RECOVERY OF PYRUVIC ACID FROM FERMENTATION BROTH:

PROCESS DEVELOPMENT AND MODELING

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Abstract. Besides the production process, the product recovery must be also taken into consideration – not only because of its usually high contribution to the total process costs but also on account of the necessity of integrating it on-line into the production process if the product is inhibitory. In the case of pyruvate, some indications have been published that high extra-cellular pyruvate concentrations > 500 mmol dm^{-3} might cause a significant inhibition of microbial pyruvate synthesis. Hence, pyruvate process development should consider strategies for the separation of the product and to purify it as well.

Ion exchange and solvent extraction followed by distillation are common methods for pyruvate separation from cell-free fermentation broth. However, the low exchange capacity of resins and the use of concentrated acids as eluents makes the application of ion-exchange for pyruvate isolation very difficult and not attractive in terms of sustainable process development as well as in commercial retrospect.

However, the charge-dependent ion-separation in the electric field of electrodialysis is regarded as a promising approach for the downstream processing of organic acids from fermentation broth. It has been widely applied for production of table salts, organic acids, amino acids and sugar demineralization as well as for blood treatment and wine stabilization. Electrodialysis represents one of the most important membrane processes for environmentally clean technology in biochemical industries.

To develop a pyruvate production process with integrated product separation using the production strain *Escherichia coli* YYC202 *ldhA::Kan*, was motivated by the working hypothesis that high extra cellular pyruvate concentrations might be inhibitory. To separate pyruvate from fermentation broth a fully integrated continuous process has been developed. In this process electrodialysis was used as a separation unit.

Additionally, mathematical model to represent the ion and water transport behavior of an electrodialysis process for concentrating pyruvic acid under the influence of different current density was developed. The model validation will be demonstrated in the work.

Keywords: *Escherichia coli*, pyruvate, electrodialysis, product recovery

Introduction

The carboxylic acid pyruvate represent without doubt one of the most important metabolites in central metabolism, due, for instance, to its significance for the phosphotransferase (*pts*) dependent glucose uptake, its role as a precursor for amino acid synthesis, overflow metabolism and so on. Recently, the importance of pyruvic acid as a reactant in the *E. coli* network was stressed by identifying it as one of the mostly connected metabolite (apart from common coenzymes)[1].

Apart from its significance in metabolism, pyruvate might not be known as a commercially interesting building block for chemical synthesis. However, it serves as an effective starting material for the synthesis of many drugs, agrochemicals and nowadays in the food industry as a fat burner. It is also valuable substrate for the enzymatic production of amino acids such as L-tryptophane, L-tyrosine and L-dihydroxyphenylalanine (L-DOPA)[2].

Due to the importance of and the great demand for pyruvate, different processes for the production of pyruvic acid have been developed in the past. They include both the classical chemical approach and biotechnological methods. Pyruvic acid is produced on an industrial scale by classical approach including dehydration and decarboxylation of tartaric acid[3]. Although this process is simple to realize, it is not cost-effective and does not fulfill modern demands sustainable "green chemistry" processes with low energy input. An alternative approach to reduce production cost and to fulfill environmental constrains at the same time is the use of biotechnological methods. There are basically three different approaches: enzymatic, resting cells and fermentation methods[4]. In comparison to the other approaches, fermentation methods offer the opportunity to produce pyruvate from the sustainable, low-cost substrate glucose with high product/substrate yield while at the same time avoiding the co-production of unwanted by-products like H_2O_2, as is usually the case when enzymatic methods are employed.

Pyruvate process development, therefore, started, by using the recombinant strain *Escherichia coli* YYC202 ldhA::Kan[5,8], which is completely blocked in its ability to convert pyruvate into acetyl-CoA or acetate. Consequently, acetate is required for growth on glucose minimal medium. By controlling acetate and glucose feed rate, a series of lab scale fed-batch experiments were performed. At optimal process conditions a final pyruvate titer (c_P) higher than 62 g dm^{-3}, a space-time yield (STY) of up to 42 g dm^{-3} d^{-1} and pyruvate/glucose molar yield ($Y_{P/G}$) of 1.11 mol mol^{-1} were achieved[6,8]. Experimental evidence was gathered that high extra cellular pyruvate concentration inhibits the process. To face this problem with process engineering means, repetitive fed-batch experiments with cell retention were performed[7,8]. Molar $Y_{P/G}$ was improved up to 1.70 mol mol^{-1} and STY was increased more than 300 % and has reached 145 g dm^{-3} d^{-1}.

To separate pyruvate from fermentation broth fully integrated continuous process with *in situ* product recovery has been developed[7,8]. In this process electrodialysis was used as a separation unit. Under optimum conditions a (calculated) final pyruvate titer higher than 79 g dm^{-3} was achieved. The aim of our work is to develop an empirical model of the electrodialysis process, to estimate model parameters and, finally, to validate a model.

Materials and Methods

Electrodialysis

The experimental equipment for electrodialysis used a three-compartment water splitting electrodialysis (WSED) unit (Goema, Germany) with a membrane stack having four cells pairs. The three-compartment WSED stack consisted of anion-exchange, cation-exchange and bipolar membranes (Tokuyama Co., Japan) with a total effective membrane area of 0.56 m^2.

An electric power unit (Rohde & Schwarz, Germany) supplied power to the WSED stack such that a constant current in the range of 5 A was realized. The WSED consisted of four solution tanks providing the acid, the base, the feed and the electrode rinse solution. Initially the acid and the base compartments were filled with demineralized water. All streams were recycled using centrifugal pumps (Iwaki Co., Japan) at a flow rate of 60 dm^3 h^{-1}. pH (Methrom, Germany) and conductivity (WTW, Germany) of the acid, the base and the feed

solutions were measured continuously. 0.5 mol dm^{-3} Na_2SO_4 was used as the electrolyte solution.

Mathematical model of the electrodialysis process

A simple mathematical model representing the ion and water transport behavior of the electrodialysis process to concentrate pyruvic acid under the influence of different current (current density) was developed. It was assumed that the amount of the water transferred from the feed compartment (FC) to acid compartment (AC) is directly proportional to the current density applied. The water transport trend form the FC to the AC is expressed in the form of Equation 1:

$$V_{AC}^{t} = V_{AC}^{0} + \Delta V_{AC} \quad \textbf{1}$$

where V_A^t is the volume of AC at time t, V_A^0 the initial volume of AC and ΔV_A the volume of water being transported from the FC to AC. The term ΔV_A could be replaced by the term $(\alpha + \beta \cdot i) \cdot t$ represents the amount of the water being transferred from the FC to the AC (Equation 2). Both α and β terms are called phenomenological coefficients[9], and i is current density.

$$V_{AC}^{t} = V_{AC}^{0} + (\alpha + \beta \cdot i) \cdot t \quad \textbf{2}$$

The rate of ion transport was determined by evaluating the pyruvate concentration in the AC at any time t (Equation 3)[9].

$$c_{AC}^{t} = \frac{\left(A + B \cdot c_{FC}^{0}\right) \cdot I \cdot t + c_{AC}^{0} \cdot V_{AC}^{0} \cdot F}{V_{AC}^{t} \cdot F + (\alpha + \beta \cdot i) \cdot t \cdot F + B \cdot I \cdot t} \quad \textbf{3}$$

where A and B are ion transport rate constants, c_{FC}^0 is initial pyruvate concentration in the FC, I is current, c_{AC}^0 is initial pyruvate concentration in the AC, V_{AC}^0 is the initial volume of the AC, and F is Faraday constant. Equation 3 is derived from the different equations for calculation of current efficiency (Equations 4 and 5).

$$CE = \frac{\left(c_{AC}^{t} \cdot V_{AC}^{t} - c_{AC}^{0} \cdot V_{AC}^{t}\right) \cdot F}{I \cdot t} \quad \textbf{4}$$

$$CE = A - B \cdot \left(c_{AC}^{t} - c_{FC}^{0}\right) \quad \textbf{5}$$

Strain, medium, cultivation and analysis

Strain, medium, cultivation and analysis for pyruvate production are the same as already described[7,8].

Results and Discussion

Effect of current on the performance of electrodialysis

Preliminary experiments using a synthetic solution (25 g dm^{-3} pyruvate in water) were performed in order to study the effect of current on the performance of the electrodialysis process (Figure 1). Experimental results are summarized in Table 1.

The power supply was set at a constant current in the range of 2 – 10 A. Experiments were terminated when the conductivity of the feed solution was 1 mS cm^{-1}. Current efficiencies higher than 91 % and pyruvate recovery higher than 91 % were achieved in each experiment. The lowest current efficiency was observed in the experiment at constant current of 10 A. If the electrodialysis is operated at a current density beyond the limiting value, the current efficiency significantly decreases, and excessive energy is also dissipated for splitting water. However, in the range tested up to 10 A no limiting current could be found, and there was no sudden change observed in resistance at 25 g dm^{-3} of pyruvate. Anyhow, lower current efficiency in the experiment at a constant current of 10 A cannot be explained by limiting current[10].

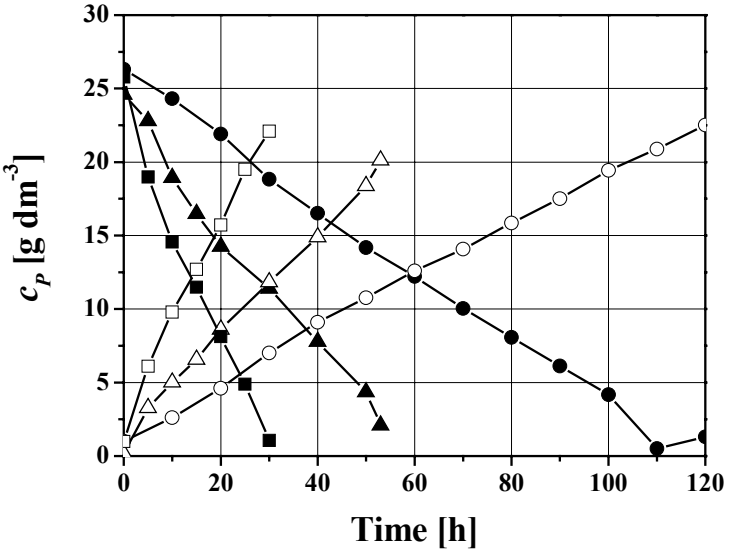

Figure 1 Pyruvate concentration (c_P) in the electrodialysis experiments for different currents; 2 A - ●, 5 A - ▲, and 10 A - ■ (closed symbols – pyruvate concentrations in the feed compartment; open symbols – pyruvate concentration in the acid compartment)

Table 1 Comparison of results for electrodialysis of pyruvate model solution, for different currents

Current [A]	Pyruvic acid recovery [%]	Current efficiency [%]	Energy consumption [kWh kg^{-1}]	Volumetric productivity [g dm^{-3} h^{-1}]
2	95.0	97.0	1.3	11.8
5	91.5	96.3	1.4	22.5
10	94.4	91.3	1.7	45.2

High current efficiency leads to a reduction in the membrane area, and low energy consumption to a reduction in the operating costs. 20 % higher energy consumption for recovery of 1 kg of pyruvic acid in the experiment at a constant current of 10 A was a reason for performing of further experiments at lower constant current even volumetric productivity was approximately 2 fold higher (Table 1).

Effect of medium components on the performance of electrodialysis

Preliminary experiments using a synthetic solution (48.0 g dm^{-3} pyruvate in water) and fermentation broth (42 g dm^{-3} pyruvate) were performed in order to study the effect of medium components on the performance of the electrodialysis process (Figure 2). Experimental results are summarized in Table 2. Experiments were carried out at a fixed current (5 A) for the whole period. Each experiment was stopped when the stack voltage increased to a maximum value of 40 V to prevent a possible membrane rupture. Current efficiencies higher than 99 % and 91 % pyruvate recovery were achieved in the experiment with the model solution. Compared to the best result 30 % recovery decrease was observed by the use of cell-free fermentation broth (CFB) with a similar current efficiency.

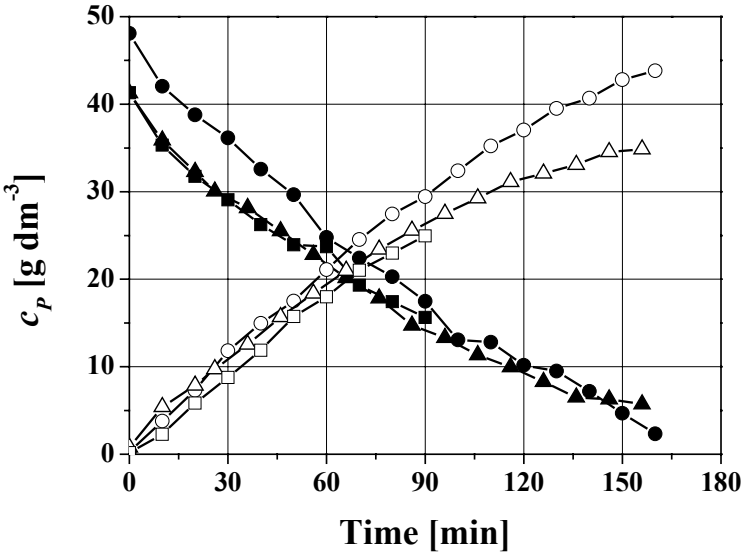

Figure 2 Pyruvate concentration (c_P) in the electrodialysis experiments for pyruvate model solution - ●, cell-free fermentation broth (CFB) - ▲, and cell- and protein-free fermentation broth CPFB - ■ (closed symbols – pyruvate concentrations in the feed compartment; open symbols – pyruvate concentration in the acid compartment)

Table 2 Comparison of results for electrodialysis of pyruvate model solution, cell-free fermentation broth and cell- and protein-free fermentation broth

	Feed medium initial pyruvate [g dm^{-3}]	Acid medium final pyruvate [g dm^{-3}]	Pyruvic acid recovery [%]	Current efficiency [%]
Model solution	48.0	43.8	91.2	99.4
CFB	41.3	24.9	60.2	99.8
CPFB	41.3	34.8	84.3	95.6

However, when fermentation broth was used, higher voltage levels and an accelerated voltage increase were measured indicating membrane fouling by high-molecular medium components like proteins, cell debris etc. Hence, an ultra-filtration step (cut-off 10 kDa) was installed for protein separation, which resulted in an 84 % pyruvate recovery achieving 90 % current efficiency. We concluded that cell- and protein-free broth (CPFB) should be used for electrodialysis. However, slightly reduced pyruvate recovery and current efficiency compared to the model solution (most presumably due to salts in the fermentation medium) must be

taken into account. Additionally, in experiments with cell- and protein-free fermentation broth glucose rejection of 95 % was observed. The final pH of the recycled feed solution in each experiment was higher than 3, which ensured sufficient amounts of pyruvic acid ions ($pKa_{pyruvate} \cong 2.5$) in the feed[11].

Ion and water transport modeling in electrodialysis

The rate of water transport could be evaluated from the slope in relationship between the water being transferred and the time. As was observed the amount of the water transported was directly proportional to the current density (Figure 3). By mean of linear regression parameters α and β (Equation 2) were estimated (Table 3) from experimental results.

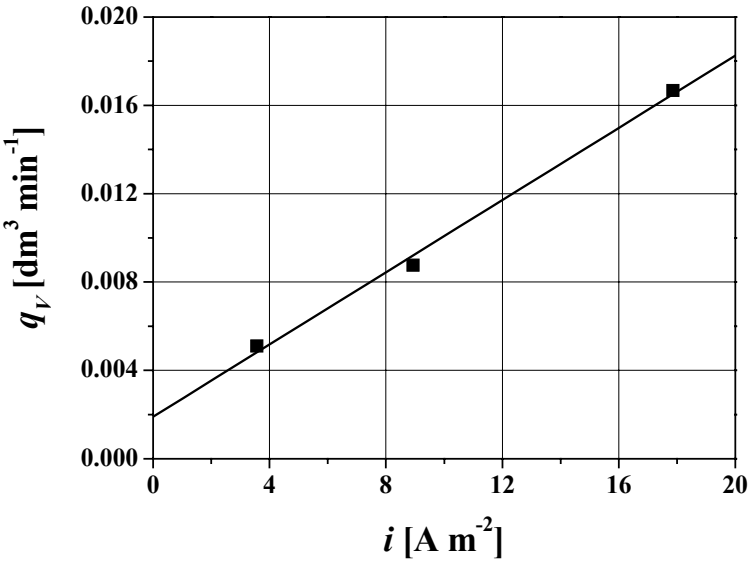

Figure 3 The relationship between the water transport rate and the current densities

Parameters A and B (Equation 3, Table 3) were estimated by the non-linear regression analysis from the data set obtained in the electrodialysis of the pyruvate model solution at 10 A. It should be mentioned that different ion exchange membranes as well as different solutions would affect the value of constants A and B[9]. Therefore, these constants should be determined experimentally for different ion exchange membranes and solutes used.

Table 3 Overall phenomenological coefficient of water transport and ion transport rate constant electrodialysis of the pyruvic acid

α [dm^3 min^{-1}]	β [dm^3 m^2 min^{-1} A^{-1}]	A [mmol A^{-1} min^{-1}]	B [A^{-1} min^{-1}]
$1.91\ 10^{-3} \pm 0.63\ 10^{-3}$	$8.17\ 10^{-4} \pm 0.54\ 10^{-4}$	$3.78\ 10^{5} \pm 1.20\ 10^{5}$	152 ± 122

In order to verify the developed water and ion transport model for the electrodialysis of pyruvic acid, the calculated values obtained from the model simulation were compared to the collected data from experiments at different current. Both calculated as well as experimental results for the concentration of pyruvic acid in the acid compartment are plotted as shown in Figure 4.

The graph indicates that the developed water and ion transport model is reliable to describe the transport behavior for this electrodialysis system even for a different current (current density's). This model could be used for the prediction of the other condition for water and ion transport phenomena under similar operating conditions (same type of ions, same membranes and membrane area).

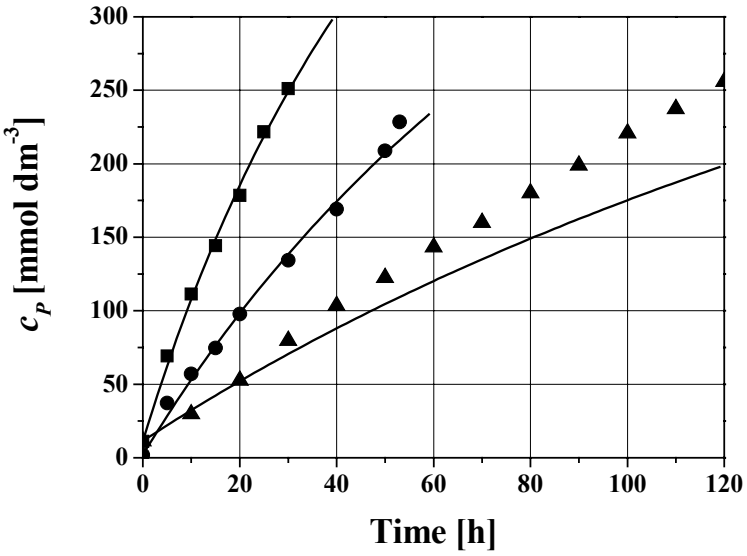

Figure 4 Variation results between both calculated (—) and measured data (10 A - ■, 5 A - ●, 2 A - ▲) for pyruvic acid concentration in the acid compartment for the different current applied

In situ *Product Recovery (ISPR) Approach with Fully Integrated Electrodialysis*[7]

It was planned that three streams should left the electrodialysis unit: the pyruvic acid reduced fermentation permeate, the pyruvate enriched acid stream and NH_4OH enriched base stream. The pyruvic acid reduced fermentation permeate contained glucose with some nutrients and could be reused in a continuous recycling process. Consequently, a reduction of raw material costs was expected. NH_4OH produced in the three-compartment electrodialysis could also be reused to decrease the amount of the fresh base needed for pH titration. Based on the preliminary experiments, it was expected that pyruvate could be concentrated in the acid stream thus allowing a simplified purification later on. Fermentation suspension was first ultra-filtrated (cut-off: 500 kD) to produce a cell free permeate which was pumped through a second ultra-filtration unit (cut-off: 10 kD) to separate proteins and cell debris. Then cell and protein free fermentation solution entered the electrodialysis. Fermentation permeate with reduced pyruvic acid was recycled into the bioreactor through a sterile micro-filtration unit to prevent a possible contamination by the non-sterile pyruvate separation process. A constant flow of the NH_4OH containing base stream was pumped into the bioreactor to support pH regulation.

It can be stated that the fully integrated separation of pyruvic acid via electrodialysis was successfully realized[7,8]. Pyruvate was concentrated in ED up to 48.4 g dm^{-3} with purity higher than 95 %. Only 7% of glucose were also co-separated, which is comparable to the results of the preliminary experiments. If the total amount of pyruvate produced is referred to the real working volume of the bioreactor, a final concentration of about 79 g dm^{-3} would have been achieved.

Conclusion

The use of electrodialysis (ED) has shown that this approach is well suited for separating pyruvate from fermentation broth – even as a fully integrated *ISPR* approach. Although these results are promising, further *ISPR* studies using ED should focus on the unwanted co-separation of other medium components to enable a long-term pyruvate production. Using ED for pyruvate separation should be favored with respect to a sustainable process development because it offers the opportunity to separate pyruvate without using solvents, to concentrate the product and to minimize process waste-water.

Developed model of the electrodialysis process could be used for the prediction of the other condition for water and ion transport phenomena.

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