

A semi-empirical mathematical model useful for describing the relationship between carbon dioxide, pH, lactate and base in a bicarbonate-buffered cell-culture process

Michael J. Gramer¹ and Tad Ogorzalek

Protein Design Labs, Inc., 9450 Winnetka Ave. N., Brooklyn Park, MN 55445, U.S.A.

The purpose of the present study was to develop a quantitative relationship between the primary factors of state affecting pH control in a bicarbonate-buffered medium. Starting with the Henderson–Hasselbach equation, several assumptions led to the following equation:

$$L = B_T - s \cdot dCO_2 \cdot 10^{(pH-pK)}$$

where L is the lactate concentration (mM), B_T is the total amount of base added (mM), s is the solubility of CO_2 (mM/%), dCO_2 is the dissolved CO_2 concentration (%) and pK is the acid ionization constant for bicarbonate. This equation appropriately described the relationship of these factors when using bicarbonate, carbonate and HCl (as a lactic acid surrogate) in water. However, the equation required modification to describe the relationship in cell culture medium, due presumably to the presence of other buffers and components; the final form of the equation from an empirical fit in the absence of cells was:

$$L = B_T - 0.88 \cdot dCO_2^{0.79} \cdot 10^{(pH-6.38)}$$

This equation was tested against actual cell culture data, from inoculum preparation in a T-flask through a 10 000-litre fed-batch bioreactor, by comparing the lactate concentration calculated from base, pH and dCO_2 data with that actually measured in the bioreactor using a YSI 8500 SELECT™ Biochemistry Analyzer (YSI Inc., Yellow Springs, OH, U.S.A.). In every case, the calculated and actual lactate concentrations were in good agreement. The equation was useful for isolating the mechanisms leading to varied base addition across 2-, 600- and 10 000-litre-scale bioreactors. This procedure enables a new approach for quantitatively evaluating and understanding factors associated with bioreactor pH control.

Introduction

Bioreactor pH control is critical for maintaining consistent growth, metabolism, productivity and product quality [1–8]. As the cell density increases in the bioreactor, there is

an increase in lactic acid production. The lactic acid is neutralized by sodium bicarbonate and other buffers in the medium. In order to maintain pH, the CO_2 concentration is initially reduced in the gas phase, thus reducing the dCO_2 (dissolved CO_2 concentration) and resulting in an increase in pH. At some point, the amount of CO_2 being generated by the culture (from lactic acid neutralization and from respiration) exceeds the amount necessary to control pH, and additional base, such as sodium carbonate, is added for additional buffering capacity.

When scaling up a mammalian cell culture process, there is often an increased concentration of dCO_2 at larger scales [9–15]. Elevated dCO_2 can alter cell growth, cell metabolism, product titre and product quality either directly or through the increased osmolality associated with the increased base addition required for pH control [15–24].

Cellular metabolism is complex, and a number of acids and bases are produced and consumed in a cell culture process. As such, it is difficult to predict net acid or base production that must be neutralized for pH control. Others have suggested that the lactate production rate could be predicted during the middle of a run assuming a 1:1 stoichiometric relationship between lactate production and base addition [25,26]. However, before base addition is initiated, this approach is not applicable. Furthermore, this procedure is not applicable to situations where lactate is re-adsorbed by the cells, which is a case of net acid consumption.

High concentrations of lactic acid can be toxic to mammalian cell growth, either directly or through the increased osmolality associated with base addition to neutralize the acid [27–31]. As a result of these issues, a number of approaches have been implemented to minimize lactate formation [32–39].

The Henderson–Hasselbach relationship has been applied to cell culture medium for use with sodium bicarbonate, a major buffering component that maintains equilibrium

Key words: antibody production, bioreactor, carbon dioxide, osmolality, pH control, scale up.

Abbreviations used: $CO_{2,g}$, CO_2 gas; dCO_2 , dissolved CO_2 ; dCO_2 , dissolved CO_2 concentration; DO , dissolved O_2 concentration.

¹ To whom correspondence should be addressed (email michael.gramer@pdl.com).

with dCO_2 [5,14,17,19,25,40,41]. Several issues complicate the use of this equation for conditioned cell culture media. First, base is typically consumed by the cell culture process as a result of cellular acid production. When bicarbonate is consumed by acid neutralization, it is converted into CO_2 and must be driven from the bioreactor for pH control. As a result, the actual base concentration changes throughout the course of cell culture due to cellular metabolism. Secondly, the rate of dCO_2 removal can be limiting, requiring that additional base be added to the culture, which again results in a change in base concentration. Thirdly, other buffers are typically present in the medium, and this may influence the acid–base relationship between bicarbonate, dCO_2 and pH. As a result of these issues, the standard Henderson–Hasselbach equation has limited use for describing a cell culture process.

When conditions are modified, it can be difficult to determine cause–effect relationships due to the interplay of changes in pH, dCO_2 , base and acid–base metabolism of the cells. The purpose of the present study was to explore the development of a mathematical approach to relate these factors in a cell culture process. Development was initiated using the Henderson–Hasselbach equation, which describes the equilibrium relationship between sodium bicarbonate, pH and CO_2 . This equation required modification in order to account for other medium components, resulting in a semi-empirical relationship between sodium bicarbonate, pH and CO_2 . Next, the equation was modified to account for production of lactic acid, while ignoring other possible drivers of pH, assuming that 1 mol of lactic acid consumes 1 mol of bicarbonate. Lastly, the equation was modified to allow for addition of sodium carbonate throughout the run. The resulting equations were first tested theoretically using model systems and then against actual cell culture data from inoculum preparation in a T-flask through a 10 000-litre bioreactor.

Materials and methods

Media

The cell culture media used in the present study were proprietary formulations that were chemically defined and animal-component-free. The medium used for inoculum preparation and for initial inoculation into the bioreactors was termed basal medium. Sodium bicarbonate was added to the basal medium at a concentration of 29 mM. Medium added during the run was termed feed medium. Feed medium was a concentrated source of nutrients and did not contain sodium bicarbonate.

Inoculum preparation

An antibody-producing recombinant NS0 cell line was used for the present study. Cells were scaled up in T-flasks and

roller bottles in incubators at 37 °C and 7.5% CO_2 . When necessary, cells were additionally scaled up in 8-litre spinners (in incubators at 37 °C and 5% CO_2) and in bioreactors (controlled at pH 7.0 and 37 °C). Cells were propagated in basal medium.

Bioreactor control

The basic bioreactor setpoints were the same at all scales. The pH was maintained at 7.0 using $CO_{2,g}$ (CO_2 gas) injection or sodium carbonate addition (0.9–1 M). The temperature was maintained at 37 °C by circulating ethylene glycol or water through the bioreactor jacket. The total gas flow, including air plus oxygen, was maintained at a constant level by mass flow controllers, the values of which depended on scale. The bioreactor was initially sparged with 100% air and the DO (dissolved oxygen concentration) decreased freely until reaching 30%. Thereafter, the DO was controlled at 30% using O_2 flow up to a maximum level depending on the scale (75–85% of gas flow rate). Agitation was used in cascade with O_2 flow to increase oxygen transfer as the culture progressed.

Bioreactor sampling, testing and feeding

The following off-line measurements were made from each sample: glucose and lactate concentration [YSI 8500 SELECT™ Biochemistry Analyzer; YSI Inc., Yellow Springs, OH, U.S.A.]; dCO_2 , DO and pH (ABL5 blood–gas analyser); viable cell density and cell viability (Cedex Automated Cell Culture Analyzer using Trypan Blue; Innovatis Inc., Malvern, PA, U.S.A.); osmolality [Precision Systems (Natick, MA, U.S.A.) freezing-point-depression-measuring instrument]; and antibody titre (in-house Protein A HPLC method). The pH probe (glass electrode from Mettler-Toledo, Columbus, OH, U.S.A.) was calibrated before inoculation using an off-line sample. The pH probe was calibrated again during the run if the off-line sample was more than 0.04 pH units different from the on-line sample. The DO probe (galvanic electrode from Mettler–Toledo) was calibrated to 100% before inoculation and was not calibrated thereafter. The bioreactor was initially filled to 40% of the working volume with basal medium (bioreactor sizes referred to in the present study are working volumes). Cells in basal media were inoculated to bring the total inoculation volume in the range of 50–52% of the working volume. The inoculum seed train was sampled each time just before passage; the 2-litre bioreactors were sampled once daily, and the 600- and 10 000-litre bioreactors were sampled twice daily. The bioreactor was fed fixed volume amounts of feed medium once a day, starting on the second day; the fixed amounts were proportional to the bioreactor working volume to bring the final volume 92–93% to after 10 days.

Reporting of $d\text{CO}_2$

As stated, the $d\text{CO}_2$ was measured using a blood-gas analyser. The analyser provides a reading in terms of mmHg, which is the equilibrium partial pressure of the gas phase rather than the actual millimolar concentration in the liquid phase. For convenience, this number was converted into %CO₂ using a factor of 100%/760 mmHg. The %CO₂ number was then used in the equations for $d\text{CO}_2$.

Experiments without cells

All experiments in the absence of cells were performed in 2-litre bioreactors with 2-litres of solution. The total gas flow rate (air plus CO₂) was set to 200 standard cm³/min with agitation at 150 rev./min and temperature at 37 °C. For each solution, the CO₂ flow rate was adjusted over a range of approx. 0.5–20% of the total gas flow rate; the actual range used depended partly on the base concentration being tested such that the pH did not vary outside of 6.7–7.8. A sample was taken for off-line determination of pH and $d\text{CO}_2$ after each condition reached equilibrium based on the reading from an on-line pH probe.

Results

Run profiles at varying scale

Typical fed-batch run profiles for lactate, pH, base, $d\text{CO}_2$ and osmolality are shown for 2-, 600- and 10 000-litre scales in Figure 1. Over the first several days, CO₂ was injected into the bioreactor to control pH. During that time, the $d\text{CO}_2$ profile was similar across the scales. Around day 3, the CO₂ flow in the gas phase was off and the $d\text{CO}_2$ profile in the bioreactor was determined by rate of cellular production versus rate of removal, which varied with scale; at increased scale, lower volumetric airflow led to increased $d\text{CO}_2$. Base addition began shortly after the point of cessation in CO₂ flow. The increased $d\text{CO}_2$ led to increased base addition, leading in turn to higher osmolality. However, despite very different $d\text{CO}_2$ concentrations from days 3 to 5, the amount of base added was similar between the 600- and 10 000-litre scales.

Standard Henderson–Hasselbach equation

In order to better understand the factors affecting base addition, analysis was initiated by using equilibrium equations for bicarbonate and $d\text{CO}_2$ (assuming that the bioreactor can be modelled as a pseudo-steady state). When CO_{2,g} is sparged through an aqueous medium, it dissolves into the medium ($d\text{CO}_2$; eqn 1), combines with water to form carbonic acid (H₂CO₃; eqn 2), and dissociates to form bicarbonate (HCO₃⁻; eqn 3) and a hydrogen ion, and further dissociates to form carbonate (CO₃²⁻; eqn 4) and an additional

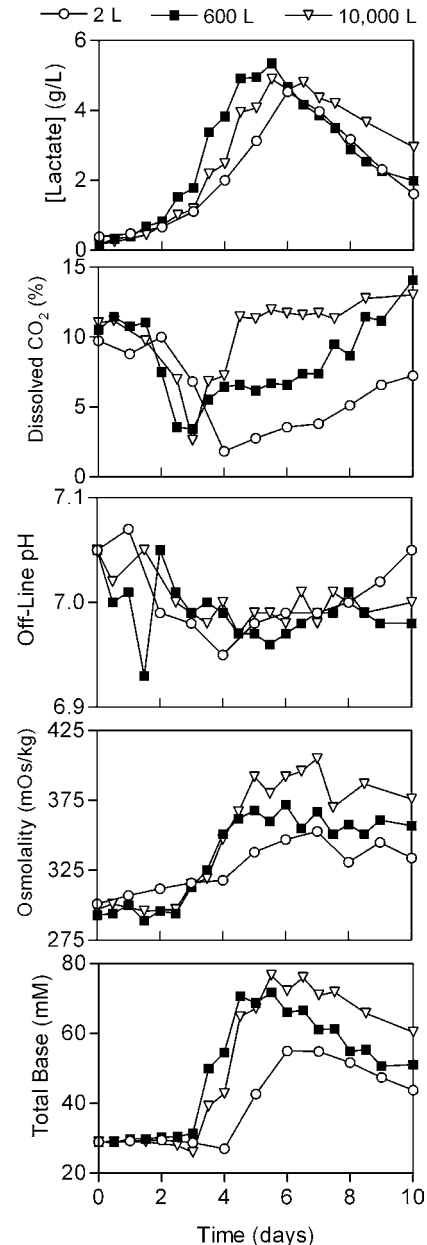
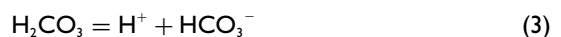


Figure 1 Effect of scale on fed-batch bioreactor data associated with pH control

hydrogen ion [41].



Several factors simplify the equations relating these chemical species at equilibrium in a bioreactor. First, the pK of the second hydrogen ion (eqn 4) is above 10, and as a result, the

amount of carbonate present in the medium can be ignored. Secondly, H_2CO_3 readily dissociates into bicarbonate plus a hydrogen ion. Thirdly, at equilibrium the amount of dissolved CO_2 is proportional to the amount of CO_2 in the gas phase as provided by Henry's Law. Taken together, the Henderson–Hasselbach equation is typically expressed in a form similar to eqn (5) where the bicarbonate concentration (B) is in mM, the $d\text{CO}_2$ is in %, and s is a solubility factor converting % CO_2 partial pressure into mM CO_2 in the liquid phase (mM/%). Eqn (5) was rearranged to provide a form useful for experimental evaluation (eqn 6):

$$\text{pH} = \text{pK} + \log[B / (s \cdot d\text{CO}_2)] \quad (5)$$

$$10^{(\text{pH}-\text{pK})} = (B / d\text{CO}_2) / s \quad (6)$$

For practical application of eqns (5) and (6), the solubility (s) and the pK are not mathematically independent; the two could be lumped together as one constant for the purposes of the present paper. However, when converting from a logarithmic form of the equation into an exponential form, this would leave an isolated 10^{pH} , a large number that is difficult to deal with. For convenience, the pK was assumed to be 6.38 based on previous literature [25]; the solubility was then determined through experimentation. According to eqn (6), a plot of $10^{(\text{pH}-6.38)}$ versus $B/d\text{CO}_2$ should provide a straight line of slope $1/s$ and an intercept of 0. As a control, and to evaluate s , this relationship was examined at three different concentrations of bicarbonate in water while varying the CO_2 level in the gas phase. As shown in Figure 2, all three concentrations of bicarbonate fell on the same line providing an intercept near 0 and a solubility (s) of 0.39 mM/%; this solubility was in line with expectations [40].

Incorporation of lactate production

Cells produce lactic acid, which is known to drive decreases in pH in uncontrolled cultures. Lactate has a pK of 5.5; since bioreactors are controlled near pH 7, 1 mol of lactic acid production requires approx. 1 mol of base for neutralization [26]. Assuming that lactate is the dominant metabolite driving changes in pH control and assuming that bicarbonate is the dominant base in the system, then 1 mol of lactic acid produced would consume 1 mol of bicarbonate, which would leave in the form of CO_2 . The effective base concentration is then $B-L$, where B is the original amount of bicarbonate added (mM) and L is the lactate concentration (mM). A similar approach was presented to evaluate the theoretical effect of lactic acid production on medium pH [40]. Placing this relationship into eqn (6) leads to eqn (7). Eqn (7) can then be rearranged to calculate the lactate concentration from the process data (eqn 8). These relationships were tested using 29 mM bicarbonate in water and a constant $\text{CO}_{2,g}$ flow rate by adding HCl as a surrogate for

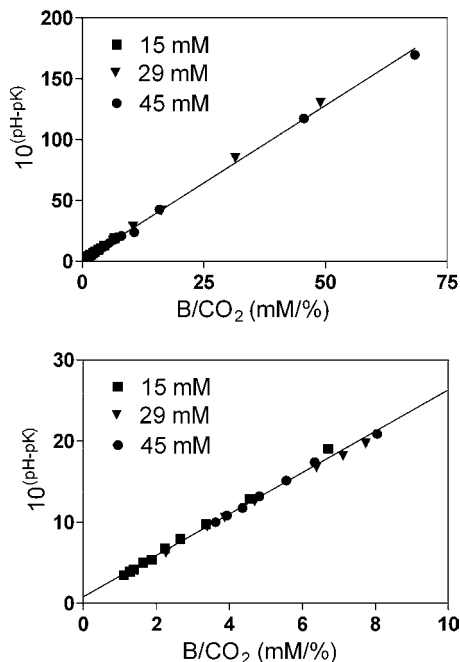


Figure 2 Application of eqn (6) to analysis of sodium bicarbonate in water

The sodium bicarbonate concentration (B) was 15, 29 or 45 mM. The $d\text{CO}_2$ concentration was varied by adjusting the flow rate of $\text{CO}_{2,g}$. A sample was taken for off-line determination of pH and $d\text{CO}_2$ (%). The entire data set was fit to a single line as shown. The upper panel is the entire data set, while the lower panel is the same graph magnified for better presentation of data near the origin. The data fit theory well with an intercept near zero and a slope of 2.547, providing a solubility parameter of $s = 0.39$ mM/% (assuming a pK of 6.38).

lactic acid production in the absence of cells. After each HCl addition, an off-line sample was taken for determination of $d\text{CO}_2$ and pH. When all the data, including those from Figure 2 and those from acid addition, were plotted on the same graph using eqn (7), all data fell on the same line with the same slope as shown in Figure 2 (results not shown). Alternatively, the theoretical lactate (or, more correctly, HCl) concentration was calculated from eqn (8); this calculated concentration was predictive compared with the actual amount of acid added (Figure 3).

$$10^{(\text{pH}-6.38)} = [(B - L) / d\text{CO}_2] / s \quad (7)$$

$$L = B - s \cdot d\text{CO}_2 \cdot 10^{(\text{pH}-6.38)} \quad (8)$$

Incorporation of base addition

Starting on approximately day 3 in the bioreactor, a 0.9–1 M solution of sodium carbonate was added to control pH. Theoretically, 1 mol of sodium carbonate (C ; mM) should act as 2 mol of bicarbonate (B ; mM) according to eqns (1)–(4), leading to a total amount of base added (B_T ; mM) as described in eqn (9). Incorporating this into eqn (7) gives the final theoretical form desired as eqn (10). To test

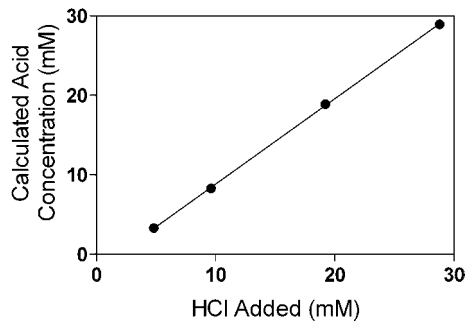


Figure 3 Calculated versus actual acid addition to sodium bicarbonate in water

The sodium bicarbonate concentration was 29 mM and CO₂ flow was kept constant. HCl was added as a surrogate for lactic acid in the absence of cells. Off-line samples were taken after each acid addition for determination of pH and dCO_2 . The acid concentration was calculated according to eqn (8) and plotted against the actual amount of acid added. The best-fit line shown on the graph has a slope of 1.07 and an intercept of -2 mM, which was comparable with a theoretical slope of 1.0 and intercept of 0.

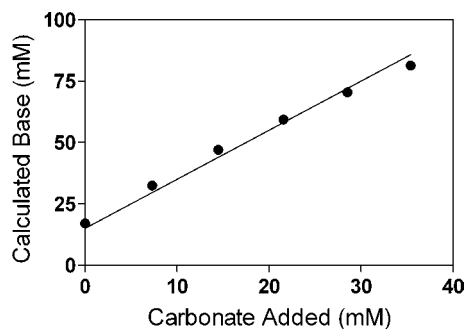


Figure 4 Evaluation of effective base concentration for carbonate addition

The medium initially contained 15 mM of sodium bicarbonate. Carbonate was added and an off-line sample was taken for determination of pH and dCO_2 , which were used to determine the calculated base concentration using eqn (11). Carbonate was expected to act equivalently as two bicarbonates. The line shown is the theoretical expectation with an intercept of 15 mM and a slope of 2. The calculated base concentration was in line with theoretical expectations.

the assumptions in this section, carbonate was added to a 15 mM solution of bicarbonate in water using a constant CO₂ flow rate, and the total base concentration was calculated from off-line readings using eqn (11). As shown in Figure 4, the calculated base concentration followed the theoretically expected line with a slope of 2.

$$B_T = B + 2 \cdot C \quad (9)$$

$$10^{(pH-6.38)} = [(B_T - L)/dCO_2]/s \quad (10)$$

$$B_T = s \cdot dCO_2 \cdot 10^{(pH-6.38)} \quad (11)$$

Application to basal medium

Up to this point, all data were generated using a minimal system of sodium bicarbonate, carbonate, HCl and water, all of which provided good controls. The next step was

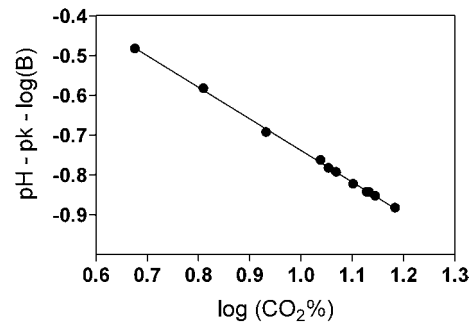


Figure 5 Test of basal medium without cells

Basal medium with 29 mM sodium bicarbonate did not fit eqn (6) (results not shown). Eqn (6) was rearranged as eqn (12); the data fit this equation with a straight line (correlation coefficient of 0.999), although the slope was 0.79 instead of the theoretically expected 1.0. In addition, the intercept gave a solubility of 0.88 mM/% instead of the ideal value of 0.39 mM/% that was obtained in the bicarbonate/water system. This fit (eqn 13) forms the basis of the semi-empirical relationship provided in eqn (14).

to evaluate whether cell culture medium could be similarly characterized. In an experiment similar to that for Figure 2, basal medium containing 29 mM sodium bicarbonate was evaluated. Unfortunately, the data did not fit eqn (6). Interestingly, however, if the data were analysed according to a re-arranged form of eqn (6), as shown in eqn (12), the data fit a straight line as expected (Figure 5). However, if cell culture medium behaved similarly to the ideal conditions with sodium bicarbonate and water, the theoretical slope of the line should be -1 with an intercept of $\log(1/0.39) = 0.41$. In contrast, the best-fit line for medium provided a slope of -0.79 with an intercept of 0.054 (eqn 13). The exact reason for the non-ideal relationship was explored further. The basal medium also contains 15 mM of HEPES, another strong buffer. When 15 mM HEPES was added to sodium bicarbonate in water, the data essentially fit eqn (13) (results not shown). These results suggest that the non-ideal, semi-empirical correlation determined in eqn (13) is likely due to the presence of HEPES and perhaps other buffers in the medium.

$$(pH - pK - \log B) = \log(1/s) - \log(dCO_2) \quad (12)$$

$$(pH - 6.38 - \log B) = (0.054 - 0.79) \cdot \log(dCO_2) \quad (13)$$

Application to cell culture data

In order to evaluate the correlations for cell culture, eqn (13) was converted back into a more useful form, with the lactate and carbonate modifications incorporated as determined previously, and the equation was solved for lactate concentration (eqn 14). Historical cell culture data were then analysed by comparing the actual measured lactate concentration with that calculated from process data using eqn (14). Because each production run was fed-batch process, the total amount of base added (B_T) was adjusted depending on the bioreactor volume (eqn 15), where B_0 is the initial

amount of bicarbonate in the basal medium (29 mM), V_0 is the initial bioreactor volume (50% of working volume in L), C_s is the stock concentration of carbonate (900–1000 mM), V_s is the volume of carbonate stock solution added to the bioreactor up to the day of calculation (L), and V_T is the total volume in the bioreactor on the day of calculation (L).

$$L = B_T - 0.88 \cdot d\text{CO}_2^{0.79} \cdot 10^{(\text{pH}-6.38)} \quad (14)$$

$$B_T = (B_0 V_0 + 2 \cdot C_s V_s) / V_T \quad (15)$$

The inoculum seed train was first evaluated from T-flasks, roller bottles and spinner flasks (Figure 6). Under these conditions, the total base concentration was 29 mM and there were no additional solutions added (such as carbonate or feed medium). In every case, the calculated lactate concentration accurately reflected the measured lactate concentration. These results suggest that the assumptions used to generate eqn (14) were reasonably valid under these conditions.

Next, data were evaluated from bioreactors at 2-, 600- and 10 000-litre scales (Figure 6). Over the first two days, conditions in the bioreactors were essentially identical with those in the inoculum seed train, with the exception of active pH control in the bioreactors. As such, the fact that lactate predictions were accurate over the first two days was not surprising. The first feed began on day 2 of the culture and base addition began on day 3 and continued through to day 7. The lactate concentrations were rising over this time and the calculated lactate concentrations were an accurate reflection of the measured lactate concentrations. These results confirm that the assumptions used to generate eqn (14) were reasonable. One somewhat surprising aspect was that the correlation held true later in the culture, during a time when lactate was actually re-adsorbed by the cells. Furthermore, by the end of the culture, almost 50% of the medium was feed medium, which differed substantially from the basal medium in composition.

Altogether, these results demonstrate that eqn (14) can provide useful and predictive information across a wide array of scales and conditions, in spite of the semi-empirical nature of the equation. Interestingly, the same equation was useful for predicting the lactate concentration for two other cell lines that used similar, but not identical processes or media (results not shown).

Determining the relative contribution of pH, lactate and $d\text{CO}_2$ to base addition

Eqn (14) can be used to explain the base addition profiles shown in Figure 1. The procedure is exemplified in Table 1 using day-5 data, where the base concentration was 42.6 mM at the 2-litre scale, 69.5 mM at the 600-litre scale, and 69.8 mM at the 10 000-litre scale. As a control, the theoretical amount of base addition was calculated at each

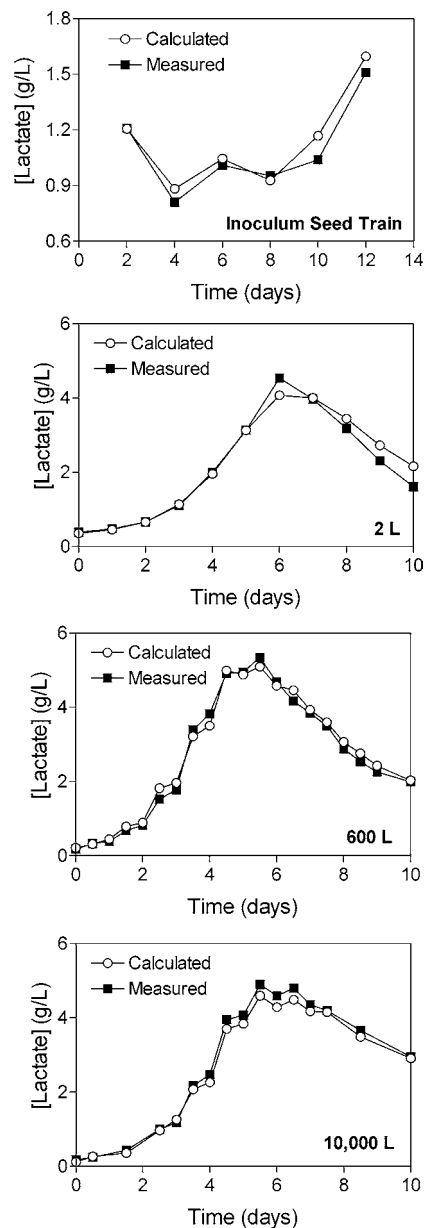


Figure 6 Evaluation of lactate prediction in various scale cell culture devices

Lactate concentrations were estimated from eqn (14) and were converted from mM into g/l for the Figure. In the inoculum seed train, cells were in T-flasks on days 0–2, in roller bottles on days 2–8, and in spinners on days 8–12. Three other scales were evaluated including 2-, 600- and 10 000-litre bioreactors (the data correspond to Figure 1). The lactate concentrations calculated from the off-line CO_2 and pH data along with on-line readings of base (carbonate) addition demonstrated good agreement with actual concentrations of lactate measured using the YSI Biochemistry Analyzer. These data were analysed for a single run at each scale, although the results and conclusions were similar for other runs or averaged runs.

scale from the pH, $d\text{CO}_2$ and lactate data; these calculations agreed well with the actual amount of base used (Table 1). To determine why more base was added at the larger scales, the base addition calculation at 600 or 10 000 litres was modified

Table 1 Determining the relative contribution of pH, lactate and dCO₂ to excess base addition at increased scale (using day 5 data from Figure 1)

Parameter	Value		
Bioreactor scale (litres)	2	600	10000
Lactate (g/l)	3.13	4.95	4.07
pH	6.98	6.97	6.99
dCO ₂ (%)	2.76	6.18	11.32
Actual base (mM)	42.6	68.7	67.2
Calculated base (mM)	42.6	69.5	69.8
Base if pH was same as 2 litres (mM)		69.8	69.2
Difference (mM)		-0.3	0.6
Base if lactate was same as 2 litres (mM)		49.3	59.3
Difference (mM)		20.6	9.9
Base if CO ₂ was same as 2 litres (mM)		62.7	53.2
Difference (mM)		7.2	16.0

by alternately substituting the pH, dCO₂ or lactate data from the 2-litre condition into the calculation. There was little difference in pH across the scales, so pH differences accounted for less than a 1 mM difference in base contribution (Table 1). At the 600-litre scale, increased lactate contributed to a 20.6 mM increase in base addition, while increased dCO₂ contributed to an additional 7.2 mM of base addition (Table 1). At the 10000-litre scale, increased lactate contributed to a 9.9 mM increase in base addition, while increased dCO₂ contributed to an additional 16 mM of base addition (Table 1). Thus the amount of base addition was similar at the 600- and 10000-litre scales, but the relative contributions of lactate and dCO₂ were different at each scale.

Sensitivity analysis

Error in calculation of lactate concentration can arise due to inaccurate addition of base or inaccurate measurement of pH or dCO₂. Reasonable estimates of these errors were assumed (base 2% error; pH 0.03 pH units error; dCO₂ 10% error) and the influence of each error was determined around a single point of pH 7.0 with a base concentration of 29 mM (Figure 7). In each case, the absolute error in lactate calculation was relatively small (< 0.2 g/l). However, the absolute error either remained the same or increased with decreasing lactate concentration (especially with pH error); thus the percentage error increased as the lactate concentration decreased. These results suggest that care should be taken when interpreting results at low lactate concentration, especially if pH measurement error is large.

Discussion

Different scales and process conditions may lead to ambiguity when analysing data associated with pH control

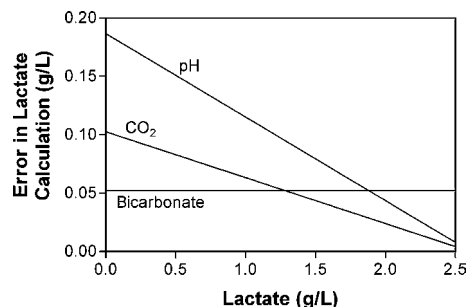


Figure 7 Theoretical determination of error in lactate calculation due to inaccurate addition of base (2%) or inaccurate measurement of pH (0.03 pH units) or dCO₂ (10%)

The influence of each error as a function of lactate concentration was determined around a single point of pH 7.0 with a base concentration of 29 mM assuming dCO₂ is adjusted as necessary to control pH.

due to the interplay of the associated process and metabolic parameters. The present paper provides a useful method to more critically evaluate the data. Although the final result was an empirical equation, the correlation has a foundation in, and is traceable to, basic physical, chemical and biological principles. Furthermore, eqn (14) has proven useful for diagnostic and predictive purposes on several occasions as described in the following examples.

First, data from a 100-litre pilot bioreactor did not correlate well with data shown in Figure 1 (results not shown). At the 100-litre scale, the average amount of base addition was higher than expected and the standard deviation was relatively large. When eqn (14) was applied at the 100-litre scale, the data fit well up until base addition commenced. Upon closer examination, the likely cause of the problem was determined as poor resolution in the amount of base added compared with the measurement method (the size of base tank was large relative to amount of base used).

Secondly, the equation was applied to analysis of an out-of-trend lactate number (results not shown). The measured lactate concentration was approx. 2-fold higher than expected, but the sample was no longer available for retesting. When eqn (14) was applied, all data in the run fit eqn (14) except the out-of-trend lactate number. Eqn (14) suggested that the actual lactate concentration was in-line with historical expectations, supporting the conclusion that the sample was likely diluted incorrectly before analysis.

Thirdly, this approach was applied to evaluation of technology transfer success from site to site and from one scale to another (results not shown). In particular, eqn (14) was useful for evaluating changes in process control procedures and whether sensors were providing appropriate readings.

Future work will be aimed at combining the relationship derived here with a model for CO₂ transfer across the different scale bioreactors. Such a combination could provide a powerful model of the bioreactor system. For example,

poor growth may be evident at a larger scale because of elevated osmolality, which results from increased base addition owing to increased dCO_2 . These models could be used to target an acceptable osmolality by first calculating an acceptable amount of base addition, which, in turn, is used to determine an acceptable level of dCO_2 from eqn (14). An appropriate airflow or agitation rate to achieve this dCO_2 is then determined from the CO_2 transfer model.

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