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Development of an electrochemical membrane bioreactor for succinic acid production and in situ separation with engineered *Yarrowia lipolytica* cultivated on municipal biowaste hydrolysate

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ABSTRACT

A novel electrochemical membrane bioreactor (EMB) integrating succinic acid (SA) production and in situ separation in the anode compartment through an anion exchange membrane was employed in fed-batch fermentations with *Y. lipolytica* PSA02004 using hydrolysates from the organic fraction of municipal solid waste (OFMSW) as feedstock. The initiation of electrolysis cell operation and the reduction of pH from 6 to 5.5 at 30 h in a 6.7 L bioreactor improved the SA production efficiency, resulting in 66.7 g_{SA}/L, 0.51 g/g yield, 0.78 g/(L·h) productivity, high coulombic efficiency (66.2%) and relatively low electricity consumption for SA separation (2.6 kWh/kg_{SA}). The recirculation of the fermentation broth in the cathode compartment and the OH^- produced by water reduction reduced NaOH consumption (35.4%) for pH control during fermentation. The fermentation was efficiently replicated in a 30 L bioreactor with a low membrane surface area (100 cm^2) electrolysis cell, but it failed with a higher membrane surface area (702 cm^2) electrolysis cell indicating that yeast cell viability, cell design and EMB configuration are important aspects for process scale-up. SA crystals were purified, at 99.95% purity and 95% yield, from the anolyte solution via activated carbon treatment, evaporation, crystallization and drying. Cell removal via centrifugation and acidification stages were not required as in conventional SA purification processes. The produced SA crystals were suitable for the production of polyester polyols for polyurethane urea dispersions applications.

1. Introduction

In 2020, the production of Municipal Solid Waste (MSW) in Europe was ca. 226 million t [1] with the organic fraction (OFMSW) being 30–40% (or 20–80%) [2]. According to the EU Directive 2018/851, the OFMSW contains biodegradable biomass and food waste originated from parks, gardens, offices, households, various catering services and the food industry. The wide variation of free sugars and polysaccharides (30–60%) in the OFMSW depends on the region and the origin of the biowaste [3]. Sugar-rich OFMSW hydrolysates have been used widely for fermentative production of various bio-based chemicals and polymers (e.g. succinic acid, lactic acid, polyhydroxyalkanoate), hydrogen

Abbreviations: OFMSW, Organic fraction of municipal solid waste; MSW, Municipal solid waste; DSP, Downstream separation and purification; IEM, Ion exchange membrane; AEM, Anion exchange membrane; CEM, Cation exchange membrane; EMB, Electrochemical membrane bioreactor; FAN, Free amino nitrogen; IP, Inorganic phosphorous; $k_L a$, Volumetric oxygen mass transfer coefficient; DCW, Dry cell weight; TCA, Tricarboxylic acid cycle; DO, Dissolved oxygen; SA, Succinic acid; AA, Acetic acid; LA, Lactic acid; PUDs, Polyurethane urea dispersions.

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and biogas [2,4–8]. Considering an average 35% of OFMSW content in MSW, 75% moisture content, 50% OFMSW availability for bio-based chemical production and 45% carbohydrate content in OFMSW, around 4.45 million t of sugars would be available as carbon sources for bioprocess development. The widespread availability of this feedstock in EU countries creates an important feedstock for bioprocess development towards bio-based chemical production.

Despite the high prospects of industrial succinic acid production and the significant investment efforts starting around 2009, many of those failed due to the limited industrial interest to use this intermediate into final products [9]. After the bankruptcy of BioAmber, LCY Biosciences acquired its succinic acid production plant in Sarnia (Ontario, Canada) and it is now producing 18,000 t with plans to increase production to 30,000 t [10]. One of the problems that hinders its market growth is the higher market price of bio-based succinic acid ($2.9/kg) than the combined bio- and fossil-based succinic acid ($2.5/kg) [2]. Conventional bioprocesses rely on the utilization of commercial carbon sources (e.g. corn-derived glucose syrup) and nutrients. The utilization of crude hydrolysates derived from agro-industrial side streams could lead to lower ($1.6–1.9/kg) succinic acid production cost [11]. If OFMSW is used as fermentation feedstock, the profitability potential could be enhanced further due to the OFMSW management fee that varies depending on the country and the region ($35–118/t) [12]. In the fermentation and downstream separation and purification (DSP) stages, neutralization of fermentation broth using NaOH or other bases, centrifugation to remove microbial biomass, decoulorisation of the broth using activated carbon and acidification or esterification of the succinate salts produced during fermentation increase the succinic acid production cost [13]. Novel technologies should be developed to minimise the DSP unit operations. Furthermore, engineered yeast cultures (e.g. *Yarrowia lipolytica*) have been developed to produce succinic acid at lower pH than bacterial cultures [14].

Electrochemical membrane extraction in aqueous solutions mediates the transportation of ions across ion-exchange membranes using an electrical potential as driving force. Ion exchange membranes (IEMs) have been extensively used in water desalination and electrolysis processes. Monopolar IEMs consist of crosslinked polymer chains negatively charged in cation exchange membranes (CEM) or positively charged in anion exchange membranes (AEM). The fixed charge in each type of membrane allows for the selective migration of cations in CEMs and anions in AEMs, while the fixed functional groups inhibit the transportation of anions in CEMs and cations in AEMs [15,16]. When an electric current is applied, water splitting electrolysis protons (H^+) are formed at the anode compartment of the electrolysis cell, while simultaneously hydroxide ions (OH^-) are formed at the cathode compartment [17,18]. By applying a constant current between the cathode and the anode chambers (e^- from the anode to the cathode), an equivalent charge flux of anions must be transferred through the anion exchange membrane (AEM) [19]. The following reactions occur [17,20,21]:

Cathode (water reduction): $H_2O + e^- \rightarrow ½\ H_2 + OH^-$
Anode (water oxidation): $½\ H_2O \rightarrow e^- + H^+ + ½O_2$

An integrated electrochemical membrane bioreactor (EMB) could be used for simultaneous organic acid production and in situ separation. The fermentation broth is continuously recirculated through the cathode compartment of the membrane electrolysis cell where the carboxylate product is dissociated due to OH^- generation and, thus, it can be transferred through an AEM into the anolyte by the charge of balance forces. At the anode compartment, the pH is low (~1.5) because of the electrochemical production of H^+, and carboxylates are protonated (undissociated) [22,23]. In this integrated system, the metabolic efficiency of some microorganisms could be enhanced due to direct or indirect provision of reductive energy into the metabolic pathway, the productivity could be increased due to organic acid removal from the broth and the fermentation pH could be controlled minimizing base addition (e.g. NaOH) for pH regulation [23,24]. Furthermore, carboxylate acidification and concentration in the anode compartment could minimise DSP stages as cell removal and acidification are no longer needed.

Membrane electrolysis has been previously utilized to extract volatile fatty acids from acidogenic fermentation as well as lactic acid and succinic acid from bacterial fermentations [23–28]. Xu et al. [23] used a membrane electrolysis cell to separate medium chain carboxylic acids with *n*-caproic acid and *n*-caprylic acid being more than 90% of the oil composition the production of which reached 6.8 mL/day. Recovery of crystalline succinic acid from pH neutral aqueous solutions by electrochemical extraction, back-extraction and crystallization steps have reached a purity higher than 94% [29]. However, in this case, a cation exchange membrane was used preventing the simultaneous separation of succinic acid from the fermentation broth [29]. In a similar manner, Gausmann et al. [30] achieved more than 99% itaconic acid purity by extracting the itaconic acid from fermentation broth with an electrochemical extraction process.

An EMB using AEM has been employed in succinic acid production from spent sulphite liquor via fermentation using the bacterial strain *Basfia succiniciproducens* leading to 45% higher succinic acid production, 15% higher yield, 32% higher productivity and 19.3% lower NaOH consumption than conventional cultures [24]. However, the highest coulombic efficiency of succinic acid separation was only 12–13% and 16% when glucose- and xylose-based media were used, respectively [24]. This is attributed to the presence of competing anion species (e.g., bicarbonate) in the fermentation broth due to CO_2 supply during bacterial cultures. Similarly, Pateraki et al. [31] evaluated the effects of a cathodic bioelectrochemical system on metabolic response and succinic acid production by the bacterial strain *Actinobacillus succinogenes*, improving succinic acid production (36.4 g/L) and productivity (0.81 g/(L·h)) by 18% and 20%, respectively. The oleaginous yeast strain *Rhodosporidium toruloides* was evaluated in cathodic and anodic electrofermentation at different pO_2 values, showing improved microbial oil production and increased (35%) saturated fatty acid content [32].

This work presents the implementation of the EMB for integrated succinic acid production and separation using a genetically engineered *Y. lipolytica* strain cultivated on OFMSW hydrolysates. The operation of the electrolysis cell has been evaluated using synthetic organic acid solutions and different pH values. A dual pH strategy has been employed to enhance succinic acid production efficiency in the EMB. Fed-batch fermentations in the EMB were carried out in 6.7 L and 30 L scale followed by purification of succinic acid crystals, which were subsequently evaluated for the production of polyester polyols as building blocks for polyurethane urea dispersions (PUDs). The novelty of this study lies on the demonstration that the proposed EMB system enhances succinic acid production efficiency with simultaneous succinic acid separation, acidification and concentration in the anode compartment at high coulombic efficiency, while the required purity of succinic acid crystals can be achieved with lower DSP stages than conventional processes.

2. Materials and methods

2.1. Raw material

The OFMSW hydrolysate used in the present study was kindly provided by IMECAL S.A. (Valencia, Spain). The hydrolysate contained 84 g/L carbon source concentration (4.3%, sucrose, 73.1% glucose, 18.4% fructose, 0.9% mannose, 1.8% arabinose, 0.3% galactose and 1.2% glycerol), 650 mg/L free amino nitrogen (FAN) and 550 mg/L inorganic phosphorous (IP). The production of OFMSW hydrolysate and its composition have been presented by Stylianou et al. [3].

2.2. Electrochemical membrane extraction of SA from synthetic solutions

The impact of pH, current density and membrane selection on the

efficiency of the electrochemical membrane extraction of pure commercial succinic acid from synthetic broths and the competition between succinic acid and fermentation by-products (acetic acid) was evaluated in a membrane electrolysis cell with a membrane area of 7.5 cm^2. The pH and current densities were selected as an upper and lower limit of the actual conditions applied in the integrated bio-electrochemical fermentation. The anode compartment was fitted with Ir MMO coated Titanium electrode (1 cm^2) and the cathode compartment was fitted with a stainless-steel mesh cathode (7.5 cm^2). The catholyte was a synthetic fermentation broth (2 L) which was continuously recirculated through the cathode compartment. Two different types of membranes were compared, AEM from Membrane International (MI 700S) and AEM from Fujifilm (Fujifilm AEM type II). In order to investigate the effect of the presence of fermentation by-products on the efficiency of succinic acid separation, tests were carried out with a synthetic broth that contained succinic acid (10 g/L), acetic acid (8 g/L) and yeast extract (10 g/L). The pH of the catholyte was adjusted with NaOH to the pH of each experiment. A H_2SO_4 (3 g/L) solution (0.2 L) was used as anolyte that was continuously recirculated through the anode compartment. The separation was performed by applying a constant current density (1.5 mA/cm^2). Samples were regularly collected from the anode compartment for analysis of succinic and acetic acids. The separation tests were performed in a temperature-controlled room at 28 °C.

2.3. Microorganism and inoculum preparation

The engineered *Yarrowia lipolytica* PSA02004 yeast strain was employed for succinic acid production that has been adapted to efficiently consume glucose [33]. The yeast was preserved in cryopreservation vials at –80 °C containing liquid culture and 50% (v/v) pure glycerol. The preculture medium contained 20 g/L glycerol, 10 g/L yeast extract, 20 g/L tryptone and 20 mM disodium hydrogen phosphate (Na_2HPO_4). Inoculum was prepared by the addition of 1 mL liquid from a cryopreservation vial in 500 mL Erlenmeyer flasks containing 100 mL preculture medium. The inoculum was cultivated at 28 °C and 220 rpm in an orbital shaker incubator for 21–24 h until a yeast cell concentration of 13 – 15 g/L was reached.

2.4. Fed-batch fermentations in lab-scale EMB

Fed-batch fermentations were conducted in a 6.7 L bench-top bioreactor (Bioengineering, RALF Advanced) with 3 L initial working volume. The fermentation media contained either glucose or OFMSW hydrolysate with 120 g/L initial carbon source concentration. In the case of glucose-based fermentations, the fermentation medium was supplemented with 10 g/L yeast extract, 20 g/L tryptone and 20 mM phosphate buffer. When OFMSW hydrolysate was used, yeast extract was supplemented depending on the free amino nitrogen (FAN) content of OFMSW hydrolysate (approximately 10 g/L) in order to adjust the initial FAN concentration to around 900 mg/L. Fermentations were carried out at 28 °C, 1 vvm aeration and 10% (v/v) inoculum addition, corresponding to 1.3 – 1.5 g/L of initial concentration of yeast cells. The agitation and aeration rate were 600 rpm and 1 vvm, respectively, corresponding to a $k_L a$ value of 183.2 h^{-1}. The $k_L a$ was estimated using the methodology presented by Aroniada et al. [34]. The following two pH strategies were employed during fermentation: 1) constant pH at 6 and 2) initial pH set at 6 and gradual reduction to 5.5 at 30 h or 40 h fermentation. The gradual reduction of fermentation pH to 5.5 occurred naturally. The recirculation of the fermentation broth through the catholyte and the operation of the electrolysis cell was initiated at 30 h or 40 h. From that point onwards the fermentation pH was controlled at pH 6.0 or 5.5 by the OH^- produced in the cathode compartment supplemented with 10 M NaOH when necessary. In the fermentations conducted with commercial glucose, the feeding solution used contained 700 g/L glucose and 10 g/L yeast extract in order to maintain the sugar concertation at 20–60 g/L in the broth. When fermentations were carried out with OFMSW hydrolysate, a concentrated OFMSW hydrolysate was used with 600 g/L glucose concentration. The OFMSW was concentrated in a rotary evaporator operated under vacuum. Carbon and nitrogen sources were sterilized separately from the rest of the medium at 121 °C for 20 min prior to fermentation.

The electrolysis cell contained two compartments as described by Pateraki et al. [24]. The cathode chamber (Length × Width × Depth: 20 cm × 5 cm × 2.5 cm) was separated by the anode chamber (L × W × D: 20 cm × 5 cm × 1 cm) with a single AEM (AMI 7001, Membrane International, New Jersey, United States) with the following operating surface dimensions L × W × Thickness: 20 cm × 5 cm × 0.3 cm with 100 cm^2 working surface area. The anode compartment was fitted with a titanium electrode (20 cm × 5 cm) coated with iridium oxide (IrO_2/TaO_2: 0.65/0.35, Magneto, The Netherlands), while the cathode compartment was fitted with a stainless-steel mesh cathode (20 cm × 5 cm). Two spacers (ElectroCell A/S, Denmark) were placed between the electrodes' surface and the AEMs to prevent convection at the membrane surface. The electrodes had a distance of ca. 1 cm. The electrolysis cell was sterilised alone at 121 °C for 15 min and it was aseptically connected to the bioreactor. During sterilisation, the cathode compartment contained a 20 mM phosphate buffer solution and the anode compartment contained 10 mM K_2HPO_4 solution.

The fermentation broth was recirculated through the cathode compartment with a peristaltic pump at a constant flowrate of 3 L/h. The solution of the anode chamber was recirculated at a flowrate of 3 L/h through an external vessel with 0.5 L working volume. The extraction was performed by applying a constant current (0.57 A) and the voltage was monitored during fermentation. The temperature of the extraction vessel was ambient. A power supply unit (PL-3003D, Protek, United States) was used to apply current to the system. Samples of the fermentation broth and anolyte were periodically collected for analysis of organic acids. When the concentration of succinic acid in the extract reached 50–60 g/L it was replaced with new extract solution at the same volume, because the solubility of succinic acid is 58 g/L at 20 °C.

A fed-batch fermentation was also carried out in the 6.7 L bioreactor (Bioengineering, RALF Advanced) with 3 L initial working volume using an electrolysis membrane cell with 702 cm^2 surface area. The anode compartment was fitted with an Ir MMO coated Titanium electrode (100 cm^2), while the cathode compartment was fitted with a stainless-steel mesh cathode (100 cm^2). The anolyte was 1 L solution of 10 mM K_2HPO_4. The extraction was performed by applying a constant current of 0.57 A.

Samples were taken from the fermentation broth and the anolyte at regular intervals to determine the production of extracellular metabolites (e.g. organic acids, exopolysaccharides) and DCW as well as the consumption of sugars, free amino nitrogen (FAN) and inorganic phosphorus (IP). Fermentation samples were also observed under the microscope to ensure that there was no contamination during fermentation.

2.5. Fed-batch fermentation in 30 L EMB

Fermentations were also carried out in a 30 L bioreactor (Infors HT, Techfor-S, 30 L) with 10 L initial working volume using, as scale-up parameter, the same $k_L a$ value (183.2 h^{-1}) employed in the 6.7 L bench-top bioreactor. The same fermentation conditions were employed as described in the lab-scale fermentations. A two-stage pH regulation strategy was applied starting with pH 6 that was naturally reduced to 5.5 at 30 h, when the operation of the electrolysis cell was also initiated. The initial fermentation medium contained OFMSW hydrolysate with 120 g/L carbon sources. The feeding solution contained 700 g/L glucose and 10 g/L yeast extract and was added in pulses in order to maintain the sugar concentration in the fermentation broth at 20–60 g/L. Carbon and nitrogen sources were sterilized separately from the rest of the medium at 121 °C for 20 min prior to fermentation.

The 30 L bioreactor was integrated with either the low membrane

surface area (100 cm^2) or the high membrane surface area (702 cm^2) electrolysis cell. When the low membrane surface area electrolysis cell was used, the volume of the anolyte (10 mM K_2HPO_4 solution) was 1.6 L and the current supply was 0.57 A (5.7 $mA{\cdot}cm^{-2}$) until 122.5 h, which was thereafter increased to 1.12 A (11.2 $mA{\cdot}cm^{-2}$). When the high membrane surface area electrolysis cell was used, the volume of the anolyte was 2 L and the applied current was either 3 A or 0.57 A.

2.6. Downstream separation and purification of succinic acid

The extract solutions from the anode compartment of EMB fermentations carried out with OFMSW hydrolysates were used for the purification of succinic acid crystals following the methodology presented by Alexandri et al. [13]. The extract solution was mixed for 1 h at ambient temperature with 12.5% (w/v) activated carbon. The activated carbon was initially hydrated with double distilled water (ddH_2O) in order to decrease the succinic acid losses during anolyte solution treatment. The suspension was then filtered through a Whatman membrane filter (0.2 μm). The extract solution from the anolyte that contained 50–60 g_{SA}/L, 5–10 g_{AA}/L and 2–6 g_{LA}/L was concentrated to approximately 25% of the initial volume at 60 °C using a rotary vacuum evaporator. The crystallization step of succinic acid was carried out at 4 °C for 24 h. The succinic acid crystals were carefully washed with a cold saturated solution of succinic acid to remove impurities at 4 °C. The crystallization and water washing of SA crystals was repeated 6–10 times in order to increase the crystal purity. The IP content in the SA crystals was determined in each crystallisation/washing cycle. The obtained succinic crystals were dried at 70 °C for 24 h.

2.7. Scanning electron microscopy

The untreated membrane and the membrane after its utilization were cut into small pieces and were dried with freeze drying and sputter-coated with gold. Samples were observed and digitally photographed in a Jeol 6360 Scanning Electron Microscope at 15 kV.

2.8. Polyester polyol synthesis

Polyester polyols were synthesized according to a previously published procedure [35]. During the synthesis, the acid number was monitored to follow the progress of the reaction. The hydroxyl number of the resulting polyol was measured according to DIN 53240-1 to determine the average molecular weight.

2.9. Polyurethane urea dispersion synthesis

The polyester polyols were used in PUD synthesis according to a previously published synthesis route [35]. The reaction was monitored by measuring the decrease of free NCO groups by titration. The resulting PUDs were characterized by their average particle sizes via dynamic light scattering, their molecular weight via gel permeation chromatography, their viscosity and their non-volatile content.

2.10. Analytical methods

Biomass was estimated by measuring dry cell weight (DCW). Specifically, cells were collected by centrifugation (9000 × g, 10 min, 4 °C) and washed twice with deionized water. The sediment was dried at 100 °C for 24 h and then the sample was placed in a desiccator until constant weight. IP [36] and FAN [37] were determined with spectrophotometric methods. Sugars and organic acids were determined using a Shimadzu HPLC system with a Shimadzu RI detector and a Rezex ROA-Organic acid H^+ column. The temperature of the column was 65 °C and the mobile phase was 10 mM H_2SO_4 aqueous solution at 0.6 mL/min flow rate. Monosaccharides were also determined with a Shodex SP0810 (8.0 × 300 mm) column. The temperature of the column was 80 °C and the mobile phase was HPLC grade water at a flow rate of 1 mL/min.

Exopolysaccharides (EPS) were determined by the method described by Levander et al. [38]. Briefly, biomass was removed by centrifugation and the supernatant was treated with 12% TCA for protein precipitation followed by centrifugation. The supernatant was placed in microcon M−30 ultracentrifugation filter (Millipore) and centrifuged. The sample was washed twice and the EPS content was determined by drying the filter.

3. Results and discussion

3.1. Electrochemical membrane extraction of succinic acid

Pure commercial organic acids were used to evaluate the extraction efficiency in the electrolysis cell at varying pH, current densities and two membranes. The two tested membranes showed similar coulombic efficiencies when extracting towards an anolyte devoid of succinic acid (Fig. 1). The coulombic efficiency increased from 38.6 ± 9.7% to 75.2 ± 14.8% with the current density in the range 1.5–7.5 mA cm^{-2} when using the MI AEM, while it increased to a maximum of 79.9 ± 0.3% at 12.5 mA cm^{-2} with the AEM Fujifilm type II. The presence of succinic acid in the anolyte (10 g/L) negatively impacted the efficiency of the separation, with a maximum coulombic efficiency of 57.3 ± 0.7% observed at a current density of 7.5 mA cm^{-2} (Fujifilm type II AEM).

The pH of the catholyte in the range 4.9–5.8 did not have a significant impact on the separation of succinic acid and acetic acid (Fig. 2A), even though the pH affects the speciation of both succinic acid and acetic acid. On the other hand, high current densities had a positive effect on the fluxes of succinic and acetic acids, and the coulombic efficiencies increased in a non-proportional way, i.e. the flux of succinic

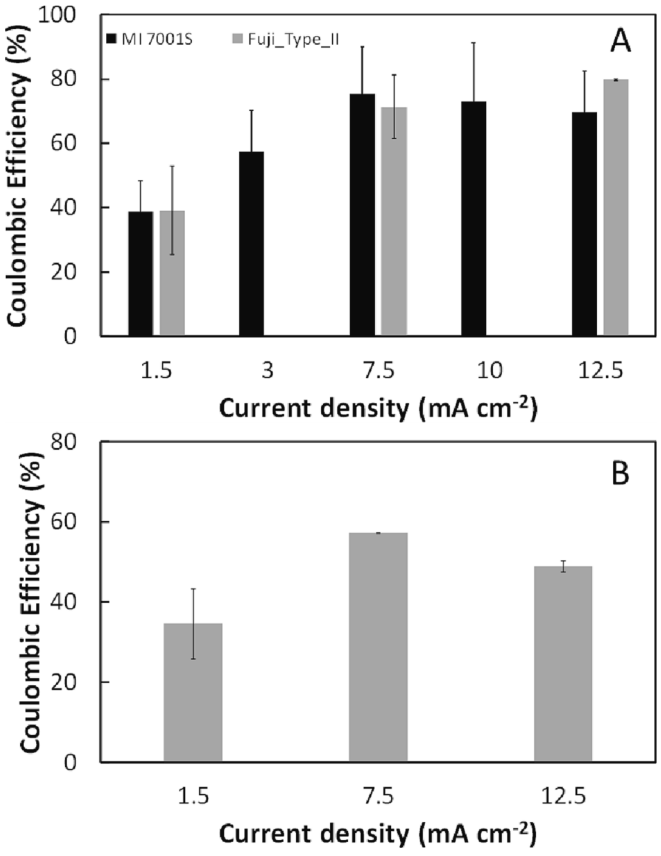

Fig. 1. Coulombic efficiency of succinic acid separation from a synthetic catholyte, without (A) and with (B) succinic acid in the extract, using the MI700S AEM and Fujifilm type II AEM.

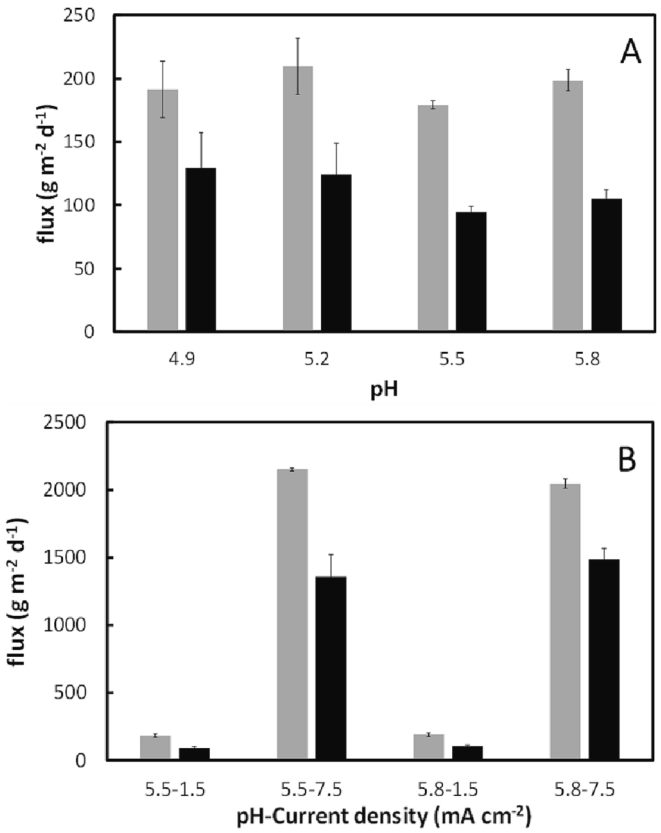

Fig. 2. Flux of succinic (grey) and acetic (black) acids as affected by the catholyte pH (A) and by the current density (B) when using Fujifilm type II AEM.

acid increased by 14-fold after an increase in current density of only 5-fold (Fig. 2B). As a consequence of this increase in flux, the coulombic efficiency increased by 3-fold (Fig. 3). The coulombic efficiency for succinic acid extraction increased from 11 to 27% and from 11.9 to 25.4%, at pH 5.5 and 5.8, respectively, when increasing the current density from 1.5 to 7.5 mA cm^{-2}. Acetic acid was extracted at slightly lower rates at the higher pH values investigated. The coulombic efficiency for separation of acetic acid was also higher at high current densities, and was extracted with coulombic efficiencies of 33.7 and 36.8% at pH 5.5 and 5.8, respectively (Fig. 3).

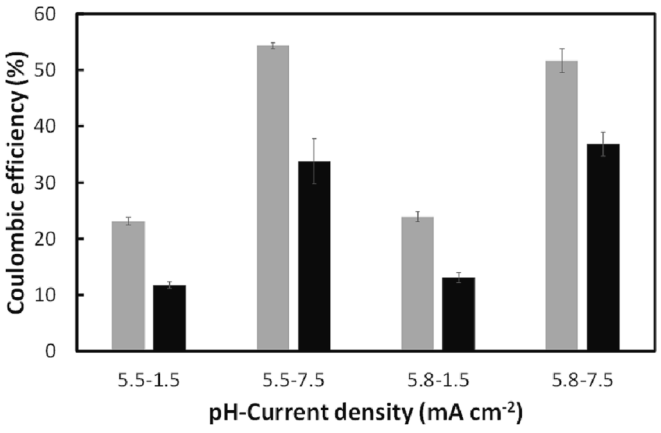

Fig. 3. Coulombic efficiency of the separation of succinic (grey) and acetic (black) acids as affected by the fermentation pH and current density, when using Fujifilm type II AEM.

3.2. Fed-batch fermentations on synthetic media at constant pH 6 in the EMB

Fermentations in the EMB with the genetically engineered *Y. lipolytica* PSA02004 were initially carried out in glucose-based media by applying the current supply (0.57 A) at the beginning of fermentation. The yeast cells were severely stressed and cell death occurred within a few hours (results not presented). Pateraki et al. [24] presented the successful production of succinic acid by the bacterial strain *B. succiniciproducens* using the same EMB where the current was applied since the beginning of fermentation. It should be stressed that *B. succiniciproducens* utilizes the reductive branch of the tricarboxylic acid (TCA) cycle for succinic acid production, while the engineered *Y. lipolytica* strain utilizes the oxidative TCA cycle. The yeast strain *Y. lipolytica* may have been stressed by the excess electrons provided via the electrolysis cell during cell growth. Furthermore, *Y. lipolytica* is an obligatory aerobe and the retention time in the cathode compartment of the electrolysis time may have led to cell death. The following fermentations were carried out by integrating the electrolysis cell with the bioreactor at a later fermentation stage.

Fig. 4A and 4B present the experimental results from a fed-batch fermentation carried out at constant pH 6 using glucose-based media where the electrolysis cell was activated at 40 h at the same current supply as above (0.57 A). The pH was regulated solely via NaOH addition up to 40 h, while thereafter it was regulated by combined NaOH addition and hydroxyl groups produced in the cathode compartment. The extract solution (500 mL) was replaced 4 times (Fig. 4B). *Y. lipolytica* produced acetic acid until 40 h, while when the electrolysis cell was activated, the acetic acid was initially extracted in the anode compartment in the first cycle and gradually disappeared after 80 h from the fermentation broth (Fig. 4A). The initial FAN concentration was 916 mg/L, while ca. 772 mg/L were consumed until 50 h after which the FAN concentration remained constant at ca. 140 mg/L. The biomass concentration was increased alongside succinic acid production reaching the highest value (37 g/L) at 88.5 h, while it was 23.6 g/L at 40 h when the electrolysis cell was activated. The yeast did not produce any EPS.

Succinic acid concentration reached 29.8 g/L at 40 h (Fig. 4A), while thereafter succinic acid was simultaneously produced by *Y. lipolytica* (Fig. 4A) and extracted in the anode compartment of the electrolysis cell (Fig. 4B). After 88.5 h, the succinic acid concentration followed a declining trend in the fermentation broth, while succinic acid was constantly extracted in the anode compartment at almost the same rate, 92.1 g/(m^2·h), at all extraction cycles (Fig. S1A). The extraction rate remained stable at all extraction cycles and membrane fouling was not observed (Fig. 7). At 88.5 h, the overall succinic acid production reached 214.2 g (Fig. 5A) with 0.34 g/g yield and 0.8 g/(L·h) productivity (Table 1). If the extracted mass of succinic acid (Fig. 4B) is added to that remained in the fermentation broth (44.5 g/L, Fig. 4A), then the overall succinic acid concentration in the fermentation broth would have been 71 g/L (Table 1). Stylianou et al. [2] presented fed-batch bioreactor fermentations of *Y. lipolytica* PSA02004 cultivated on the same glucose-based media used in this study. When the fermentation pH was controlled at 6, then 51 g/L succinic acid with 0.34 g/g yield and 0.63 g/(L·h) productivity were achieved at approximately the same fermentation duration (81 h) as the one mentioned above (Table 1), corresponding to 163.2 g succinic acid (Fig. 5A). The highest productivity and yield were considered as the most important criteria for selecting the fermentation time for comparison purposes. Thus, the EMB used in this study resulted in 39% higher succinic acid concentration and 27% higher productivity than the conventional culture. The yield remained constant indicating that in this case the power supply did not have an impact on the metabolism of *Y. lipolytica* as in the case of *B. succiniciproducens* [24]. The increased productivity should be attributed to the removal of organic acids from the fermentation broth.

The coulombic efficiency for organic acid separation were calculated

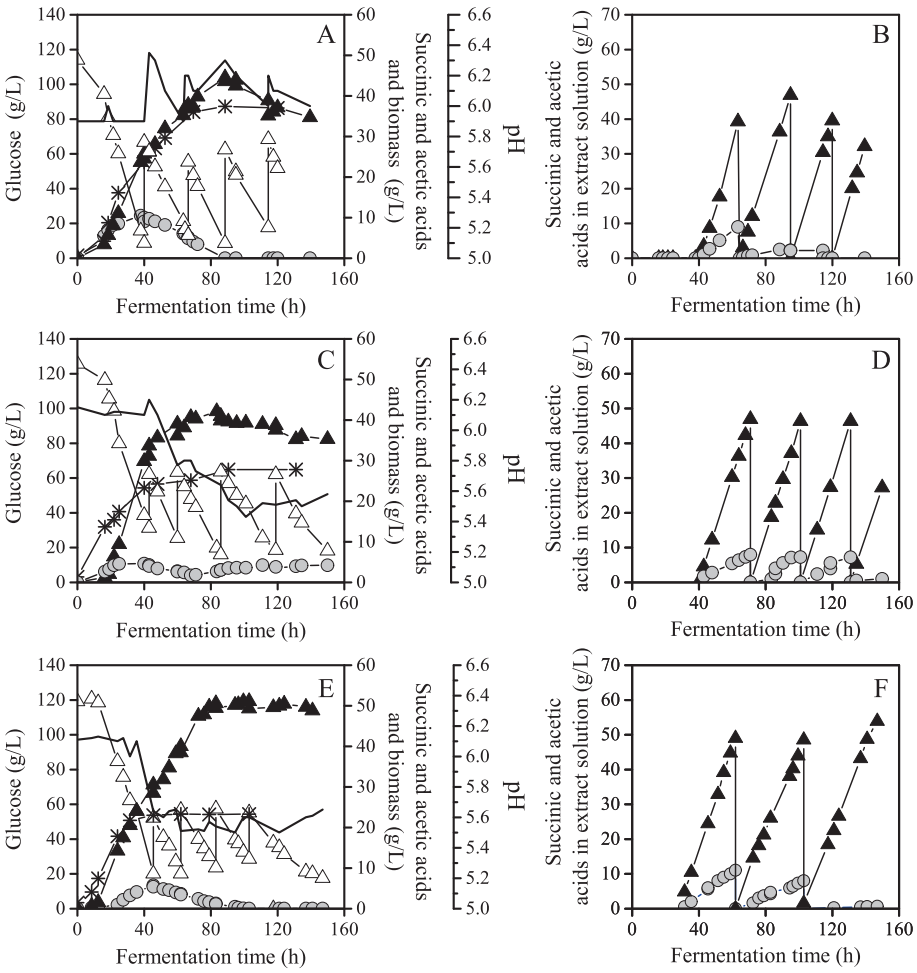

Fig. 4. Succinic acid (▲), acetic acid (●) and yeast biomass (*), glucose (△), pH (black line) in the bioreactor (A, C, E) and the anolyte extract solution (B, D, F) during fed-batch EMB fermentations under different pH strategies with glucose as carbon source. Constant pH 6 with initiation of electrolysis cell operation at 40 h (A and B), initial pH 6 gradually dropped to 5.5 with initiation of electrolysis cell operation at 40 h (C and D), initial pH 6 gradually dropped to 5.5 with initiation of electrolysis cell operation at 30 h (E and F).

on average during the separation period. The coulombic efficiencies achieved were 66.1% in the case of succinic acid and 9.3% for acetic acid (Table 2). The fluxes that were achieved through membrane electrolysis to the extract solution were 92.1 g/(m^2·h) for succinic acid and 8.4 g/(m^2·h) for acetic acid (Table 2). The extraction rate could be improved by increasing the input current density.

The same fermentation was carried out at higher current of 1 A (or 10 mA cm^{-2}) (Fig. S2). The succinic acid production until 40 h was 27 g/L with a productivity of 0.61 g/(L·h). However, when broth recirculation through the anode compartment was initiated at 1 A applied current, succinic acid production was reduced and finally stopped a few hours after the integrated system was activated. Higher current had a positive effect on the flux of succinic acid, 116.9 g/(m^2·h), through the electrolysis membrane resulting in 29.6% higher extraction rate. The sudden end of the fermentation at around 45 h could be attributed to the application of higher current, which negatively affected the viability of *Y. lipolytica*.

3.3. Fed-batch fermentations on synthetic media with two-stage pH regulation in the EMB

Stylianou et al. [2] showed that reducing the pH from 6 to 5.5 at 40 h or 30 h fermentation using the same *Y. lipolytica* PSA02004 strain leads to higher productivity and yield as well as reduced NaOH consumption for pH regulation (Table 1). The two-stage pH regulation strategy leads to optimum growth conditions at pH 6, while at pH 5.5, lower than the $pK_a2 = 5.6$ of succinic acid, up to 30% lower NaOH consumption for pH regulation is achieved.

Fig. 4C and 4D present the results obtained during fed-batch fermentation carried out at initial pH 6 followed by gradual reduction to 5.5 at 40 h, when the operation of the electrolysis cell was also initiated. In this case, the final overall succinic acid concentration was 60.6 g/L at 71 h (Table 1), considering the succinic acid concentration in the broth (Fig. 4C) and the mass of succinic acid extracted in the anode compartment (Fig. 4D), with a yield of 0.43 g/g and productivity of 0.87 g/(L·h). The yield and productivity were improved by 26% and 9%, respectively, as compared to the fermentation conducted at constant pH 6 (Fig. 4A and 4B). The application of the electrolysis cell resulted in 20.2% higher succinic acid concentration, 5% higher yield and 26% higher productivity than the conventional fed-batch fermentation carried out under the same pH regulation strategy (Table 1) without the electrolysis cell [2]. The acetic acid concentration was 8.5 g/L and maximum biomass concentration reached 27.8 g/L. Low cell growth was observed after 40 h (Fig. 4C) contrary to the fermentation carried out at constant pH 6 where cell growth was observed until approximately 80 h.

In the two-stage regulation strategy implemented at 40 h, the coulombic efficiency of succinic acid (82.7%) and acetic acid (10%) were higher than the respective values obtained in the fermentation carried out at constant pH 6 (Table 2). The average extraction rate of succinic acid was 102.2 g/(m^2·h) (Table 2, Fig. S1B). Acetic acid production was observed until 40 h (Fig. 4C), while after this point simultaneous separation through the electrolysis membrane (Fig. 4D) and production via fermentation were observed. The extraction rate of acetic acid was significantly lower, 17.8 g/(m^2·h), than the succinic acid extraction rate (Fig. S1B).

Application of the electrolysis cell and the two-stage pH strategy at an earlier fermentation stage (30 h) resulted in further improvement of fermentation efficiency (Fig. 4E and F). Specifically, the final succinic

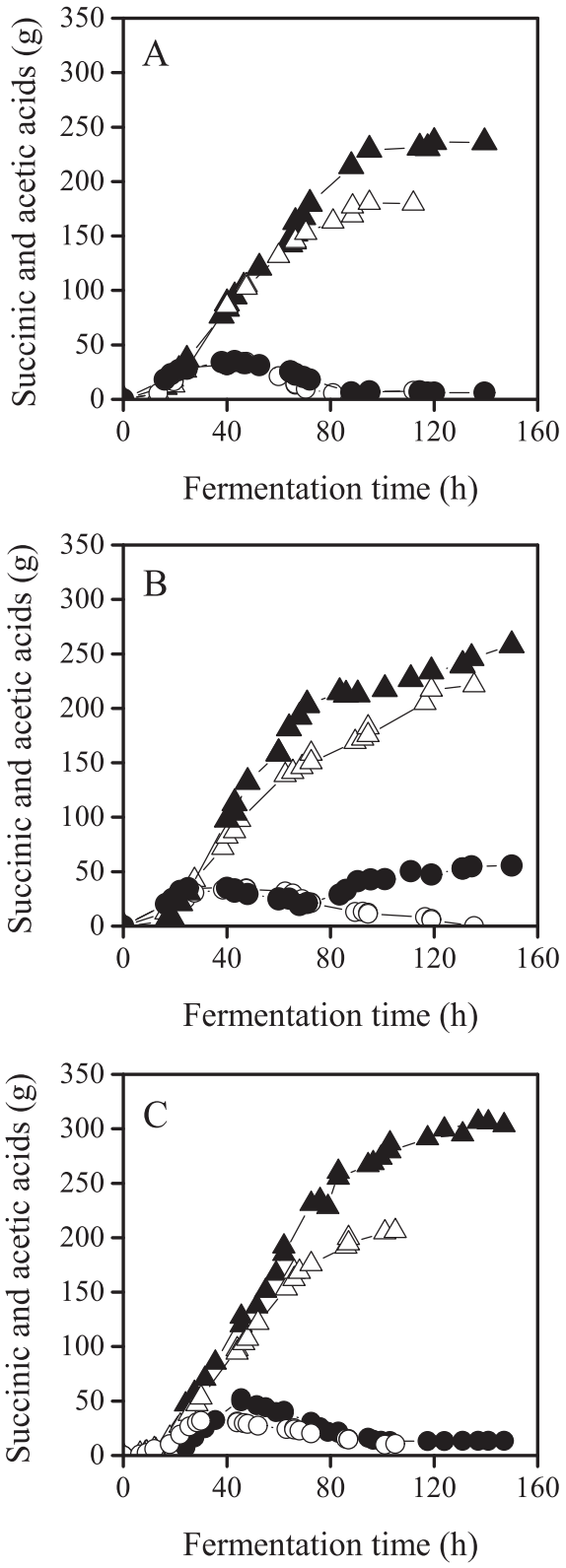

Fig. 5. Production of succinic and acetic acids during fed-batch fermentations under different pH strategies using glucose as carbon source either with electrolysis cell operation (filled symbols) or without electrolysis cell operation (unfilled symbols). Constant pH 6 with initiation of electrolysis cell operation at 40 h (A), initial pH 6 gradually dropped to 5.5 with initiation of electrolysis cell operation at 40 h (B), initial pH 6 gradually dropped to 5.5 with initiation of electrolysis cell operation at 30 h (C). Succinic acid (triangle), Acetic acid (circle).

acid concentration was 70.3 g/L with a yield of 0.48 g/g and a productivity of 0.97 g/(L·h). These results correspond to 16%, 11.6% and 11.5% improvement in final succinic acid concentration, yield and productivity, respectively, as compared to the experiment that the electrolysis cell and the two-stage pH regulation was initiated at 40 h (Fig. 4C and D). Compared to the fed-batch fermentation carried out at constant pH 6 throughout fermentation and application of the electrolysis cell at 40 h (Fig. 4A and B), 41% higher yield and 21% higher productivity were achieved. By applying two-stage pH regulation at 30 h and simultaneously integrating the electrolysis cell into the bioreactor, total succinic acid production reached 260.5 g, while total acetic acid production reached 22 g (Fig. 5C). Decreasing the pH gradually to 5.5 at 30 h led to reduced yeast cell growth (23.3 g/L) (Fig. 4E). The coulombic efficiency and extraction rate of succinic acid were 78.7% and 80.6 g/(m^2·h), respectively, while for acetic acid they were 14.9% and 11.9 g/(m^2·h), respectively (Table 2, Fig. S1C).

3.4. Fed-batch fermentations using OFMSW hydrolysate in the EMB

A fed-batch fermentation at constant pH 6 was initially carried out on OFMSW hydrolysate coupled with the electrolysis cell at 40 h (Fig. 6A and 6B) leading to 59.7 g/L succinic acid concentration with 0.4 g/g yield and 0.67 g/(L·h) productivity. OFMSW hydrolysate was supplemented with yeast extract to reach 890 mg/L initial FAN concentration. FAN consumption occurred until 45 h and remained constant at around 150 mg/L until the end of fermentation. The extract solution in the anode compartment was changed 4 times and the succinic acid, acetic acid and lactic acid average extraction fluxes were 79.5 g/(m^2·h), 2.0 g/(m^2·h) and 3.2 g/(m^2·h), respectively (Table 2, Fig. S3A). The extraction rate remained stable at all extraction cycles and membrane fouling was not observed (Fig. 7). Lactic acid was contained in the OFMSW hydrolysate and it was not produced during fermentation. The average coulombic efficiency was 73.5%, 7.3% and 3% for succinic acid, acetic acid and lactic acid, respectively (Table 2). The same fed-batch fermentation at pH 6 was also carried out by initiating the operation of the electrolysis cell at 30 h fermentation resulting in 47.5 g/ L succinic acid concentration with 0.44 g/g yield and 0.67 g/(L·h) productivity (Table 1). The overall succinic acid production efficiency achieved in this culture was similar to the one achieved in the fermentation where the operation of the electrolysis cell was initiated at 40 h (Table 1). The average coulombic efficiency for succinic acid (56.8%) and the succinic acid extraction flux (76.6 g/(m^2·h)) were lower than the fermentation where the operation of the electrolysis cell was initiated at 40 h (Table 2).

Fig. 6C and 6D present the fed-batch fermentation results carried out with OFMSW hydrolysate where the two-stage pH regulation and initiation of electrolysis cell operation at 40 h were implemented. This fermentation resulted in 47.4 g/L succinic acid with a yield of 0.44 g/g and a productivity of 0.67 g/(L·h) (Table 1). The final acetic acid concentration was 7.7 g/L. Fig. 6E and 6F present the fed-batch fermentation results carried out with OFMSW hydrolysate where the two-stage pH regulation strategy and the operation of electrolysis cell was initiated at 30 h. The final succinic acid production was 66.7 g/L with 0.78 g/(L·h) productivity and 0.51 g/g yield, which are higher than all previous fermentations carried out on OFMSW hydrolysate (Table 1). The succinic acid concentration and yield were improved by 23% and 16%, respectively, as compared to the culture conducted under the same operating conditions without the integration of the electrolysis cell (Table 1). The coulombic efficiency and the extraction flux for succinic acid achieved in this fermentation was 66.2% and 90 g/(m^2·h) (Fig. S3D), respectively (Table 2).

Fig. 8 presents the total mass production of succinic and acetic acids during fed-batch fermentations carried out on OFMSW hydrolysate with or without electrolysis cell operation under different pH strategies. It is obvious that the application of the integrated system and the two-stage pH regulation strategy increased the overall mass production of succinic

Table 1
Succinic acid production efficiency by metabolic engineered *Yarrowia lipolytica* PSA02004 integrated with the electrochemical system.

Substrate	pH	Current ON	Succinic acid (g/L)	Acetic acid (g/L)	Yield[1] (g/g)	Productivity (g/(L·h))	Literature
Glucose	Regulated at 6	40 h	71.0	0.47	0.34	0.80	This study
	Two-stage 6 to 5.5 (40 h)	40 h	60.6	8.5	0.43	0.87	
	Two-stage 6 to 5.5 (30 h)	30 h	70.3	9.4	0.48	0.97	
	Regulated at 6	–	51.0	2.0	0.34	0.63	[2]
	Two-stage 6 to 5.5 (40 h)	–	50.4	0.0	0.41	0.69	
	Two-stage 6 to 5.5 (30 h)	–	54.0	3.4	0.44	0.82	
OFMSW hydrolysate	Regulated at 6	40 h	59.7	1.23	0.40	0.67	This study
	Regulated at 6	30 h	47.5	4.30	0.44	0.67	
	Two-stage 6 to 5.5 (40 h)	40 h	47.4	7.7	0.44	0.67	
	Two-stage 6 to 5.5 (30 h)	30 h	66.7	8.23	0.51	0.78	
	Regulated at 6	–	48.7	2.60	0.37	0.49	[2]
	Two-stage 6 to 5.5 (40 h)	–	52.1	0.0	0.45	0.82	
	Two-stage 6 to 5.5 (30 h)	–	54.4	3.0	0.44	0.82	

[1] The yields were calculated based on the quantities (g) of sugars and organic acids produced and consumed during fermentation, added via feeding and removed via sampling. The volumes considered included the volume of the fermentation broth, samples removed, feeding solution added and NaOH added.

Table 2
Coulombic efficiency and extraction fluxes of organic acids during fed-batch fermentations in EMB and electricity consumption expressed as kWh per kg succinic acid extracted.

Substrate	pH	Current ON	Coulombic efficiency of SA (%)	Coulombic efficiency of AA (%)	Coulombic efficiency of LA (%)	SA flux (g/(m^2·h))	AA flux (g/(m^2·h))	LA flux (g/(m^2·h))	kWh/kg$_{SA}$
Glucose	Regulated at 6	40 h	66.1	9.3	–	92.1	8.4	–	2.5
	Two-stage 6 to 5.5 (40 h)	40 h	82.7	10	–	102.2	17.8	–	2.3
	Two-stage 6 to 5.5 (30 h)	30 h	78.7	14.9	–	80.6	11.9	–	3.0
OFMSW hydrolysate	Regulated at 6	40 h	73.5	7.3	3	79.5	2.0	3.2	2.8
	Regulated at 6	30 h	56.8	4.4	4.2	76.6	3.9	8.6	3.4
	Two-stage 6 to 5.5 (40 h)	40 h	60.8	7.6	3.2	94.3	11.4	6.1	2.1
	Two-stage 6 to 5.5 (30 h)	30 h	66.2	7.5	1.9	90.0	10.5	3.5	2.6

acid. The highest succinic acid production (223 g at 86 h) was achieved in the fermentation where pH reduction to 5.5 and initiation of electrolysis cell operation was conducted at 30 h (Fig. 8C).

The electrochemical membrane extraction process could improve the conventional industrial fermentation processes, achieving continuous product separation and recovery of the final product through the membrane and reducing at the same time the NaOH addition for pH maintenance during fermentation. Over the past decades, several studies have attempted to apply electrical potentials to fermentative microbial cultures in order to assist the cell metabolism and enhance the formation of the target product [39,40]. The application of electrolysis cells with cation exchange membranes in succinic acid production with the bacterial strain *A. succinogenes* has been reported by Zhao et al. [41] using corncob hydrolysate and Park et al. [42] using glucose-based media. Although the electrically generated reducing power led to improved succinic acid production efficiency, the use of cation exchange membranes did not allow the simultaneous acidification, separation and concentration of succinic acid in the anode compartment. Electrochemical extraction of carboxylic acids with an AEM from fermentation broths to the anode compartment prevents product inhibition during fermentation [27]. The EMB with AEM used in this study has also been employed by Pateraki et al. [24] for succinic acid production by *Basfia succiniciproducens* leading to 15% higher yield and 33% less NaOH consumption for pH control during fermentation. Membrane electrolysis was implemented for acetic acid production by Verbeeck et al. [43] using an AEM allowing the recovery of clean and concentrated acetic acid from CO_2 achieving lower power input and cost by external hydrogen injection.

In situ electrochemical membrane extraction of organic acids coupled with fermentation carried out using complex waste streams has led to the production of mid-chain fatty acids with purity higher than 80% [19,28]. Carvajal-Arroyo et al. [44] reported the production of medium chain carboxylic acids using a 3-compartment membrane electrolysis cell with direct electrochemical membrane extraction of oil from the fermentation broth.

3.5. NaOH requirements and electricity consumption

The requirements for sodium hydroxide to regulate pH during fermentation is one of the most important environmental burdens leading to increased global warming potential and abiotic depletion potential in the case of *Y. lipolytica* fermentation [2]. The growth of the yeast strain *Y. lipolytica* PSA02004 is observed throughout fermentation when the pH is maintained at 6, while it stops when the pH is reduced to 5.5, which is slightly below the pK_a2 where both dissociated succinate salts HAS^- and SA^{2-} are present in the fermentation broth [2,18]. The production of succinic acid by *Y. lipolytica* PSA02004 is maintained at pH 5.5. The production of succinic acid at low pH could provide a significant advantage in downstream separation and purification, while the regulation of fermentation pH at higher values increases the NaOH requirement and subsequently acid consumption for acidification in downstream separation and purification (e.g. for cation exchange resins regeneration) [2,13].

The OH^- produced in the cathode compartment led to reduced NaOH consumption when the EMB was employed as compared to fermentations carried out in the bioreactor alone (Fig. 9A and 9B). The NaOH consumption in the EMB fermentation conducted at constant pH 6 with initiation of electrolysis cell operation at 40 h using glucose (152 g NaOH) or OFMSW hydrolysate (162 g NaOH) was 11.6% (Fig. 9A) and 3.6% (Fig. 9B) lower than the respective fermentations conducted in the

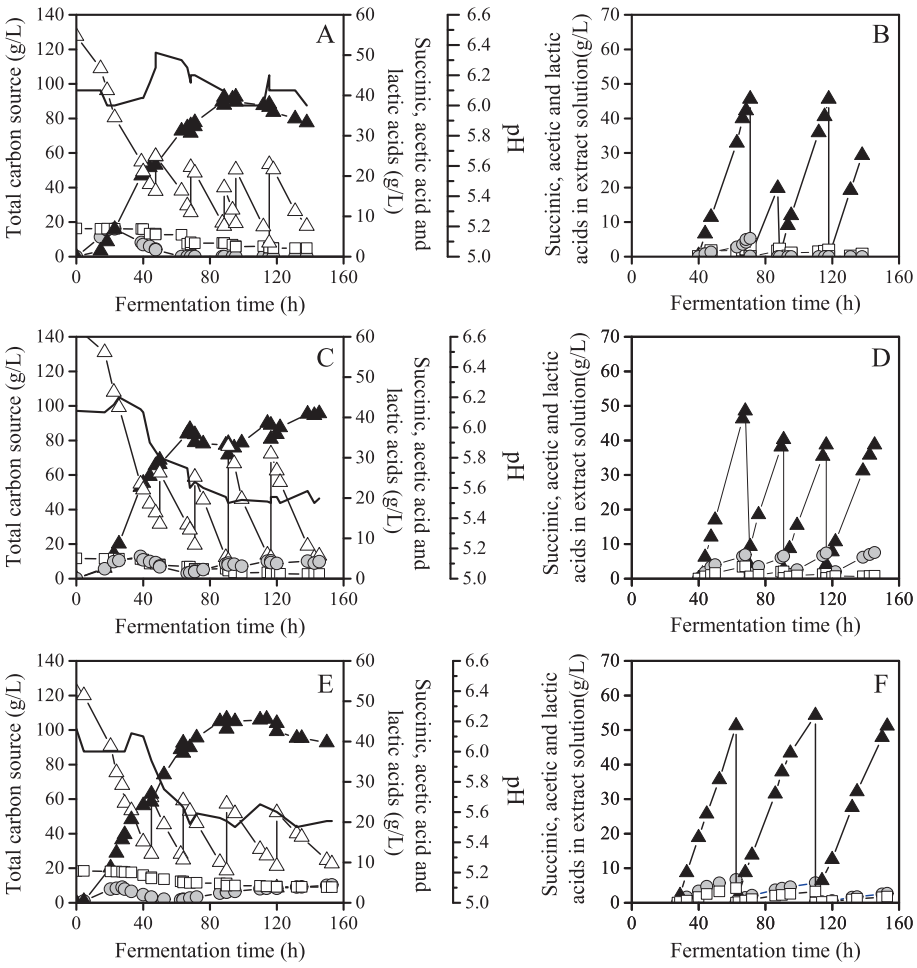

Fig. 6. Succinic acid (▲), acetic acid (●), lactic acid (□), yeast biomass (*), total carbon source (△) and pH (black line) in the bioreactor (A, C, E) and the anolyte extract solution (B, D, F) during fed-batch EMB fermentations under different pH strategies with OFMSW hydrolysate. Constant pH 6 with initiation of electrolysis cell operation at 40 h (A and B), initial pH 6 gradually dropped to 5.5 with initiation of electrolysis cell operation at 40 h (C and D) and initial pH 6 gradually dropped to 5.5 with initiation of electrolysis cell operation at 30 h (E and F).

bioreactor alone (the NaOH consumption in these fermentations has been reported by Stylianou et al. [2]).

Stylianou et al. [2] showed that the two-stage pH regulation strategy led to significant reduction in NaOH consumption (up to 43%) as compared to fermentations conducted at pH 6. When this strategy was combined with the EMB, the NaOH reduction achieved was even higher. Specifically, when the two-stage pH strategy and the electrolysis cell were initiated at 40 h, 26% and 25.2% lower NaOH consumption were observed in the case of glucose and OFMSW hydrolysate, respectively as compared to fermentation carried out with the bioreactor alone. When the electrolysis cell was initiated at 30 h, the reduction of NaOH was 36.3% and 35.4% in the case of glucose and OFMSW hydrolysate, respectively.

The electricity consumption in the electrolysis cell for succinic acid separation was 2.1 – 3.4 kWh/kg_{SA} (Table 2). The lowest electricity consumption (2.1 kWh/kg_{SA}) was achieved in the fermentation conducted with OFMSW hydrolysate where the pH dropped to 5.5 and the operation of the electrolysis cell was initiated at 40 h.

The results presented above demonstrate that the electricity consumption and the NaOH reduction achieved in the integrated EMB are promising for further process development. The combination of OFMSW hydrolysate usage with the electricity-driven succinic acid extraction in the EMB and the two-stage pH regulation strategy may potentially enable sustainable production of bio-based succinic acid with reduced environmental impact.

3.6. Fed-batch fermentations in 30 L EMB

The first fed-batch fermentation in the 30 L EMB was conducted with the low membrane surface area (100 cm^2) electrolysis cell using OFMSW hydrolysate and pH regulation to 5.5 with simultaneous initiation of electrolysis cell operation at 30 h (Fig. 10A and 10B). The current supply was 0.57 A (5.7 mA cm^{-2}) until 122.5 h, while thereafter it was increased to 1.12 A (11.2 mA cm^{-2}). The results presented in Fig. 10 demonstrate that succinic acid production and separation was efficient until 122.5 h at 0.57 A current supply with an average extraction flux of 87.3 $g_{SA}/(m^2 \cdot h)$ (Fig. S4). Until 122.5 h, the average coulombic efficiency was 67% for succinic acid, 11% for acetic acid and 6% for lactic acid. The average succinic acid extraction flux and coulombic efficiency achieved in the 30 L bioreactor were similar to those achieved in the respective fermentation in the bench-top bioreactor (Table 2). When the applied current was increased to 1.12 A, the average extraction flux and coulombic efficiency of succinic acid were increased to 105.5 $g/(m^2 \cdot h)$ (Fig. S4) and 84%, respectively. However, 21 h after the application of the higher current supply, the fermentation stopped as it was observed in the bench-top bioreactor. Succinic acid production reached 60.4 g/L with 0.47 g/g yield and 0.74 g/(L·h) productivity at 82 h considering the succinic acid present in both the fermentation broth and the anolyte. Acetic acid production occurred before the initiation of electrolysis cell operation and thereafter it was extracted in the anolyte without any additional production. Lactic acid was present in the OFMSW hydrolysate and it was not produced during fermentation.

The small membrane surface area electrolysis cell used in the previous fermentation does not allow high extraction rates from a 30 L bioreactor. For this reason, the subsequent fermentation was carried out with a higher membrane surface area (702 cm^2) electrolysis cell at higher applied current (3 A). Succinic acid production progressed well until the initiation of the electrolysis cell operation at 30 h, but

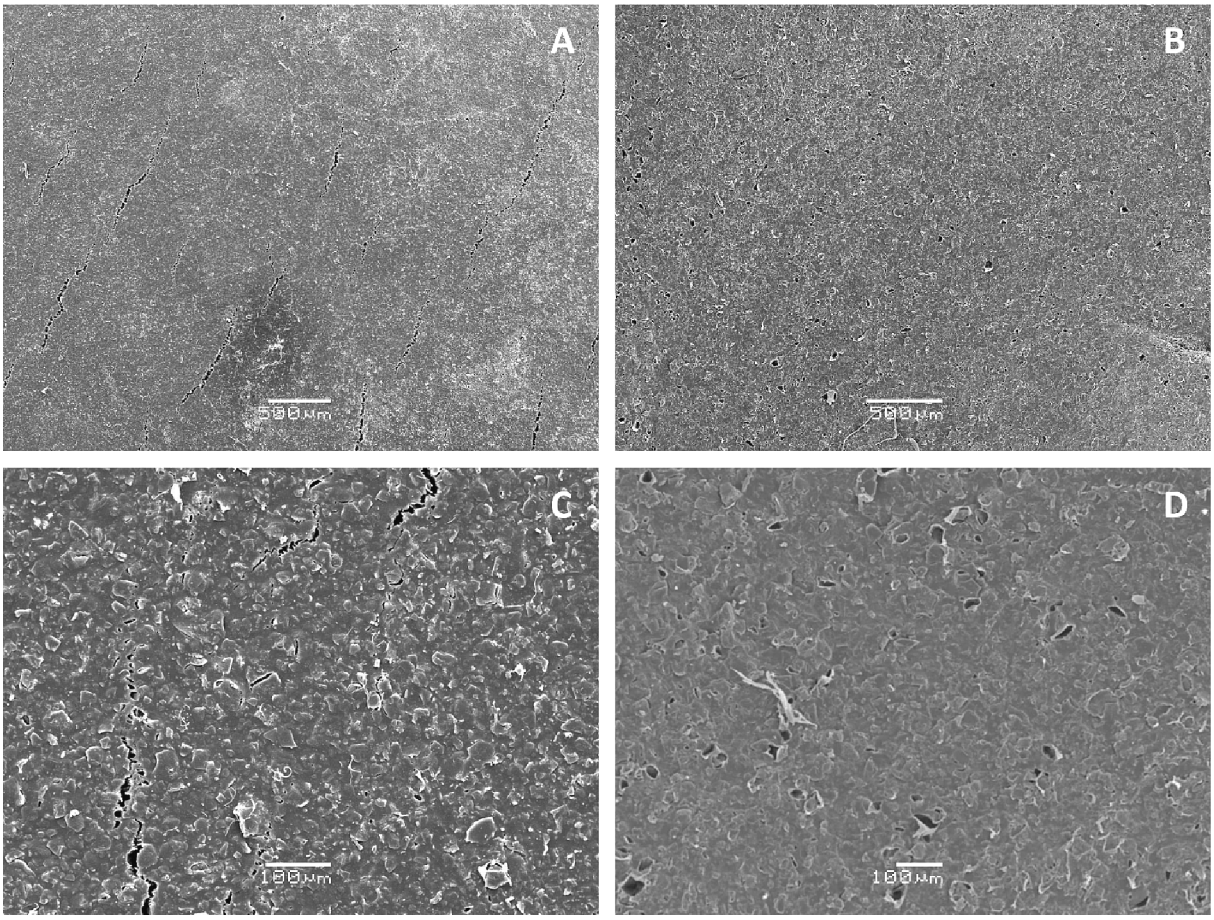

Fig. 7. Scanning electron microscopy for unused membranes (A: 500 µm and C: 100 µm) and membranes after utilisation in the succinic acid fermentation integrated with the electrochemical cell (B: 500 µm and D: 100 µm).

thereafter succinic acid production efficiency was significantly reduced leading to only 21.2 g/L succinic acid concentration at 49 h (Fig. S5A). The same fermentation was carried out in the 30 L EMB with the high membrane surface area (702 cm^2) electrolysis cell at 0.57 A applied current leading also to low succinic acid production efficiency and yeast cell death (results not presented).

The high membrane surface area electrolysis cell was also used in the 3 L working volume bioreactor where yeast cell death and low succinic acid production efficiency was also observed (results not presented). The sudden yeast cell death was indicated by the sudden increase of the DO from 0% to 85% after the initiation of electrolysis cell operation at 30 h.

Based on the results presented above, the electrolysis cell design, the integrated EMB configuration and the applied current are crucial aspects in process scale-up to ensure prolonged yeast cell viability with simultaneous high succinic acid extraction rates. Future studies should evaluate further these aspects. For instance, the application of multiple electrolysis cells integrated with a large-scale bioreactor operating in parallel could lead to higher membrane exchange area at low current. An alternative approach could be the retention of yeast cells in the bioreactor via membrane filtration preventing the recirculation of the yeast cells through the cathode compartment. In this way, the yeast cells will not be stressed by high currents and low oxygen availability in the cathode compartment.

3.7. Succinic acid recovery and purification

The succinic acid rich solution extracted in the anode compartment was used for the purification of succinic acid crystals, which were subsequently used for the production of polyester polyols as precursors for the production of PUDs. The anolyte solution was treated via activated carbon, filtration, vacuum evaporation, crystallisation and drying. Activated carbon was used because the succinic acid crystals produced without this processing step led to the production of polyester polyols the properties of which did not meet the required specifications. The remaining liquid stream after crystallization that contained non-precipitated succinic acid was recirculated in the evaporation step in order to reduce succinic acid losses and increase the recovery yield. Vacuum evaporation leads to acetic acid removal and succinic acid concentration facilitating succinic acid crystallisation in the following step.

Besides carboxylate anions, other anions (e.g. phosphate) pass through the AEM from the fermentation broth to the anolyte extract solution. To assess the purity of the SA crystals produced after each crystallisation and water washing cycle, the IP content was determined (Fig. S6) demonstrating a negligible IP content after 6 cycles. Furthermore, lactic acid and acetic acid content was negligible in the purified SA crystals (results not presented). The dried succinic acid crystals had 99.95% (w/w) purity, while the recovery yield was ca. 95%.

3.8. Polyester polyol and polyurethane urea dispersion synthesis

The use of succinic acid in polyester synthesis resulted in similar polyol properties as with commercial bio-based succinic acid and fossil-based adipic acid. In all syntheses, the acid number went down to approximately 1 mg KOH/g. For the EMB-SA-based polyol, an OH number of 64.7 mg KOH/g was measured corresponding to an average molecular weight of 1734 g/mol.

Table 3 presents the properties of PUDs produced with polyester

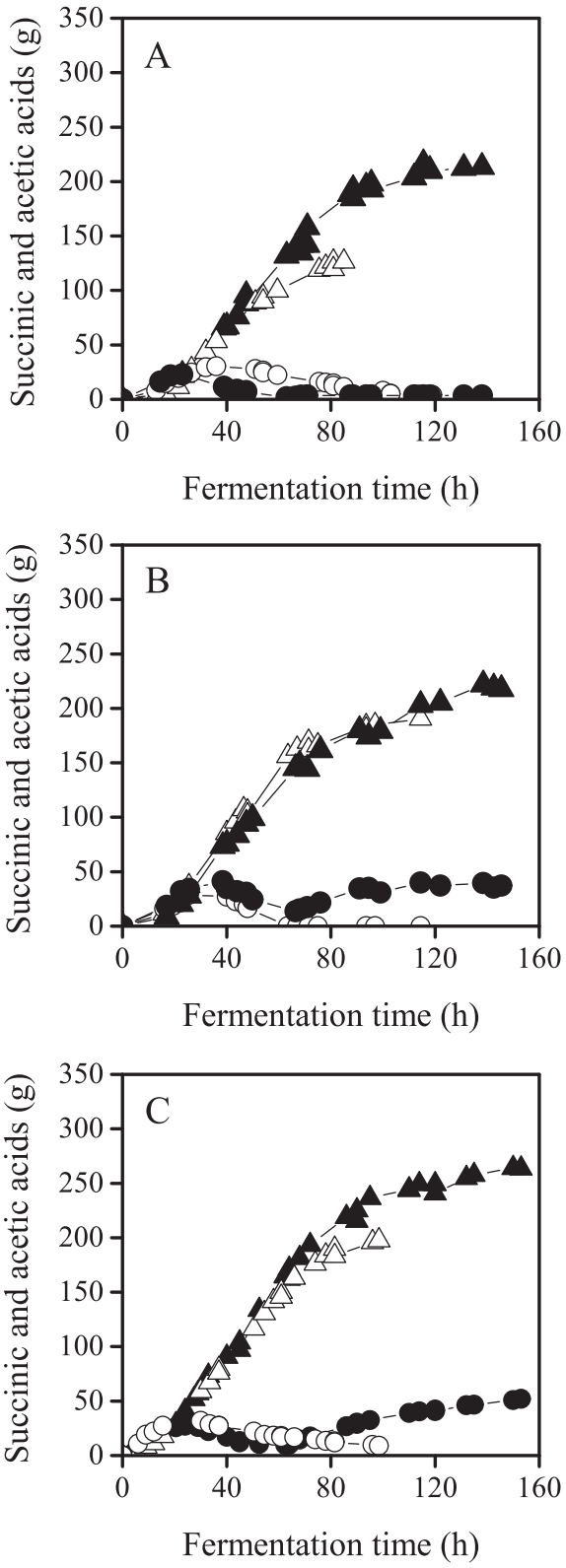

Fig. 8. Production of succinic acid and acetic acid during fed-batch fermentations under different pH strategies using OFMSW hydrolysate either with electrolysis cell operation (filled symbols) or without electrolysis cell operation (unfilled symbols). Constant pH 6 with initiation of electrolysis cell operation at 40 h (A), initial pH 6 gradually dropped to 5.5 with initiation of electrolysis cell operation at 40 h (B) and initial pH 6 gradually dropped to 5.5 with initiation of electrolysis cell operation at 30 h (C). Succinic acid (triangle), Acetic acid (circle).

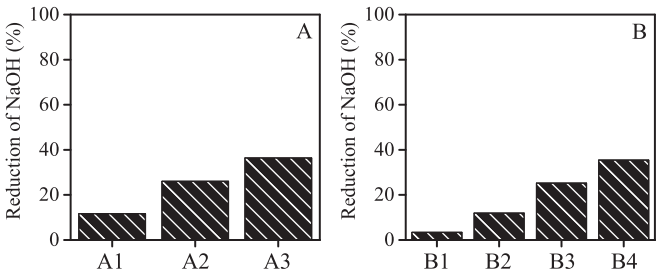

Fig. 9. Reduction of NaOH consumption in fed-batch EMB fermentations using glucose (A) or OFMSW hydrolysate (B) as compared to the respective conventional fermentations carried out in the bioreactor alone. (A1, B1) constant pH 6 with initiation of electrolysis cell operation at 40 h, (A2, B3) initial pH 6 gradually dropped to 5.5 with initiation of electrolysis cell operation at 40 h, (A3, B4) initial pH 6 gradually dropped to 5.5 with initiation of electrolysis cell operation at 30 h, (B2) constant pH 6 integrated with the electrolysis cell at 30 h.

polyols using commercial succinic acid, fossil-based adipic acid or succinic acid crystals obtained in this study via EMB cultures. In the prepolymerisation step, the speed of NCO decrease with the EMB-SA polyester polyol was comparable to the syntheses with polyols made from commercial SA or adipic acid. The EMB-SA-PUDs properties have negligible differences with the PUDs produced with commercial succinic acid and fossil-based adipic acid. The results indicate that the OFMSW-EMB-derived succinic acid has the adequate quality for PUDs production. However, when the activated carbon treatment step was not used, then PUDs properties produced from OFMSW-EMB-derived succinic acid did not meet the specifications.

The aforementioned results demonstrate that the EMB-based process requires less unit operations than the conventional DSP for the production of succinic acid crystals with the required purity for the production of polyester polyols. In the conventional DSP where succinic acid is purified from fermentation broths containing succinate salts, yeast cell removal is carried out by centrifugation and succinic acid is obtained via acidification with cation-exchange resin treatment. Thus, the use of both acidic and alkaline solutions is minimised.

4. Conclusions

An EMB using AEM and OFMSW hydrolysates can be efficiently used for simultaneous production, in situ separation, acidification and concentration of succinic acid in the anolyte extract solution. Thus, renewable electricity consumption in the proposed process could replace alkali and acid consumption, improve succinic acid production efficiency and reduce unit operations for SA purification as compared to conventional SA production processes. For instance, using both the EMB and the two-stage pH regulation strategy in OFMSW hydrolysate based cultures leads to 63.1% lower NaOH consumption than the conventional fed-batch fermentation carried out at constant pH 6. The introduction of electrification in chemical production will facilitate the transition to a sustainable chemical industry. The sustainability of the proposed process especially with respect to the environmental impact will be presented in a forthcoming publication.

Authors contribution

Eleni Stylianou and Jose M. Carvajal-Arroyo performed the experiments. Jakob Marbach performed the polyester synthesis and Sebastian Dörr performed the PUD synthesis. Eleni Stylianou, Jose M. Carvajal-Arroyo, Chrysanthi Pateraki, Apostolis Koutinas, Dimitrios Ladakis and Korneel Rabaey designed the experiments. Apostolis Koutinas and Korneel Rabaey supervised the experiments. Eleni Stylianou, Jose M. Carvajal-Arroyo, Chrysanthi Pateraki, Apostolis Koutinas and Korneel Rabaey wrote the manuscript. Apostolis Koutinas, Korneel Rabaey, Carol Sze Ki Lin and Vera Eßmann revised the manuscript. All authors read and approved the final manuscript.

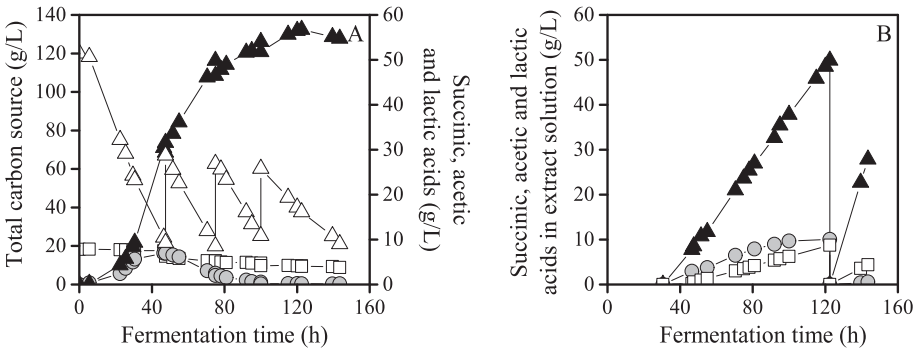

Fig. 10. Total carbon source (△), succinic acid (▲), acetic acid (●) and lactic acid (□) in the bioreactor (A) and the anolyte extract solution (B) during fed-batch EMB fermentation in 30 L bioreactor carried out at initial pH 6 gradually dropped to 5.5 and initiation of the low membrane surface area (100 cm^2) electrolysis cell operation at 30 h.

Table 3
Properties of PUDs produced from commercial succinic acid, fossil-based adipic acid and OFMSW-derived succinic acid obtained in this study via EMB fermentations.

PUDs	Particle size (nm)	M_n (g/mol)	M_w (g/mol)	Viscosity (mPa s)
Commercial bio-based SA	122	$3.40{\cdot}10^4$	$1.17{\cdot}10^5$	102
Fossil-based adipic acid	177	$3.03{\cdot}10^4$	$8.6{\cdot}10^4$	87
EMB-SA	130	$3.68{\cdot}10^4$	$1.47{\cdot}10^5$	59

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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