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Metabolite analysis of Clostridium acetobutylicum: Fermentation in a microbial fuel cell

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厭氧菌(Clostridium acetobutylicum) 的代謝物分析：微生物燃料電池的發酵

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abstract 摘要

Microbial fuel cells (MFCs) were used to monitor metabolism changes in Clostridium acetobutylicum fermentations. When MFCs were inoculated with C. acetobutylicum, they generated a unique voltage output pattern where two distinct voltage peaks occurred over a weeklong period. This result was markedly different to previously studied organisms which usually generate one sustained voltage peak. Analysis of the fermentation products indicated that the dual voltage peaks correlated with glucose metabolism. The first voltage peak correlated with acidogenic metabolism (acetate and butyrate production) and the second peak with solventogenic metabolism (acetone and butanol production). This demonstrates that MFCs can be applied as a novel tool to monitor the shift from acid production to solvent production in C. acetobutylicum.

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微生物燃料電池(MFCs)是用於監看**厭氧菌**(Clostridium acetobutylicum)發酵時的新陳代謝。當 MFC 被植入厭氧菌中時，它產生一種獨特的電壓輸出，其形式是在一周內產生兩種不同的電壓高峰。這個結果與從前的有機體研究只產生一種持續的電壓高峰有顯著的不同。發酵產品的分析指出雙電壓高峰與葡萄糖代謝息息相關。第一道電壓高峰與**產酸**的代謝(醋酸鹽與酪酸鹽生產)有關，而第二道電流高峰與**溶劑性菌株(solventogenic)**的新陳代謝(丁醇以及丙酮生產)**有關**。這示範了 MFCs 對於有關厭氧菌的溶解產生可以成為新穎的監看工具。

1. Introduction 介紹

MFCs present an unprecedented opportunity to reclaim energy directly from organic waste. The power density of an MFC is low compared to chemical or enzymatic fuel cells and the energy efficiency has been reported to range from 2% to 50% (Logan, 2008); however, MFC power is generated through natural metabolic processes while degrading a wide range of organic matter. Furthermore, based on the selection of organisms these cells produce traditional fuel chemicals as by-products of fermentation such as hydrogen, methane, and short chain alcohols, which can in turn power a conventional generation system. Since MFCs can simultaneously process waste and generate power, they can potentially increase the efficiency of municipal, manufacturing, or military systems while preventing environmental contamination from untreated waste (Logan, 2008; An et al., 2009).

MFCs展現了史無前例可直接回收有機廢料的機會。經報導指出，MFC的能量密度較化學燃料電

池或是酶燃料電池的效率低2~50% (Logan, 2008)。然而，MFC能源是經由天然新陳代謝過程，一連串有機物質的降質。甚至，基於有機體的選擇，這些電池經由發酵後的副產品製造出傳統的化學燃料，例如氫、甲烷、些微的酒精，其常見於傳統的電力產生系統。由於MFCs可同時處理廢料以及產生能源，它在提升內政、生產或軍事系統的效率有相當的潛力，又同時能防止環境遭受廢料的汙染(Logan, 2008; An et al., 2009).

The obligate anaerobe Clostridium acetobutylicum was used in industrial fermentations to produce the commodity chemicals acetone, butanol, and ethanol until the early part of the 20th century (Jones and Woods, 1986). The search for renewable fuel sources has revived interest in this organism since it can ferment complex carbohydrate sources found in many agricultural and industrial wastes (Qureshi et al., 2006). In batch fed fermentations C. acetobutylicum exhibits biphasic metabolism where during the initial acidogenic growth phase the cells produce high levels of butyrate and acetate. An accumulation of fermentation products and a drop culture pH causes the cells to enter the solventogenic growth phase where butyrate and acetate are converted to butanol and acetone, respectively (Jones and Woods, 1986). Previous studies have shown that C. acetobutylicum reduces artificial redox mediators and that this process alters the metabolic fluxes (Peguin and Soucaille, 1996). Additionally, we have demonstrated that MFCs inoculated with Clostridium cellulolyticum require artificial redox mediators for current production and these mediators alter metabolism (Sund et al., 2007). While it has been reported that C. acetobutylicum can be used in an MFC with the redox mediators methlyene blue and resazurin (Mathuriya and Sharma, 2009), here we demonstrate that C. acetobutylicum can generate current in MFCs without the addition of redox mediators and current output of MFCs can be used to monitor acidogenic and solventogenic metabolism. The exact mechanism of electron transfer by C. acetobutylicum is not known but the time constant of the current generation of the system is very different from that reported earlier, where a simple discharge was recorded for multiple common MFC redox mediators (Sund et al., 2007). Other researchers have shown that MFCs can be used to measure metabolic output (Biffinger et al., 2008; Favre et al., 2009), however this is the first instance of MFC use for sensing changes in an organism's metabolic pathway.

厭氧菌(Clostridium acetobutylicum) 一直到20世紀初期還是用於工業發酵製造化學商品：丙酮、丁醇以及乙醇的必要厭氧性生物。(Jones and Woods, 1986) 可重新使用的燃料資源研究重新燃起人們對這個有機體的興趣，因為它可以發酵許多農業以及工業廢棄物中所見的複雜碳水化合物來源。(Qureshi et al., 2006). 當最初的產酸成長使電池逐漸產生高量的**酪酸鹽**以及**醋酸鹽**，這一連串厭氧菌的發酵過程當中呈現了雙向的新陳代謝。一些發酵產品的累積，以及一滴培養菌使電池進入生**溶劑性菌株(solventogenic)**成長使**酪酸鹽**以及醋酸鹽分別逐漸轉換成丁醇以及丙酮(Jones and Woods, 1986)。之前的研究指出**厭氧菌(C. acetobutylicum)** 減少人造的**氧化還原反應中介物(redox mediators)**，以及這個過程改變新陳代謝流動(Peguin and Soucaille, 1996)。此外，我們已為目前的生產以及這些改變新陳代謝的中介物示範了MFCs植入**cellulolyticum 桿菌(Clostridium cellulolyticum)**需要人工**氧化還原反應中介物(redox mediators)** 的過程 (Sund et al., 2007).
然而已有報導指出**厭氧菌(C. acetobutylicum)** 跟**氧化還原反應中介物(redox mediators)** - 藍色**亞甲基(methlyene)**以及**刃天青(resazurin)**可以用於**微生物燃料電池(MFC)** (Mathuriya and Sharma,

2009)，我們在此示範厭氧菌(C. acetobutylicum)可以在MFC中產生電流而不需要氧化還原反應中介物以及MFCs的電流輸出的情況下監看**產酸(acidogenic)**以及**溶劑性菌株(solventogenic)** 新陳代謝。厭氧菌(C. acetobutylicum)確切的電子轉移結構目前還未知，但電流產生的系統時間跟先前報導的相當不同，諸如在許多常見的MFC氧化還原反應中介物當中所記錄的簡易釋放(Sund et al., 2007)。其他研究者已經示範MFCs可以用於測量新陳代謝輸出(Biffinger et al., 2008; Favre et al., 2009)，然而這是第一個MFC應用在感應有機體新陳代謝路徑的例子。

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Current generation 電源產生

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2. Methods 方法

2.1. Reagents and biological materials 試劑以及生物素材

Culture growth and MFC operation, both in the anode and cathode chambers, was achieved with Clostridial growth medium (CGM) based on the recipe previously developed by Wiesenborn et al. (1988).

Wiesenborn et al. (1988)先前開發**桿菌生長培養基(Clostridial growth medium (CGM))** 的秘訣(同時在陽極以及陰極箱體) 成就了培養菌以及微生物燃料電池運作。

A 500 mL solution of CGM contained 25 g glucose, 2.5 g yeast extract, 1 g asparagine, 1 g $(NH_4)_2SO_4$, 0.5 g NaCl, 0.174 g $MgSO_4$, 5 mg $FeSO_4\_7H_2O$, 5 mg $MnSO_4\_H_2O$, 0.375 g K_2HPO_4, 0.491 g $KH_2PO_4\_3H_2O$, and 100 lL antifoam C emulsion.

一個500mL的CGM包含 25g 葡萄糖、2.5g 酵母提取物、1g天門冬素、1g (氨氮) 硫酸銨、0.5g 氯化鈉、0.174 g 硫酸鎂、 5 mg 硫酸亞鐵_7H2O、5mg 硫酸錳_ H2O、0.375 g 磷酸氫二鉀、0.491 g 磷酸氫二鉀_3H2O 以及100 lL 防泡C乳膠。

Cultures of C. acetobutylicum (ATCC824) were prepared from spore suspensions by 10 min of heat shock at 80 _C and overnight incubation in CGM under anaerobic conditions at 35 _C. The culture was then diluted with CGM by a ratio of 1:5 before inoculation into MFCs. All chemicals were purchased from Sigma–Aldrich (St. Louis,MO) and were molecular biology grade or the highest grade available.

厭氧菌 (ATCC824)培養菌，由孢子懸浮液以 80°C 在10分鐘的熱震動以及整晚在CGM中以35°C的厭氧狀態中潛伏而產生。在植入MFCs之前，培養菌會被桿菌生長培養基以1:5稀釋。所有的化學製品都是從Sigma–Aldrich(St. Louis,MO) 購買而來，而且是以分子的生物等級或最高等級

取得

2.2. MFC design/data collection 微生物燃料電池設計/ 資料收集

MFCs with graphite electrodes were constructed similarly to those described in the literature (Milliken and May, 2007) and as previously described by the authors (Sund et al., 2009).

微生物燃料電池以及石墨電極的建構與(Milliken and May, 2007)的著作相似，而在先前由作者(Sund et al., 2009)所描述。

After assembly, each chamber was filled with 20 mL of CGM. The anode hambers were secured with rubber septa and the cathode chambers with a loose glass cap. Before inoculation, the electrodes, housings, and media were assembled and autoclaved. The MFCs were placed in a 35 _C incubator, linked to a 10 kO resistor, and allowed to discharge for a minimum of 120 min. The anode chambers of the MFCs were inoculated with 1 mL of the diluted C. acetobutylicum culture.

集合之後，每一個箱體被注入 20mL的CGM，陽極箱體由石製隔片掩護，而陰極箱體由鬆散的玻璃套掩護，在植入之前，電極、遮蔽、培養基被集合而且真空加熱。MFCs被放置在35 °C的細菌培養器，跟一個10 kO 電阻器做連結，並且允許釋放至少120分鐘。MFCs的陽極箱體被植入1 mL 稀釋的厭氧菌培養菌

The anode compartments were sealed with silicone septa and vented with 12-gauge syringe needles fed through a Millex-GP 0.2 lm sterile syringe filter unit (Millipore,Bedford, MA) through a length of plastic Tygon tubing into a flask of nitrogen-sparged water.

陽極隔間被矽樹脂隔間密封，用12口徑的注射針經由Millex-GP 0.2 1m 消毒後的針筒濾器(Millipore,Bedford, MA) 開孔注射入塑膠聚乙烯管至裝有氮氣水的燒瓶中。

This prevented buildup of metabolic gasses in the anode chamber while also preventing contamination of the chamber with O_2 or ambient microbes. The anode was entirely submerged in media and placed in direct contact with the lump graphite while the cathode was submerged just below the surface of the media in the cathode chamber. The potential across a 10 kO resistor was measured and recorded every 10 s via a DAQPad-6016 and a custom LabView_ VI (National Instruments, Austin,TX).

這防止新陳代謝氣體在陽極箱體中產生，同時防止氧氣汙染箱體周遭的微生物。陽極整個被培養基淹沒並直接放置在石墨塊上；而陰極被淹沒在陰極箱體的培養基表面下。透過10 kO電阻器可以測量並記錄每10秒的電能經由DAQPad-6016以及特製的LabView_ VI (National Instruments, Austin,TX).

2.3. Quantification of fermentation products 發酵產品的量化

Fermentation products were quantified using a previously reported **high performance liquid chromatography** (HPLC) technique. (Ehrlich et al., 1981) At several points during the MFC run, 200 lL aliquots were withdrawn from the anode chambers, filtered through a Montage PCR centrifugal filter device (Millipore, Bedford,MA), and stored at _20 _C pending HPLC analysis. An Aminex HPX-87H organic acid analysis column (Bio-Rad, Hercules, CA) heated to 30 _C was used for all separations. The samples were stored at 4 _C until ready for injection at volumes of 20 lL each. Each

HPLC signal was analyzed for the presence of peaks corresponding to glucose, acetate, ethanol, butyrate, and butanol. These data were quantified using standardized concentration gradients (data not shown) on the HPLC column using HPLC grade reagents (Sigma–Aldrich, St. Louis, MO).

發酵產品可以用先前報導過的**高效能液體色層分析技術(HPLC)** 量化(Ehrlich et al., 1981)。
在MFC實驗的幾個時間點，從陽極箱體中取出200 lL的量置入**Montage PCR 離心過濾機**過濾(Millipore, Bedford,MA)，並儲存至20°C等待HPLC分析。
所有的MFC數據都會獲得分別四份，而發酵情形會經由pH值以及HPLC分析做監看。樣品會被1200系列HPLC技術 (Agilent Technologies 公司) 的**多重波長偵測器(MWD)**以250奈米監看並以**可折射的指數探測器(RID)**做分析。
將一個Aminex HPX-87H 有機酸分析柱體(Bio-Rad, Hercules, CA) 加熱至30°C來做所有的分隔。樣品會被儲存至4°C直到做好分別20 lL 的注射。個別的HPLC訊號分析出幾個高峰與葡萄糖、醋酸鹽、乙醇、酪酸鹽以及丁醇一致。這些數據都被HPLC柱體上的**標準濃縮梯度(數據未顯示)**使用HPLC分級試劑做量化(Sigma–Aldrich, St. Louis, MO)。

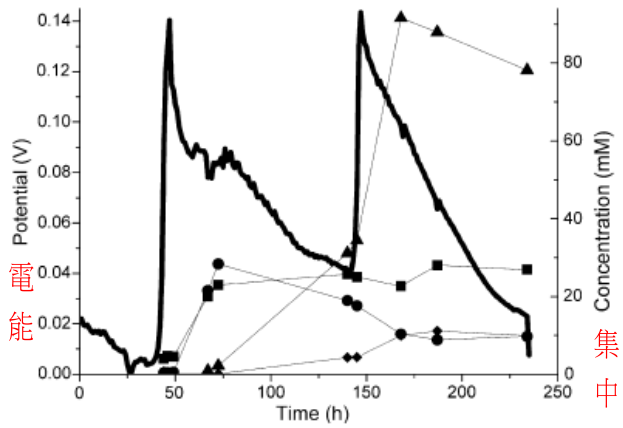

Fig. 1. Plot showing the time course of the potential (-Solid Line-) measured across a 10 kX resistor for a representative MFC containing C. acetobutylicum in the anode chamber. Note the two peaks generated by C. acetobutylicum. The plot also includes the concentrations of acetate (-Square-), butyrate (-Circle-), ethanol (-Diamond-),and butanol (-Triangle-) in the anolyte of this MFC through the time course of the experiment.

圖1，標繪圖表示了電能的時間路程(實線)經由10 kX電阻器測量在陽極箱體中典型包含了**厭氧菌**的**MFC**，紀錄**厭氧菌**製造的兩個尖峰。標繪圖同時包含整個實驗**醋酸鹽(方塊)**，**酪酸鹽(圓圈)**、**乙醇(菱形)**、以及**丁醇(三角形)**在 MFC的陽極液內集中的時間歷程。

3. Results and discussion 結果與討論

Batch fed MFCs inoculated with C. acetobutylicum were monitored by measuring current as a voltage across a 10 kO resistor. There were two current peaks which occurred over a period of approximately 7 days. The initial current peak occurred approximately 40 h after inoculation and the second peak occurred approximately 150 h after inoculation (Fig. 1). In previous experiments other organisms exhibited a single peak and decay (Crittenden et al., 2006; Sund et al., 2007; Sund et al., 2009). HPLC quantification of the metabolic products of C. acetobutylicum in the MFCs showed a close correlation between concentration of metabolites and current output (Fig. 1). A few hours after the flow of current started, butyrate and acetate concentrations rose sharply while butanol concentration remained close to zero. After the initial peak, current output slowly decreased and butyrate concentration leveled while butanol concentration began to rise rapidly. The trend was similar for acetate and ethanol although acetate concentrations leveled rather than decreased. The data shows that the concentrations of butyrate, acetate, ethanol, and butanol match their expected trends, with the acid generation occurring most prevalently during the first voltage peak and alcohol generation occurring noticeably in the second voltage peak. The concentration trends match the shift in metabolism and utilization

of butyrate.

一組注射了**厭氧菌**的MFCs藉由電阻器用測量電流的方式監看。在大約7天的期間內有兩個電流高峰產生，最初的電流高峰在注射後大約40小時候發生，而第二個高峰在注射後約150小時發生(圖一)。在先前的實驗，其他有機體呈現單一尖峰以及衰退(Crittenden et al., 2006; Sund et al., 2007; Sund et al., 2009)。厭氧菌新陳代謝產品的HPLC量化，在MFCs的新陳代謝集中和電流輸出中呈現了相近的關聯性(圖1)。在電流開始的幾小時候，酪酸鹽以及醋酸鹽集中快速的提升，而丁醇集中依然趨近於零。在一開始的尖峰過後，電流輸出緩慢的減少，而酪酸鹽持平，丁醇集中卻開始級數的上升。醋酸鹽和乙醇的趨勢很相似，雖然醋酸鹽集中持平而不是減少。數據顯示了酪酸鹽、醋酸鹽、乙醇以及丁醇的集中跟他們預期的走向相呼應，在第一道電壓尖峰產生了最高量的酸以及在第二道電壓尖峰產生時酒精明顯的增加。集中的趨勢跟新陳代謝以及酪酸鹽應用的轉換相呼應。

Fig. 2 shows that a rapid drop in the medium's glucose concentration occurs during the first measured voltage peak and slows during the intermediate period; this suggests the metabolism is slower due to excess butyrate decoupling the extracellular proton gradient. There is another rapid glucose consumption phase during the second measured voltage peak. The rate of glucose consumption was the most rapid when the fuel cell was producing the most current indicating that the two processes are coupled. Butyrate production rate was the highest during the first current peak while butyrate/butanol production rates were highest during the second current peak, (Figs. 1 and 2 summarized in Table 1) demonstrating that current output correlates with metabolic activity. During the initial current peak, butyrate and acetate concentrations rose sharply while the concentration of butanol remained close to zero indicating that the cells were in the acidogenic growth phase. Butanol concentrations rose dramatically in the intermediate period between the two peaks indicating that the culture was in the solventogenic growth phase.

圖二，顯示了葡萄糖培養基集中率的急速下降，在第一次測量到的電壓尖峰時發生。並在中間的時候趨於緩慢。這表示著新陳代謝在酪酸鹽過度的細胞外質子去耦時會較緩慢。在第二個測量到的電壓尖峰產生了另一個急速的葡萄糖消耗階段。葡萄糖消耗的比例在燃料電池製造最多電流的時候是最快的，這表示這兩個程序是互相聯結的。酪酸鹽在第一道電流尖峰期間製造最多的比例，而丁醇在第二道電流尖峰製造了最高的比例(表格1由圖. 1 和 圖2做彙整)，前兩個反應表示了電流輸出與新陳代謝活動互相呼應。在第一道電流尖峰，酪酸鹽以及醋酸鹽集中極速的提升而丁醇依然趨近於零，這指出電池正在產酸成長階段。丁醇集中在兩個尖峰之間中期極速的提高指出培養菌正在溶劑性菌株成長階段。

We see further support for the metabolic shift in Fig. 2 where the pH drops through the initial voltage peak and increases in the time frame when butyrate and acetate would be utilized for solvent production. The open circuit voltages (OCV) of four independent MFCs are shown in the supplemental materials. OCV data also exhibits two peaks, although they are less pronounced than those measured by passing the generated current though a resistive load. The two peaks are of a similar timeframe as Fig. 1, with some smoothing due to averaging, indicating that this phenomenon is still linked to the culture's metabolism. The OCV exhibits significantly higher magnitude, 0.65 V which is approximately 0.2 V higher than previously reported studies with ***Shewanella oneidensis*** in identical hardware (Sund

et al., 2009). It is suspected that this is due to the high redox potential of C. acetobutylicum ferredoxins involved in the process of converting pyruvate to acetyl-CoA. (Guerrini et al., 2008) This shift in E_0 is most likely the cause of the OCV difference between the two genera.

我們參考之前圖2.新陳代謝轉變的輔助，pH值在一開始的電壓高峰下降，然後當酪酸鹽以及醋酸鹽被運用在溶劑製造時增加。開放電路電壓(OCV)在四個獨立的MFCs呈現在添加的素材上，OCV數據也呈現了兩個尖峰，雖然他們比起電阻器附載產生的電流測量數據較不明顯。如圖一，兩個尖峰都在相似的時間週期，由於平均數而變得比較平均，指出這個現象依然跟培養菌的新陳代謝有關。OCV數據呈現了相當重要的高量，0.65 V較之前***Shewanella oneidensis***在相似硬體的研究報告還要高出0.2 V (Sund et al., 2009)。這很有可能是因為**厭氧菌鐵蛋白(C. acetobutylicum ferredoxins)**的氧化還原電勢，包含在丙酮酸鹽的轉換成acetyl-CoA的過程中(Guerrini et al., 2008)。這個轉變在E0最有可能是OCV中兩種差異的原因。

The data presented agrees with previous reports that initial fermentation of glucose by C. acetobutylicum is acidogenic where glucose is oxidized to lactate, butyrate, and acetate (White, 2007). Butyrate is lipophilic so it migrates from the outside of the cell to the cytosol (Monot et al., 1984); this eventually causes a disturbance in the proton gradient and impedes metabolism. The organism compensates for this decreased extracellular pH by shifting metabolism to the solventogenic phase and is capable of converting the acetate to acetone and butyrate to butanol thus moderating the pH. In nature these phases are capable of oscillating, but in the closed system of a batch fed fuel cell we did not see the shift back to the acidogenic phase under these experimental conditions. Fig. 1 shows this process where there is a lag in the metabolism during the shift from acidogenic to solventogenic phases, correlating with a lag in the current generation.

呈現的數據都與先前的報告一致表示著最初**厭氧菌**的葡萄糖發酵氧化成乳酸鹽、酪酸鹽以及醋酸鹽進而製造出產酸。酪酸鹽易溶解油脂，所以它由電池的外部轉移到細胞質液(Monot et al., 1984)；這最後造成的質子梯度的混亂並妨礙新陳代謝。有機體藉由轉換新陳代謝成**溶劑性菌株**階段補償了細胞外pH值的降低，而它可以把**醋酸鹽**轉換成為**丙酮**，把**酪酸鹽**轉換成**丁醇**，藉此監看pH值本質上，這些階段可以游移，但在最近的燃料電池植入系統，我們並沒有在這些實驗情形中看到產酸階段。圖1，呈現了這個過程中在新陳代謝時有個延遲，在產酸階段轉換成溶劑性菌株階段時電流的產生與延遲有所關連。

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4. Conclusions 結論

The data presented a direct correlation between voltage output and metabolic phase. This correlation between electrical output and solvent formation makes C. acetobutylicum an ideal candidate for studying biofuel production and cellular metabolism. In addition to the current generated by the MFC itself, the organism also produces H_2 gas, which may then power a conventional hydrogen fuel cell. The acetone, butanol, and ethanol generated as by-products of fermentation may be separated and used as biofuels or industrial solvents. By consolidating the functions of waste

management, renewable power generation, and solvent production, C. acetobutylicum fuel cells have the potential to reduce organic wastes and increase opportunities to convert those wastes to usable energy. This communication demonstrates that the metabolic activity of C. acetobutylicum can be tracked via current generation in an MFC. Current generation has proven to be reproducible and repeatable and was validated through measurements of pH, glucose consumption, and metabolite generation. This phenomenon could be utilized to design an inexpensive autonomous system consisting of graphite electrodes, an electrical load and a recordable voltmeter to track C. acetobutylicum fermentations. With MFC-based monitoring, time and resources would be saved by reducing the need for processing multiple HPLC samples and the need to rely on pH probe calibration over extended periods of time in complex matrices would be negated. In the near term, these fuel cells could be utilized as a research tool for metabolic studies where the current response of microbial fuel cells would be extremely useful. With continued development, future monitoring systems for bioproduction become possible.

數據呈現出電壓輸出與新陳代謝階段有直接的關連。這個電子輸出以及溶解組成之間的關聯使得**厭氧菌**在生物燃料製造研究以及細胞組成新陳代謝中成為理想的候選者。除了MFC本身產生的電流，有機體也同時製造氫氣，這很可能驅動常見的氫燃料電池。丙酮、丁醇以及乙醇等發酵的副產品可能被分離並使用在生物燃料或是工業溶劑。藉由統合廢棄物管理、再生能源產生以及溶劑生產，**厭氧菌**燃料電池具有減少有機體浪費的潛力，並增加轉化這些廢棄物成為可用能源的機會這個交流呈現了**厭氧菌**的新陳代謝活動可以藉由MFC產生的電流來追蹤，電流產生已透過pH質測量、葡萄糖消耗以及新陳代謝產生的方式證實是有效果的既可再生且能持續的。這個現象可以被應用在設計價格低廉的自生系統，其包含石墨電極、電子附載以及可記錄的伏特計用來追蹤**厭氧菌**發酵。藉由MFC為基礎設計的監視技術，時間跟資源將可以節省下來，減少製造多重HPLC樣品的需求，以及需要額外的時間在依賴pH探針測定複雜母體的需求將不再需要。在短期內，這些燃料電池將可以當作研究工具應用在新陳代謝研究微生物燃料電池的電流反應時將會相當有用，在持續的發展，未來製造監看生化製造系統將成為可能

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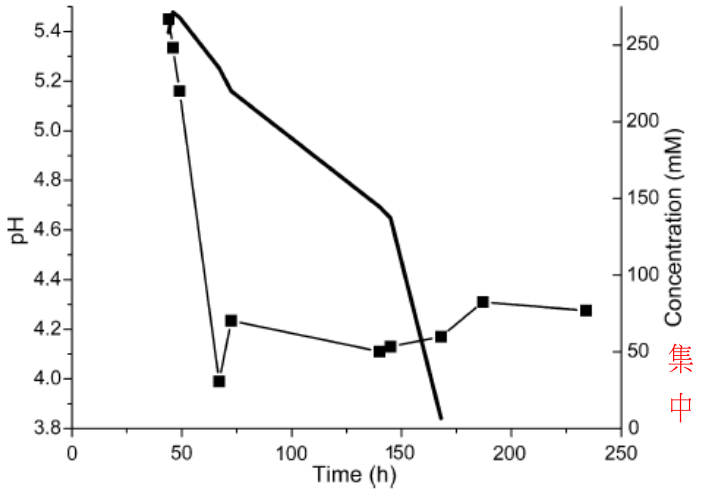

Fig. 2. Plot showing the time course of glucose utilization (-Solid Line-) and pH (- Square-) during the C. acetobutylicum fermentation in an MFC. Note that the concentration of glucose is from a representative MFC while the pH is an average of a total of six fuel cells (three trials of two fuel cells in each trial).

圖.2 標繪圖顯示了葡萄糖應用的時間歷程(實線)，以及pH值(方塊)。在MFC中的**厭氧菌**發酵過程，記錄下葡萄糖在傳統MFC的集中，而pH值在六個燃料電池(兩個燃料電池分別的三個個別的試驗)中的平均值。

Table 1

Glucose utilization and production of butyrate and butanol (mM/h) in the MFC anolyte, calculated from chromatography data. Measureable quantities of butyrate started at about 50 h and butanol at about 67 h. Combined butyrate/butanol production is indicative of overall butyrate production.

從色譜中所計算出的葡萄糖應用以及酪酸鹽和丁醇在MFC陽極液中的產生(mM/h)數據，可測量的酪酸鹽數量在大約50H的時候開始，丁醇在大約67H的時候，結合的酪酸鹽和丁醇產生指出了整個酪酸鹽發生。

Time (h)	Glucose utilization 葡萄糖應用 (mM/h)	Butyrate production 酪酸鹽產生 (mM/h)	Butanol production 丁醇產生 (mM/h)	Butyrate + butanol production 酪酸鹽和丁醇產生 (mM/h)
46–49	1.1	–	–	–
49–67	1.8	1.2W	–	1.2
67–72.5	2.7	1.2	0.2	1.5
72.5–140	1.1	_0.1	0.4	0.3
140–145	1.5	_0.3	0.7	0.4
145–168	5.7	_0.3	2.5	2.2
168–187	0.4	_0.1	_0.2	_0.3
187–234	0.0	0.0	_0.2	_0.2