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Application of bipolar electro dialysis to *E. coli* fermentation for simultaneous acetate removal and pH control

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Abstract The application of bipolar electro dialysis (BPED) for the simultaneous removal of inhibitory acetate and pH control during *E. coli* fermentation was investigated. A two cell pair electro dialysis module, consisting of cation exchange, anion exchange and bipolar membranes with working area of 100 cm² each, was integrated with a standard 7 l stirred tank bioreactor. Results showed that BPED was beneficial in terms of in situ removal of inhibitory acetate and a reduction in the amount NH₄OH used for pH control. In batch and fed-batch BPED fermentations, base additions were decreased by up to 50% in both cases compared to electro dialysis (ED) fermentations with pH controlled at 6.7 ± 0.1. Consequently, the final biomass (34.2 g DCW l⁻¹) and recombinant protein (5.5 g l⁻¹) concentrations obtained were increased by up to 37 and 20%, respectively.

Keywords Acetate removal · Bipolar electro dialysis · *E. coli* TG1 · pH control

Introduction

E. coli is widely used for the production of recombinant enzymes and therapeutic proteins. One of the factors limiting the productivity of *E. coli* fermentations is the formation of acetate which causes inhibition of cell growth and recombinant protein synthesis (Han et al. 1992). A number of approaches exist to overcome this problem (Eiteman and Altman. 2006), the most widely used being controlled feeding of the carbon source (Akesson et al. 2000). Recently we have demonstrated the benefits of electro dialysis (ED) applied to *E. coli* fermentation to reduce the concentration of acetate in the media and hence increase both cell growth and the specific recombinant protein production (Wong et al. 2009). Concerns remain however regarding the volume of NH₄OH added for pH control which could also be toxic to the cells (Muller et al. 2006). A potential solution is the use of bipolar electro dialysis (BPED) which can hydrolyse an aqueous salt solution under the influence of electrical current to produce H₂ and OH⁻. In principle this approach could be used to directly acidify or basify the fermentation broth without the addition of extraneous chemicals. This technology has been widely used in the production of

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organic acids (Huang et al. 2007; Jaime-Ferrer et al. 2008), amino acids (Grib et al. 1998) and in the field of environmental protection (Koter and Warszawski 2000). From an operational perspective, the cost of acid or base could be reduced and the process could have reduced requirements with regard to treatment of the spent broth.

The aim of this work is therefore to demonstrate the principle of BPED fermentations and compare cell growth and protein production to our previous studies on batch and fed-batch fermentations with or without ED application (Wong et al. 2009). To the best of our knowledge no previous study has reported simultaneous acetate removal and pH control of *E. coli* fermentation by BPED.

Materials and methods

The *E. coli* strain, culture media, equipment and operating conditions used for shake-flask, batch and fed batch fermentations were the same as described previously (Wong et al. 2009), as were all the analytical protocols. All cultures of *E. coli* TG1 pGLO were induced at early exponential phase with 0.25% (w/v) L-arabinose for the synthesis of recombinant green fluorescent protein (GFP). Results represent typical profiles from at least duplicate independent fermentations.

Bipolar electro dialysis module and fermentations

Figure 1 shows a schematic diagram of the BPED system used. The module consists of six equal volume compartments fitted with two electrodes at each end, supported by one-cation exchange, two-anion exchange and two-bipolar membranes (Neosepta, Tokuyama Corporation, Japan) having a total effective membrane area of 200 cm². Prior to operation, the module was chemically sterilised with 1 M NaOH. When BPED was applied, the fermentation broth was circulated through compartment F at 500 ml min⁻¹, while 2 l of 2 M Na₂SO₄ flowed through compartments A, R and C at the same rate. Automatic pH control was achieved using a proportional-integral-derivative (PID) controller, linking measured pH with applied current. When the pH fell below the set point of 6.7, due to the production of acetic acid, the power output was increased

accordingly, up to a maximum current of 5 A and a maximum voltage of 40 V. This resulted in the generation of OH⁻ from the bipolar membrane in compartment F as well as removal of acetate ions from the fermentation broth in compartment F to compartment R via the anion exchange membrane. When the pH returned to pH 6.7, the current was switched off, so the pH remained constant, such that there would not be any removal of ions from compartment F.

The use of BPED thus allows pH control without direct acid or base addition. In the media formulation used, some NH₄OH addition is required as a nitrogen source (Korz et al. 1995). In order to facilitate this, the dynamic of the PID controller used for BPED unit operation was reduced by 20%, so that the rate of hydroxyl ions produced was decreased. Consequently, some NH₄OH was added by the conventional pH control system of the fermenter but at a rate more closely linked to the nitrogen requirements of the cells rather than that required for pH control.

Results and discussion

Ammonium toxicity

To demonstrate the impact of ammonium toxicity on the cells, a small scale preliminary experiment was performed involving additions of 10, 20 and 30 g NH₄Cl l⁻¹ to separate 100 ml shake-flask cultivations. These amounts were indicative of the quantity of ammonium used for pH control in our previous batch and fed-batch fermentations (Wong et al. 2009). Figure 2 gives an indication of ammonium toxicity on growth of the *E. coli* TG1 cells. Once NH₄Cl was added at mid-exponential phase, there was an immediate effect on the cells resulting in a reduction of cell growth rate. Addition of 30 g NH₄Cl l⁻¹ led to a 50% reduction in the final biomass concentration. These data suggest that the ammonium concentrations in the broth should be maintained below 10 g l⁻¹.

Batch and fed-batch BPED fermentations

The results of batch and fed-batch BPED fermentations are presented in Figs. 3 and 4, respectively. Reproducible trends for cell growth, acetate accumulation and recombinant protein production were

Fig. 1 Principle of bipolar membrane electro dialysis for simultaneous in situ removal of acetic acid from *E. coli* fermentation broth and bioreactor pH control: *AEM* anion-exchange membrane; *BPM* bipolar membrane; *A* anode compartment; *R* recovery compartment; *F* fermentation compartment; *C* cathode compartment

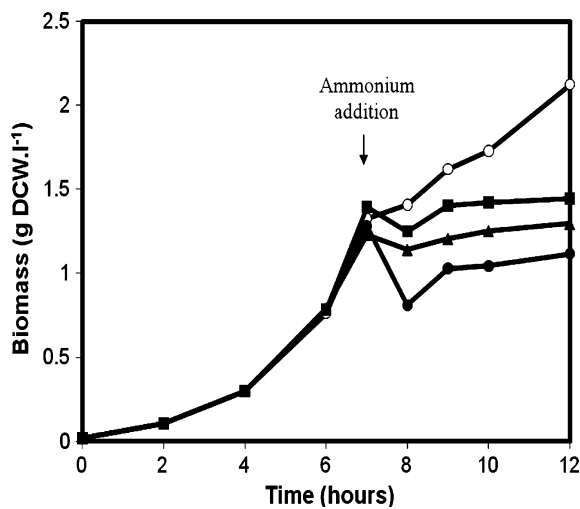
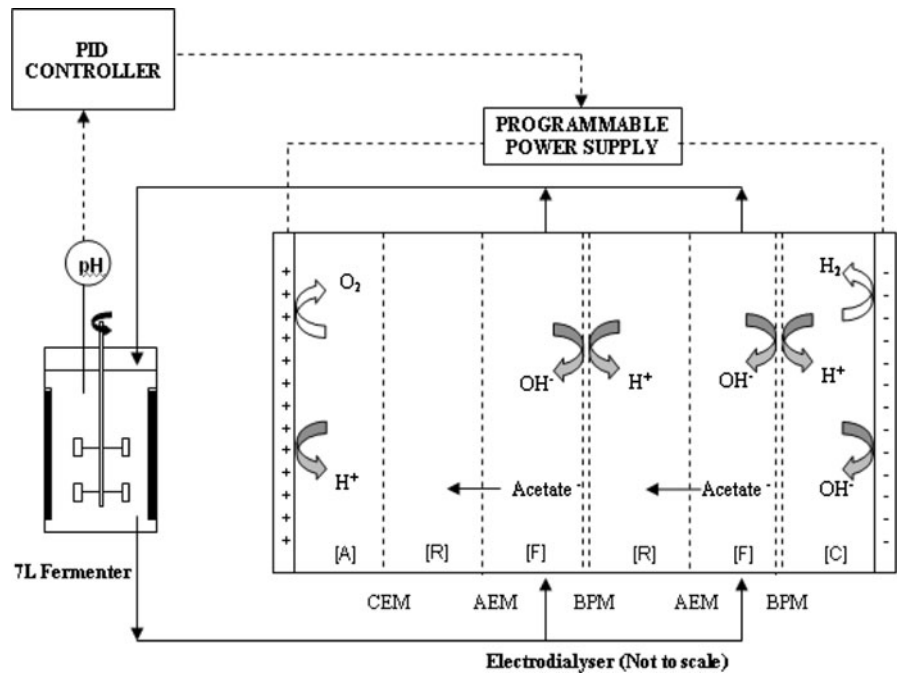


Fig. 2 Effect of ammonia on growth of *E. coli* TG1 pGLO in shake-flask fermentations with additions of 0 (open circle), 10 (filled square), 20 (filled triangle) and 30 (filled circle) g NH₄Cl l⁻¹. Arrow indicates time of ammonium chloride addition

obtained in replicate independent experiments. In batch BPED fermentations, BPED was applied after the lag phase at 6 h and arabinose was added to induce GFP synthesis at early exponential phase. The biomass and protein concentrations continued to increase to 15.4 and 3.3 g l⁻¹ respectively at 18 h.

Similar trends can be observed for the initial phase of the fed-batch BPED fermentation. When exponential feeding started at 10 h, biomass and protein concentrations increased linearly up to 30 h, reaching final concentrations of 34.2 and 5.5 g l⁻¹ respectively. Figures 3b and 4b indicate the corresponding base additions and power output of each experiment. These are important parameters of BPED performance and are discussed further in the next section.

Comparison of ED and BPED fermentations

Table 1 summarises the results of the BPED fermentations performed here and compares them to our previous results from batch and fed-batch fermentations with or without application of conventional electro dialysis. The presence of acetic acid retards cell growth and inhibits recombinant protein production (Han et al. 1992). Consequently we were able to show that the application of conventional ED for in situ acetate removal enhanced cell growth and recombinant protein synthesis (Wong et al. 2009). A limitation of the earlier ED studies was that the anion exchange membrane area was limited to 100 cm². It is evident in this work that by doubling the anion exchange membrane area to 200 cm², the rate of acetate ion removal is increased and so the

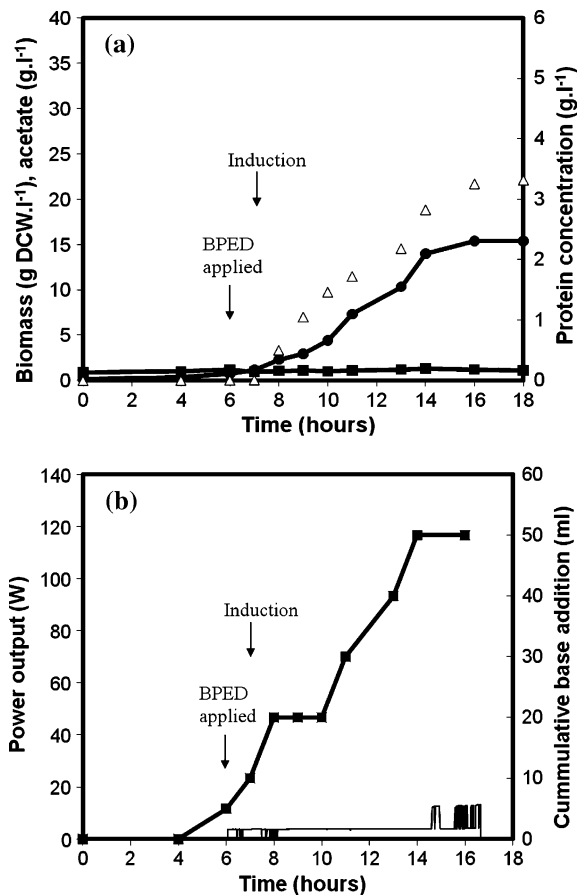


Fig. 3 Bipolar electrodiagnosis batch fermentation kinetics of *E. coli* TG1 pGLO. **a** Off-line data for biomass (filled circle), acetate (filled square) and recombinant protein, GFP (open triangle) concentration. **b** Power output (dash) and base addition (filled square). Arrows indicate times of induction and BPED application

accumulated acetate concentrations were reduced by up to 50%.

In addition to removal of acetate, however, ED also removed OH^- from compartment F to R and as much as 50% extra base addition was required for pH control. In contrast to the ED fermentations, the additional PID controller used in the BPED system led to the rate of ion removal being proportional to the amount of acetate present so fewer OH^- were removed. This conservation of OH^- in compartment F and simultaneous generation of OH^- for pH control from the bipolar membrane resulted in reduction of base addition by up to 60% in batch and 71% in fed-batch mode respectively. In addition, by providing a less toxic environment for cell growth and further

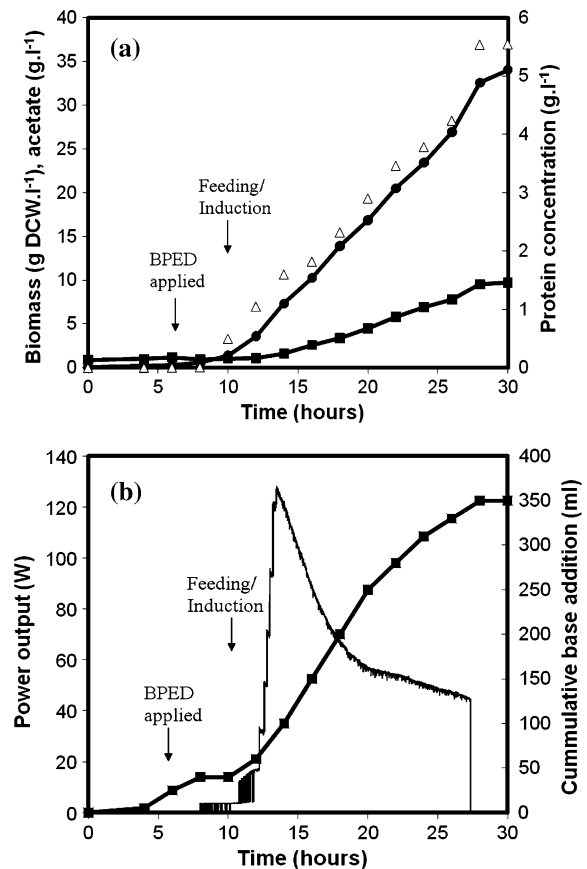


Fig. 4 Bipolar electrodiagnosis fed-batch fermentation kinetics of *E. coli* TG1 pGLO. **a** Off-line data for biomass (filled circle), acetate (filled square) and recombinant protein, GFP (open triangle) concentration. **b** Power output (dash) and base addition (filled square). Arrows indicate times of induction, feeding and BPED application

reductions in acetate accumulation, there was an increase in biomass yield of up to 37% in fed-batch fermentation and an increase in recombinant protein production of up to 20%.

With respect to operation of the ED/BPED units reduced energy consumption is important as it leads to a reduction in operating cost and reduced environmental footprint of the process. ED fermentations operated at a fixed current of 2 A with a voltage range of 35–40 V, so the power output was fixed at 70–80 W. From Figs. 3b and 4b, the calculated average power output for BPED batch and BPED fed-batch fermentations were 6.5 and 59 W, respectively. Based on the ED power consumption data there appears to be a specific power consumption of the order of 10 kW m^{-3} . However, this is due to the

Table 1 Comparison of batch and fed-batch *E. coli* TG1 pGLO fermentation kinetics with standard electro dialysis (ED) application and application of bipolar electro dialysis (BPED)

Fermentation conditions	[Biomass] (g DCW l ⁻¹)	[Protein] (g l ⁻¹)	[Acetate] (g l ⁻¹)	Volume of base addition (ml)	References
Batch	13.6	2.9	5.5	100	Wong et al. (2009)
ED batch	12.3	3.1	2.1	125	Wong et al. (2009)
BPED batch	15.4	3.3	1.3	50	This work
Fed-batch	30.5	5.0	25.1	800	Wong et al. (2009)
ED fed-batch	24.9	4.6	18.8	1200	Wong et al. (2009)
BPED fed-batch	34.2	5.5	9.7	370	This work

small scale and experimental nature of the ED device used. The lower energy consumption is a major advantage of BPED over ED. Furthermore, due to the lower power output with BPED, electromigration only took place when acetate was present, so energy was not wasted in the removal of other ions. This is even more significant at industrial scale where energy consumption and base used can be major cost factors.

Conclusions

This work has demonstrated for the first time the use of a PID controller system for bioreactor pH control and simultaneous in situ removal of inhibitory acetate by bipolar electro dialysis. BPED was able to remove up to 75% of acetate formed from the fermentation broth. Furthermore, it served to provide base pH control and therefore the total volume of base addition was decreased by 50%. Consequently, there were increases in biomass yield of up to 37% in fed-batch fermentation and in recombinant protein production of up to 21%. We conclude that this approach could therefore be applied beneficially to a wide range of *E. coli* fermentations for production of recombinant enzymes and therapeutic proteins.

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