as a precursor to industrial products. Currently, four principal markets are known for this chemical platform:

1)surfactants and detergents;

2) ion chelator to prevent corrosion of metals;

3) food industry, as flavouring and antimicrobial agent;

4) pharmaceutic field, as antibiotics amino acids and for vitamin production [1].

Its production has a volume between 30000 and 50000 tonnes per year [2]. Nowadays SA is mainly produced by crude oil, starting from n-butane/butadiene via maleic anhydride. Due to its independence of petroleum, environmental benefit and reduction in CO_2 emissions, the biotechnological production of SA from renewable feedstocks is gaining increasing attention in the last years. SA is an intermediate of the tricarboxylic acid cycle and one of the end products of the anaerobic metabolism. However the bio-based SA production is still not competitive with the petrochemical one especially on the costs level, due to a low SA concentration obtained by fermentation and the difficulty to recover SA from the fermentation broth at high yields and purity. Indeed it is estimated that 60-70% of the total production cost is accounted by the downstream recovery process, while 20-25% by the fermentation and the remaining 10-15% is allocated to feedstocks [3,4]. For this reason, in order to decrease the cost of the bio-SA technological process, extensive efforts have been made to improve the SA biotechnological production. They have principally concerned on one side the improvements in the upstream technology to reach higher yields and selectivity by development of biocatalysts, and on the other side the optimization of the downstream technologies for the separation of SA from the aqueous fermentation broths [5]. As far as sustainability is concerned, several methods of purification and separation, including electrodialysis, ion exchange, precipitation, reactive extraction, have been studied and developed. One of the drawbacks of the most separation technologies concerns the large amount of waste materials, derived by the presence of succinic salt in the upstream part. From 2008, many companies and joint ventures such as Myriant, Everdia, BioAmber, DSM&Roquette joint venture, Reverdia, BASF and Succinity have shown interest on the conversion of purified sugars to SA at industrial scale. As result, at least two industrial plants with a capacity of 4000 MT/year and 10000 MT/year have set up and they are currently running [5]. This work aims to provide an integrated process based on the biotechnological production of SA using a lignocellulose biomass (Arundo donax) as raw material for the synthesis of a biodegradable plastic, i.e.

poly(butylene succinate) (PBS); in fact one of the main challenges is the development of industrially feasible fermentation processes exploiting fermentable sugars from such feedstocks. Among natural producers used for SA production, Basfia succiniciproducens [1] was used in this study to conduct batch and fed-batch experiments in anaerobic conditions up to the 150 L scale demonstrating the possibility of using hydrolysed A.donax as inexpensive raw material [1]. The novelty lies in the type of raw material used, a harvested waste, to produce added value chemicals such as PBS. PBS was synthesized using SA, recovered and purified directly from its fermentation broth through the downstream protocol developed and optimized in this study.

Methods

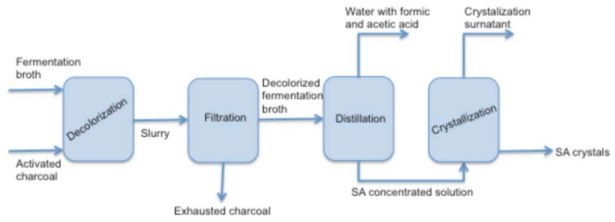
Fermentation

Fermentation experiments were performed on a Biostat C (150 L total volume), working volume of 70 L (Sartorius Stedim; Melsungen, Germany). B.succiniciproducens BPP7 [1] working cell bank was first amplified in 0.25 L bottles overnight on MH medium with glucose as C-source and then transferred to a 3 L tank containing the same medium for the preinoculum preparation. After 16±1 h the broth (2-5% v/v) was transferred to the Biostat C reactor to carry out the main fermentation. Pre-pilot scale fermentations were conducted at 37°C on A.donax hydrolysate [1] supplemented with glucose-free MH medium in a 1:1 (v/v) ratio and run for 48 h. The culture was sprayed with CO2 at 0.1 vvm and the agitation speed was set to 100 rpm. A constant pH of 6.5 was maintained via automated addition of 30% v/v NH_4OH and 30% v/v H_2SO_4. After the batch phase, a concentrated feed of pure glucose and xylose was added to the broth at a rate of 0.5-0.8 g/L·h for about 24 h. Samples were withdrawn from the reactors at regular time intervals for the determination of dry cell weight, substrate consumption and acids production.

Downstream process - Succinic acid recovery

The downstream process applied is illustrated in Figure 1.

Figure 1- SA recovery process.



After post-fermentation ultrafiltration, the broth was decolorized at room temperature by a treatment with activated carbon (amount of 6% with respect to the surnatant), in a tank of 150 L under continuous stirring for 3 h. Then a slurry was passed through a filtering system composed by 15 plates covered by a paper filtering sheet (thickness 720-820 µm), to remove the dispersed activated carbon from liquid. The colourless broth was distilled at 40°C under vacuum to obtain a higher concentration in SA (> 100 g/L). After that, concentrated sulphuric acid was added to the broth to reach a pH of 2.0 Then, the crystals of SA were obtained keeping the solution at 4°C for 24 h and then by filtering through Whatman paper and drying under vacuum at 40°C. After measurement of the SA concentration by HPLC analysis, the yield of SA was determined as follows:

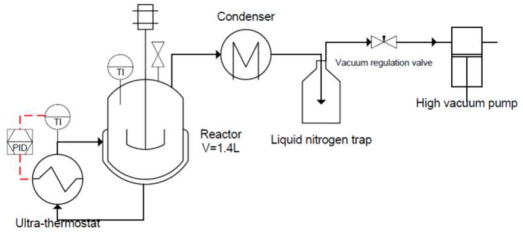
$$Yield\ (\%) = \frac{Succinic\ acid\ in\ the\ recoverd\ crystals\ (g)}{Succinic\ acid\ in\ the\ initial\ fermentation\ broth\ (g)}$$

The purity of recovered SA was determined by comparison with pure SA through DSC analysis.

PBS synthesis

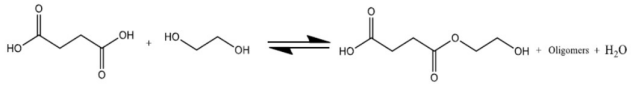
The PBS synthesis has been performed in a 1.4 L stirred tank vessel (see device sketch in Figure 2). The reactor is charged with the desired amount of succinic acid/1,4-butanediol and temperature is adjusted by using a heating oil bath regulated by an ultra-thermostat. The vessel is equipped with a thermocouple, used to measure the temperature inside the reactor, a valve for sample withdrawn/catalyst or reactant loading and a distillation system necessary to remove only the diol or water formed as the reaction proceeds. The refrigerator is then connected to a vacuum trap, loaded with liquid nitrogen, and a vacuum pump, with related regulation valve.

Figure 2- Experimental device for PBS synthesis.

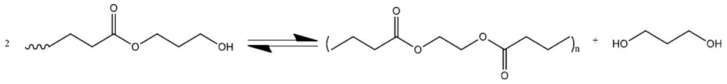


The reaction is performed in two steps [6]:

(i) load of the reactants, in molar ratio SA:1,4- butanediol 1:2, and heating the system at the desired temperature at atmospheric pressure, then waiting 2 h in order to perform the esterification reaction as follows:



(ii) adding Titanium (IV) butoxide (TBT) catalyst and stirring for 6 h under vacuum (10 Pa) to perform the polycondensation reaction as follows:



The product is eventually discharged and quickly quenched in a water bath. By following the described experimental procedure, two different PBS were prepared starting from either commercial or bio-based succinic acid. The details of the adopted experimental conditions are reported in Table 1. The samples were characterized in terms of molecular weight distribution.

Table 1- Summary of the adopted experimental conditions for PBS synthesis.

Test	SA	SA [g]	1,4-butanediol [g]	Catalyst [g]
1	Commercial	236	360	3.6
2	Bio	200	305	3.1

Discussion and Results

Fermentation

Fermentation experiments on pre-pilot scale were performed on diluted A.donax hydrolysate [1] in order to lower the initial concentration of toxic molecules released during the hydrolytic process such as acetic acid

and other phenolic compounds. Overall experiments resulted in a titre of succinic acid ranging between 22 and 25 g/L after 48 h of growth with a yield of consumed C sources of about 0.63±0.3 g/g. These data well compare with previous literature on the development of viable industrial scale processes starting from lignocellulosic economically convenient feed-stocks.

Downstream process- Succinic acid recovery

Succinic acid recovery from real fermentation broth, produced by lignocellulosic feedstock, was carried out. The results, in terms of SA concentration related to each step of the downstream process, are summarised in Table 2. The fermentation broths were treated by decolourization with activated charcoal and distillation. The following losses in SA were observed in each step: 6% of SA was adsorbed on charcoal, 20% was lost in the non-optimized filtration and a part in the crystallization for thermodynamic reasons (about 30 g/L of SA are left in the crystallization surnatants). For the runs reported in Table 2, SA yields were in the range 37-50% while the purity was 98.8- 99.6%. These values of purities were determined by HPLC analysis and confirmed by DSC. These results are very interesting considering the nature of the feedstock, a lignocellulosic waste, which involves a production of a fermentation broth rich in impurities and poor in SA. Moreover, the results highlighted: 1) highly pure SA can be also obtained starting from lignocellulosic biomass; 2) improvements of each step of the downstream process may allow increasing the final yield in SA.

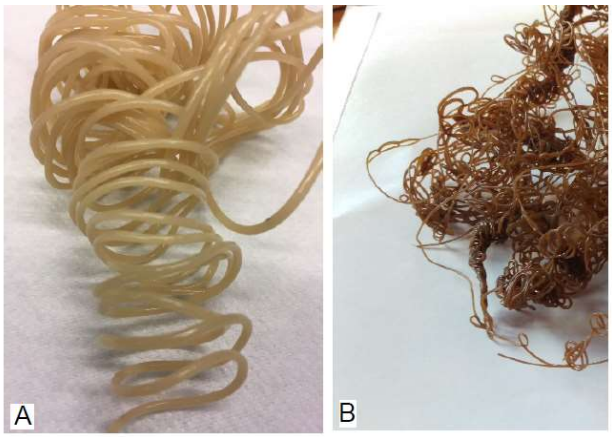
Table 2- SA concentration for each step of the downstream process and yield and purity of final SA.

	Initial		After treatment with carbon and filtration	After evaporation		Final		
Tank	SA concentration [g/L]	Volume [L]	SA Concentration [g/L]	SA Concentration [g/L]	Volume [L]	SA amount [g]	Yield [%]	Purity [%]
1	25.0	40	-	92.5	15	500	50.0	98.8
2	23.4	72	18.9	115.8	14	1000	39.7	98.4
3	26.9	70	25.2	111.6	9	700	37.1	99.6

PBS synthesis

For each experiment, 250 g of PBS were obtained. The synthetized PBS using either commercial or bio-based succinic acid are rather similar in appearance even if the bio-PBS presented a slightly darker color (see Figure 3). The difference of colour can be attributed to the small impurity present in the adopted bio-succinic acid.

Figure 3- PBS samples obtained starting from: A. commercial succinic acid; B. bio-succinic acid.



The two samples were characterized in terms of molecular weight distribution, performing gel permeation chromatography (GPC) analysis. The results are reported in Table 3.

Table 3- GPC analysis results.

Test	M_n [g/mol]	M_w [g/mol]	M_p [g/mol]	PI [-]	μ [Pa·s]
1	15345	60610	55824	3.950	0.5393
2	13364	56922	58794	4.259	0.5491

As it can be seen, the two samples show very similar physical properties in terms of molecular weight, polydispersion index (PI) and intrinsic viscosity. This fact clearly demonstrates that possible bio-succinic acid impurities do not negatively affect the polymerization reaction. Moreover, the system can be considered reproducible and longer reaction times or lower vacuum could lead to products characterized by higher molecular weight and lower PI.

Conclusions

In this work an integrated process based on the biotechnological production of SA, starting from a lignocellulosic biomass (Arundo donax), for the synthesis of PBS was studied and developed. The SA separation and purification procedure was developed and integrated to the fermentation process by a vacuum distillation at pH=4 to remove the volatile byproducts after a treatment with activated charcoal, followed by a final crystallization from mother solution. PBS was synthesized using SA, recovered and purified directly from its fermentation broth through the downstream protocol developed and optimized in this study. The synthesized polymers showed physical properties comparable with a commercial PBS derived by crude oil, so highlighting the suitability of the proposed process.

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