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Stoichiometric Flux Balance Models Quantitatively Predict Growth and Metabolic By-Product Secretion in Wild-Type *Escherichia coli* W3110

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Flux balance models of metabolism use stoichiometry of metabolic pathways, metabolic demands of growth, and optimality principles to predict metabolic flux distribution and cellular growth under specified environmental conditions. These models have provided a mechanistic interpretation of systemic metabolic physiology, and they are also useful as a quantitative tool for metabolic pathway design. Quantitative predictions of cell growth and metabolic by-product secretion that are experimentally testable can be obtained from these models. In the present report, we used independent measurements to determine the model parameters for the wild-type *Escherichia coli* strain W3110. We experimentally determined the maximum oxygen utilization rate (15 mmol of O_2 per g [dry weight] per h), the maximum aerobic glucose utilization rate (10.5 mmol of Glc per g [dry weight] per h), the maximum anaerobic glucose utilization rate (18.5 mmol of Glc per g [dry weight] per h), the non-growth-associated maintenance requirements (7.6 mmol of ATP per g [dry weight] per h), and the growth-associated maintenance requirements (13 mmol of ATP per g of biomass). The flux balance model specified by these parameters was found to quantitatively predict glucose and oxygen uptake rates as well as acetate secretion rates observed in chemostat experiments. We have formulated a predictive algorithm in order to apply the flux balance model to describe unsteady-state growth and by-product secretion in aerobic batch, fed-batch, and anaerobic batch cultures. In aerobic experiments we observed acetate secretion, accumulation in the culture medium, and reutilization from the culture medium. In fed-batch cultures acetate is cometabolized with glucose during the later part of the culture period. Anaerobic batch culture is observed to primarily secrete the by-products acetate, ethanol, and formate. The flux balance model was found to quantitatively predict the time profiles of cell density and glucose and by-product concentrations in the above-described experiments. Taken together, the experimental data and model predictions presented show that observed growth and by-product secretion of wild-type *E. coli* are consistent with stoichiometrically optimal pathway utilization. Flux balance models can thus be used to describe prokaryotic metabolic physiology, and they can be applied to bioprocess design and control.

The ability to quantitatively describe metabolic fluxes through metabolic networks has long been desired (10). However, this endeavor has been hampered by the need for extensive kinetic information describing enzyme catalysis within the living cell. Detailed information about all the enzymes in a specific metabolic network is not available, perhaps with the exception of the enucleated human erythrocyte (6). This dilemma has recently been partially resolved by the development of flux balance-based metabolic models (3, 12, 17, 19, 24). The flux balance approach relies on metabolic stoichiometry, metabolic requirements for growth, and optimality principles (19, 20). These models quantitatively describe steady-state flux distributions in metabolic network. Since metabolic transients are typically rapid, the flux balance models are applicable to most experiments that involve cell growth, including bioprocess situations in which process transients are much longer than those associated with metabolism.

Flux balance models have provided valuable insights into prokaryotic metabolic physiology (18). A balance between metabolic fluxes has been used to formulate hypotheses explaining the phenomena of acetate secretion in *Escherichia coli* (9) and ethanol production in *Saccharomyces cerevisiae* (15). Flux balance models provide a quantitative analysis of metabolic pathway utilization under different environmental conditions. For example, switches in pathway utilization that lead to the secretion of by-products are interpreted in terms of optimal coupling between energy and redox metabolism and the achievement of optimal growth rates (18). From a bioprocessing standpoint flux balance models have been used to predict optimal process conditions and feed strategies (21). However, to date no direct experimental verification of the predictions of flux balance models has been carried out.

Here we address the secretion of metabolic by-products by a nearly wild-type *E. coli* strain under various culture conditions and relate experimental observations to predictions made by a flux balance model. Much of the theoretical framework and insights relevant to an understanding of this work have been published previously (18). Here we report data concerning *E. coli* growth on glucose under aerobic chemostat, batch, and fed-batch conditions as well as anaerobic batch conditions. We show that the flux balance model with an objective of optimizing the growth rate is an appropriate representation of metabolism under these conditions, and furthermore we show that when the initial conditions of the culture are known the flux balance model is able to predict the time profiles of growth and by-product concentration in the culture media for batch and fed-batch cultures.

MATERIALS AND METHODS

Culture. An *E. coli* K-12 strain, W3110 (ATCC 27325), was used for all the experiments. The strain has been described as

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being nearly wild type, and it is able to grow on glucose mineral medium. Defined M9 medium (Na_2HPO_4 [6 g/liter], KH_2PO_4 [3 g/liter], NaCl [0.5 g/liter], NH_4Cl [1 g/liter], $MgSO_4$ [2 mM], $CaCl_2$ [0.1 mM], $FeCl_3$ [0.01 mM]) was used for all the experiments with 2 g of glucose per liter except for the fed-batch experiments, in which glucose was continuously added as described below. A temperature of 38°C was maintained for the culture in the bioreactor as well as in the incubator.

Bioreactor setup. Batch, fed-batch, and chemostat experiments were performed in a water jacketed bioreactor (Pegasus). The temperature was controlled at 38°C by using a water bath (Haake, Berlin, Germany). Medium inflow in chemostat experiments was achieved by using a peristaltic pump, and it was measured volumetrically at the outlet. Glucose solution was injected with a syringe pump in fed-batch experiments. Cell density was measured either with a Coulter Counter (Model ZM; Coulter Electronics Inc., Hialeah, Fla.) or by optical density at 600 nm with a UV 160 spectrophotometer (Shimadzu, Kyoto, Japan) and calibrated with the dry weight. A dry weight calibration of 0.32 g (dry weight)/optical density at 600 nm was obtained.

Gas sparging was achieved by using three type-D sintered glass spargers (bore size, 10 to 20 μm; Ace Glass Inc., Louisville, Ky.). A small bubble size that helped keep dissolved oxygen above 50% saturation in aerobic experiments was obtained. The gas flow rate was measured with Gilmont rotameters (Gilmont Instruments, Inc., Great Neck, N.Y.) in the inlet and outlet. The oxygen percentages in the inlet and outlet gases were measured by using fuel cell-based oxygen analyzers (Systech model 2000 [Illinois Instruments Company Inc., McHenry, Ill.] and Micro-Oxymax [Columbus Instruments International Corporation, Columbus, Ohio]). Both instruments produced similar results.

Analytical assays. Glucose and lactate concentrations were measured by using a YSI glucose lactate analyzer model 2000 (Yellow Springs Instruments Inc., Yellow Springs, Ohio). Lactate secretion was not observed in any of the experiments reported. Acetate was measured by enzymatic conversion to acetyl phosphate and reaction with hydroxylamine (7, 8, 11). Ethanol, formate, and succinate were measured by using available enzymatic kits (Boehringer Mannheim Corporation, Indianapolis, Ind.).

Flux balance model. A metabolic steady state is assumed, in which the metabolic pathway flux leading to the formation of a metabolite and that leading to the degradation of a metabolite must balance, which generates the flux balance equation (3, 13):

$$S \cdot v = b \tag{1}$$

where S is a matrix comprising the stoichiometry of the catabolic reactions, v is a vector of metabolic fluxes, and b is a vector containing the net metabolic uptake by the cell. Equation 1 is typically underdetermined, since the number of fluxes (or metabolic pathways) normally exceeds the number of metabolites. Thus, a plurality of solutions exists and a particular solution may be found by using linear optimization by stating an objective and seeking its maximal value within the stoichiometrically defined domain. In other words, specifying an objective such as optimization of the growth rate would determine the specific metabolic pathway utilization that would fulfill the objective.

Objective. A maximal growth objective was used in the present study (19, 20); it is shown in equations 2 and 3:

$$\text{Minimize } Z = -V_{gro} \tag{2}$$

$$\sum_{\text{all } M} d_M \cdot M \xrightarrow{V_{gro}} \text{biomass} \tag{3}$$

where d_M represents the requirements in millimoles per gram of biomass of the M biosynthetic precursors and cofactors for biomass production. These metabolic requirements for growth are based on biomass composition (5, 20). As noted below, we also incorporated a scaling factor to allow for strain-specific variations in biomass and maintenance. V_{gro} is the growth flux (grams of biomass produced), which with the basis of 1 g (dry weight) per h reduces to the growth rate (grams of biomass produced per gram [dry weight] per hour). Minimizing the objective function, Z, thus maximizes the growth rate, V_{gro}.

In order to fulfill the objective of optimizing the growth rate, we allowed the model to secrete unlimited amounts of the by-products acetate, ethanol, formate, lactate, and succinate in all simulations. Thus, the amount of by-product secreted is determined by the objective of optimizing the growth rate. We then compared predictions of growth rates and by-product levels simulated by the model with experimental data.

Algorithm for prediction of transient changes in external concentrations. For the prediction of the time profiles of consumed and secreted metabolites in batch and fed-batch experiments, we divided the experimental time into small time steps, Δt. The initial concentration values were specified. Starting with the first time step, the flux balance model was used to predict concentrations for the next step by using the following iterative algorithm.

(i) Substrate concentration (S_c) (millimoles per liter) is determined from the substrate concentration predicted for the previous step (S_{co}) plus any additional substrate provided in a fed-batch mode:

$$S_c = S_{co} + \frac{\text{supply} \cdot \Delta t}{\text{volume}} \tag{4}$$

(ii) The substrate concentration is appropriately scaled to define the amount of substrate available per unit of biomass per unit of time (millimoles per gram [dry weight] per hour):

$$\text{Substrate available} = \frac{S_c}{X \cdot \Delta t} \tag{5}$$

where X is the cell density.

(iii) The flux balance model is used to evaluate the actual substrate uptake (S_u) (this may be less than the amount of substrate available), the growth rate (μ), and potential by-product secretion.

(iv) Concentrations for the next time step are calculated from the standard differential equations:

$$\frac{dX}{dt} = \mu X \rightarrow X = X_o \cdot e^{\mu \Delta t} \tag{6}$$

$$\frac{\partial S_c}{\partial t} = -S_u \cdot X \rightarrow S_c = S_{co} + \frac{S_u}{\mu} X_o (1 - e^{\mu \Delta t}) \tag{7}$$

For the algorithm shown above, we considered both glucose and by-products as substrates that can be used by cells. Thus, as a result of implementing the above algorithm, we predicted the concentration time profiles of cells, glucose, and by-products.

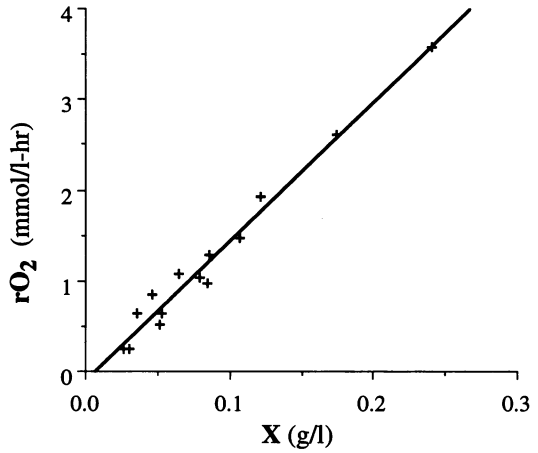

FIG. 1. Maximum enzymatic capacity for oxygen utilization determined from batch experiments as the slope of the best fit, 15 mmol of O_2 per g (dry weight) per h. The data are a composite from three batch experiments in which oxygen consumption was measured in the gas phase. The correlation coefficient (r^2) was 0.975. rO_2, millimoles of oxygen consumed by 1 liter of culture volume in 1 h.

RESULTS

In order to apply the flux balance model we first needed to determine the strain-specific parameters. The fully specified model was then used to compare predictions with experimental data.

Strain characterization: experimental determination of model parameters. It is necessary to establish a finite upper limit on the rate of metabolism per unit (dry weight) of the bacterium. We thus determined the maximum enzymatic limits of oxygen and glucose uptake rates from batch experiments for *E. coli* W3110. We also derived the biomass scaling and maintenance requirements, which are strain- and temperature-specific.

Oxygen uptake rate. The enzymatic capacity of the cell to consume oxygen was determined by performing batch experiments. During the exponential growth phase of a batch culture, when the glucose substrate is present in excess, the growth rate is at a maximum. In such a situation bacterial cells consume the maximum amount of oxygen possible (1).

The flux balance model also predicts that the maximal oxygen consumption rate occurs during exponential batch culture. On the basis of a linear regression of the experimental data shown in Fig. 1 the maximum oxygen utilization rate was determined to be 15 mmol of O_2 per g (dry weight) per h.

Glucose uptake rate. The flux balance model requires that a maximum glucose utilization rate be specified for metabolic simulations of batch and fed-batch cultures in order to restrict the metabolic capacity of a single cell to a finite upper limit. The enzymatic capacity for glucose utilization is determined as the ratio of the growth rate to the biomass yield in batch experiments. For aerobic batch cultures we have determined this value to be 10.5 mmol of Glc per g (dry weight) per h (Fig. 2). Similarly, for anaerobic cultures the maximum glucose utilization rate was determined to be 18.5 mmol per g (dry weight) per h (Fig. 3).

Maintenance and biomass requirements. The metabolic requirements for biomass synthesis have been defined on the basis of previously reported composition analyses (5, 20). In order to fully describe biomass generation in the model we needed to include the metabolic maintenance requirements of the bacterium as well as account for strain-specific differences in biomass composition.

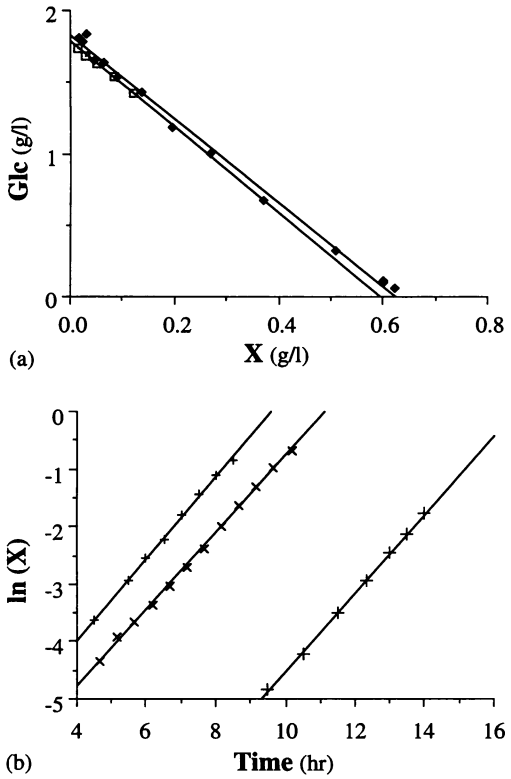

FIG. 2. Maximum aerobic glucose utilization rate (10.5 mmol of Glc per g [dry weight] per h) determined from batch experiments as the growth rate (0.68 h^{-1}; r^2 = 0.999) divided by the biomass yield (0.064 g [dry weight] per mmol of Glc; r^2 = 0.995). The plots are composites from batch experiments.

Maintenance requirements were determined by the standard approach (14) of plotting glucose uptake as a function of the growth rate in a chemostat. As shown in Fig. 4, a fit of the model to the experimental plot gives us the non-growth-associated maintenance (18) as the y-intercept, which is equal to 7.6 mmol of ATP per g (dry weight) per h.

Growth-associated maintenance (18) was determined by the closest fit of the discontinuity in slope predicted by the model to the experimental data of Fig. 4. The flux balance model predicts that such a discontinuity in slope must exist at a point where oxygen becomes limiting and acetate is secreted. Growth-associated maintenance accounts for energy depleting activities such as protein turnover that increase with increasing growth rates. For strain and experimental conditions described here, we have determined the growth-associated maintenance requirement to be 13 mmol of ATP per g of biomass. This maintenance requirement adds to the ATP energy requirements for biomass synthesis.

Biomass requirements for growth have been estimated for *E. coli* (5) on the basis of composition analyses. To apply the flux balance model, we found it necessary to modify these biomass requirements, which may reflect strain-specific differences. Thus, a scaling factor of 1.3 (i.e., 30% higher biomass requirements) was used to obtain the best fit of the predicted slope to experimental data (Fig. 4). Therefore, the flux balance model for our *E. coli* strain requires a definition of biomass requirements that is 30% higher than that reported previously (5). It

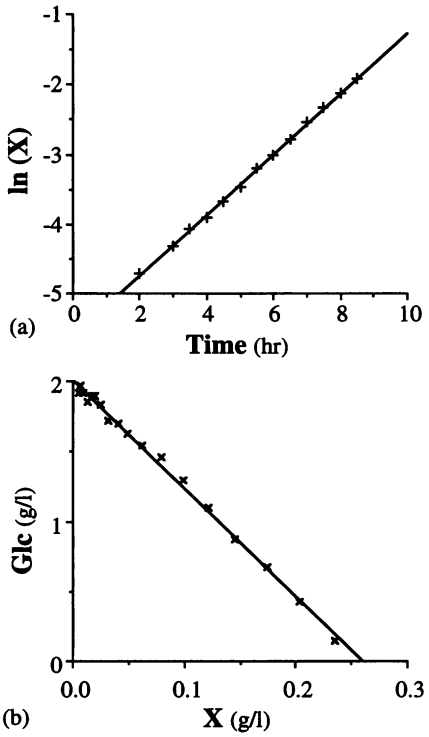

FIG. 3. Maximum anaerobic glucose utilization rate (18.5 mmol of Glc per g [dry weight] per h) determined from batch experiments as the growth rate (0.43 h^{-1}; r^2 = 0.999) divided by the biomass yield (0.023 g [dry weight] per mmol of Glc; r^2 = 0.996).

should be pointed out that the biomass composition in terms of specific biosynthetic precursors for our strain may be different from the composition determined previously for a particular strain (5). However, it has been shown that the flux balance model is not highly sensitive to demands for any particular

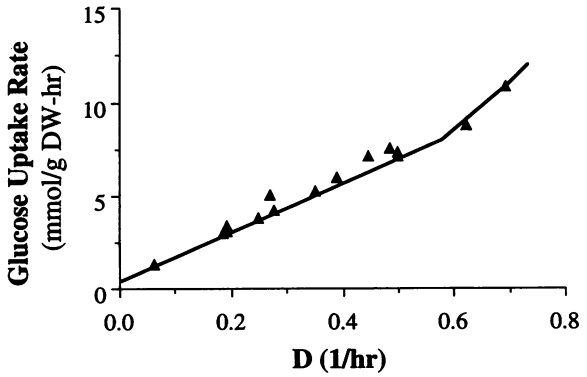

FIG. 4. Determination of biomass and maintenance requirements on the basis of chemostat experiments. There were no mineral limitations in the chemostat. The solid line denotes the best fit of data from the flux balance model to the experimental datum points shown. The non-growth-associated maintenance requirement determined from the y-intercept is 7.6 mmol of ATP per g (dry weight [DW]) per h, while the growth-associated maintenance requirement is defined as 13 mmol of ATP per g (dry weight). In addition, biomass requirements are scaled to be 30% higher than the defined values reported previously (5) to account for strain-specific differences. D, dilution rate in the chemostat.

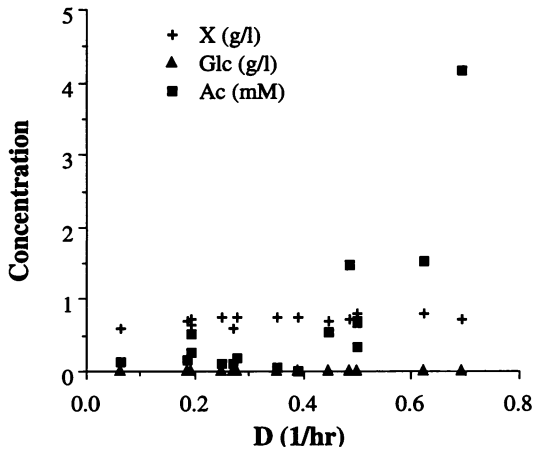

FIG. 5. Analysis of aerobic chemostat culture showing cell density, glucose concentration, and acetate concentration as functions of the dilution or growth rate. The chemostat was not limited for minerals. D, dilution rate in the chemostat.

biosynthetic precursor (20). Thus, the nonspecific scaling factor of 1.3 is expected to take into account any discrepancy in the biomass composition.

From the model specifications described above we have arrived at a total ATP requirement for biomass production that is equal to 70 mmol of ATP per g of biomass ([41 mmol for biomass synthesis + 13 mmol for maintenance] × 1.3 [scaling factor] = 70 mmol total), a value that compares favorably with the value (71 mmol of ATP per g of biomass) obtained for the closely related organism *Aerobacter aerogenes* (16).

Stoichiometrically optimal by-product secretion. The flux balance model is formulated by using stoichiometric information concerning the metabolic pathways and the metabolic requirements for growth. The model utilizes the principle of optimal growth rate in order to determine the metabolic pathway utilization. We now report a series of experiments whose data can be directly compared with predictions made by the model.

E. coli cells were grown in an aerobic chemostat at several different dilution rates. Cell density and glucose and by-product concentrations in the outlet from the culture vessel were measured and are plotted in Fig. 5. At low dilution rates we observed that all the glucose was consumed without the secretion of any by-product. Increasing the dilution rate above a critical value resulted in the secretion of acetate as a by-product. Lactate secretion was not observed in any of the experiments reported here.

The glucose uptake, oxygen uptake, and acetate secretion rates at different dilution rates were computed on the basis of data presented in Fig. 5. Simulation using the flux balance model was carried out by specifying a glucose uptake rate and determining the optimal rates of growth, oxygen uptake, and by-product secretion. The measured and predicted rates were directly compared (Fig. 6). The flux balance model was found to quantitatively simulate both the onset of acetate secretion and the increase in the acetate secretion rate with increasing dilution rates. Switches in metabolic flux distribution lead to the secretion of metabolites, and a detailed explanation for these switches can be obtained by flux balance analysis (18). At high growth rates the cells are enzymatically limited for oxygen, which acts as a redox sink. This limitation causes a change in the usefulness of metabolic intermediates to the cell.

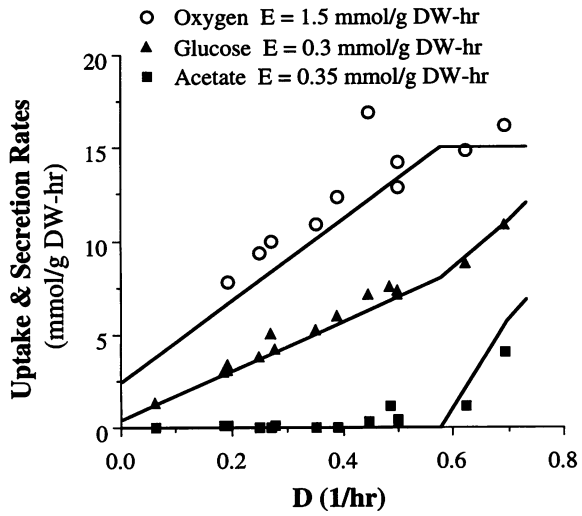

FIG. 6. Analysis of aerobic chemostat culture showing the glucose and oxygen uptake rates and the acetate secretion rate as functions of the dilution or growth rate. The chemostat was not limited for minerals. The solid lines represent the flux balance model simulations. E, average deviation between predictions of the model and experimental measurements; DW, dry weight.

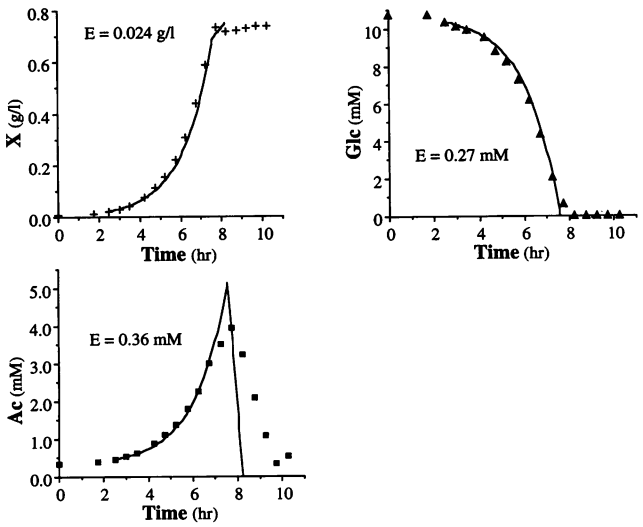

FIG. 7. Analysis of aerobic batch culture showing time profiles of cell density, glucose concentration, and acetate (Ac) concentration. The culture was not limited for minerals. The solid lines are the flux balance model predictions of the time profiles for the culture. E, average deviation between predictions of the model and experimental measurements.

Acetate is no longer useful to the cell, and it is secreted as a redox sink.

Batch and fed-batch cultures. We now consider the predictive ability of the flux balance model to describe batch and fed-batch cultures. In order to simulate the time course of concentration changes in the culture medium we discretized time into small segments, in each of which, we assumed, the metabolic steady state was maintained.

Aerobic batch culture. Bacterial cells were cultured in defined M9 medium with glucose as the carbon source under aerobic conditions. Figure 7 shows experimental data from a typical aerobic batch culture. Also shown are predictive simulations from the flux balance model. The predictions were carried out as described in Materials and Methods by specifying the initial conditions for the model.

The flux balance model was able to accurately predict time profiles for the cell density as well as the glucose uptake rate (Fig. 7). The model was also able to predict acetate secretion and accumulation in the medium. The model accurately predicted the reutilization of acetate, but it was unable to quantitatively account for the time required to make the transition from glucose consumption to acetate consumption. Transitions between different pathway utilizations are a function of genetic regulatory processes. The flux balance model incorporates only metabolic pathway information and thus cannot predict the time course of genetic regulation.

Enzymatic limitations on acetate uptake rates may also have significance in limiting the reconsumption of acetate. We have not determined the enzymatic limits on acetate uptake. The current specification of the flux balance model allows unlimited acetate consumption. However, there is a broader enzymatic limit of oxygen uptake that indirectly restricts acetate consumption. Thus, an oxygen uptake limit of 15 mmol of O_2 per g (dry weight) per h corresponds to an acetate utilization limit of 11.3 mmol of acetate per g (dry weight) per h.

Aerobic fed-batch culture. We next investigated the applicability of the flux balance model to fed-batch cultures. Fed-batch cultures were initiated by inoculating bacteria into defined mineral medium without a carbon source. Glucose as the carbon source was continuously fed to the culture by using a syringe pump. As with the batch experiments, the lag phase of growth was ignored for the simulations. The initial concentrations and the glucose feed rate were inserted into the predictive algorithm in order to obtain time profiles of cell density and glucose and by-product concentrations.

Let us first consider the case of a culture inoculated with bacteria at a relatively high cell density and fed with glucose at a relatively low feed rate. Figure 8 shows the experimental and predicted time profiles of such a culture. A high degree of correlation between the experimental and predicted time

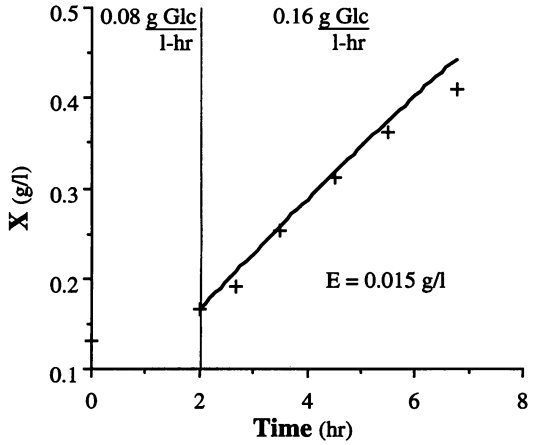

FIG. 8. Aerobic fed-batch culture with continuous glucose injection at the rates indicated. The culture was not limited for minerals. The time profile of cell density is shown, with predictions of the model represented as the solid line. Glucose and acetate concentrations were zero for both experiments and predictions of the model. E, average deviation between predictions of the model and experimental measurements.

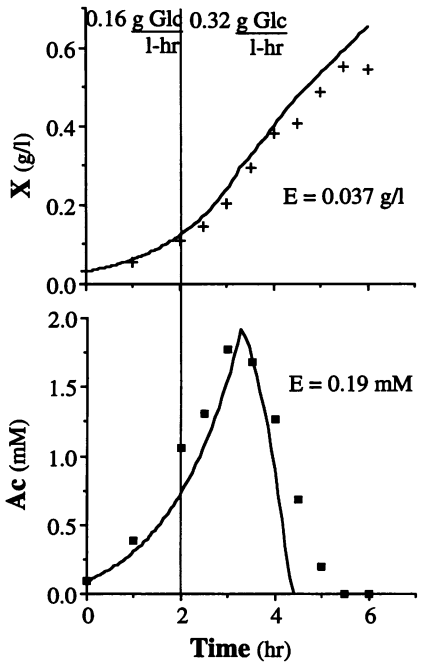

FIG. 9. Aerobic fed-batch culture with continuous glucose injection at the rates indicated. The culture was not limited for minerals. The time profiles of cell density and acetate (Ac) concentration are shown, with predictions of the model represented as solid lines. Glucose concentrations were zero both in experiments and in predictions of the model. E, average deviation between predictions of the model and experimental measurements.

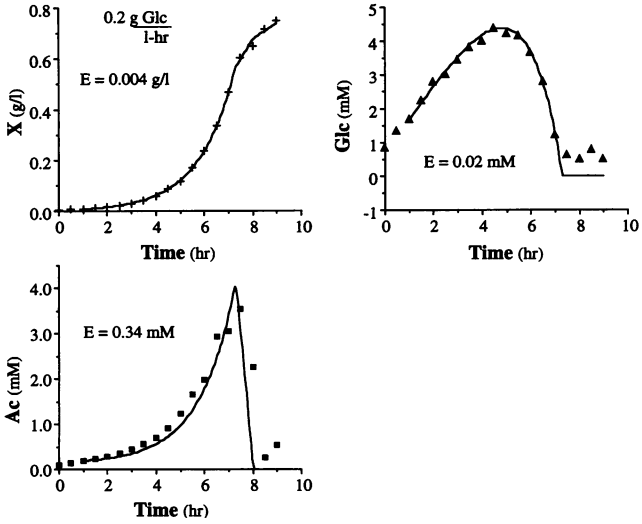

FIG. 10. Aerobic fed-batch culture with continuous glucose injection at the rates indicated. The culture was not limited for minerals. The time profiles of cell density, glucose concentration, and acetate (Ac) concentration are shown, with predictions of the model represented as solid lines. E, average deviation between predictions of the model and experimental measurements.

profiles of cell concentration was obtained. Neither experiments nor model predictions showed any accumulation of glucose and acetate.

Next, consider a fed-batch culture with a somewhat higher glucose feed rate relative to the cell density. Again the model was able to predict the time profile of cell density (Fig. 9) as well as that of acetate concentration in the medium. Glucose was not found to accumulate in the culture medium, which was also predicted by the flux balance model.

Acetate was observed to accumulate in the early part of the culture, and it was reconsumed later in the culture. In the early part of the culture the cell density was low compared with the glucose supply rate. Thus, the growth rate was high and acetate was secreted in a manner similar to that observed for the chemostat cultures (Fig. 6). In the later part of the culture the cell density was high enough to consume all glucose supplied as well as to consume the acetate accumulated in the medium. Thus, we observed a depletion of acetate from the medium. It is interesting to note that in the later part of the culture glucose and acetate were metabolized simultaneously.

Finally, let us consider a fed-batch culture for which the inoculum density was quite low. Figure 10 shows experimental and predicted time profiles of cell density, glucose concentration, and acetate concentration in the culture medium. Once again the flux balance model was found to accurately predict the experimental observations.

At low cell densities an accumulation of glucose in the culture medium was observed (Fig. 10). Glucose accumulation is explained as a result of feeding the culture with glucose in excess of the enzymatic uptake limit of the cells. Later in the culture as the cell density increased the cells were able to consume all the glucose supplied as well as to deplete the glucose accumulated in the culture medium.

Again the accumulation of acetate in the culture medium was observed (Fig. 10). As cell density increased and glucose was depleted from the culture, the cells were able to metabolize the acetate that was accumulated in the culture medium along with the continuously fed glucose.

Anaerobic batch culture. Since *E. coli* is a facultative organism, we also considered the ability of the flux balance model to predict time profiles for anaerobic cultures. Experiments were conducted in a manner similar to that for the aerobic batch experiments, with the exception that a nitrogen atmosphere was used.

Figure 11 shows the typical time profile of cell density and by-product concentrations. Once again the solid lines represent the predictions of the model for the culture. Three major by-products, acetate, ethanol, and formate, were found to be secreted and to accumulate in the medium. We observed a high degree of correlation between the predictions of the flux balance model and the experimental data obtained for the anaerobic batch culture.

The by-product succinate was also experimentally observed to be secreted at a low rate. The accumulation of succinate in the culture medium was quite small (less than 1 mM at the end of culture). The flux balance model however does not predict any succinate secretion. This appears to be a small deviation from optimal metabolism under anaerobic conditions.

DISCUSSION

Flux balance models have been developed to describe steady-state metabolic pathway utilization. These models are based on three separate bodies of knowledge: metabolic stoichiometry, metabolic requirements for growth, and optimality principles. The first two are fairly well established, while the third represents an assumed objective that the cell strives to attain. In general, such objectives may be hard to state. However, under the experimental conditions used here,

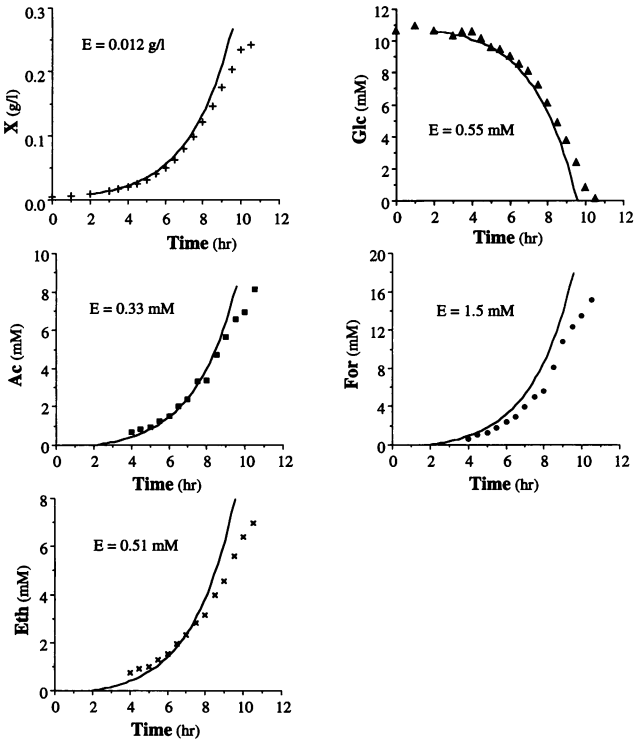

FIG. 11. Anaerobic batch culture showing the time profiles of cell density and various by-product concentrations. Solid lines represent the model predictions of the model. E, average deviation between predictions of the model and experimental measurements; Ac, acetate; For, formate; Eth, ethanol.

namely, nutritionally rich environments, the assumption that growth rates are optimized is consistent with evolutionary principles.

Although stoichiometric models have provided insight into metabolic physiology and optimal bioprocess conditions (18, 21), the predictions they yield have not been directly experimentally verified. In this report we provide quantitative comparisons of experimental and theoretical results. We first measured strain-specific parameters in independent experiments. Then, we used the fully specified model to predict the behavior of *E. coli* W3110 in a chemostat and in batch and fed-batch cultures. A detailed sensitivity analysis of the flux balance model formulated here is reported elsewhere (22).

The flux balance model was specified for the wild-type strain W3110 by determining the enzymatic capacity limits of oxygen and glucose utilization. In a broader sense specification of such limits prevents a single cell from displaying an infinite metabolic capacity—a physical impossibility. In addition, maintenance requirements and strain-specific biomass scaling were determined on the basis of chemostat experiments.

With a glucose substrate the bacterial culture was found to secrete and reutilize by-products in specific situations. Cells in the aerobic chemostat were shown to secrete acetate when grown at a rate above a specific growth rate in a manner similar to that reported previously (2). We have shown here that the flux balance model can explain the secretion of acetate by using the constrained-optimization logic reported previously (9).

We have developed a predictive algorithm for time profiles of metabolism in the unsteady state of batch and fed-batch culture. The algorithm divides the culture time into small steps and assumes the existence of a steady state in each small time step. The ability of the algorithm to predict time profiles for cell density, glucose concentration, and by-product concentrations in the culture medium provides a verification for the validity of the flux balance model and demonstrates its usefulness for bioprocess engineering.

Some deviations from the predictions of the model were observed during transitions in the utilization of metabolic pathways, such as the change from glucose metabolism to acetate metabolism. It was observed that the transition was less abrupt for fed-batch cultures, resulting in quantitatively better predictions by the model for fed-batch transitions.

It should be recognized that the flux balance model is a model of metabolism and does not incorporate the dynamics of regulation. As the culture environment changes, through such a process as the depletion of a specific carbon source, the flux balance model predicts a change in metabolic pathway utilization. However, in order to actually achieve the new metabolic pathway utilization several regulatory changes have to be made both at the genetic level and at the enzymatic level. The time required for these regulatory changes is not taken into consideration in the flux balance model. Thus, the flux balance model is able to accurately predict the occurrence of metabolic transitions, but it has trouble in circumstances in which regulatory changes require time. Thus, for example, the flux balance model is inappropriate for simulating the lag phase in cultures.

An interesting phenomenon observed in the aerobic fed-batch cultures is that of acetate reconsumption in the presence of a glucose feed. It is generally assumed that the presence of glucose represses the utilization of other substrates. In contrast, we observed in both experiments and the predictions of the model that a sufficiently high cell density can result in the simultaneous consumption of glucose and acetate. Cometabolization of glucose and acetate has been reported previously (23).

An important question concerning the observation of cometabolism is that of clone-specific metabolism. Culture in a glucose-limited chemostat, starting with a single clone of *E. coli* (4), has been shown to result in the generation of polymorphisms. Clones isolated from the chemostat were found to have different metabolism rates for glucose and acetate. The cometabolism of glucose and acetate observed in the fed-batch experiment (Fig. 9 and 10) could be the result of either single-cell cometabolism or clone-specific metabolism, with different clonal populations metabolizing glucose and acetate. However, since the experiments only last a few hours, one may reasonably assume that genetic mutants cannot be significant unless they are present in the inoculum. One may also formulate arguments for clone-specific metabolism as a result of regulation within the same genetic clone. These questions form an interesting area for future research.

Applicability of the flux balance model to the experimental conditions described in this report demonstrates the importance of stoichiometrically optimal metabolism. It would be of interest to further develop the model and investigate its experimental applicability under a wider range of conditions. Validation of the flux balance model has far-reaching ramifications for the interpretation of metabolic physiology, pathway utilization, strain design, and bioprocess development and control (9, 18, 21).

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