Case Study

Electrochemical Systems for the Recovery of Succinic Acid from Fermentations

This work was carried out during a two year Innovate UK grant funded project. Electrodialysis methods were investigated to reduce product inhibition and boost product yields through *in situ* product recovery (ISPR) from bench top fermentations. The fermentation studied was succinic acid production by the bovine rumen bacterium *Actinobacillus succinogenes* strain 130Z[1,2].

Succinic acid yields of 50-60g/l have been reported for this bacterium[3]. However, fermentation also yields mixed acid byproducts (acetic, formic, lactic, propionic and pyruvic acids in various proportions) with associated inhibition of batch fermentation and increasing costs of product purification. Continuous addition of base to fermentations can be used to alleviate the pH inhibition, but this increases material and process cost and dilutes the final product.

Materials and equipment:

1. Electrodialysis

Prototype C-Flow ED 10x18, 2-compartment with 4 unit cells. In conventional ED, cell was assembled with Neosepta AMX and CMX membranes. In EDBM, the cell was assembled with Neosepta CMB and BP-1E membranes. In ED for ISPR, the cell was assembled with Neosepta CMB and AHA membranes. All membranes were supplied by Eurodia.

The ED cell was connected to a 16V, 20A programmable Elektro Automatik power supply.

In all experiments, 500ml of 0.5M sodium sulphate was used for the electrode rinse. 1M NaOH was used for the BASE stream. In ED for ISPR, the NaOH in the BASE stream was depleted with time, so it was periodically replaced with fresh 1M NaOH.

In conventional ED, the dilute and concentrate streams were initially a mixture of representative acidic fermentation products.

All streams were recirculated from their holding vessels to the ED cell and back by Cole Parmer peristaltic pumps.

In ED for ISPR, a pH probe was placed in-line between ED cell and the return to the holding vessel. A pH controller switched a relay to turn the power supply on and off according to a pH set point of 6.8.

Prior to connection with the fermenter, all reservoirs of the electrodialysis unit were filled with 500ml 1M NaOH and this solution recirculated through the cell for 30min to sterilise it. The compartments were were then washed with autoclaved deionised water to remove any excess base. Valved quick disconnect couplings were used to connect the electrodialysis unit to the fermenter, and these were sterilised using 70% ethanol prior to connection.

2. Fermentation

Actinobacillus succinogenes strain 130Z (available from culture collections ATCC 55618, DSMZ 22257).

Trypticase soy broth (TSB)

Trypticase soy agar (TSA)

Glucose

Corn Steep Liquor (solids removed)

Carbon dioxide

0.2micron air filter

Antifoam, propylene glycol

Software controlled Applikon Bio Reactor (3litre capacity with stirrer, CO2 and glucose feed, pH and temperature control).

Analytical method

HPLC column: MetaCarb 67H 300x6.5mm

Column temp: 35degC

Eluent: 0.05N H2SO4

RI detector

Injection volume: 10microlitre

Flow rate: 0.8ml/min

Run time: isocratic for 13min

Culture of *Actinobacillus succinogenes*

All incubations were carried out at 37degC under a CO2 atmosphere.

1. A freeze-dried vial of *Actinobacillus succinogenes* DSMZ 22257 was rehydrated in sterile TSB and streaked on TSA petri dishes.
2. A single isolated colony froma fresh culture plate was used to inoculate 80ml sterile TSB in a Duran bottle; incubated overnight at 30rpm and 37degC in a gas jar flushed with CO2 for 5min prior to incubation. This culture was the inoculum for the fermentation (subject to prior check by HPLC, optical density and Gram stain).

Fermentation media

1.5litres of TSB based medium with 8%w/v glucose and 10%w/v corn steep liquor was prepared. 0.3ml of antifoam was added once all the media components were in the fermenter (ut before addition of inoculum). The pH was adjusted to 6.8 by automatic addition of 40%w/v NaOH.

Fermentation

The fermenter was operated at 500rpm impeller speed, at a CO2 flow rate of 1vol/vol/min.min^{-1} at 37degC. 40%w/v NaOH was used to maintain the pH at 6.8±0.5. Samples were taken at 2-3hrs intervals and assayed for organic acid composition and glucose content.

A semi-continuous mode fermenter operation was also assessed with succinate removal by ED and an additional glucose feed to maintain glucose levels at approx. 50g/l.

Electrodialysis – downstream processing

Initial experiments were performed to determine optimal conditions for succinic acid recovery using mixtures of representative acidic fermentation products reported for batch fermentations of *A.succinogenes* (Table 1).

Table 1 Typical organic acids present at end of batch fermentation[3].

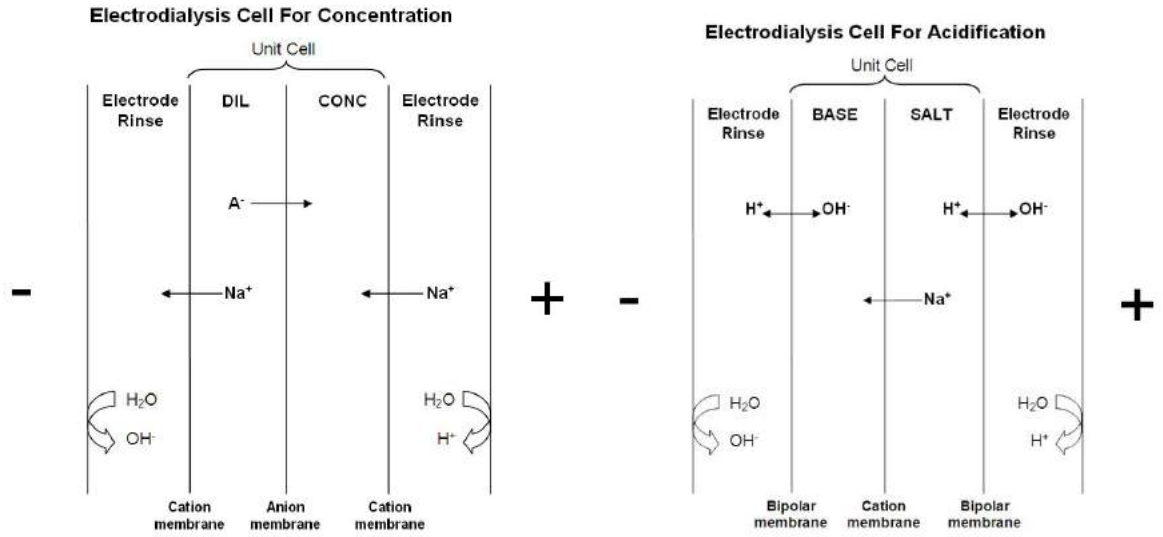
	Succinate	Acetate	Formate	Propionate	Pyruvate
Typical final concentration (g/l)	67.2	12	8.7	2.5	4.3
Formula weight (g/mole)	118	60	46	74	88
molarity	0.57	0.2	0.19	0.03	0.05
pK_a1	4.16	4.75	3.75	4.87	2.49
pK_a2	5.61				
Solubility	76.9	miscible	miscible	miscible	miscible

In electrodialysis, ions migrate through ion-exchange membranes due to an imposed electric field. The flux of an ion through the membrane is dependent upon the bulk concentration of the ion and its mass transport to the membrane surface.

In this case, succinate has the highest concentration, however other anions will compete. Some selectivity can be achieved by altering the pH so that it is below that of the pK_a of acetic and propionic acids. At pH4.6, acetic and propionic acids are protonated and therefore neutral and won't migrate under the electric field. Also, succinic acid is singly charged at this pH and so less charge per mole is required to transport the ion than if it were doubly charged. Pyruvate and formate are charged at pH4.6, however the lower solubility of succinic acid indicates it could be selectively recovered by precipitation from concentrated solutions.

Recovery of succinic acid from batch culture supernatant can be performed by adjustment of pH to 4.6 followed by two successive ED steps (Fig.1). First, the dilute succinate solution was concentrated by conventional two-compartment ED. As only ions from the broth migrate, this step also partly purifies the product. Next the concentrated succinate solution was acidified by two-compartment ED with bipolar membranes to make succinic acid and NaOH. The NaOH can then be recycled to the fermenter for pH control.

Fig.1 Succinic acid production by electrodialysis, showing membrane configurations for concentration by conventional ED and acidification by ED with bipolar membranes.



In practice, 95% of total succinate could be removed from fermentation broths using downstream ED processing. This was combined with >90% reduction of other contaminating acids in the product stream. The process cost (ED) for recovery of succinate by this method is estimated to be £0.12/kg based on energy costs for industrial users.

Some pretreatment of the fermentation broth is required before ED processing in order to protect the membranes, which involves filtration to remove cells and particulate matter from supernatants and ion-exchange to remove cations Mg^{2+} and Ca^{2+}.

Electrodialysis – *in situ* product recovery

ED can also be used to couple continuous product recovery to the fermentation process. In this case, ED is performed at pH6.8, which reduces selectivity for succinate removal. However, the production of other acids was suppressed compared to levels seen in batch mode fermentation of this organism, effectively improving the product specification. A feed of cells and culture medium is circulated to an ED cell (Fig.2), where succinate is continuously removed into the BASE stream and the residual culture containing cells and nutrient is recirculated back into the fermenter vessel. The pH of the fermentation was controlled using a relay on the pH controller to switch the power supply to the ED cell on and off. A glucose feed was used to maintain cell productivity. In this case, the continuous removal of succinate resulted in overall product yield and more efficient conversion of glucose to succinate.

Features of both batch and continuous fermentation systems are summarised in Fig.3.

Fig.2 Continuous succinic acid removal from fermentation by electrodialysis

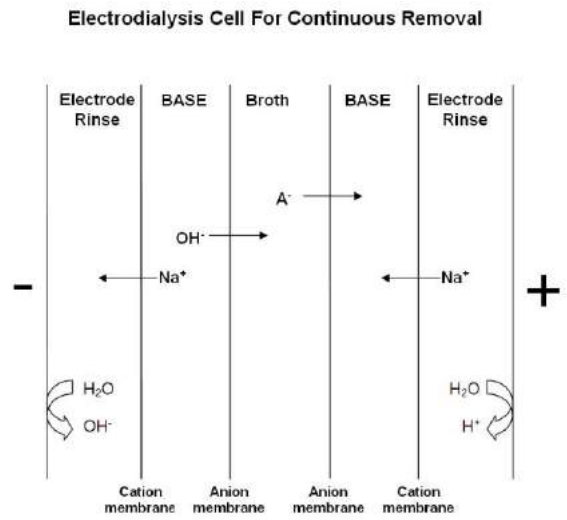
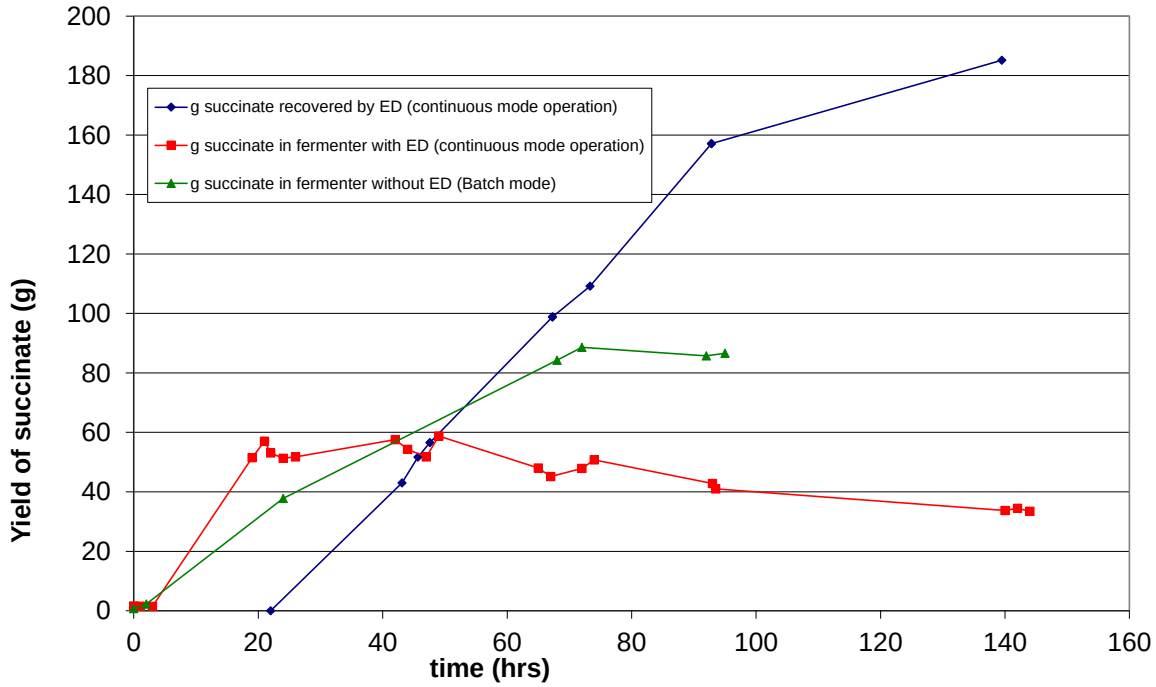


Fig.3 Succinate yields for batch and continuous cultivation of *A.succinogenes* in a batch fermenter with product recovery by two-step ED (batch system) or single-step ED (continuous system). Yields were estimated by HPLC in each case.



Conclusion

ED can be used as a processing aid for selective recovery of succinic acid from fermentations, both as a downstream batch mode recovery system and as a method of *in situ* product removal (ISPR) from continuous or semi-continuous production systems. The latter has some promising features which could enhance biomass productivity and simplify product recovery (Table 2).

Table 2 Yields and process control of batch and continuous mode methods for succinate recovery by ED

Product Recovery	Down stream process	Process with continuous product recovery
Total succinate produced	59 g	218 g (185 g recovered by ED system plus 33 g in fermenter)
Product recovery mode	Post batch fermentation, cell free liquor	Continuous product removal during fermentation process
Process volume control	Process limited by volume changes on base addition (eventually diluting product).	Fermentation volume control improved (base recycling feasible, acid removal, glucose feed)
Product production control	Process limited by closed state (succinate production in log phase, followed by mixed acid in later phases). A complex and changing multiphase pattern of acid production made optimum 'end point' estimation difficult.	Increased glucose consumption and acid removal associated with increased succinate production ('steady state'). Conversion of glucose to product efficiency improved by >20%

In principle, the ED system concepts used here could also be applied to other reaction systems for specific product recovery and/or improvement of product yields where final yields in closed systems are currently unfavourable.

A more rigorous evaluation of cell productivity using the ISPR system would require more complex analysis of nutrient requirements and controls of nutrient feeds to ensure that cell viability and maximal succinic acid productivity can be maintained over extended periods.

References

1. Guettler, M.V.; Rumler, D. and Jain, M.K. *Int. J. Syst. Bacteriol*. **1999**, *49*, 207-216.
2. van der Werf, M.J.; Guettler, M.V.; Jain, M.K. and Zeikus, J.G. *Arch. Microbiol*., **1997**, *167*,
   332-342.
3. Guettler, M.V.; Jain, M.K. and Rumler, D. *US PCT Appl*. **1996**, US 5 573 931.