Purification of fermentation broths produced from industrial candy waste towards separation and recovery of bio-succinic acid

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Abstract

Succinic acid is considered as a top value-added platform chemical which can be used for various derivatives such as polymers, cosmetics, component for pharmaceutical and food industries. Several studies have been conducted concerning the production of bio-succinic acid, however, often, they do not use real industrial waste and/or are not able to achieve high concentrations of bio-succinic acid towards making the process viable for economic exploitation In this direction, two fermentation broths (B1 and B2) were produced with the use of different real industrial candy waste streams with the purpose of achieving high succinic acid production, in order to separate it afterwards with methods of purification. This study focuses on evaluating various purification methods and applying different steps to investigate and determine a proper downstream sequence for the examined broths. An appropriate and effective downstream sequence was determined and defined, with succinic acid rejection values ranging from 13.8 % to 17.52 %.

1. Introduction

Up until now, the major production of succinic acid derives through petrochemicals (Cheng *et al*. 2012) which contributes to greenhouse emissions and has multiple implications for the environment. Nevertheless, as climate change is becoming more and more of a concern, environmental restrictions take place to apply more sustainable practices and turn away from petroleum-based material sources to achieve environmental-friendly processes. The development of a cost-effective industrial bio-succinate production through fermentation relies on the utilization of industrial waste streams. Alternative material sources for succinic acid production have become quite popular in the biorefinery framework by utilizing biomass and other process-derived waste materials (Putri *et al.* 2020). They can provide the necessary substrate for its production in an optimal manner which is self-sustaining and not harmful to the environment. In general, the succinic acid separation and purification process for further exploitation, can be divided in the following steps: i) microbial cell removal, ii) concentration and clarification (protein removal), iii) succinic acid separation and concentration and, iv) purification and final crystal formation. In most cases, though, fermentation broths produced using real industrial waste present different and complex compositions and characteristics, which rises the challenge to find suitable purification methods (Kumar et al.) with the purpose of isolating the bio-succinic acid for its further exploitation as a chemical component. Therefore, extensive investigation is required to find an efficient downstream process with several technologies, which is often achieved through membrane separation techniques.

The aim of this research was to examine, identify ways and sequences to purify, separate and recover the bio-succinic acid from the rest of the components in the broths. Different methods, membranes and sequences were tested in order to assess suitable and effective methods to purify these particular real broths, derived from industrial candy waste fermentations.

2. Materials and Methods

2.1 Experimental trials

A series of tests with different steps was executed for the investigation of the two real broths' purification. The process was comprised of trials, starting the investigation from the simplest step, evaluating the effect and then adding more steps accordingly, until a proper sequence was determined to purify the broths. All trials were performed in duplicate. The total tests are presented below in table 1.

Table 1. Different sequence trial tests.

Broth	Trials	Process steps
B1	1	Ultrafiltration (1000 kDa)
B1	2	Centrifugation→Ultrafiltration (1000 kDa)
B1	3	Centrifugation→Activated carbon (2.5% w/w) + Microfiltration (1.2 μm)→ Nanofiltration (150-300 Da)
B1	4	Centrifugation→Activated carbon (2.5% w/w) + Microfiltration (1.2 μm)→Ultrafiltration→ Nanofiltration (150-300 Da)
B2	1	Microfiltration (0.2) → Nanofiltration (150-300 Da)
B2	2	Ultrafiltration (50k Da) → Nanofiltration (150-300 Da)
B2	3	Centrifugation→ Microfiltration (0.2 μm)→ Ultrafiltration (50000 kDa) → Nanofiltration (150-300 Da)
B2	4	Centrifugation→Activated carbon (2.5%) + Microfiltration (1.2 μm)→ Microfiltration (0.2)→Ultrafiltration (50000 kDa)→Nanofiltration (150-300 Da)

2.2 Analytical methods and analysis

Centrifugation of the samples to remove solids was performed with Eppendorf centrifuge 5425 at 10000 rpm for 10 minutes. The membrane screening and testing was performed with the method of dead-end filtration in 15 ml Amicon cells. Prior to each trial, membranes were washed and pre-compacted with ultrapure water to reach constant water permeability (Mancini *et al*. 2022). Pressure was applied with pure N_2 injection in the headspace of the cells which was controlled with a pressure gauge and varied from 0.1 bar to 4 bar depending on the membrane used for each test. Agitation of the samples was

differentiating depending on the volume left in the cell (50-200 rpm).

Membranes mentioned in Table 1 were: 1) Ultrafiltration with Alfa-Laval 1000 kDa and 50000 kDa, 2) Microfiltration with Alfa-Laval 0.2 μm and 3) DK membrane for nanofiltration with 150-300 Da pore size. Prior to the nanofiltration step, pH of the samples was adjusted to 7.0 to retain the succinic acid in the retentate.

For the activated carbon step, Sigma-Aldrich activated charcoal was used, followed by microfiltration 1.2 μm (Whatman Filter pads), with vacuum pump Vacuubrand GMBH – ME1. Samples were collected before, during and after each step for analysis. For the composition analysis, HPLC Shimadzu Nexera XR was used, with HPLC organic Acid Analysis Column Aminex® HPX-87H Ion Exclusion Column (Vigato *et al*. 2022).

3. Results and discussion

Taking into account the complexity (color, microbial cells, proteins and pigments) of the two real broths' composition, the trials seemed to be successful regarding their clarification and purification. The process of adding new downstream steps to retrieve better results after each test was an effective way to evaluate the progress of the process and determine a proper sequence for the purification of the broths. Different steps and higher pore size membranes were tested to improve the performance of the process and remove pigments that negatively affect the filtrations, avoiding the clogging of the membranes. Nonetheless, high membrane fouling, followed by low membrane performance and low permeate flow was observed, even with establishing the full sequence of the investigated steps that effectively clarified the broths, produced from two different organic real waste streams. Minimal rejection of succinic acid was observed, with values of 13.8 % and 17.52 % for B1 and B2, respectively, along with low byproduct (other acids) elimination, due to the use of dead-end filtration mode operation and the inability to achieve higher operating pressures (reached max 4 bar).

4. Conclusions

The investigation of this work lead to some interesting findings regarding the broths' purification that were produced from two different organic real waste streams (industrial candy waste). This work defined and determined an appropriate downstream sequence that was able to clarify (removing color, rejecting pigments, proteins) the complex broths. The low ability, though, to separate and isolate succinic acid from the rest of the byproducts (other acids) suggests that a different operational strategy is required. In particular, the use of crossflow filtration (passing the solution along the surface of a membrane and not vertically like dead-end filtration that was performed in the current work**)** is deemed essential with the addition of recirculation of the liquid for optimal results, along with nanofiltration equipment that can withstand high operating pressures (8-60 bar), where nanofiltration membranes have optimum performance. Afterwards, to retrieve the succinic acid for exploitation, a final step of crystallization is required either by using a crystallizer or a spray drier.

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