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Novel Control Strategies for Catabolite Repressible Fermentations

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1. Abstract

Because of the problem of catabolite repression, a significant class of industrially important fermentations, *e.g.* enzyme and antibiotic production, are controlled by the fed-batch policy in which the catabolite concentration is maintained at a low, constant, and acceptable level. An improved policy, the pulsed batch policy, is proposed which takes advantage of the significantly accelerated production rates observed when a microorganism adapts from one environment to another. Experimental and modeling results are presented for the β-galactosidase from *Escherichia coli* case.

2. Introduction

Extremely intricate control mechanisms exist in the metabolism of a microbial cell to allow it to efficiently utilize nutrients. One of the jobs of the fermentation engineer is to control the adjustable parameters of the fermentation such that the microorganism diverts a large part of its metabolism away from its own reproduction and metabolic needs and into excess production of commercially valuable biochemical products. The production of many of these products is controlled at the genetic DNA level by several regulatory mechanisms. One of the more important of these is called "catabolite repression". The exact mechanism of catabolite repression is not fully understood, even by microbial physiologists. The symptoms of catabolite repression are that when a microorganism is given an excess of nutrients, especially sugars, such that its own energy level is high, it will divert most of this energy into its own physical growth and reproduction and stop making excesses of most of the noncatabolic biochemicals, *e.g.* enzymes, antibiotics, and other secondary metabolites. In the fermentation industry, it is common to circumvent this problem by one of two control strategies: the two-stage control policy or the fed-batch control

policy.

According to the two-stage control policy, the biomass is grown first on a substrate mixture that may include a small amount of repressive catabolite. Next the product is produced in the second stage on second mixture that does not contain any repressive catabolite. Example applications of this policy include α-amylase, glucose isomerase, and penicillin acylase production (Crueger and Crueger, [1982]). The drawback of this approach from a productivity standpoint is that one must limit the growth rate of the biomass in the first stage since the presence of excess catabolites causes permanent repression that manifests itself in the production phase.

According to the fed-batch control policy, the catabolite is slowly and steadily metered into the fermentor such that the microorganisms are maintained at a low, constant,and acceptable energy level thus allowing the simultaneous growth and production of the desired biochemical. Example applications of this policy include β-galactosidase production (Lundell, [1982]) and penicillin production (Crueger and Crueger, [1982]). As with the two-stage strategy, one must limit productivity to prevent the onset of repression.

By choosing either strategy, one has assumed that a maximum energy content exists that effectively constrains the production rate. For example, consider the data of Gendron and Sheppard, [1974] as shown in Figure 1. Here β-galactosidase gene expression is compared against the glucose concentration during exponential growth phase, and one can see why one would choose to omit glucose from the medium formulation (as in the two-stage policy) or why one would steadily feed glucose at a low concentration (as in the fed-batch policy).

However biological systems have long been observed to have some behaviors that make one question this assumption of a fixed constraint. Namely, it is not unusual for biological systems to return to different and perhaps otherwise

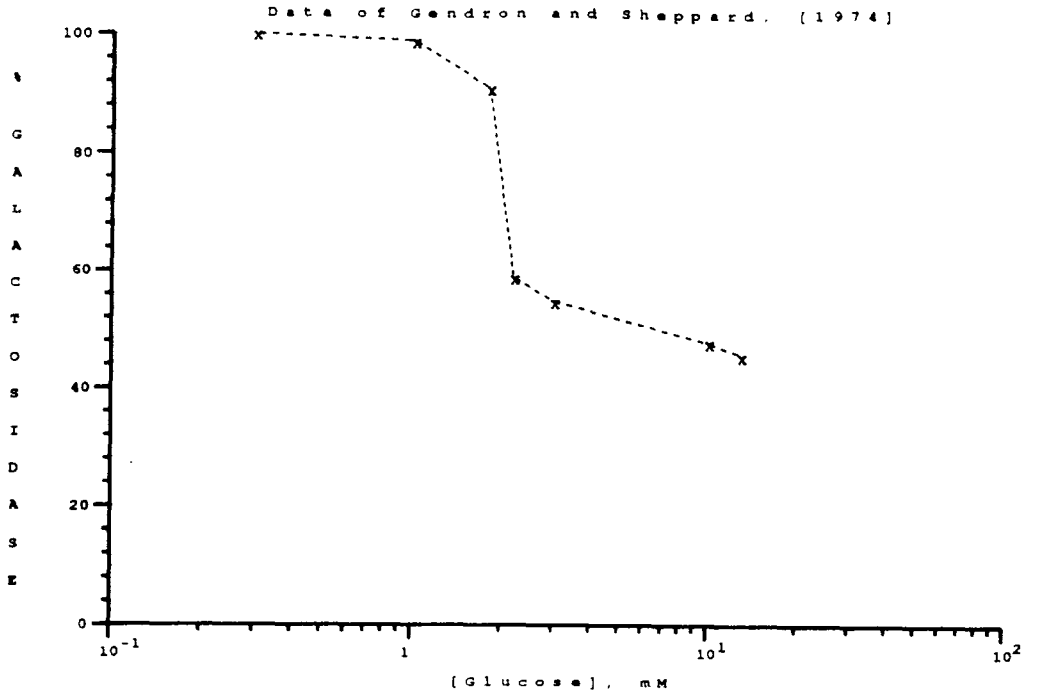

Fig.1. Steady Growth Repression of β-Galactosidase Expression by Glucose

unreachable steady production rates following some type of forcing. Further the dynamics of such forcings tend to be very nonlinear and may be advantageous. In view of these observations, the question was asked whether a trajectory of nonconstant specific feeding rates would be attractive.

3. Microbiological Background

While the problem of catabolite repression was recognized in 1900 (Dienert, [1900]), a mechanistic understanding of this cellular control system(s) remains to be uncovered. For the purpose of this paper, catabolite repression is defined according to the convention of Wanner *et al.*, [1978]. That is, catabolite repression is an active inhibition of catabolic gene expression that is independent of the individual operon regulatory controls, that is exerted on the promoter region of the individual operons, and that is brought about by the presence of

catabolites in the medium. Thus catabolite repression is a subset of the glucose effect (Epps and Gale, [1942]) which also includes the phenomenon of inducer exclusion.

Today it is generally accepted that in bacteria such as *E. coli*, a major regulon involves the interaction of cyclic 3',5'-adenosine monophosphate (cAMP), catabolite activator protein (CAP), and the promoters of the individual operons. Whether this regulon is a subset of a larger carbon and energy regulon remains a question (Magasanik and Neidhardt, [1987]). Within the cAMP-CAP regulon, cAMP is the metabolic signal; CAP is the regulator; and the promoters are the regulated processes. For example, Lis and Schleit, [1973] reported that the *lac* operon was 50% expressed at a external cAMP concentration of 0.38 mM while the *ara* operon was 50% expressed at a external cAMP concentration of 0.86 mM. During the 1970's, it was felt that cAMP was the sole metabolic signal that regulated catabolite repression; however, so many counterexamples of this hypothesis were discovered that this position has been reconsidered (Wanner *et al.*, [1978]).

The general mechanism by which the level of intracellular cAMP is controlled by extracellular catabolites is moderately understood. There is agreement that the level of cAMP is primarily controlled by its rate of synthesis, that there is a minor control by the rate of excretion, and that the rate of degradation of cAMP is negligible (Ullmann and Danchin, [1983]). However this work questions the "minor" control exerted by the rate of excretion in that over 99% of the total cAMP is typically extracellular (Matin and Matin, [1982]). Thus minor changes in the rate of excretion could have major effects on the intracellular concentration of cAMP. This position is discussed in more detail by Minderman, [1989].

The detailed control mechanism(s) over the rate of synthesis and the rate

of excretion are poorly understood. In that drastic differences exist between *in vivo* and *in vitro* measurements of adenylate cyclase activity (Peterkofsky and Gazdar, [1974] and Joseph *et al.*, [1982]), one must conclude that the modulation of adenylate cyclase activity via inhibitors and/or activators is more significant that the modulation of adenylate cyclase synthesis. Indeed there is some thought that the phosphorylated form of the phosphotransferase system enzyme III^{Glc} may be an activator of adenylate cyclase (Postma, [1987]). Further one can speculate that catabolite modulator factor (CMF) (Ullmann *et al.*, [1976]) may also be a repressor of adenylate cyclase activity since the role of CMF has not been definitively determined (Dessein *et al.*, [1978]). In addition, Peterkofsky and Gazdar, [1979] have shown that the breakdown of the proton motive force by active carbohydrate transport correlates with the rate of *in vivo* cAMP synthesis; thus they speculated that a "membrane maintenance factor" may interact with adenylate cyclase. The negative regulation of *cya* operon expression by cAMP-CAP has been shown to be insufficient to explain the observed modulation of *in vivo* adenylate cyclase activities (Roy *et al.*, [1988], Kawamukai *et al.*, [1985]; Aiba, [1985]; and Joseph *et al.*, [1982]). The search for a mechanistic understanding of the control of intracellular cAMP levels remains an active area of research.

4. Transient Reactor Operation– Theory

Researchers have experimented with the idea of a transient operation of a bioreactor for many years, but there have been few implementations where the transient operation was considered for nonbiomass production. Primarily transient applications have focused on changes in cell growth in a chemostat (for example: Goochee *et al.*, [1985]; Rice and Hempfling, [1985]; Pickett and Bazin, [1979]). For the most part, these transient operations have been useful

in the study of process dynamics.

In the case of *E. coli*, the premise that intracellular cAMP concentrations correlates with the carbon and energy regulon control suggests that there may be physiological reasons why a transient operation would be superior to a more traditional steady specific rate operation. Suppose one were to pose the reactor feeding optimization problem,

$$\underset{\{u_k\}}{max} \quad P \tag{1}$$

where P is the reactor productivity and $\{u_k\}$ is the set of possible feeding control policies for the time set $\{t_k \mid k = 0, 1, 2, \ldots n\}$. For a product which is regulated by catabolite repression, this optimization problem can be approximated by the following,

$$\underset{\{u_k\}}{max} \quad \overline{[\mathrm{cAMP}]}_i \tag{2}$$

where $\overline{[\mathrm{cAMP}]}_i$ represents the average intracellular cAMP concentration over time. Of course the validity of this approximation depends on the strength of the metabolic signal on the promoter operon of interest; in the case of the *lac* operon in the wild-type *E. coli*, this approximation is quite reasonable.

Due to the lack of a general and complete model of catabolite repression in *E. coli*, one cannot formally solve this feeding optimization problem. Nevertheless some microbiological observations suggest that a transient operation may be superior to a steady specific rate operation. Even in the original postulate of the role of cAMP in *E. coli*, Makman and Sutherland, [1965] reported a cAMP increase of a factor of five after the cells entered **stationary phase** after growth on glucose. This increase was short-lived, and it disappeared as the cells died. While there have been significant measurement problems in quantifying this observation, the basic observation has been supported by others through the years. Peterkofsky and Gazdar, [1974] reported a factor of two increase in the *in vivo* adenylate cyclase activity after the cells entered station-

ary phase following growth on 1.0 g/L glucose. Joseph *et al.*, [1982] reported a ten-fold increase in the total cAMP concentration after the cells entered stationary phase following growth on 0.36 g/L glucose. Unfortunately there have not been any well-accepted reports of how the corresponding intracellular cAMP concentration varies under these conditions. Nevertheless there appears to be unquestionable evidence that there is a significant boost in intracellular cAMP concentrations during the early stages of stationary phase.

Clearly this observation hints that the optimal feeding policy might involve a cycle between growth phase and stationary phase. A tradeoff exists between maximizing the intracellular cAMP concentration and maximizing the specific growth rate in order to maximize productivity. The next Section presents some preliminary experimental work which was aimed at determining whether this premise was real or not. Then Section 6 presents an improved structured model of catabolite repression in *E. coli*; as existing models of this system were found to be inadequate. Finally Section 7 presents a preliminary comparison between a fed-batch strategy and a pulsed-batch strategy. In both the experimental and simulated examples, a significant productivity increase results from the pulsed-batch operation.

5. Validation Experiments

To demonstrate that a pulsed-batch strategy was technically feasible and to begin to quantify the economic justification of such a strategy, some experiments were completed to validate the premise. The primary goal of these experiments were simply to show that the stationary phase production boost was real.

5.1. Materials and Methods

5.1.1. Organism

The wild-type strain of *Escherichia coli* (ATCC 11775) was selected for the production of β-galactosidase. The master culture was stored as freeze-dried pellets according to the procedure of Gherna, [1981]. The working culture was stored on nutrient agar (Diffco) plates at 4°C.

5.1.2. Medium and Growth Conditions

The basal salt medium of Dobrogosz, [1965] was used for most experiments with glucose and/or lactose added in specified quantities. The inoculum was prepared in 250 mL Bellco "Triple Baffled" shake flasks at 37°C and 250 rpm. The production experiments were carried out in either a 2.8 L Nalgene polycarbonate Fernbach flask or in a 14 L Virtis fermentor (Model 43-100). In either case, the production temperature was maintained at 37°C; and the pH was controlled at 7.00 ± 0.02 by a pH controller (model 45AR, Chemtrix). Dissolved oxygen was manually set well in excess of the critical level.

5.1.3. Analytical Procedures

The biomass concentration was determined from both dry weight measurements and turbidity measurements. The dry weight samples were removed from the fermentor and stored in a 4°C water bath for later analysis. These samples were then centrifuged and washed with deionized water at 4°C, and the pellets were flushed to aluminum drying pans. After drying overnight in an oven, the dry weight values were calculated. The turbidities were determined both off-line (at 590 nm) and on-line (at 540 nm), and these measurements were used to eliminate dry weight outliers and to reduce the dry weight standard errors. These procedures are detailed in Minderman, [1989]. The β-galactosidase activity was determined by a semi-automatic procedure developed by Minderman,

[1989] based on the colorimetric procedure of Lederberg, [1950] as modified by Novick and Weimer [1957]. The statistics of the semi-automated assay were found to be significantly better than those of the manual assay.

5.2. Experimental Results

5.2.1. Lactose Pulse

Because lactose is the natural substrate of the β-galactosidase system, a simple comparison between a pulsed strategy and a constant specific rate strategy was made using lactose as the sole substrate. Figure 2 illustrates the time series of enzyme and biomass concentrations for an experiment in which the batch was started on 1.0 g/L lactose and pulsed to 2.0 g/L lactose as soon as stationary phase was detected (at a time of 1.9 hrs). The feed concentration of lactose was 20%, so the volume change in the reactor was negligible. The jumps in enzyme concentration are easier to visualize in Figure 4 where the observed enzyme concentrations are normalized and compared with those of a constant 1.0 g/L lactose strategy. From this figure, one can see that a 26% increase in enzyme concentration resulted from using a simple pulse strategy.

5.2.2. Mixed Glucose and Lactose Pulse

Because glucose is one of the stronger catabolic repressors, one could speculate that the corresponding cAMP recovery might also be very strong. This situation would then allow one to make a trade-off between the repressive effects and the growth rate/possible economic advantages of glucose over the inducing substrate. Figure 3 illustrates a similar experiment to that of Figure 2 except that a 50/50 mixture of glucose and lactose was pulsed to a final concentration of 0.8 g/L at the time that stationary phase was detected (at a time of 1.9 hrs).

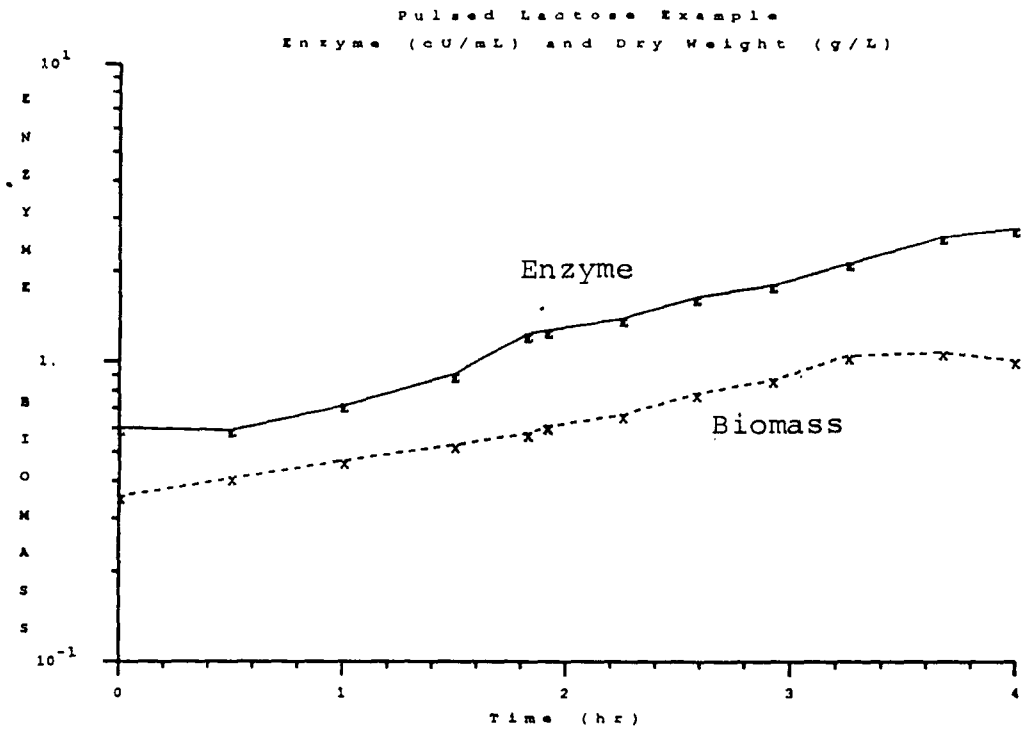

Fig.2. Pulsed-Batch Lactose Experimental Example

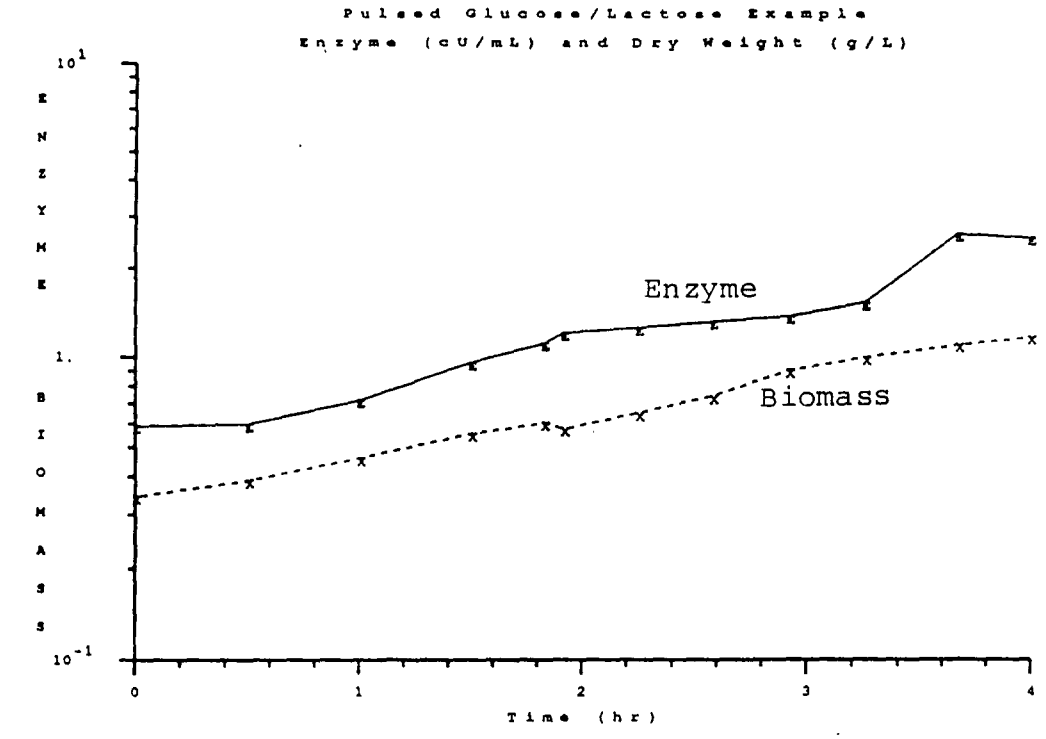

Fig.3. Pulsed-Batch Glucose/Lactose Experimental Example

Again the results are easier to visualize in Figure 4 in which the normalized enzyme concentrations are compared with the same base case as Figure 2. The important feature to note is that although the repressive effect of glucose is strong, the system does "catch up" to the base case before the cells begin to die. This result would allow one to use a mixture of glucose and inducing substrate in the event that the inducing substrate was significantly more expensive than glucose.

As nonminimal media are often used in industrial situations, this experiment was repeated except that the basal medium was supplemented with 3.0 g/L yeast extract (Diffco). The results indicated a 47% increase in final enzyme titre over the conventional approach (data not shown).

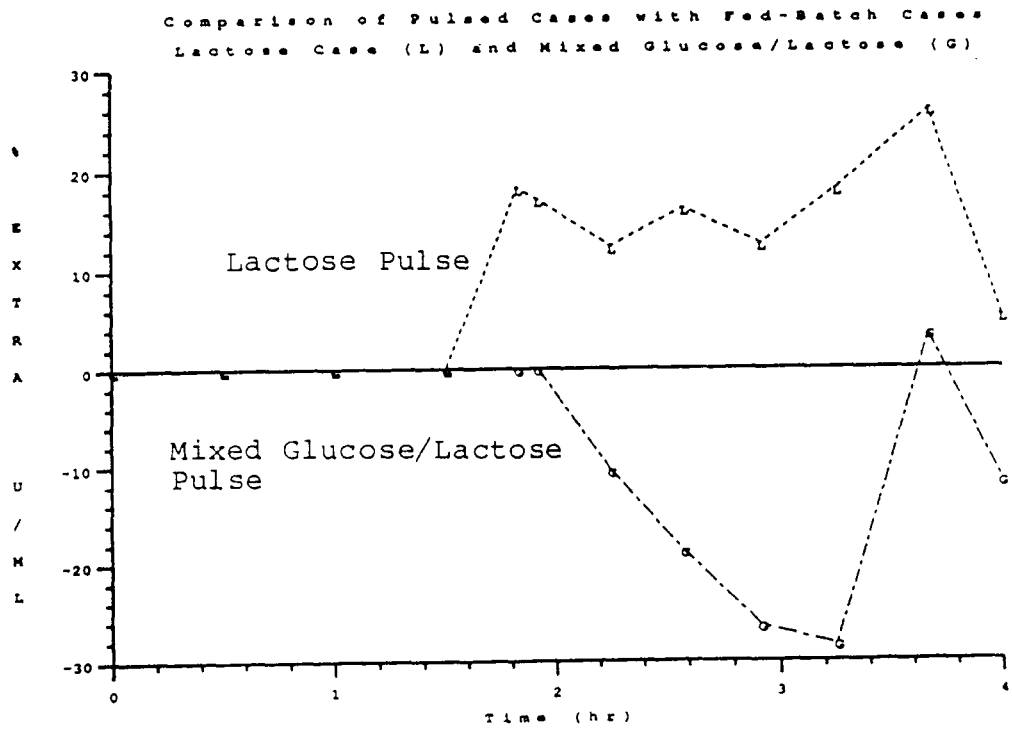

Fig.4. Comparison of Pulsed Examples with Fed-Batch Strategy (S.P. of 1.0 g/L Lactose)

5.2.3. Discussion

These results indicate that the predicted benefits from a pulsed-batch reactor feeding strategy are realizable. Other experiments have been completed which repeatedly indicated that improved productivity results from the pulsed-batch strategy. Other benefits such as lower raw material usage, lower reactor cycle time, and lower energy consumption have been observed (data not shown). It was felt that to effectively and systematically compare the two feeding strategies (fed-batch vs pulsed-batch), a model was needed to predict the additional validation experiments and the desired control trajectories. Thus the validation experiments did confirm that the pulsed-batch strategy is an attractive one; and they illustrated the need for a complete model of the system in order to plan appropriate experiments.

6. A Unified Model of Catabolite Repression in *E. coli*

The progress in biochemical engineering modeling of the phenomenon of catabolite repression in *E. coli* has been limited by the progress of the microbiologists in developing a mechanistic understanding of this phenomenon. Numerous measurement and observability problems have hindered this search for a mechanism; reviews by Ullmann and Danchin, [1983] and by Pastan and Adhya, [1976] address some of these problems. Nevertheless enough microbiological observations exist such that one can propose a plausible mechanism which is consistent with many of these observations on a macroscopic scale. Such a unified mechanism is presented below. Because of the limited space at this meeting, a complete description and discussion of the model is not presented here; the interested reader is referred to Minderman, [1989].

A brief review of previous models of catabolite repression systems is appropriate as some components of the model were based on these previous results. During the latter presentation of the new model, the differences between this

model and previous models will be highlighted. Perhaps the first significant contribution to repression modeling was by Yagil and Yagil, [1971] who presented a model of the negative induction process of the *lac* operon as described by the Jacob and Monod, [1961] theory. Imanaka and Aiba, [1977] continued this approach with the presentation of a model of the cAMP promotion process. An unsolved problem has been how to predict the intracellular cAMP concentration as a result of the bulk substrate environment. Previous workers such as van Dedem and Moo-Young, [1975]; Imanaka and Aiba, [1977]; Kaushik *et al.*, [1979]; and Vieth *et al.*, [1982] have all presented various correlations between intracellular cAMP levels and a specific substrate (usually glucose) level. Not until the work of Ray *et al.*, [1987] have attempts at a mechanistic model of cAMP synthesis been made. These latter workers presented structured equations for inducer exclusion, for III^{Glc} inhibition of intracellular cAMP synthesis, and a correlation between catabolite modulator factor (CMF) and specific growth rate which was postulated to act as a competitive inhibitor of cAMP for CAP (Dessein *et al.*, [1978]). This last model successfully represented the input-output relationship of β-galactosidase production on a mixed glucose/lactose substrate in a chemostat. While all of these previous models were shown to adequately represent the reported input-output characteristics of a given example system, the unfaithful prediction of reasonable intracellular cAMP levels suggested the need for a unified model of catabolite repression for *E. coli*.

A unified structured model for catabolite repression in *E. coli* has been developed based on the mechanism proposed in Figure 5. For simplicity, this model is illustrated for the case of growth on a single (but not arbitrary) substrate. However the model is not limited to this situation; and Minderman, [1989] discusses the application of this model to the mixed substrate case. As previously stated, only the key features of this model which differ from previous

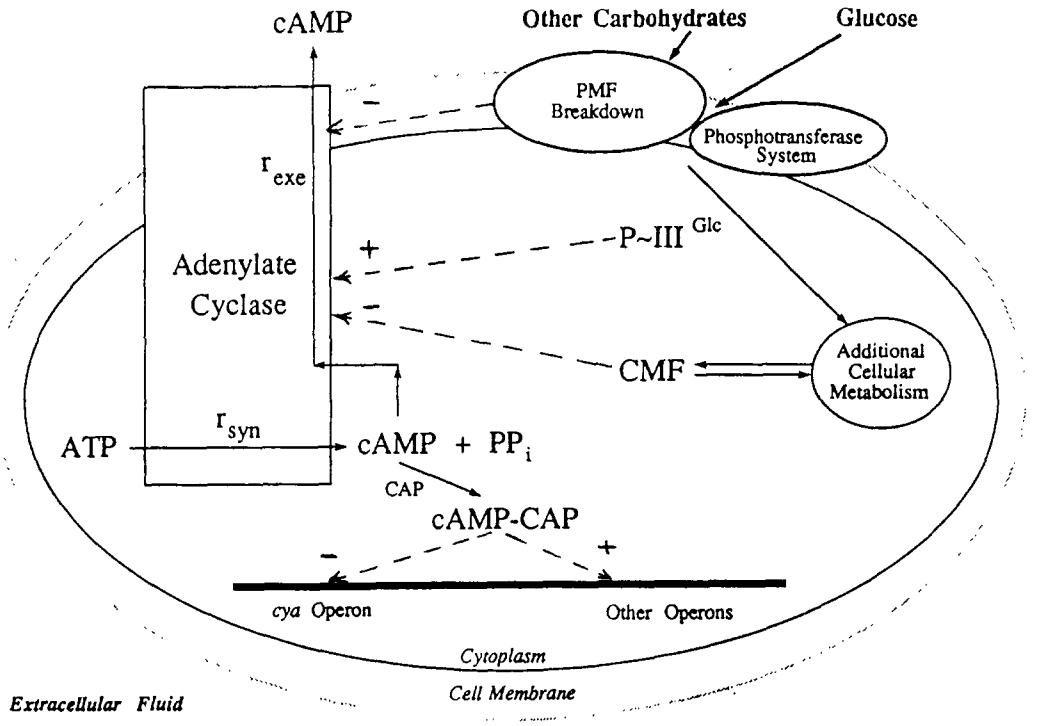

Fig.5. Proposed Mechanism for Intracellular cAMP Regulation

efforts are presented below.

1. Both the synthesis rate and the excretion rate of cAMP are modeled. This approach is essential since up to 99% of the total cAMP can be extracellular (Matin and Matin, [1982]); thus minor changes in the rate of excretion have a significant effect on the intracellular concentration of cAMP.
2. Although the true role of catabolite modulator factor (CMF) is not known, this work proposes that CMF is an inhibitor of adenylate cyclase synthetic and excretory activity. This postulate directly conflicts with the suggestion by Dessein *et al.*, [1978] that CMF is a competitive inhibitor of cAMP binding to CAP; however, the supporting arguments presented by these workers were not irrefutable. This newer postulate allows for the explanation of different intracellular cAMP concentrations at different growth rates during balanced growth (Epstein *et al.*, [1975]; Harmon and Botsford, [1979]; and Joseph *et al.*, [1982]).

3. The breakdown of the proton motive force by active carbohydrate transport is postulated to alter the rate of cAMP excretion. This position is consistent with the findings of Peterkofsky and Gazdar, [1979].

4. The phosphorylated form of III^{Glc} is presented as an activator of adenylate cyclase activity. Again this position directly conflicts with previous engineering work (Ray *et al.*, [1987]), but this approach more correctly reflects current microbiological theories (Postma, [1987]).

5. A method of modeling intracellular cAMP as a negative repressor of the *cya* operon is presented that is consistent with the recent microbiological observations of Roy *et al.*, [1988]; Kawamukai *et al.*, [1985]; Aiba, [1985]; and Joseph *et al.*, [1982].

6. A different method of handling maintenance is presented which postulates the existence of a limiting growth component (S^*) that competitively inhibits the uptake of the true substrate (S). This model more faithfully predicts the combination of balanced growth and transition growth into stationary phase while maintaining the substrate K_s near its true value.

The primary equations in this model are presented in Table 1. Using literature data, the parameter values of the model were identified and are given in Minderman, [1989]. Since the model is still being tested, it is inappropriate to discuss the parameter values and their significance at this time. However the preliminary evaluation of the model structure is very encouraging. In Figure 6, the model is compared with the experimental batch data of Joseph *et al.*, [1982] for a glycerol and a glucose batch. While the glycerol example may be considered as a calibration set, the glucose example illustrates of the **predictive** capabilities of this model. Note that the parameter values were not readjusted for the glucose case; rather the parameter values were identified from other independent experiments. Although the prediction of total cAMP is not as good as

one would like, the prediction of the reported intracellular cAMP concentration is quite good.

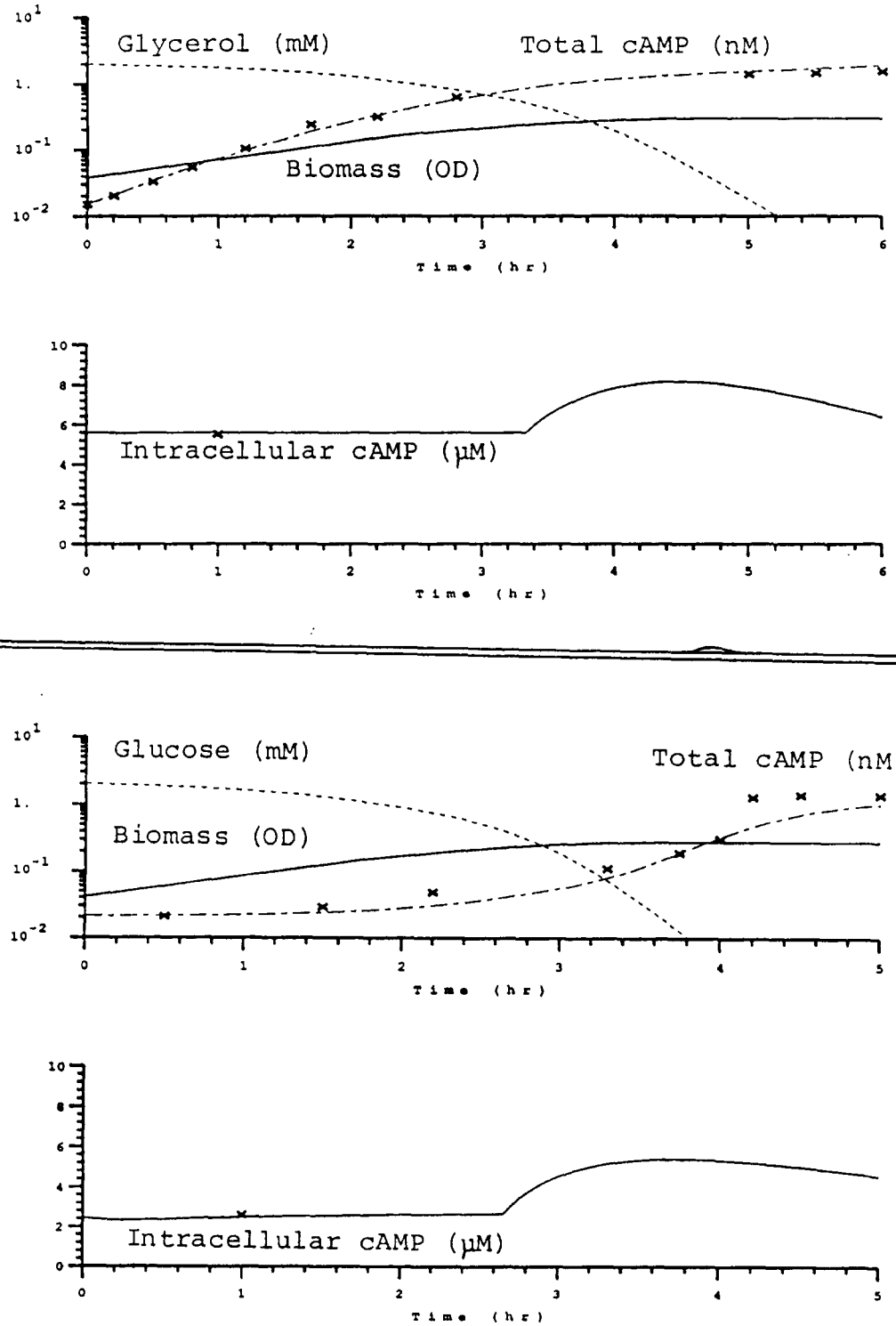

Fig.6. Comparison of Model Predictions and Data of Joseph et al., [1982]

In summary, the unified model of catabolite repression seems consistent with many microbiological observations and seems to give reasonable intracellular cAMP predictions. Certainly this unified model seems "good enough" given the lack of microbiological understanding and data for this system (Ullmann and Danchin, [1983]).

7. Simulated Comparison Between Fed-Batch and Pulsed-Batch

To illustrate the pulsed-batch strategy on a larger scale, a multiple pulse case is compared with a reasonable fed-batch case in Figure 7. The set point for both the fed-batch and the pulsed-batch cases was 0.5 g/L lactose, and the active constraint was 30 g of total lactose. From Figure 7, one can see that the pulsed-batch strategy results in 120% additional β-galactosidase by increasing the batch time by ninety minutes. However, the resultant productivity is still better (40%) than the fed-batch case.

8. Conclusions

For catabolite repressible fermentations, a pulsed-batch reactor feeding control strategy has been presented which has been shown to be superior to the traditional fed-batch approach for physiological reasons. This strategy has been demonstrated for the case of β-galactosidase production in *E. coli*; and the results, both simulated and experimental, have been very encouraging. Significantly increased final product concentrations were observed as were smaller but still significant increases in productivity. Depending on the economics of a particular fermentation, one could use this strategy to lower the raw materials cost while maintaining a fixed final product concentration. Clearly the optimization of this type of control strategy is very problem specific.

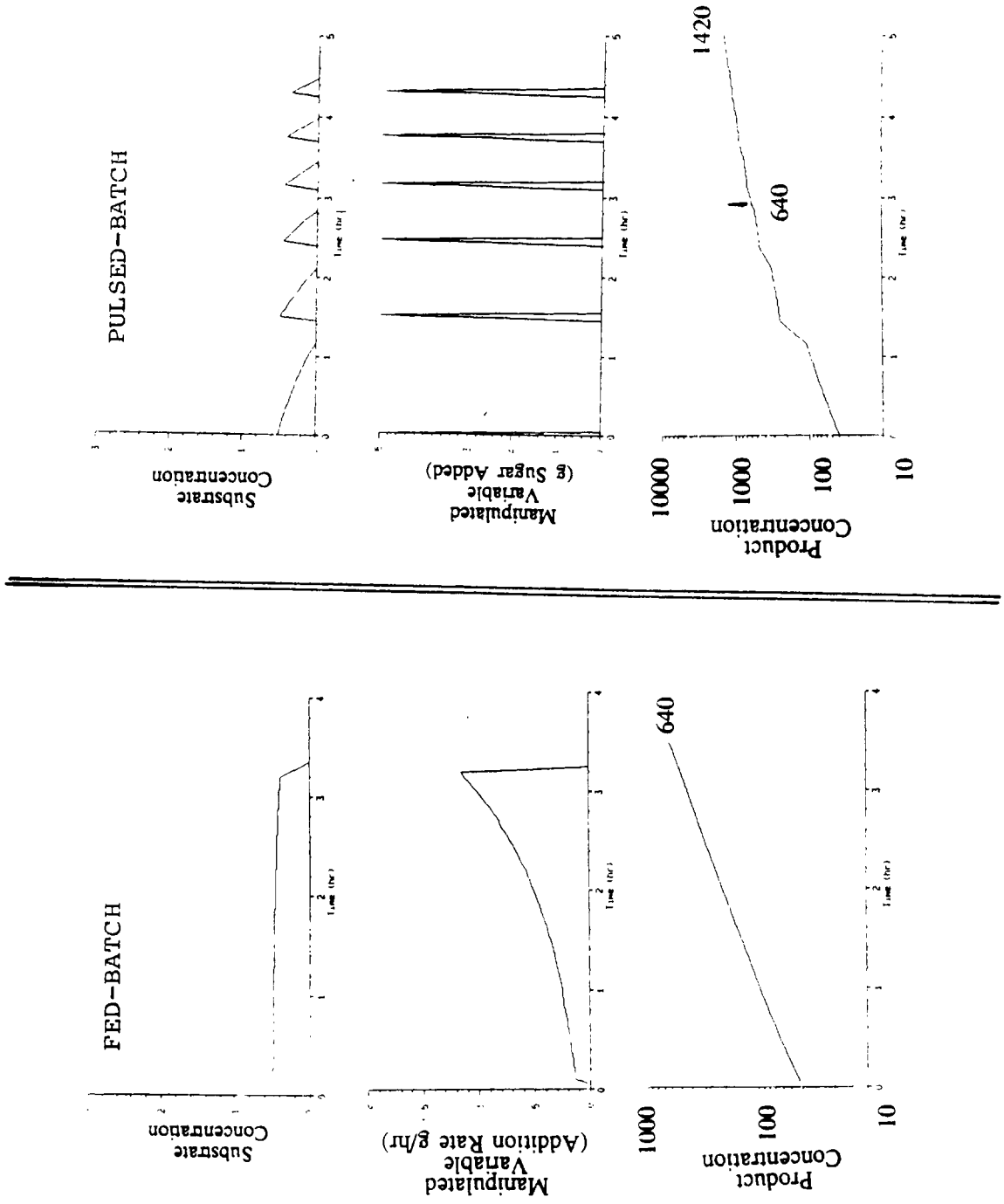

Fig.7. Simulated Comparison between Fed-Batch and Pulsed-Batch

Because catabolite repression is a global control system in a cell, it is tempting to speculate that these findings are more general than for this particular example. Certainly two process characteristics that may be necessary for the successful application of this strategy were present in this example.

1. The primary product was controlled by a repression of the metabolic signal by excess cellular energy.
2. The product was stable in time.

Additional necessary process characteristics remain to be uncovered.

The authors do not feel that this control strategy is limited to *E. coli* or to other bacteria in which cAMP correlates with the degree of catabolite repression. Catabolite repression is a general problem to many microorganisms, and it is not obvious why cAMP is a necessary condition for the pulsed-batch strategy to be attractive. Other microorganisms certainly use different metabolic signals, thus the generalization of the necessary condition for attractiveness of this strategy should be the relationship between the **history** of the repressive substrate concentration and the metabolic signal concentration. Considerable work remains in order to verify this hypothesis.

Table 1: Summary of Model for a Catabolite Repressible Fermentation

Bulk concentrations:

$$\dot{X} = -DX + \mu X$$

$$\dot{S}_b = D(S_f - S_b) - \frac{\mu}{Y_{x/b}}X$$

$(+r_{\beta G,G}X$ when S_b is glucose)

$$\dot{S}^* = D(S_f^* - S^*) - \frac{\nu}{Y_{x/*}}X$$

$$\dot{A}_b = -DA_b + r_{exe}X$$

Intracellular "energy" terms:

$$\dot{E} = \frac{\mu}{Y_{ATP}}\phi_1 - k_{-ATP}\phi_2 E$$

$$\dot{A} = (r_{syn} - r_{exe}) - k_{-cAMP}A$$

$$\dot{C} = \phi_3 - (k_{-CMF} + \mu)C$$

Membrane proteins:

$$\dot{Y} = k_Y f_{g,lacR} f_{g,lacP} - k_{-Y}Y$$

$$\dot{P} = \frac{\mu}{Y_{III}}\phi_4 - k_{-III}P$$

Other cytoplasmic concentrations:

$$\dot{L} = r_{L,t} - r_{\beta G,L}$$

$$\dot{I} = r_{\beta G,I}$$

$$\dot{Z} = k_Z f_{g,lacR} f_{g,lacP} - k_{-Z}Z$$

Specific reaction rates:

$$\mu = \frac{\mu_{max} S_b}{K_{S_b}(1. + (1-\nu)/K_{I,S^*}) + S_b}$$

$$\nu = \frac{S^*}{K_{S^*} + S^*}$$

$$r_{L,t} = \frac{v_{max,1} L_b}{K_{s,L} + L_b}Y + h_L(L_b - L)$$

$$r_{\beta G,L} = -\frac{(a+b)L}{L + \alpha I + \beta}Z$$

$$r_{\beta G,I} = \frac{(bL - \alpha cI}{L + \alpha I + \beta}Z$$

$$r_{\beta G,G} = \frac{aL + \alpha cI}{L + \alpha I + \beta}Z$$

$$r_{syn} = f_{c,CMF} f_{g,cAMP} f_{P3} P \frac{v_{max,s} E}{K_{s,E} + E}$$

$$r_{exe} = f_{P3} P \chi \frac{v_{max,e}}{1. + C/K_{I,CMF}} \frac{A}{K_{s,A}(1. + A_b/K_{I,A_b}) + A}$$

9. Acknowledgements

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10. Nomenclature

A	intracellular cAMP concentration (nM)
A_b	extracellular cAMP concentration (nM)
a	β-galactosidase catalytic constant for lactose consumption (hr^{-1})
b	β-galactosidase catalytic constant for allolactose formation (hr^{-1})
C	catabolite modulator factor concentration (-)
c	β-galactosidase catalytic constant for allolactose consumption (hr^{-1})
D	dilution rate (hr^{-1})
E	intracellular ATP concentration (mM)
f_a, i	fraction of activity altered by component i
f_g, i	fraction of operon expression altered by component i
h	mass transfer coefficient (hr^{-1})
I	intracellular inducer concentration (mM)
K	equilibrium constant
k	reaction kinetic coefficient
L	intracellular lactose concentration (mM)
L_b	extracellular lactose concentration (mM)
P	productivity (e.g., U/L/hr)
	total concentration of both forms of III^{Glc} enzyme (mg/g)
r	rate of chemical reaction
S_b	substrate concentration (mM)
S^*	rate limiting substrate concentration (-)
t	time (hr)
u_k	a control move made at time t_k
v	Cell volume (1.0 μL)
v_{max}	maximum reaction velocity in enzyme kinetic expression

X	biomass concentration (g/L)
Y	*lac* permease concentration (mg/g)
$Y_{i/j}$	yield coefficient of component i from component j
Z	β-galactosidase concentration (mg/g)

Greek Symbols

α	part of Michaelis constant of β-galactosidase for allolactose
β	Michaelis constant of β-galactosidase for lactose
μ	biomass specific growth rate
μ_{max}	maximum biomass specific growth rate
ν	specific uptake rate of rate limiting substrate
ϕ	arbitrary functions that force constant cellular levels during growth phase
χ	correlation between substrate and PMF breakdown

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