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An electrokinetic bioreactor: using direct electric current for enhanced lactic acid fermentation and product recovery

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Abstract—The microbiological production of organic acids by fermentation processes is growing in commercial importance. However, the removal of product and pH control are two main issues that limit the technical and commercial viability of such processes. A laboratory scale bioreactor combining conventional electrodialysis and bipolar membrane electrodialysis has been developed for in situ product removal and pH control in lactic acid fermentation. The electrokinetic process enabled removal of the biocatalytic product (lactic acid) directly from the bioreactor system, in a concentrated form, as well as enabling good pH control without generation of troublesome salts. Moreover, end-product inhibition of glucose catabolism was reduced, resulting in a greater generation of the end-product lactic acid. An automatic pH sensor and current application system was developed and successfully implemented for lactic acid fermentation in the electrokinetic bioreactor.

1. Introduction

Lactic acid has been produced commercially by fermentation since 1883.[1] The major application of lactic acid is in the food industry as an additive and preservative. Other applications include use as a pharmaceutical intermediate, lactate ester, which is an alternative solvent to glycol ether. Lactic acid-derived polymers are becoming increasingly important because of their application within drug delivery systems and their biodegradable and thermoplastic nature means that they can be produced as high volume biodegradable plastics for packaging and other applications. However, this potential can only be realised if the cost of production is competitive on a global scale.[2–5]

Lactic acid is produced microbially from a variety of feedstocks by fermentation using lactic acid bacteria (*Lactobacilli*). In the conversion of glucose to lactic acid by homofermentative *Lactobacilli*, two molecules of lactic acid are produced for every molecule of glucose consumed. pH control of this organic acid fermentation is crucial; in the absence of such pH control, the final lactic acid concentration may be less than half of that obtained with pH control.[6] Conventionally, calcium carbonate has been used to neutralize the pH during lactic acid fermentation, producing calcium lactate. Ammonium hydroxide and sodium hydroxide have also been used for neutralization, however the cost of sodium hydroxide for the neutralization of the process is dominant in the operational cost in the fermentation step.[7] As a result of pH neutralization, the product is an organic acid salt. After fermentation, the process of separating the product from the medium and converting the salt to an organic acid is complicated, involving precipitation and acidification using a mineral acid (sulfuric acid). These steps contain the major economic hurdles for organic acid production. Moreover, the processing produces large quantities of an effluent containing high concentrations of salts. For instance, in the case of calcium carbonate neutralization, one ton of gypsum by-product will be produced for every ton of lactic acid produced.[2]

A low cost, environmentally sustainable process for organic acid downstream processing is desirable. Many investigations have been carried out into the separation, concentration and purification of organic acids from fermentation broth.[1,2] Among these, conventional electrodialysis has been developed for the separation, purification and concentration of organic acid salts from a fermentation broth, leading to economic product formation and low environmental impact of the downstream processing. Conventional electrodialysis is a membrane separation technology that uses ion-exchange membranes under the influence of direct current for separating and concentrating ions in solution. It has been widely used for separating and concentrating organic acid salts from fermentation broths.[2,8] Bipolar

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electrodialysis involves water splitting within a bipolar membrane, which is a laminate of cation and anion exchange membranes, with the efficient generation of protons and hydroxyl ions, thus producing acid and base from the salt solution with high energy efficiency.[9–11] Bipolar electrodialysis, integrating conventional electrodialysis with bipolar membrane water splitting, provides a potentially attractive complement to organic acid fermentation, enabling the separation, purification and concentration of salts and converting these salts into acid and base without producing effluents containing high concentrations of salts or gypsum, thereby avoiding discharge to the environment. Another advantage of bipolar membrane electrodialysis is that the base produced can be recycled and used for neutralization of the fermentation process.[2,10–12]

There is the potential that separation and product recovery can be applied in situ, during the fermentation process. As lactic acid fermentation is product inhibited, the removal of lactic acid produced in the fermentation medium will relieve this inhibition.[1] Studies of product removal following fermentation have been carried out in a number of systems by means of extraction,[13] dialysis[14] or conventional electrodialysis[15] as well as bipolar membrane electrodialysis.[16] The in situ extraction of lactic acid is possible, but it has yet to be demonstrated economically.[1] Conventional electrodialysis during fermentation with the cathode located in the fermentation compartment damages the bacterial cells if they come into direct contact with the electrodes. Therefore, an ultrafiltration step must be used before electrodialysis to prevent the cells from contacting the electrode.[17] A second issue for the microbiology is that of pH control in the fermentation chamber during electrodialysis. This can be partially achieved by electrodialytic removal of the acid, however the pH remained lower than optimal because some acid remained in the medium.[15,18] In order to maintain precise pH control, an additional pH control system with addition of NaOH solution was incorporated into the electrodialysis fermentor.[18]

Bipolar membrane electrodialysis has been used as a single unit operation for industrial organic acid production,[19] however, when coupled to fermentation, it is currently used as a separate second downstream stage for acid formation and base recycling.[9,10] In this format it follows conventional electrodialysis and enables recycling of base generated in the fermentor, leading to the production of the organic acid salt in the medium, which will also need to be converted into acid in a downstream process.

The aim of the current research is to combine the advantages of both conventional electrodialysis and bipolar membrane electrodialysis within the bioreactor configuration for lactic acid fermentation. This electrokinetic membrane bioreactor couples fermentation directly with both in situ lactic acid separation and product concentration. The use of a positively charged buffering system (bis-Tris) in the fermentation and concentration chambers leads to the migration of the negatively charged lactate ion alone into the concentration chamber. There is in situ pH control through removal of lactate and the direct use of hydroxyl ions produced by bipolar electrodialysis. The potential benefits of the bioreactor include relief of product inhibition, and hence an increase in yield, plus separation

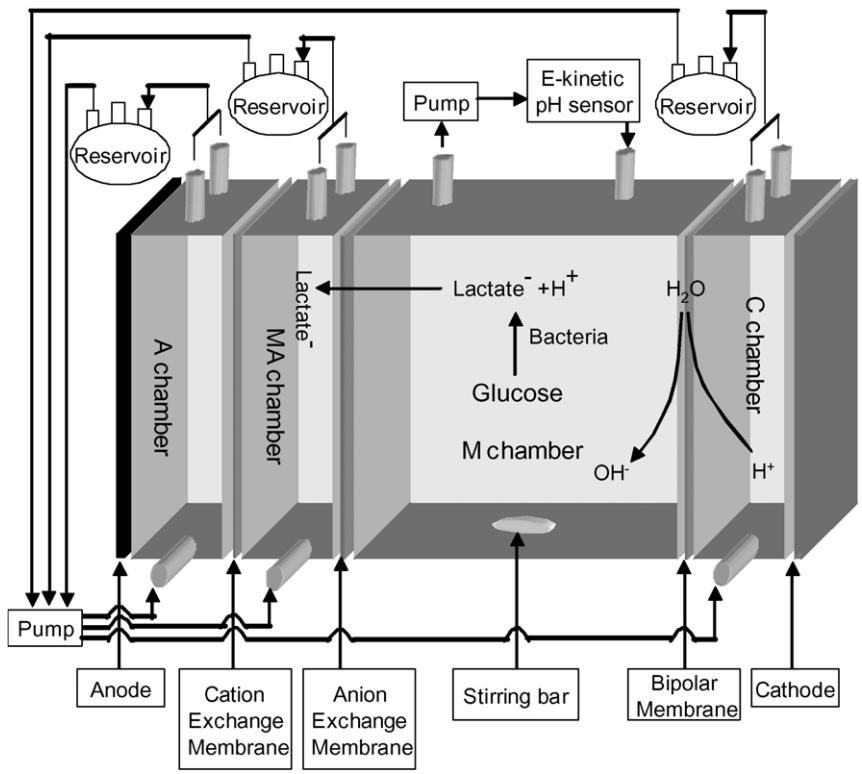

Figure 1. Electrokinetic bioreactor Each compartment has a circulation loop and reservoir for pH control, sampling and product recovery. Chambers A, MA, C and M were recirculated at 120 ml/min using a peristaltic pump with Norprene tubing (Cole-Parmer Instrument Co. Ltd, London, UK). The anode and cathode were connected to a computer-controlled DC power supply, with a pH sensor in chamber M connected to a computer interface.

and concentration of lactic acid and direct use of hydroxyl ions produced by water splitting to neutralize pH without the requirement for additional acid or base. pH control is coupled to the power supply to enable lactic acid to be removed as it is produced.

2. Results

2.1. Bioreactor design and electromigration of lactic acid in the electrokinetic bioreactor utilising bipolar electrodialysis

The electrokinetic bioreactor consisted of four chambers, labelled A, MA, M and C (Fig. 1). These chambers were separated by membranes, and the complete system was sealed by means of double O-rings between chambers and the system was clamped together. Chambers A and C were adjacent to the anode and cathode, respectively. Chambers A, MA, and C had a total capacity of 100 ml, however they were each connected to separate reservoirs, which enabled a total volume of 500 ml solution to be recirculated through each chamber. The reservoir for chamber MA contained a pH control system for monitoring and control of pH. Chamber M had a total capacity of 1400 ml. A cation exchange membrane (Nafion 450, DuPont Fluoroproducts, Fayetteville, USA) separated chambers A and MA, an anion exchange membrane (AMH, Tokuyama Co. Ltd., Tokyo, Japan) separated chambers MA and M, and a bipolar membrane (BP-1, Tokuyama Co. Ltd., Tokyo, Japan) separated chambers M and C. Chamber M was stirred at 250rpm using a magnetic stirring bar. The anode was fabricated from titanium-plated steel, and the cathode was a stainless steel plate. They were both square and had a working surface area of 11×8.5 cm^2. Chamber A and its reservoir contained 500 ml 0.1 M phosphate buffer, initially at pH 6.5. Chambers MA and C (including their reservoirs) each contained 500 ml 0.1 M bis-Tris buffer, starting pH 8.5 and 6.5, respectively. Chamber M contained 1400 ml 0.1 M bis-Tris buffer, pH 6.5, 20% (w/v) glucose. Harvested bacterial cells were resuspended in this medium to a density as indicated in the text for each bioreactor run. The anode and cathode were connected to a DC programmable power supply (PL-P, Thurlby Thandar Instruments Ltd. Cambridgeshire, UK). Within bipolar electrodialysis, pH control was achieved through water splitting and migration of hydroxyl ions into chamber M and through electromigration of lactate across the anion exchange membrane into chamber MA.

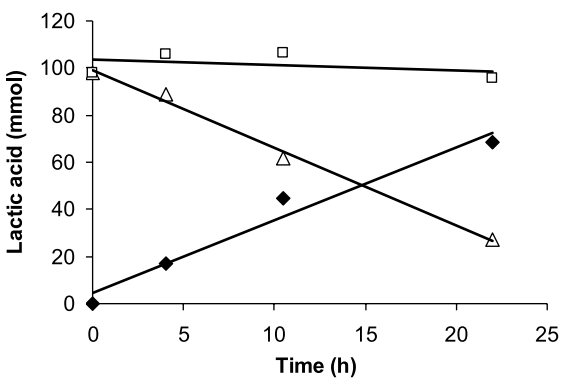

Figure 2. Lactic acid migration in the electrokinetic bioreactor with bipolar electrodialysis Bioreactor configuration and buffer content were as described in Section 4.1. Lactate (79.3 mM) migrated from chamber M (△) into chamber MA (◆) giving total lactic acid in the bioreactor (M+MA) (□) as determined by ion chromatography. The current density was fixed at 0.86 mA cm^{-2}. pH in chamber MA was controlled at pH 8.5 by addition of 4 M NaOH.

An initial experiment was conducted using buffer and lactic acid alone (i.e. no bacteria or glucose) to ensure that lactic acid migrated across the anion exchange membrane and could be recovered in compartment MA. The initial concentration of lactic acid in the buffer solution was 79.3 mM (10 g lactic acid in 1.4 l). Electromigration was observed, and a mass balance of 95% recovery of lactic acid was maintained as it migrated from chamber M across the membrane into chamber MA (Fig. 2). The current efficiency under these conditions was approximately 100%.

2.2. Lactic acid fermentation in the electrokinetic bioreactor with the application of current part way through the fermentation process

The bioreactor configuration and buffers in chambers A, MA, C and M were the same as described in Section 2.1. When glucose (20 g/l) was added to harvested cells and the fermentation was allowed to proceed in the absence of an applied current, lactic acid accumulated in chamber M giving a total of 81 mmol after 86 h, with some diffusion into compartment MA to a total of 93 mmol in the reactor (Fig. 3). After 86 h, the current was applied and lactic acid began to migrate into chamber MA. The amount of lactic acid in M remained constant to 106 hours and thereafter decreased significantly to approximately 10 mmol after a further 20 hours, whilst the amount of lactic acid in MA increased to a final level of 130 mmol after 48 h of applied current. In the control bioreactor, lactic acid production was very similar to that in the electrokinetic bioreactor up to 86 h (Fig. 4). However, after 86 h, there was very little further increase in lactic acid production, such that total lactic acid production in the electrokinetic bioreactor was

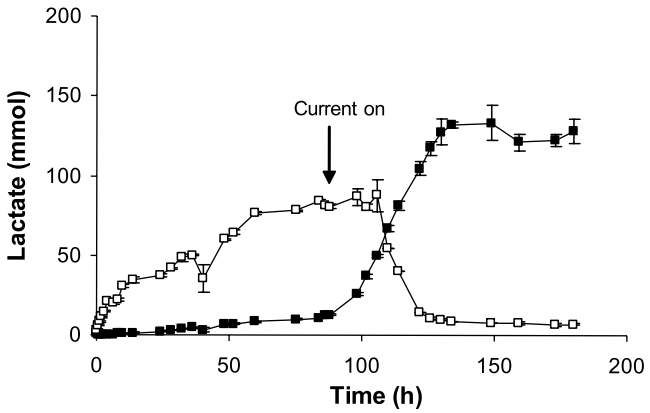

Figure 3. Lactic acid production in the electrokinetic bioreactor, with application of current after 86 h and no pH control. The configuration of the electrokinetic bioreactor and operating conditions are described in Sections 2.1 and 2.3, respectively. Starting glucose concentration was 111 mM (20 g/l) and applied current density was 1.8 mA/cm^{-2}. The pH in chamber M was not externally controlled. Lactate in chambers M (□) and MA (◆) was determined by ion chromatography. Harvested cells were used at a concentration of 0.87 g dry cell weight/l in both bioreactors. Values are the means of three determinations±SEM.

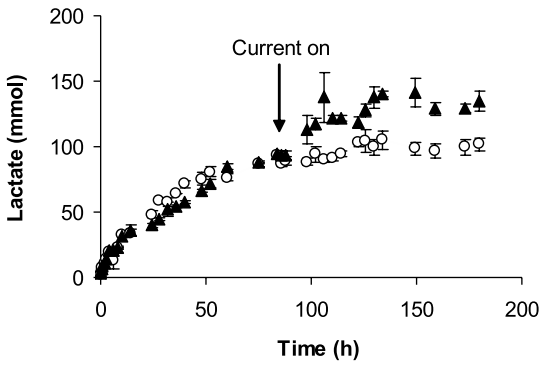

Figure 4. Total lactic acid production in the electrokinetic and control bioreactors without pH control. Lactic acid, measured as lactate, in the electrokinetic (▲) and control (○) bioreactors was determined by ion chromatography. Bioreactor configurations, operating conditions and harvested cells used were the same as in Figure 3. Values are the means of three determinations±SEM.

approximately 34% higher in comparison with the production in the control bioreactor (Fig. 4). The average lactic acid production rate over 180 h was 0.86 mmol/h/g dry cell weight for the electrokinetic bioreactor and 0.64 mmol/h/g dry cell weight for the control bioreactor. The increased lactic acid production also correlated to differences in glucose consumption in the electrokinetic and control bioreactors (Fig. 5). The total mass balance of glucose to lactic acid conversion was 57% for the electrokinetic bioreactor and 46% for the control bioreactor at the end of the fermentation.

As a result of lactic acid production, the pH in both bioreactors had decreased to approximately pH 4 after 86 h. After the current was switched on, the pH in chamber M in the electrokinetic bioreactor increased dramatically and finally stabilized at approximately pH 12 (Fig. 6).

2.3. pH controlled lactic acid fermentation in an electrokinetic bioreactor

Having show that the application of current could recover the pH in the fermentation chamber, leading to increased lactic acid production, it was necessary to control the applied current such that the pH could be maintained at a

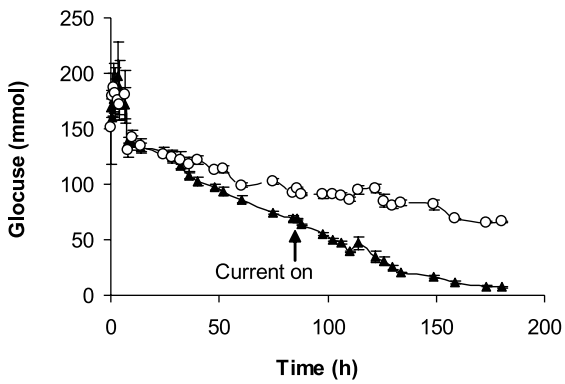

Figure 5. Glucose content in the electrokinetic and control bioreactors without pH control. Glucose content in the electrokinetic (▲) and control (○) bioreactors was determined by a glucose oxidase-kit (Section 4.5). Bioreactor configurations, running conditions and harvested cells used were the same as in Figure 3. Values are the means of three determinations± SEM.

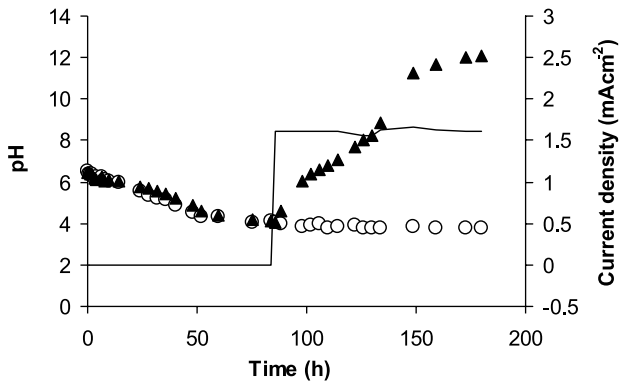

Figure 6. pH levels in the electrokinetic and control bioreactors during fermentation and applied current density for the electrokinetic bioreactor without pH control. pH in the EK bioreactor (▲) and control bioreactor (○) was determined using a microelectrode. Bioreactor configurations, operating conditions and harvesting of cells used were the same as in Figure 3.

suitable value. Manual pH control was achieved by increasing the applied current when the pH fell below pH 6.5, and decreasing or switching off the current when the pH increased above pH 6.5. The pH in the control bioreactor was controlled at pH 6.5 using a pH controller and addition of 4 M NaOH. The lactic acid production rate was 0.78 mmol/h/g dry cell weight in the electrokinetic bioreactor and 0.51 mmol/h/g dry cell weight for the control bioreactor (averages calculated over a period of 197 h). The production of lactic acid in the electrokinetic bioreactor, proceeded at a constant rate to 100 h and continued to increase to 197 h, giving a maximum production of 168 mmol, whereas in the control reactor lactate production stopped after 100 hours, with a maximum production of 112 mmol (Fig. 7). pH was maintained at pH 6.5 in both bioreactors. The increased production of lactic acid in the electrokinetic bioreactor may be due to the removal of lactic acid from chamber M into chamber MA and due to the different conditions of the pH control systems (Fig. 8). Moreover, a higher concentration of lactic acid was achieved in chamber MA (approximately double) at the end of the fermentation compared to that in chamber M

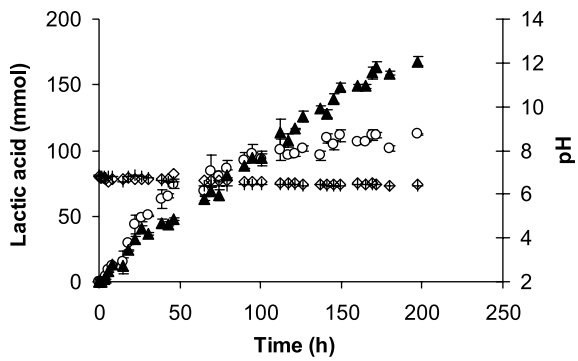

Figure 7. Lactic acid production within an electrokinetic bioreactor with manual pH control. Lactic acid levels in the electrokinetic bioreactor (▲) and control bioreactor (○) were determined by ion chromatography. The pH in the electrokinetic bioreactor (+) was controlled by manually adjusting the current according to changes in pH in chamber M. The pH in the control bioreactor (◇) was controlled using a conventional pH controller and addition of 4 M NaOH. Both bioreactors were controlled at pH 6.5. Harvested cells were used at 0.79 g dry cell weight /l in both bioreactors. Values are the means of three determinations ±SEM.

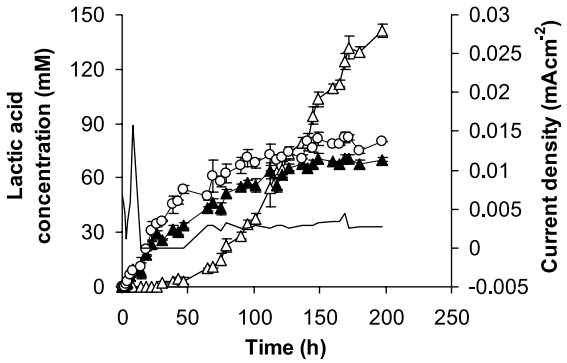

Figure 8. Lactic acid concentrations in chambers M and MA of the electrokinetic bioreactor and in the control bioreactor together with current density applied to the electrokinetic bioreactor with manual pH control. Lactic acid concentrations (mM) in chambers M (▲) and MA (△) in the electrokinetic bioreactor and in the control bioreactor (○) were determined by ion chromatography. Bioreactor configurations and other conditions, including current (—) were the same as in Figure 7. Values are the means of three determinations±SEM.

(Fig. 8). The total mass balance of glucose to lactic acid conversion was 61% for the electrokinetic bioreactor and 46% for the control bioreactor.

2.4. Lactic acid fermentation using the electrokinetic bioreactor and bipolar electrodialysis with automatic pH control

To increase the effectiveness of the bipolar electrodialysis system, manual pH control was replaced with an automatic pH controller with which applied current was linked to measured pH. In this experiment, the bioreactor configuration and operating conditions were the same as in Section 2.3, except that the pH in the electrokinetic bioreactor was controlled by an automatic pH controller set to pH 6.5 (Section 4.1). In the control bioreactor, the pH was maintained at the same value by addition of sodium hydroxide.

Lactic acid migrated electrokinetically into chamber MA from chamber M and the concentration of lactic acid in chamber MA at the end of the fermentation was approximately 20 times higher than that in chamber M (Fig. 9). The

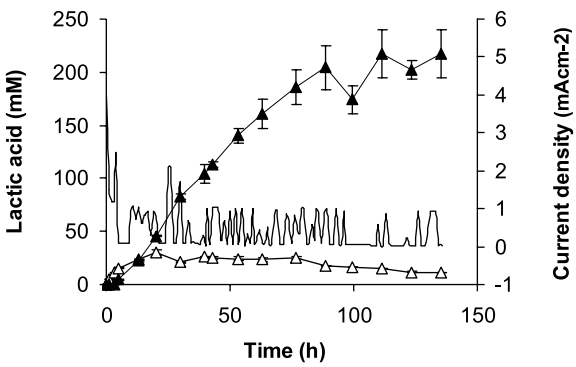

Figure 9. Lactic acid concentration during fermentation in the electrokinetic bioreactor with automatic pH control. Lactic acid concentration (mM) in chambers MA (▲) and M (△) was determined by ion chromatography. Current density (—)was supplied by a computerized automatic pH control system. Harvested cells were 1.3 g dry cell weight/l. Values are the means of three determinations±SEM.

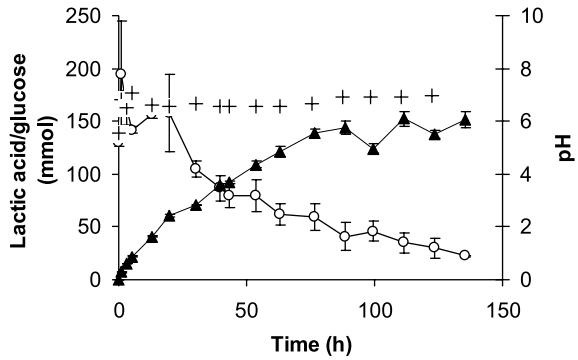

Figure 10. Total lactic acid, glucose and pH in the electrokinetic bioreactor during fermentation under automatic pH control. The total lactic acid (▲) combined from chambers MA and M, was determined by ion chromatography. Current was supplied by a computerized automatic pH controller system. The results are means of three determinations±SEM.

average rate of lactic acid production in the electrokinetic bioreactor was 0.61 mmol/h/g dry cell weight over 135.5 h. The yield was 62% of glucose consumed. The pH was maintained constant throughout the experiment (Fig. 10).

3. Discussion and conclusion

Under the application of DC current, it has been demonstrated that the negatively charged lactate ion generated by fermentation in chamber M of the electrokinetic bioreactor migrated across the anion exchange membrane into the concentrating chamber (MA). This electromigration and concentration of lactic acid was coupled with maintenance of the pH at near neutral conditions in contrast to the otherwise acidic condition in the fermentation medium in the absence of electrokinetics. In the electrokinetic bioreactor, higher lactic acid production was also achieved. This was coupled to higher yield when pH was maintained at the same level as in the control bioreactor (to which no DC current had been applied). This is most likely due to overcoming product inhibition in the electrokinetic bioreactor as the lactate generated was removed to a separate chamber. The health, as measured by culturability, of the bacteria during the electrokinetic process was not affected by the application of current at different levels up to 1.6 mA/cm^{-2} even after 150 h (data not shown).

Since pH control did not rely only on the removal of the acid produced in the electrokinetic bioreactor, the hydroxyl ion produced by water splitting at the bipolar membrane, which migrated into chamber M, also played an important role. This approach of combining the advantages of conventional electrodialysis and bipolar water splitting technology could be used to precisely control the pH while the acid migrated into MA. The pH could even be controlled at a value higher than neutral without addition of base. This is not possible by conventional electrodialysis and removal of the lactic acid alone.[18] This feature is important because conductivity of the organic acid was strongly dependent on the pH during electrodialysis.[20] Control of the pH at an optimal level is desirable both for the bacterial fermentation and for a maximum rate of electrodialysis to occur. Moreover, the direct use of the hydroxyl ions produced from bipolar membrane water splitting to control pH means that, in

contrast to other production processes, the lactic acid salt is not produced in the fermenting media.[15,18] There is also the potential to recover the accumulated lactic acid in chamber MA in its protonated form as it combines with protons migrating from chamber A into MA.

This bioreactor changes the conventional method of pH control through addition of a base to that of applying a current to control the pH. It is much simpler to control the system by this approach, which can also be automated very effectively. The overall benefit from the electrokinetic bioreactor was extremely positive in that it was a one stage integrated process, with in situ pH control, integral product (lactic acid) removal in a concentrated form, and overall enhancement of yield. The approach taken in this study is potentially applicable in a wide range of biocatalytic and fermentation processes.

4. Experimental

4.1. Bioreactor configuration and its running conditions

The electrokinetic bioreactor configuration is described in Section 2.1. The pH in chamber MA was controlled at pH 8.5 by means of a pH controller (Electrolab Ltd, Gloucestershire, UK) with addition of 4 M NaOH. In all experiments, the pH in chambers A and C was not controlled. The pH in chamber M was controlled through a combination of water splitting and migration of hydroxyl ions into chamber M, and electromigration of lactic acid across the anion exchange membrane into chamber MA.

As a further development of the process, automatic pH control (Fig. 1. electrokinetic pH controller) was later developed. A pH probe was fitted into a recirculating loop attached to chamber M in the bioreactor. This pH probe was connected to a computer programmed with software that controlled a programmable power supply unit. When the pH fell below a set value, due to production of lactic acid, the power supplied was increased, and when the pH rose to above another set value, the power supplied was decreased.

Control experiments were conducted in a 2 liter flask or in a bioreactor in which chamber M was isolated such that no current was applied. Control experiments always used the same batch of cells as used in the bioreactor and were carried out simultaneously and under the same conditions of volume, temperature and stirring speed.

4.2. Microorganisms and culture conditions

Lactobacillus rhamnosus NCIMB 6375 was obtained as a freeze-dried culture from NCIMB Ltd. (Aberdeen UK). The culture was resuscitated in MRS broth (Merck Ltd, Poole, UK) and stored on MRS agar plates and as glycerol stocks at −70 °C.

A single colony from an MRS agar plate was innoculated into 50 ml MRS broth in a 250 ml conical flask. The flask was incubated in a shaking incubator for 48 h at 30 °C, 100 rpm. An aliquot of 20 ml of the culture was then used to innoculate 500 ml of the same medium in a 2 l flask which was incubated until the cells had grown to late exponential phase (approximately 18 h). Cells were harvested by centrifuging at 3840*g* (Beckman JH-2 centrifuge with JA-14 rotor, Beckman Instruments, Bucks, UK) at 4 °C for 25 min and washed twice with 0.1 M bis-Tris buffer (pH 6.5) and re-suspended in the same buffer to a total volume of approximately 1 l. The bacterial suspension was divided into two equal parts, one part for the experiment in the electrokinetic bioreactor and one for the control bioreactor.

4.3. Fermentation conditions

A final volume of 1.4 l harvested cell suspension (in 0.1 M bis-Tris buffer, pH 6.5) containing glucose at a concentration of 20 g/l was added to chamber M and the control chamber. Fermentation commenced with the addition of glucose. Samples were withdrawn at each time point for analysis.

4.4. Determination of bacterial cell density and culturability

Bacterial cell density was determined by measuring optical density (OD) at 600 nm using a spectrophotometer (Thermo UNICAM, Cambridge, UK) and correlated to dry cell weight using a reference curve. For dry cell weight determination, cell suspensions were dried at 105 °C overnight in pre-weighed glass weighing vessels. Bacterial colony forming units (cfu's) were determined by serial dilution in sterile water and plating onto MRS agar. Colonies were counted following incubation for 24 h at 30 °C.

4.5. Sample analysis

The individual pH values for samples were determined using a semi-micro electrode (Merck Ltd., Poole, UK) attached to a pH meter (Jenway 3305, Essex, UK). Samples were centrifuged for 3 min at 10,000 rpm using a micro-centrifuge (Labnet, Berkshire, UK). Supernatants were stored at −20 °C for future analysis.

Glucose was measured using either a glucose oxidase-based system (GOD-PERID, Roche Diagnostics Corp., Indiana, USA) or a hexokinase-based system (Gluco-Quant Glucose/HK Assay, Roche Diagnostics Corp, Indiana, USA).

Lactic acid was analysed by ion chromatography using an Anion Dual 2 column (Metrohm, Buckingham, UK). The mobile phase was 2.4 mM $NaHCO_3$ and 2 mM Na_2CO_3 at a flow rate of 0.8 ml/min. Lactic acid was determined by conductivity detection and was quantified according to a standard curve.

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References and notes

1. Litchfield, J. H. *Adv. Appl. Microbiol.* **1996**, *42*, 45–85.
2. Datta, R.; Tsai, S.-P.; Bonsignore, P.; Moon, S.-H.; Frank, J. R. *FEMS Microbiol. Rev.* **1995**, *16*, 221–231.
3. Liu, Y.; Yuan, M.; Deng, X. *Eur. Polym. J.* **2003**, *39*, 977–983.
4. Brannon-Peppas, L. *Int. J. Pharm.* **1995**, *116*, 1–9.
5. Viljanmaa, M.; Södergård, A.; Törmälä, P., *Int. J. Adhes. Adhes.* **2002**, *22*, 219–226.
6. Amrane, A.; Prigent, Y. *Appl. Microbiol. Biotechnol.* **1994**, *40*, 644–649.
7. Åkerberg, C.; Zacchi, G. *Bioresource Technol.* **2000**, *75*, 119–126.
8. Chukwu, U. N.; Cheryan, M. *Appl. Biochem. Biotechnol.* **1999**, *77-79*, 485–499.
9. Bailly, M.; Roux-de Balmann, H.; Aimar, P.; Lutin, F.; Cheryan, M. *J. Memb. Sci.* **2001**, *191*, 129–142.
10. Tsai, S.-P.; Datta, R.; Henry, M.; Halpern, Y.; Frank, J. R. *Memb. Technol.* **1999**, *1999*, 8–12.
11. Franken, T. *Memb. Technol.* **2000**, *2000*, 8–11.
12. Xu, T.; Yang, W. *J. Memb. Sci.* **2002**, *203*, 145–153.
13. Yannavar, V. M.; Wang, D. I. C. *Biotechnol. Bioeng.* **1991**, *37*, 716–722.
14. Friedman, M. R.; Gaden, B. L. *Biotechnol. Bioeng.* **1970**, *12*, 961–974.
15. Hongo, M.; Nomura, Y.; Iwahara, M. *Appl. Environ. Microb.* **1986**, *52*, 314–319.
16. Siebold, M.; Frieling, P. v.; Joppien, R.; Rindfleisch, D.; Schügerl, K.; Röper, H. *Process Biochem.* **1995**, *30*, 81–95.
17. Nomura, Y.; Yamamoto, K.; Ishizaki, A. *J. Ferment. Bioeng.* **1991**, *71*, 450–452.
18. Vanktaveesuk, P.; Tonokawa, M.; Ishizaki, A. *J. Ferment. Bioeng.* **1994**, *77*, 508–512.
19. Bailly, M. *Desalination* **2002**, *144*, 157–162.
20. Novalic, S.; Okwor, J.; Kulbe, K. D. *Desalination* **1996**, *105*, 277–282.