Electronic Supplementary Information

Expanding the product spectrum in mixed-culture fermentation of organic solid waste through operational control

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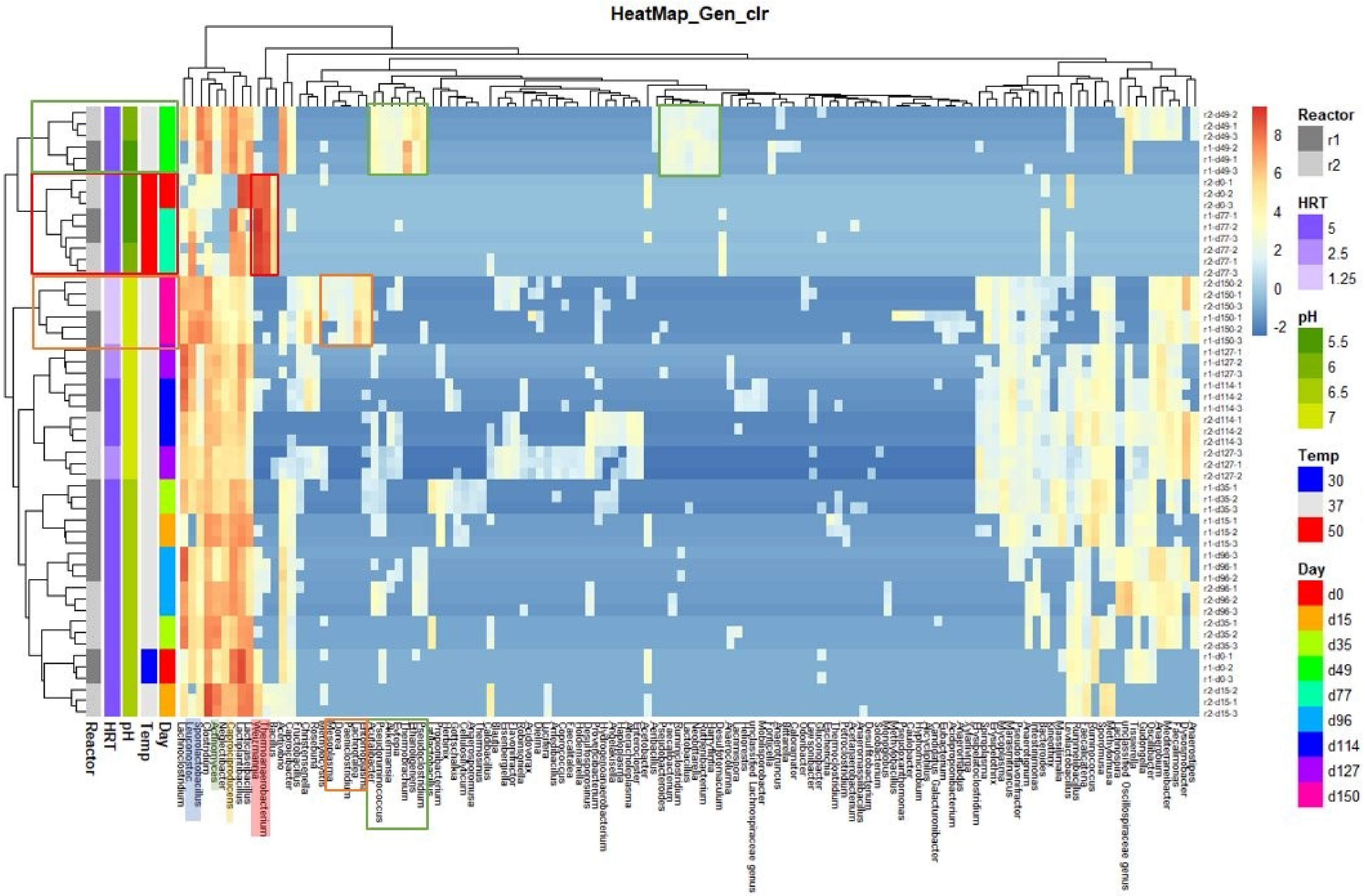

Fig. S1 Heatmap of the microbiome composition on the genus level.

Downstream processing - electrodialysis with bipolar membrane (EDBM)

Experimental. The EDBM process for the separation and concentration of carboxylic acids was carried out using a 3-chamber laboratory electrodialysis system (base – salt – acid), which allowed for the separation of carboxylic acids (simultaneously converting them from sodium salt to acid form) and the regeneration of NaOH. The diagram of the EDBM stack used is shown in Fig. S2. The electrodialysis system consisted of a single cell with alternating ion exchange membranes: bipolar membrane (BPM) – cation exchange membrane (CEM) – anion exchange membrane (AEM) – bipolar membrane (BPM), separated by a 0.8 mm poly(ethylene) thick spacer and placed between the cathode and anode made of platinum (Pt) coated titanium. The electrodes were connected directly to the S-LS-59 laboratory power supply (Stamos, Germany). The active surface area of each membrane was 64 cm². The detailed characteristics of the ion exchange membranes used in the EDBM process are presented in Table S1.

Before the EDBM process, to reduce membrane fouling, the effluent collected in Phases VI and VII was pre-treated using centrifugation at 4500 RPM for 15 min, followed by microfiltration (MF). The MF was conducted using a lab-scale membrane setup with a seven-channel tubular ceramic membrane (Tami, France), made of TiO_2, with a molecular weight cut-off (MWCO) of 0.8 µm and an active membrane area of 0.06 m². Initially, 1 L of the centrifuged feed solution was introduced into the working tank and stirred until a stable state was achieved. The cross-flow system operated in a semi-closed configuration, with recirculation of the retentate to the feed tank and simultaneous collection of the purified permeate fraction. The MF process was conducted at a constant feed temperature of 25 ± 2 °C, under a transmembrane pressure (TMP) of 0.2 MPa and a feed flow rate of 15 L h^{-1}.

Thereafter, a pre-treated fermentation broth with an initial volume of 0.7 L was introduced into the diluate chamber. Deionized water with an initial volume of 0.2 L was introduced into the acid and base chamber, while an electrolyte solution of sodium sulphate at a concentration of 0.3 M (0.5 L) was introduced into the electrode chambers (anolyte/catholyte). All solutions were recirculated in a closed system using a multi-channel peristaltic pump WT600S (Baoding Lead Fluid Technology Co., Ltd, China) at a constant flow rate of 15 L h^{-1}. The EDBM process was conducted in galvanostatic mode at a constant current density of 234.4 A m^{-2}, with the voltage (±0.01 V) across the electrodialysis stack monitored throughout. During the EDBM process, changes in pH (±0.01), conductivity (±0.01 mS cm^{-1}), and temperature (±0.1°C) of each working solution were measured using the CX-601 Multifunction Device (Elmetron, Poland). Samples of each solution were collected at predetermined time intervals, while changes in the solution volumes in individual chambers were monitored with an accuracy of ± 0.02 L.

The NaOH concentration in the base solution obtained after the EDBM process was determined by potentiometric titration using a 703 Ti Stand titrator (Metrohm, Poland). For each measurement, 10 mL of water was mixed with 1 mL of the base solution sample and titrated with 1 mol L^{-1} HCl.

Calculations. To evaluate the efficiency of the implemented EDBM process, the following parameters were calculated: the removal ratio (R), current efficiency (CE), and the total specific energy consumption required to produce 1 kg of acids (E), using equations (1-3):

$$R = \left(1 - \frac{C_{dil}^{t} \cdot V_{dil}^{t}}{C_{dil}^{0} \cdot V_{dil}^{0}}\right) \cdot 100\% \qquad (1)$$

where: R – removal ratio, %; C^t_{dil} – component concentration in the diluate solution at time t, L; V^t_{dil} - volume of diluate solution at time t, L; C^0_{dil} – initial component concentration in diluate solution, g L^{-1}; V^0_{dil} – initial volume of diluate solution, L;

$$CE = \frac{F \cdot z \cdot V \cdot \Delta C_{dil}}{N \cdot I \cdot \Delta t} \cdot 100\% \qquad (2)$$

where: CE – current efficiency, %; F –Faraday constant (96485), C mol^{-1}; I – current, A; z – electric charge; V – diluate volume, L, ΔC_{dil} – change in component concentration in diluate solution, mol L^{-1}; N – number of cells;

$$E = \int_0^t \frac{U \cdot I}{\Delta m} dt \qquad (3)$$

where: E – specific energy consumption needed to produce 1 kg of acid, kWh kg^{-1}; U – voltage, V; Δm – change in mass in the concentrate solution, g; t – time, h.

Results and discussion

Product recovery using electrodialysis with bipolar membrane (EDBM). An essential aspect of implementing biotechnological processes on an industrial scale is the development of effective methods for separating, concentrating, and purifying selected bioproducts.[1] It is still a challenge, considering the complex composition of the effluents produced during the fermentation using mixed cultures. Therefore, in this part of the study, we proposed a method for simultaneous recovery of MCCA and succinic acid from fermentation effluent using multifunctional electrodialysis with a bipolar membrane (EDBM) process. The use of an electrodialytic stack with the membrane configuration BPM-CEM-AEM-BPM allows not only the separation of low-molecular-weight ionic compounds from those that occur in the effluent solution in the non-ionic form but also the concentration and simultaneous electro-conversion of acid salts to their acidic form as well as regeneration of the alkaline solution. [2]

The completion time of the MCCA and succinic acid recovery using the EDBM was equal to 180 min, and it was determined by the conductivity value of the effluent and the voltage drop value on the EDBM stack. During the EDBM process, the conductivity of the effluent solution (Fig. S3A) decreased from the initial value of approx. 16 mS cm^{-1} to a value below 1 mS cm^{-1}, indicating a significant depletion of the effluent in charge carriers (e.g., dissociated carboxylic acids, metal cations). The lack of charge carriers in the effluent solution also causes an increase in the voltage on the electrodialysis stack, which is associated with an increase in its total resistance (Fig. S3B).[3]

The concentration values of the considered fermentation products in the effluent solution before and after the EDBM process, along with the calculated removal rate (R), are shown in Fig. S4. Undoubtedly, in the case of electrodialytic processes, the degree of removal of a low-molecular-weight metabolite depends mainly on its ionic structure and the initial concentration in the diluate solution. The initial pH of the effluent used in the EBDM process was about 6, which means the complete dissociation of carboxylic acids in the diluate solution. Therefore, the degree of removal of carboxylic acids selectively migrating by the AEM from the diluate to the acid compartment was high and ranged from 64.7% (isocaproic acid) to 100% (isobutyric). Consequently, the total removal ratio of carboxylic acids from the diluate solution after 180 min of the process was 84%. Moreover, we did not observe any change in the concentration of non-ionic components such as lactose, glucose, and i-propanol. On the other hand, the removal rate of ethanol leaking through the AEM into the acid compartment was extremely low, at approximately 1%.

Post-electrodialysis concentration levels of fermentation products in the acid compartment, in addition to the concentration factor (CF), are presented in Fig. S5. After 180 min of the EDBM process, the highest concentration value in the acid compartment, equal to 7.8 g L^{-1} (264 mM C), was achieved for succinic acid with a simultaneous high CF factor of 2.4. A CF value of around 2.5 was also achieved for lactic, acetic, and propionic acids using an appropriate initial volume ratio of diluate to acid concentrate solution. For comparison, the CF value obtained by Arslan et al. in their studies on *in-situ* carboxylate separation using EDBM during continuous mixed culture fermentation was equal to 1.5[4]. At the same time, no non-ionic fermentation products were observed in the acid concentrate in our study after the EDBM process (except for trace amounts of ethanol), which confirms the high selectivity of the AEM used. The pH value of the acid concentrate at the end of the EDBM process was 2.1 (Fig. S6). The effective electro-conversion of acid salts to their acidic form is due to the attachment of H^+ ions generated by the bipolar membrane to acidic anions transported by the AEM to the acidic compartment. This feature of the EDBM process allows the complete elimination of the acidification step using mineral acids.[5] Besides, during the EDBM process, NaOH was recovered in the alkaline compartment, which can be reused in fermentation or as a cleaning agent in the CIP procedure. As a result of the migration of Na^+ ions from the diluate solution through the CEM towards the cathode and their combination with the OH^- ion generated by the BPM, after 180 min, a base solution with a final concentration of 0.63 mol L^{-1} (pH ~14; Fig. S6) was obtained.

The total current efficiency (CE_{TOTAL}) calculated based on the migration of all acid anions by the AEM from the diluate solution to the acid compartment after 180 min was satisfactory and equal to 30.6%. The achieved CE value is higher than that obtained in the studies described by Arslan et al.[4] As referenced by the authors, the highest CE value obtained in the first hours of the experiment at the highest constant current density of 60 A m^{-2} never reached 30%. Then, over time, the CE value decreased due to the depletion of carboxylates in the reactor.[4] In the case of the EDBM process discussed here, a large part of the electrical charge is consumed by the splitting of water by the BPM. On the other hand, the value of the unit energy consumption (E) needed to produce 1 kg of acids was equal to 15.4 kWh kg^{-1}. The relatively high energy consumption value is mainly due to the high degree of acid removal achieved and the resulting increase in the total resistance of the EDBM stack.[6] The yields on carboxylic acid separation using the EDBM technique from solutions obtained in mixed culture fermentation remain limited due to the magnitude of metabolites produced and their low final concentration.[4] Furthermore, it should be emphasized that after the EDBM process, the pre-concentrated, purified, and acidified concentrate solution can be subjected to further purification steps not included in this study, using evaporative concentration and crystallization methods to separate MCCA and succinic acid.[7]

Environmental and economic considerations. Economic viability and sustainability are key for competitive biotechnological carboxylic acid production.[8] The energy consumption (E) obtained in this study (15.4 kWh kg^{-1}) is higher than values typically reported for EDBM recovery of single organic acids from pure culture fermentations (0.05-4.9 kWh kg^{-1}), synthetic mixtures (2.7 kWh kg^{-1}), or other MCF studies (5.0-7.6 kWh kg^{-1}) (Table S2). However, direct comparisons should consider the complex composition of mixed culture fermentation effluents, which contain multiple carboxylic acids at relatively low concentrations, presenting an additional challenge compared to pure culture systems with single target products at higher titers.

EDBM holds several environmental advantages, such as the simultaneous recovery of NaOH, which enables direct recycling to the fermentation process for pH control, reducing reagent costs and environmental burdens.[9] Furthermore, the electro-conversion of acid salts to their acidic form eliminates the need for mineral acid addition (e.g., H_2SO_4), which in conventional acidification processes is a primary contributor to environmental impacts across multiple categories.[8] EDBM was considered among other downstream processing methods in a comprehensive TEA and LCA study of Dickson et al. on succinic acid production in commercial-scale biorefineries.[8] Although succinic acid can be separated from the fermentation broth to a high degree, allowing microbial cell recycling, high capital and operational costs, and limited lifetime decrease its attractiveness as a downstream technology. Instead, an ion-change column was identified as the optimal purification method by the environmental indicators.[8] Furthermore, TEA and LCA of OFMSW to the lactic acid biorefinery concept showed that the electrodialysis membrane unit operations are the major cost driver, with the EDBM requiring nearly 60% of total electricity consumption (160 vs. 269 GWh per year).[10] However, EDBM was used in the modelled multiproduct biorefinery Multi Case, producing acetic and succinic acids, and dimethyl ether, proving the whole biorefinery concept, including product purification, technically and economically viable.[11] Moreover, the study by Zhou et al. shows that, based on a target product, the process configuration could be optimized to achieve high acid concentration, current efficiency, and purity, with low operating and capital costs.[12] Since 60-89% the total process energy is consumed in downstream processing, depending on a feedsock[8], switching the energy source to renewable could considerably increase the greenness of the process. As suggested by Ioannidou et al., switching from grid electricity to renewable sources reduces the global warming potential by 31–64% for organic acid production.[10] These findings suggest that optimizing downstream processing and utilizing renewable energy are critical for improving the environmental profile of bio-based carboxylic acid production.

Conclusions

The EDBM used as the downstream processing was characterized by an 84% total removal of carboxylic acids from the diluate solution. Succinic acid was a predominant component of the concentrate, reaching 7.8 g L^{-1} (264 mM C), followed by acetic acid 5.4 g L^{-1} (180 mM C), butyric acids 3.9 g L^{-1} (177 mM C), propionic acid 2.4 g L^{-1} (97 mM C) and lactic acid 3.9 g L^{-1} (130 mM C). The concentration factor of the following compounds was in the range of 2.4 to 2.5. Furthermore, a base solution of 0.63 mol L^{-1} was simultaneously recovered after 180 minutes. From an economic perspective, while the energy consumption of the EDBM process (15.4 kWh kg^{-1}) is higher than typical values for pure culture systems, the multifunctionality of the process – combining separation, concentration, electro-conversion, and alkali recovery in a single unit operation – offers potential advantages. Future optimization studies should focus on reducing energy consumption through adjustment of current density, optimization of initial acid concentrations, and volume ratios, as well as integration with renewable energy sources to enhance the environmental profile of the process.

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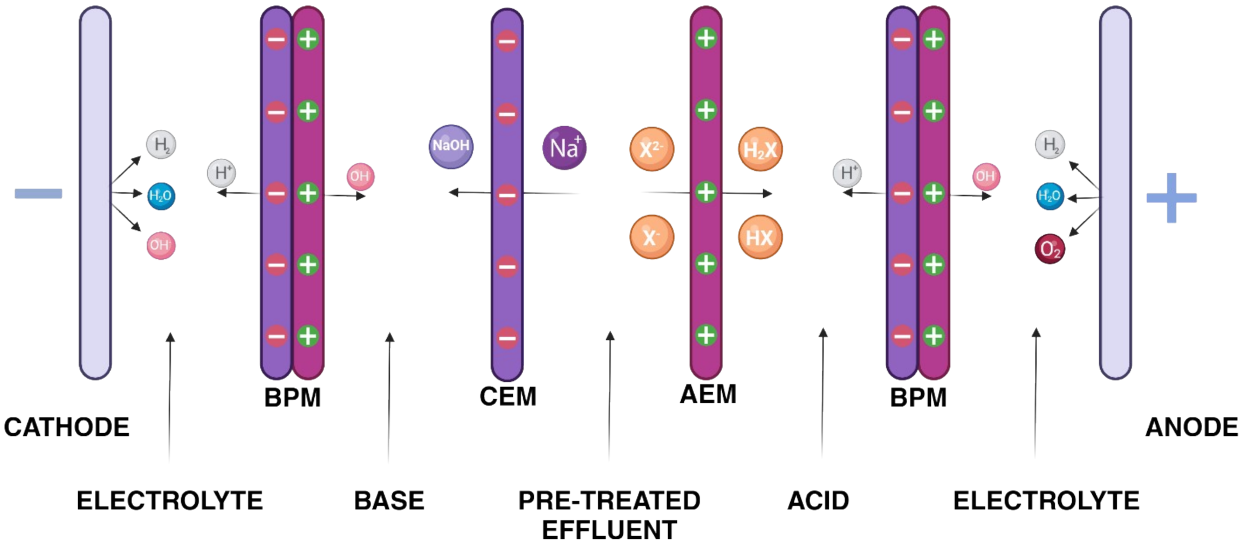

Fig. S2 The scheme of EDBM membrane stack type: BPM-CEM-AEM-BPM (bipolar membrane (BPM) – cation exchange membrane (CEM) – anion exchange membrane (AEM) – bipolar membrane (BPM)).

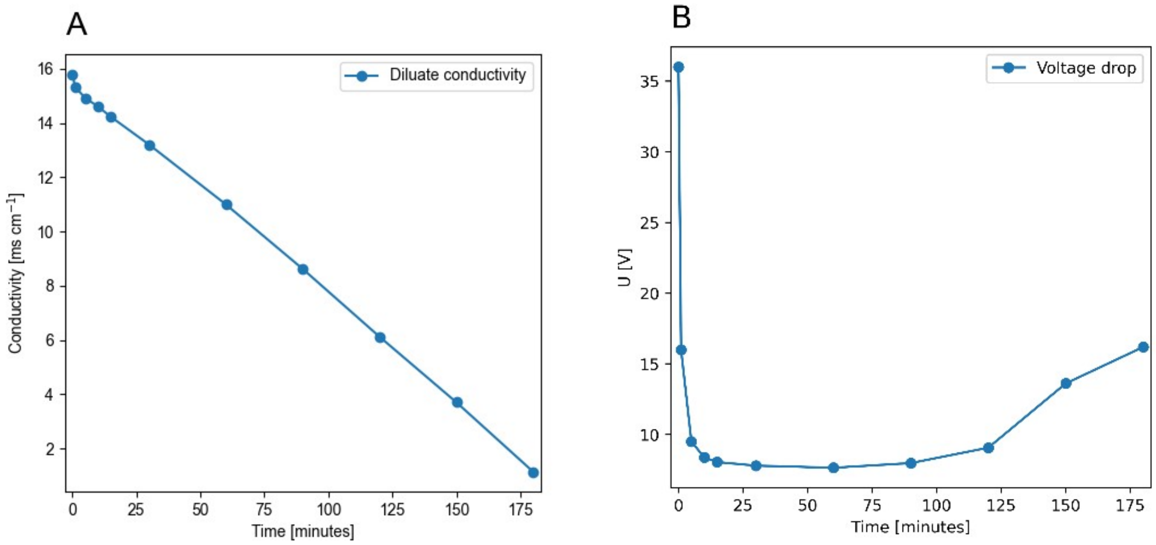

Fig. S3 Change of conductivity of the diluate solution (A) and voltage drop across the stack (B) during the EDBM process.

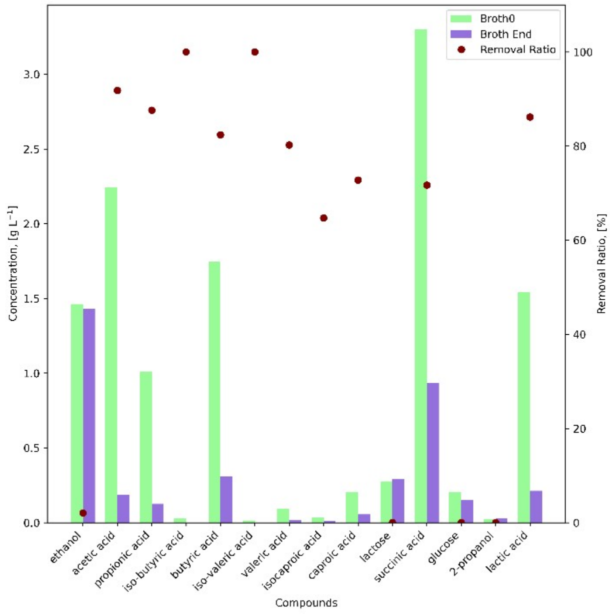

Fig. S4 Concentration values of the fermentation products in the effluent before (Broth0) and after the EDBM process (Broth End), along with their removal ratio (R).

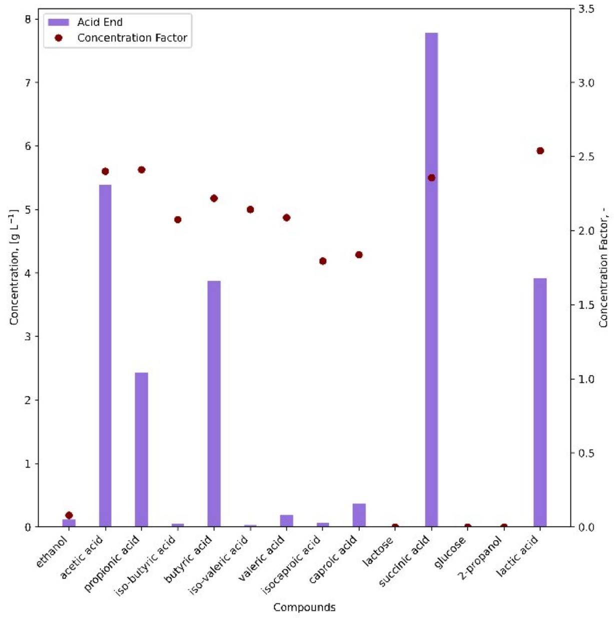

Fig. S5 Concentration values of fermentation products in the acid compartment after the EDBM process, along with their concentration factor (CF).

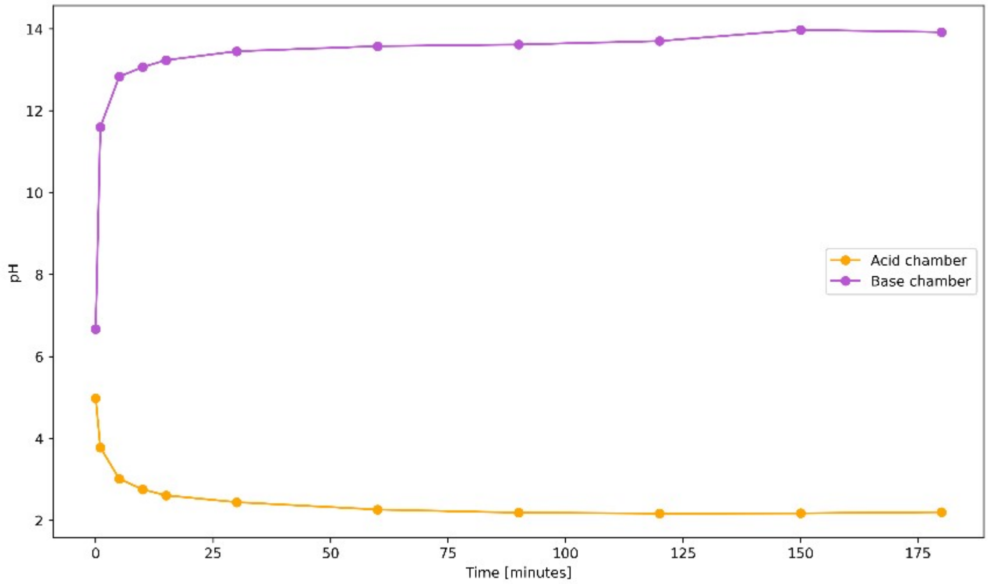

Fig. S6 Change of pH of solutions in acid and base compartments during the EDBM process.

Table. S1 Characteristics of the ion exchange membranes used

Parameter	AEM (AMH-PES)	CEM (CM-PES)	BPM (PC bip)
Producer	RALEX®, Czech Republic	RALEX®, Czech Republic	PC-Cell, Germany
Thickness	0.714 mm	0.764 mm	0.2-0.35 mm
Maximum temperature	45 °C	45 °C	50 °C
Number of transfers	KCl (0.1/0.5N)>0.95	KCl (0.1/0.5N)>0,95	-
Resistance	7.66 Ω·cm^2	<8 Ω·cm^2	-
Ion exchange capacity	1.97 mval g^{-1}	2.2 mval g^{-1}	-
Water splitting efficiency	-	-	>95
Water bursting voltage	-	-	1.1-2.2 V
pH range	0-10	0-10	0-12

Table S2 Comparison of energy consumption (E) and current efficiency (CE) for EDBM in organic acid recovery from different studies.

Application	Feed	E (kWh kg^{-1})	CE (%)	Reference
A mixture of carboxylic	MCF broth	15.4	30.6	This study

acids containing succinic acid				
A mixture of carboxylic acids	MCF broth	5.0	>30.0	[4]
A mixture of caproic, butyric, and acetic acids	MCF broth	7.6	-	[13]
Lactic acid	Pure culture fermentation broth	1.1	82.5	[14]
α-ketoglutaric acid	Co-culture fermentation broth	1.1	64	[3]
Acetic acid	Pure culture fermentation broth	0.05-0.34	9.5-22.6	[15]
Succinic acid	Pure culture fermentation broth	4.9	57.1	[16]