Biotechnology Techniques Vol 1 No 4 225-230 (1987)
Received November 10

AN AUTOMATIC, ON-LINE GLUCOSE ANALYZER FOR FEED-BACK CONTROL OF FED-BATCH GROWTH OF ESCHERICHIA COLI

Gregory W. Luli[1], Steven M. Schlasner[2], Donald E. Ordaz[1], Marc Mason[3], and William R. Strohl[1]

Departments of Microbiology[1] and Chemical Engineering[2], The Ohio State University, Columbus, OH 43210
YSI, Inc., Yellow Springs, OH 45387[3]

SUMMARY

A computer-assisted on-line glucose analyzer was developed for feed-back control of cell growth. Using this system the glucose consumption rate for Escherichia coli was determined to be linear during batch culture at 0.37 g/hr. On-line feed-back control of glucose concentration at 1.5±0.5 g/L was used with fed-batch cultures to produce 31.2 g dry weight of E. coli cells/L in 12 h.

INTRODUCTION

Recently much effort has been invested into the development of fed-batch cultivation systems for obtaining high cell concentrations of industrially important microorganisms (Allen & Luli, 1985; Pan et al., 1987; Shay et al., 1987; Suzuki et al., 1987; Zabriskie et al., 1987). Because these systems are based on feed regimens of carbon source (usually glucose), there also has been a demand for on-line chemical sensors which can be utilized for closed-loop feed-back control of the feeding of carbon sources. On-line glucose-sensing systems have been demonstrated (Cleland & Enfors, 1983; Parker et al., 1985; Ghoul et al., 1986), however, they have not yet been widely utilized. Therefore, most approaches for achieving high cell concentrations follow a desired feed-curve and are performed without direct on-line measurement of the feed substance. Previously, we described an inexpensive, Apple IIe computer-controlled fermentation system (Titus et al., 1984; Strohl et al., 1986). An on-line glucose analyzer has been added to this system to provide closed-loop, feed-back control of glucose feeding for fed-batch cultivation of E. coli.

MATERIALS AND METHODS

Organism and base medium. E. coli JM105 was grown at 37°C for 48 h on plates of SD-7 medium (see below). Seed cultures were started by loop inoculation of a colony from solid medium into 500 mL of liquid SD-7 and were incubated for 18 h at 37°C on a rotary shaker at 150 rpm.

A glucose-yeast extract-salts medium was developed for growth of E. coli based on the elemental yield analysis done by Reiling et al. (1985). This medium, denoted SD-7, contained (per liter): NH_4Cl, 7.0 g; KH_2PO_4, 1.56 g; Na_2HPO_4, 1.56 g; K_2SO_4, 0.38 g; $CaCl_2$, 0.004 g; $MgSO_4 \cdot 7H_2O$, 1.1 g; glucose 2.0 g; yeast extract, 5.0 g; and a trace

element solution, 0.8 mL. The pH was adjusted to 7.0 with 7 N NH_4OH before autoclaving. The glucose, magnesium, and trace elements were autoclaved in 100 mL of water and combined with the other components when cool. The trace elements solution was modified from that reported by Pan et al. (1987) and contained (in g per L of 5 N HCl): $FeSO_4 \cdot 7H_2O$, 40; $MnSO_4 \cdot H_2O$, 10; $AlSO_4 \cdot 18H_2O$, 28.25; $CoCl_2 \cdot 6H_2O$, 4; $ZnSO_4 \cdot 7H_2O$, 2; $Na_2MoO_4 \cdot 2H_2O$, 2; $CuCl_2 \cdot 2H_2O$, 1; and H_3BO_4, 0.5. Agar (Difco) was added to 1.5% (w/v) to prepare solid SD-7 medium.

Fermentation hardware and procedures. Growth was carried out using a 14 L New Brunswick (Edison, NJ) MF-214 fermentor equipped with automatic temperature control set at 37°C. An Apple IIe computer interfaced with a Cyborg ISAAC 9IA data acquisition system was used for computer controlled fermentation as described elswhere (Titus et al., 1984; Strohl et al., 1986). Modifications to the control software (Strohl et al., 1986) were made for this study in order to adjust various set-points and algorithms on-line. The modified BASIC control program, FERM1.2, operated at a sampling rate of 5 min.

To maintain dissolved oxygen levels above 30% of air saturation, the air flow rate was manually adjusted between 10 and 15 L/min and pure O_2 was blended up to 3.0 L/min. Agitation rate was controlled manually from 300 to 675 rpm. Dissolved oxygen (DO) and pH were monitored with a Braun MRR-1 unit using an Ingold autoclavable DO probe and a Fisher gel-filled pH probe which was sterilized with Chlorox and inserted into the cooled vessel. Control of pH at 7.0 $\pm$ 0.1 was achieved by addition of 7 N NH_4OH using direct digital control of the addition pump (Strohl et al., 1986).

Fermentation media. Batch cultivations were carried out using SD-7 medium modified by containing an initial glucose concentration of 20 g/L. Ingredients for 10 L were dissolved in 9.0 L of distilled water and autoclaved. Glucose (200 g), $MgSO_4 \cdot 7H_2O$ (11.0 g), and trace elements (8.0 mL) were added to 500 mL of distilled water, autoclaved, and added to the vessel after cooling. A 500 mL seed culture was added to bring the final volume to 10 L.

For fed-batch experiments, the SD-7 components were reformulated to support 45 g of dry cell biomass/L. To prevent precipitation at high culture densities (required to increase filtration efficiency), the $CaCl_2$ was omitted and the $MgSO_4 \cdot 7H_2O$ was reduced to a final concentration of 0.17 g/L. The culture vessel was prepared with SD-7 medium as described for batch experiments above except that the inital glucose and $MgSO_4 \cdot 7H_2O$ concentrations were 1.0 g/L and 0.085 g/L respectively. Feed solutions were delivered according to the following scheme. Feed solution #1 contained 200 g glucose and 0.085 g $MgSO_4 \cdot 7H_2O$ in 1 L of distilled water. Feed solution #2 contained 800 g glucose, 6.2 g $MgSO_4 \cdot 7H_2O$, and 30 mL trace elements in 1 L of distilled water. Feed solution #3 contained 280 g NH_4Cl, 62.4 g each KH_2PO_4 and Na_2HPO_4, 15.2 g K_2SO_4, and 20 g yeast extract in 1.2 L of distilled water. Feed solution #1 was fed until the culture density reached approximately 8.0 g of dry weight/L. Thereafter, a second pump was used to feed solutions #2 and #3 to the vessel simultaneously.

On-line glucose measurement. Glucose concentration was determined from a filtered sample of culture broth after cross-flow separation of particulate matter using a New Brunswick Scientific Megaflow TM-100 unit. The filtration system contained a 0.1 μm pore size membrane with a surface area of 10 in^2. The circulation rate was 450 mL/min and the initial flux rate was 30 mL/min at 5 lbs back pressure. Both the filtrate and retentate were returned to the vessel. A glass tee connector in the filtrate line provided access to the filtrate via a 10 cm long, 0.8 mm (I.D.) silicone tube.

The on-line glucose analysis system is shown in Figure 1. The silicone tube from the filtrate line was connected to an acrylic

two-way, two-position valve (see insert; Fig. 1). The valve had three inlets and three outlets for sample, carrier buffer (YSI Buffer 7G), and standard respectively. Two 25 µl channels were positioned in-line with either sample-plus-carrier or standard-plus-carrier by opposing solenoids. This enabled one channel to be filled while the second was placed in-line of the carrier buffer and fed to the probe unit. The sequential operation of the valve system is shown in Table 1.

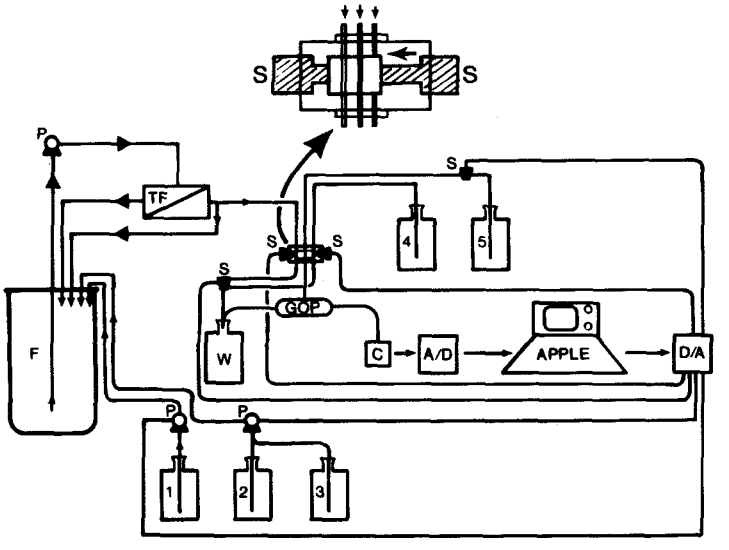

Figure 1. Schematic of computer-assisted on-line glucose feed-back control fermentation system. The large arrow indicates the blow-up diagram of the central 2-way, 2-position valve. Vessels: W, waste; #1, 2 & 3, feeds 1, 2 & 3, respectively; #4, glucose standard; #5, carrier buffer; Abbreviations: F, fermentor; P, peristaltic pump; S, solenoid; TF, tangential filter; GOP, glucose oxidase probe; C, signal conditioner; A/D, analog to digital converter; D/A, digital to analog converter.

Table 1. Sequential operation of the two-way, two chamber valve for feeding glucose standard and samples to the YSI glucose oxidase probe[a].

#	Valve position	Pump function	Valve function
1.	Carrier + standard	carrier	wash sample chamber
2.	Carrier + standard	withdrawal	load standard for calibration
3.	Carrier + sample	carrier	feed standard, obtain peak height
4.	Carrier + sample	withdrawal	load sample
5.	Carrier + standard	carrier	feed sample, obtain peak height

[a]Operation of the five sequential steps requires 80 sec.

The glucose probe had a range of 0 to 20 g of glucose/L. For the batch experiments, a glucose standard of 20 g/L was used for internal calibration, while a 10 g/L glucose standard was used for fed-batch experiments. The probe unit consisted of the same glucose oxidase probe, sample chamber, and mixing pump as used in the YSI Model 27. The probe was connected to a signal conditioner which provided -100 to +100 mV output to an ISAAC I130/I140 low level preamplifier.

Computer-assisted glucose feed-back control. The Apple IIe computer obtained the converted peak heights and operated the solenoid valves by means of an assembly language subroutine called from the BASIC control program. The control program then calculated the glucose concentration in the sample by peak height ratio.

For fed-batch experiments, the glucose feed was controlled using an analog ouput of 0-5 V to two Cole-Parmer #7534-30 peristaltic pumps, one with a #7013-20 head and the second with two #7013-20 heads. The pumps were calibrated prior to each run by feeding the glucose

solutions into a sterile 1 L flask on a Mettler PE1600 balance. Voltage output was plotted vs volume added and linear regression was performed to obtain pump slope and Y-intercept values which were entered into FERM1.2. The desired feed curve was calculated based on the glucose consumption rate obtained from batch experiments according to the following formula: $F = [V_f e^{(TS_g + I_g)}]/C_g$ [Equation 1], where F is the desired feed rate (L/h), V_f is the vessel volume (L), T is the elapsed time (h), S_g is the slope of the glucose consumption rate, I_g is the Y-intercept, and C_g is the concentration of glucose in the feed solution (g/L).

Output (0 to 5 V) to the feed pump was calculated from the pump calibration as follows: $O_u = F S_p + I_p$ [Equation 2], where O_u is the uncorrected output, S_p is the slope of the pump calibration, and I_p is the Y-intercept. The control routine then compared the actual glucose concentration to the setpoint (actual minus setpoint). If the deviation from the setpoint was outside the control window (1.5±0.5 g/L glucose), the pump rate was corrected as follows: $O_c = O_u - K_c E O_u$ [Equation 3], where O_c is the corrected output, K_c is the proportional gain, E is the glucose concentration error (in g/L), and O_u is the uncorrected output from Equation 2.

Other assays. Dry weights were determined on 30 mL samples as described previously (Strohl et al., 1986). Off-line glucose analysis was done using a YSI Model 27 analyzer calibrated with either 2.0 or 5.0 g/L glucose standards. Samples were clarified by centrifugation in a microfuge and diluted in distilled water if necessary.

RESULTS AND DISCUSSION

Medium development. The primary goal of medium development was to provide sufficient nutrients to achieve the desired cell conentration without precipitation. Reiling et al. (1985) published growth yields of E. coli relative to the major nutrient elements. Based on these yields and the medium reported by Reiling et al. (1985), SD-7 medium was formulated to support 10 g of cell dry wt/L in batch experiments and 45 g/L in fed-batch experiments. The added $CaCl_2$ was eliminated because of precipitation problems. The calcium present in the yeast extract was sufficient for batch experiments, but may have been limiting during fed-batch growth. Magnesium was reduced 10-fold from the calculated values and the amounts used were sufficient for batch experiments, but also may have been limiting in fed-batch experiments.

Analyzer calibration. Ouput from the glucose oxidase probe signal conditioner was connected to a strip-chart recorder and the peak heights measured for an initial test of the analyzer response. Five samples and five calibrations were performed using 20 g glucose/L for both. A 3.08% standard error was measured; some of this deviation was due to noise generated by the solenoid pumps. Because of this pump noise, baseline determinations were made before actuation of the solenoid pumps. With a 20.0 g/L glucose standard and samples of 0, 1, 2, 5, 10, and 20 g/L, the analyzer was run in a continuous, automatic mode. Results shown in Figure 2 demonstrate a linear response from 0 to 20 g glucose/L (n = 12; average standard error = 2.8%). The zero offset was due to the inherent electronic noise and was equivalent to a measurement of 0.15 g glucose/L.

On-line glucose analysis using the prototype analyzer described here compared closely with off-line determinations made with the widely used YSI Model 27 (Fig. 2). The sampling system provided a dead time of only four minutes, of which 2.66 minutes were required for operation of the sampling and calibration routines. This dead time is similar to that achieved by other on-line systems (Cleland & Enfors, 1983; Parker et al., 1985; Ghoul et al., 1986).

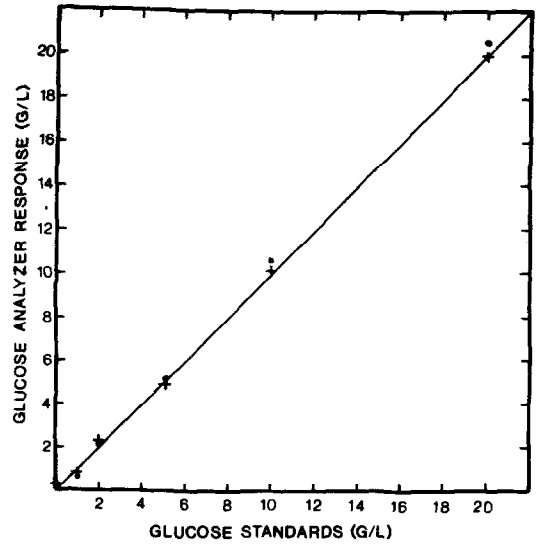

Figure 2. Linear response of on-line glucose probe (+) vs off-line glucose analysis (●) to concentrations of standard glucose solutions between 0 and 20 g/L.

Batch cultivations. The progress of E. coli growth and the glucose consumption are shown in Figure 3. For the first four hours of growth, the cells were consuming glucose at 0.37 g/hr while the growth rate was 0.69 g/h. At 4 h, the growth rate sharply decreased to 0.33 g/h and matched the glucose consumption rate for the rest of the growth period. The decrease in growth rate of E. coli in this medium was reproducibly detected by dry weight, culture turbidity, and dissolved oxygen measurements at 4 h (data not shown).

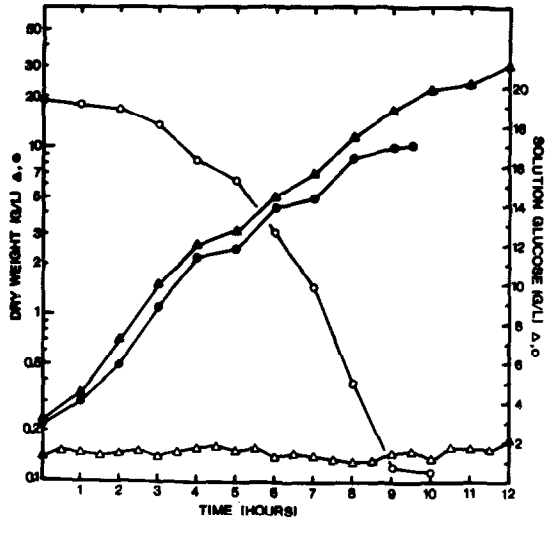

Figure 3. Growth of E. coli in batch fermentation (●) with on-line monitoring of glucose consumption (○); and growth of E. coli (▲) in fed-batch fermentation with on-line, automatic, feed-back control of glucose concentration between 1.0-2.0 g/L (△).

The medium for batch cultivation contained 20.0 g glucose/L and was formulated to support 10 g of dry E. coli cells/L. In our experiments, the final dry weight was 10.33 g/L giving an overall cell yield of 0.51 g of cell mass produced per g of glucose consumed.

Off-line and on-line glucose determinations were similar, demonstrating that the samples acquired by the on-line analyzer were representative of the bulk solution (data not shown).

Fed-batch cultivation. Cell growth and glucose concentrations are shown in Figure 3. The glucose concentration was maintained within the programmed range of 1.0 to 2.0 g/L. Initial and secondary growth rates were 0.64 g/hr and 0.37 g/hr respectively similar to the rates measured during batch experiments. Growth yield on glucose during fed-batch cultivation was 0.47 g dry weight of cells produced/g glucose consumed with a final cell density of 31.2 g dry cells/L. Further experiments are being done to optimize the medium to support higher cell densities and improve yield on glucose.

Initial (up to 4 h) and secondary (4 to 8-10 h) growth rates for batch and fed-batch cultures were similar. Such changes in growth rate have been reported for E. coli and other microorganisms (Allen & Luli, 1985; Strohl et al., 1986). Because the secondary growth rate decreased to match the glucose consumption rate, the initial faster growth rate probably was supported by components of the yeast extract which became limiting after 4 h. This also is supported by the fact that even though the growth rate decreased, the cell yield on glucose remained constant throughout the experiment. Because growth rate was shown to be independent of glucose comsumption rate, care must be taken when determining feeding strategies based on cell mass or growth rate estimates. Feeding here was based on glucose consumption rate and thus not affected by changes in growth rate.

The prototype, on-line, glucose analyzer was able to maintain glucose concentrations between 1.0 and 2.0 g/L while feeding 750 g of glucose over 12 h. This system has a shorter dead time and is less complex than previously reported systems (Cleland & Enfors, 1983; Parker et al., 1985; Ghoul et al., 1986). Also, an updated version of this system (YSI Model 2000) soon will be available commercially. This automatic analyzer has broad applications for processes where feed-back control of glucose addition is advantageous, such as the growth of E. coli, yeasts, and mammalian cell cultures.

REFERENCES

Allen, B.R., and Luli, G.W. (1985). The World Biotechnology Report. Proc. Biotech. '85 (USA) 2, 447-466.

Cleland, N., and Enfors, S. (1983). Eur. J. Appl. Microbiol. Biotechnol. 18, 141-147.

Ghoul, M., Ronat, E., and Engasser, J. (1986). Biotechnol. Bioeng. 28, 119-121.

Pan, J.G., Rhee, J.S., and Lebault, J.M. (1987). Biotechnol. Lett. 9, 89-94.

Parker, C.P., Gardell, M.G., and DiBiasio, D. (1985). Amer. Biotechnol. Lab. Sept/Oct, pp. 37-46.

Reiling, H.E., Laurila, H., and Fiechter, A. (1985). J. Biotechnol. 2, 191-206.

Shay, L.K., Hunt, H.R., and Wegner, G.H. (1987). J. Industr. Microbiol. 2, 79-85.

Strohl, W.R., Schlasner, S.M., and Lorensen, P.L. (1986). Biotechniques 4, 336-343.

Suzuki, T., Yamane, T., and Shimizu, S. (1987). Appl. Microbol. Biotechnol. 25, 526-531.

Titus, J.A., Luli, G.W., Dekleva, M.L., and Strohl, W.R. (1984). Appl. Environ. Microbiol. 47, 239-244.

Zabriskie, D.W., Wareheim, D.A., and Polansky, M.J. (1987). J. Industr. Microbiol. 2, 87-95.