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Analysis of pH Change and an Automatic pH Control with A New Function: On-Line Estimation of Acetic Acid

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The pH of microbial culture medium was calculated from equations of equilibrium, material balances for ionic components and electro-neutrality theory. Ammonium ion consumption and Acetic acid production are found out to be the major contributors for the alteration of the pH as well as the buffer capacity of the medium. By measuring the buffer capacity on-line, levels of acetic acid were estimated by a software sensor using pH signal in a fermentation process of E.coli growing in a minimal medium. The measured values of acetic acid showed good correlation to those of estimated by the software sensor.

Key words: on-line estimation on-line monitoring, fermentation system, pH control

INTRODUCTION

Biological processes can offer special control problems due to the non-linearities and time-varying dynamics but also limitations in process knowledge adds to the complexity of the biological system. Reliable on-line measurements of the process variables are critical in the monitoring and control of fermentation process [1]. Many efforts were reported to measure the important biological parameter directly by the implementation of supplemental on-line sensors. Glucose biosensors were developed and utilized for glucose level monitoring and control [2, 3]. A complicated on-line measurement system of ATP during microbial cell growth, was reported to be achieved [4]. Without using any supplemental on-line sensor system, software sensors also have been reported to estimate the state variables (e.g., biomass, substrate, or product concentrations), which are computer softwares programmed to do the on-line estimation from available direct measurements of output variables such as production or consumption gas rates or the added quantities of an acid or a base for pH regulation [5, 6]. Since precise measurement of direct parameters is critical for the development of software sensors. pH signals can be an excellent parameter for development of software sensors in terms of reliability as well as availability [1].

Automatic pH control is essential for most fermentation processes, which generates a unique pH profile comprised of series of pulse responses. The response of pH signal caused by pulse feeding of alkali or acid, reflects the buffer capacity, the rate of proton generation and mixing properties of the culture broth. In this report, the relation between buffer capacity and the ionic components of medium was mathematically calculated and an on-line estimation of fermentation process by analysing the pH profiles was carried out by the implementation of hardware prototype and development of a software program using the G-language of Labveiw™ application package. For microbial culture system. *E. coli*, known to produce acetic acid, was used as a model system and cultivated in a minimal and complex medium. Level of acetic acid and ammonia and other fermentation parameters were estimated from the pH values and pH control signal in the above system.

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THEORY

pH Change by Organic Acid Production in a Phosphate Buffered Medium

The pH change of a culture medium caused by the growth cell is mainly due to consumption of ammonia as well as organic acid production [7]. The pH of a phosphate buffered minimal medium can be calculated by an equation derived from equilibrium equations, material balance and proton condition [8], which is given by

$$[H^+] = \frac{kw}{[H^+]} - [OH^-]_{adj} + [HPO_4^{-2}]_i \left\{ \frac{2k_1k_2k_3 + k_1k_2[H^+] - [H^+]^3}{[H^+]^3 + k_1[H^+]^2 + k_1k_2[H^+] + k_1k_2k_3} \right\} + \left[\frac{HA]_p}{1 + [H^+]/k_a} + \frac{[NH_4^+]_i - [NH_3]_{consume}}{1 + [H^+]\frac{kb}{kw}} + [NH_3]_{consume} \quad (1)$$

The pH change and buffer capacity caused by phosphate consumption is not so significant as by ammonium ion consumption or acetic acid production [7]. Assumptions can be made that buffer capacity during cell growth, is mainly determined by the acid produced and the level of ammonium ion. Equation (1) can be rewritten including the present levels of acetic acid and ammonia. The buffer capacity change also can be derived from the equation given by

$$[OH^-]_{adj} = -[H^+] + \frac{kw}{[H^+]} + \frac{[CH_3COOH]_{pro}}{1 + [H^+]/ka_{AcOH}} + \frac{[NH_4^+]}{1 + [H^+]/ka_{NH_3}} \quad (2)$$

Derivatization of Equation (1) in term of pH results in buffer capacity (β=d[OH]$_{adj}$/dpH), which represent the buffer capacity change caused by the levels of ammonium ion and organic acid only and is

$$\beta = 2.303\{[H^+] + \frac{k_w}{[H^+]} + [NH_4^+]\frac{[H^+]ka_{NH_3}}{(ka_{NH_3} + [H^+])^2} + [CH_3COOH]_{pro}\frac{[H^+]ka_{AcOH}}{(ka_{AcOH} + [H^+])^2}\} \quad (3)$$

Acid production during cell growth leads to accumulation of ammonia for neutralization during automatic pH control. The relation of acetic acid production and supplemental ammonia accumulation is given by

$$[NH_4^+]_{supp} = [CH_3COOH]_{pro}\frac{ka_{AcOH}}{ka_{AcOH} + [H^+]} \quad (4)$$

MATERIALS AND METHODS

Escherichia coli K12 (ATCC 10798) was kindly provided by the Coli Genetic Stock Center (CGSC, yale USA). A minimal medium of 20 g/l glucose, 5 g/l $(NH_4)_2SO_4$, 0.5 g/l KH_2PO_4 and 0.4 g/l $MgSO_4$ was used for the cultivation of *E. coli*. Cultivations of *E. coli* were carried out in a 5-liter jar fermenter and samples were taken for analysis. Acetic acid was analysed by gas chromatography after derivatization to acetate ethylester using Chromosorb 101.

RESULT AND DISCUSSION

Implementation of Hardware Prototype and Software Programming

The 5-20 mA signal from a pH controller (Dongil, KOREA) was measured by using a 16 bit A/D convertor (Adam 4017, Advantech USA) and logged into a personal computer. The on/off control signal for pH adjustment was monitored by a digital I/O module (Adam 4050, Advantech USA) and transferred to PC via RS 485. Development of a software program using the G-language of Labveiw™ application package to calculate the buffer capacity from the duration of Alkali pumping and for the estimation of acetic acid and ammonia levels, was carried out. Subsequent fermentor runs using *E. coli* were carried out to improve and validate the software. Finally a software running on Window 95/NT™ environment, was developed and used for further studies (Fig. 1).

On-line Measurement of Fermentation Parameters

To avoid dilution of fermentation broth, pH adjustment by automatic control should be done by concentrated solution of alkali or acid, which lead to a pulse type feeding of the concentrated alkali or acid (Fig. 2). With the knowledge of pump feeding rate, alkali concentration and pulse length, the concentration of alkali fed can be calculated as

$$[OH]_{fed} = \frac{N_{alk} \cdot F_{pump} \cdot T_{pump}}{V_{broth}} \quad (5)$$

The pH shift caused by alkali feeding can be obtained by extrapolating the decreasing section of the slope of the pH response as shown in Fig. 2. Buffer capacity can

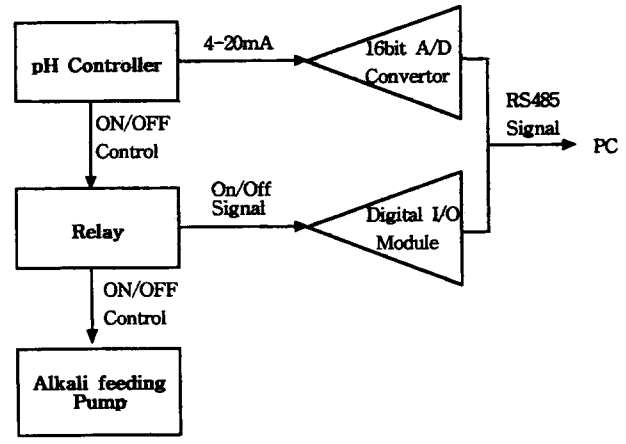

Fig. 1. Installation of A/D convertor and digital I/O module to monitor pH signal and pH control signal.

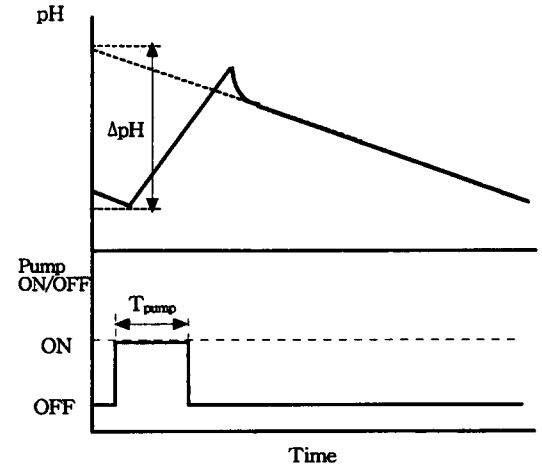

Fig. 2. Schematic of obtaining pH from pH profile in responce to pulse-type feeding of alkali.

be calculated from the pH shift by dividing the concentration of alkali fed and given by

$$\beta = \frac{[OH]_{fed}}{\Delta pH} \quad (6)$$

The change of buffer capacities during cultivation of *E. coli* in a minimal medium and in LB medium supplemented with dextrose, were monitored by the data acquisition system and the software developed. Proton generated during cultivation of *E. coli* causes pH decrease for the period between alkali pump feeding. The rate of proton generation can be obtained by dividing Equation 5. by the time period between pulse.

E. coli strain K12 was cultivated in minimal medium at 37°C and pH signal and pH control signal during the cultivation were monitored on-line. Buffer capacities as well as the rate of hydrogen ion production were calculated on-line and were plotted in Fig. 3. The profile of dry cell weight was put together to show cell growth for comparison. The rate of proton generation increased with the cell growth but retarded at the rate exponential phase. Buffer capacity gradually increased from 0.0036 M/pH to 0.0064 M/pH. Therefore, net increase of 0.0026 M/pH of buffer capacity was measured during cell growth, which implies a net increase of the amount of ionic components in the cultivation medium. The rate of proton generation seems to be related to

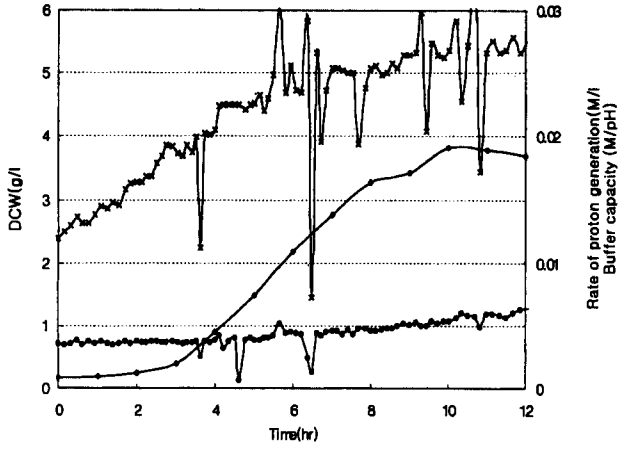

Fig. 3. Parameters monitored on line with dry cell weight. -×-; Rate of proton generation, -◆-; Dry cell weight, ○; Buffer capacity.

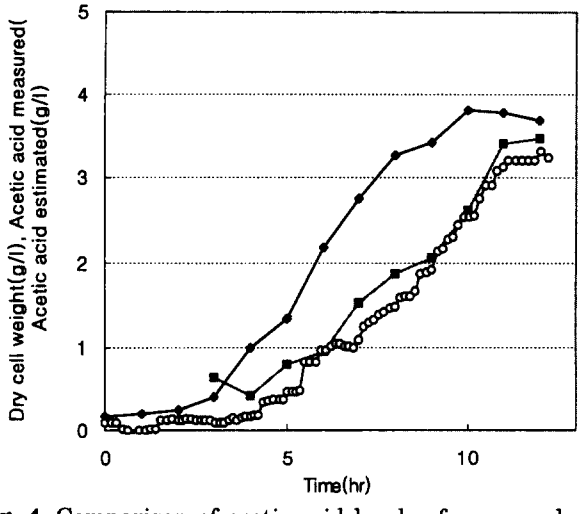

Fig. 4. Comparison of acetic acid levels of measured and estimated. -◆-; Dry cell weight, -■-; Measured acetic acid, -○-; Estimated acetic acid.

metabolic acitvity of the culture.

Estimation of Acetic Acid in *E. coli* Cultivation

Buffer capacities measured on-line can be converted to the levels of acetic acid production and ammonium ion using Equation 3 and 4. Estimation of acetic acid levels were carried out from the smoothed profiles of changes of buffer capacities, which are obtained from subtracting the increased buffer capacities during cultivation by the initial value. Acetic acid was estimated to produce upto 3.4 g/l at 12 hours of cultivation. Samples taken during the cultivation were analysed by gas chromatography. Though the profile of experimentally measured values showed slightly higher than those of estimated, the measured values of acetic acid agree well with the estimated curves as shown in Fig. 4. indicating that on-line estimation of acetic acid can be successfully estimated from the buffer capacity measured on-line.

Acetic acid production can be monitored only from the pH values and pH control signals at every time when alkalie feeding occurs. It is a very convenient and economical method of monitoring fermentation process, not reported before. It also suggests a possibility of implementation of software sensor system in industrial fermentations producing organic acid as a major product.

Ionic components other than organic acid and ammonium ion, may vary during cultivation. Carbon dioxide production will increase buffer capacity especially at high level of biomass and at low aeration rate, which should be overcome for prolonged on-line estimation. Fermentation prodmnucts as a major ionic component like amino acid or penicillin can also be monitored or estimated by the use of software after modification.

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NOMENCLATURE

$[H_2PO_4^-]_{consume}$ The quantity of dibasic phosphate ion utilized for microbial growth (M)
$[H_2PO_4^-]_i$ Initial concentration of monobasic phosphate ion provided (M)
$[HA]_p$ Organic acid produced (M)
Hco_2 Henry's law constant of carbon dioxide (M/atm)
$[HPO_4^-]_{consume}$ The quantity of monobasic phosphate ion utilized for microbial growth (M)
k_1 First acidity constant of phosphoric acid
k_2 Second acidity constant of phosphoric acid
k_3 Third acidity constant of phosphoric acid
ka_{AcOH} Acidity constant of acetic acid
k_b Basicity constant of ammonia
ka_{NH3} Acidity constant of ammonia
kc_1 First acidity constant of carbonic acid
kc_2 Second acidity constant of carbonic acid
k_w Autoprotolysis constant of water
$[NH_3]_{consume}$ The quantity of Ammonia utilized for microbial growth (M)
$[NH_4^+]_i$ Initial concentration of ammonium ion provided (M)
$[OH^-]_{adj}$ The concentration of base added for the adjustment of pH (M)
Pco_2 Partial pressure of carbon dioxide
$[CH_3COOH]$ Acetic acid produced
$[NH_4^+]_{supp}$ Ammonia fed to supplement acid production
β Buffer capacity
$[OH]_{fed}$ Alkali fed to control pH
N_{alk} Normality of alkali
F_{pump} Feed rate of alkali pump
T_{pump} Duration of pump operation
V_{broth} Volume of broth

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