Biochemical Production Capabilities of *Escherichia coli*

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Microbial metabolism provides a mechanism for the conversion of substrates into useful biochemicals. Utilization of microbes in industrial processes requires a modification of their natural metabolism in order to increase the efficiency of the desired conversion. Redirection of metabolic fluxes forms the basis of the newly defined field of metabolic engineering. In this study we use a flux balance based approach to study the biosynthesis of the 20 amino acids and 4 nucleotides as biochemical products. These amino acids and nucleotides are primary products of biosynthesis as well as important industrial products and precursors for the production of other biochemicals. The biosynthetic reactions of the bacterium *Escherichia coli* have been formulated into a metabolic network, and growth has been defined as a balanced drain on the metabolite pools corresponding to the cellular composition. Theoretical limits on the conversion of glucose, glycerol, and acetate substrates to biomass as well as the biochemical products have been computed. The substrate that results in the maximal carbon conversion to a particular product is identified. Criteria have been developed to identify metabolic constraints in the optimal solutions. The constraints of stoichiometry, energy, and redox have been determined in the conversions of glucose, glycerol, and acetate substrates into the biochemicals. Flux distributions corresponding to the maximal production of the biochemicals are presented. The goals of metabolic engineering are the optimal redirection of fluxes from generating biomass toward producing the desired biochemical. Optimal biomass generation is shown to decrease in a piecewise linear manner with increasing product formation. In some cases, synergy is observed between biochemical production and growth, leading to an increased overall carbon conversion. Balanced growth and product formation are important in a bioprocess, particularly for nonsecreted products. © 1993 John Wiley & Sons, Inc.

Key words: *Escherichia coli • amino acids • nucleotides • biosynthesis · linear optimization • metabolic fluxes • metabolic engineering •stoichiometry*

INTRODUCTION

The commercial production of biochemicals often utilizes the metabolic reactions of a microbe in order to achieve the conversion of substrate into the desired product. The subversion of microbial metabolism to overproduce the product is usually based on random mutagenesis in a selective environment along with the addition of external genetic material. This approach can result in unexpected results, particularly when metabolic regulatory systems oppose the desired changes. A rational basis for modifying cellular metabolism is the subject of the recently defined field of metabolic engineering.[1,19]

Although much research effort has focused on elucidating metabolic regulation in microbes, sufficient information is generally not available for a single cell type to characterize its dynamic behavior, the exception being the human red blood cell.[8,9] On the other hand, knowledge about the stoichiometry of biochemical pathways is relatively unambiguous and can be used to define the wider scope of the metabolic behavior of prokaryotic cells.[20] A flux balance based approach has been outlined that uses the catabolic network of the bacterium *Escherichia coli* to define the metabolic capabilities of the bacterial cell.[20,21] The regulated state of metabolism forms a subset of the stoichiometrically allowable metabolic behavior that needs to be manipulated to achieve the desired overproduction of biochemicals.

In the present work we extend the catabolic network of the bacterium *E. coli* to include its biosynthetic reactions. Thus a comprehensive representation of *E. coli's* metabolism results. The flux balance based approach is used with the combined catabolic and anabolic network to determine the capabilities of *E. coli* to convert glucose, glycerol, and acetate as substrates into amino acids and nucleotides. The optimal metabolic pathway utilization for biochemical production represents the ultimate goal of flux redistribution in a commercial bioprocess. Criteria are established that determine the metabolic constraints limiting a particular optimal solution. Metabolic constraints during the production of amino acids and nucleotides from a glucose, glycerol, or acetate carbon source are determined. We also address the industrially relevant trade-off between growth and product formation.

METHODS

Flux Balance Based Analysis

The general methods of flux balance based analysis have been outlined in the literature.[2,10,17,20] The steady state flux balance equation is

$$\mathbf{S} \cdot \mathbf{v} = \mathbf{b} \qquad (1)$$

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where **S** is the stoichiometric matrix of the metabolic network, **v** is the vector of reaction fluxes, and **b** is the net output from cellular metabolism. Equation (1) is typically underdetermined since the number of fluxes normally exceeds the number of metabolites. Thus, a plurality of solutions exists and a particular solution may be found using linear optimization by stating an objective and seeking its maximal value within the stoichiometrically defined domain.

Objective

Objective functions of maximizing growth [Eq. (2)] and the production of specific metabolites [Eq. (3)] have been used in this study:

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

and

$$\text{Minimize } Z = -v_{i,\text{drain}} \tag{3}$$

The growth flux (v_{gro}) is defined below based on biomass composition, and $v_{i,\text{drain}}$ is a flux draining the specific metabolite from the metabolic network. A simplex implementation of linear optimization is used[3] to determine the maximal solutions. The solution consists of both the maximal value as well as the flux distribution in the metabolic network.

Shadow Prices

The mathematical dual of the linear optimization problem[13] has also been evaluated to determine the dual solution. Interpretation of the dual solution as the shadow prices [Eq. (4)] provides a useful intrinsic measure of the value of a metabolic intermediate toward optimizing the objective:

$$\gamma_i = \frac{\partial Z}{\partial b_i} \tag{4}$$

Biosynthetic Network

The biosynthetic reaction pathways of *E. coli* and the associated stoichiometry are well known.[5,7,11,14] Due to the complexity of the biosynthetic reaction network, we have used several stoichiometric features[20] to reduce the size of the metabolic network and hence reduce the computational needs. The reduced biosynthetic network with major metabolic pathways is shown in Figure 1. To simplify Figure 1, we do not show the cofactor requirements for the biosynthetic paths; however, they can be obtained from Figure 2.

The stoichiometric matrix for the biosynthetic network, defined according to Eq. (1), is shown in Figure 2. The columns correspond to specific reaction pathways while the rows represent the flux balances. Thus the stoichiometric matrix provides an accurate mathematical definition of the biosynthetic network. The corresponding stoichiometric matrix for the catabolic network has been presented elsewhere.[20]

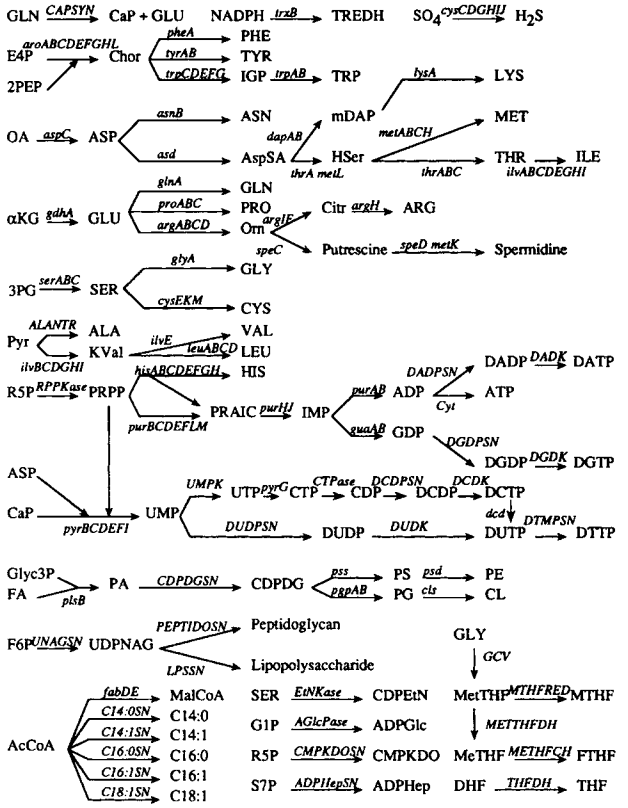


Figure 1. Biosynthetic network of the bacterium *E. coli*. The metabolic pathways have generally been designated by the *E. coli* genetic loci of the relevant enzymes. Metabolite abbreviations follow standard nomenclature.[14]

Metabolic Demands for Growth

Composition of the bacteria provides a useful definition for biomass generation[21] according to Eq. (5). The approximate chemical composition for *E. coli* B/r is known in terms of the biological monomers.[6,15] We have used the specific composition shown in Table I as a definition of biomass generation for the present analysis. Since the maximal growth solution has a low sensitivity to the individual intermediates of biosynthesis,[21] small changes in the composition are not likely to affect the results presented here:

$$\sum_{\text{all } M} d_M \cdot M \xrightarrow{v_{\text{gro}}} \text{biomass} \tag{5}$$

Maintenance Requirements

In addition to the composition based metabolic demands for growth there are also maintenance requirements in viable cells. Activities such as gradient maintenance, regulatory functions, and protein turnover are accounted for by including a maintenance energy loss in the metabolic network. A fit of the model to experimental data[12,18] yields a requirement of 23 mmol ATP/g biomass for growth associated and 5.87 mmol ATP/g DW h for non–growth associated maintenance. These two maintenance terms are also included in the flux balance based model.

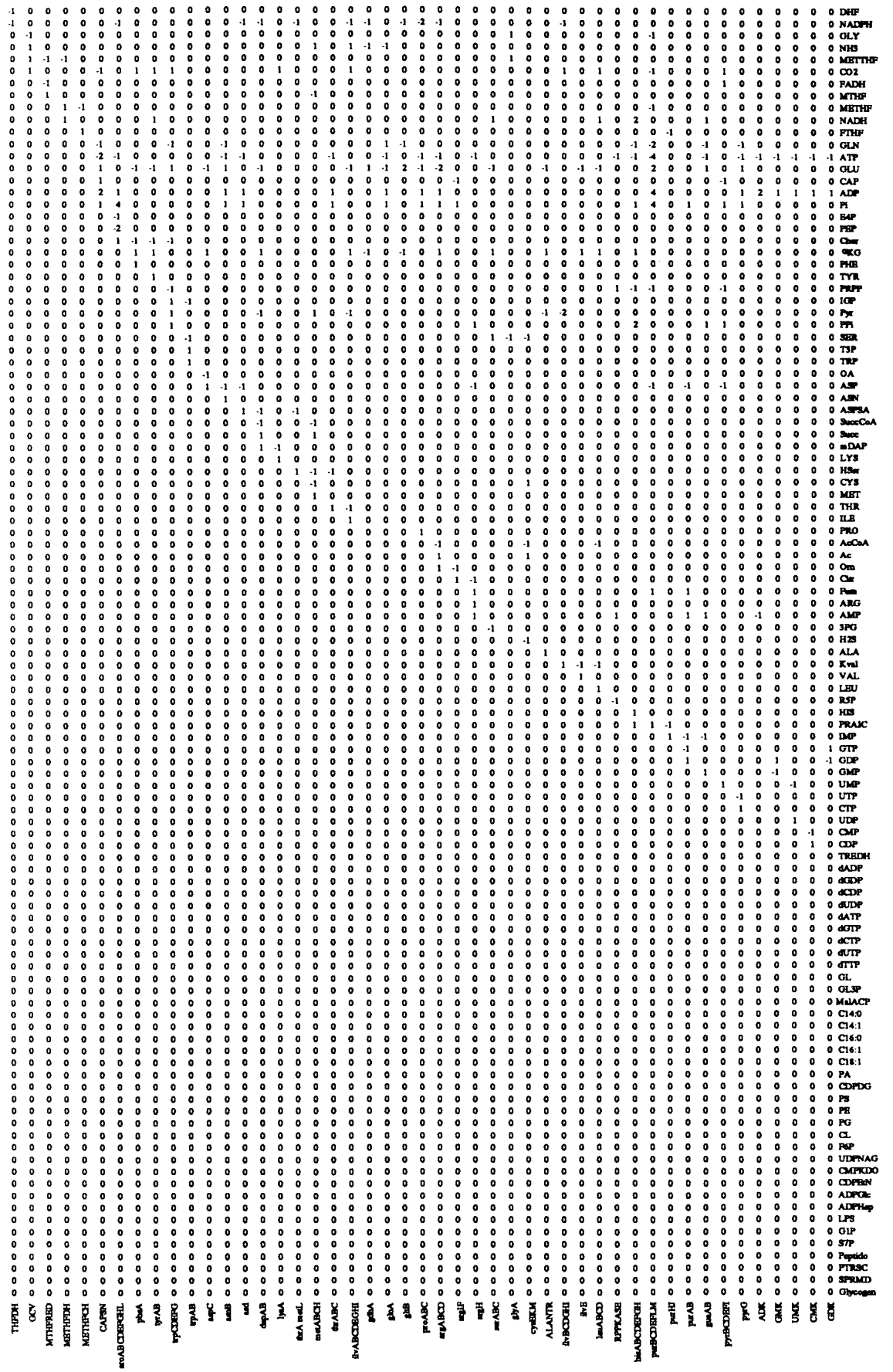


Figure 2. Stoichiometric matrix for the biosynthetic network defined according to Eq. (1). The columns represent the biosynthetic reactions while the rows represent the flux balances for the various metabolites.

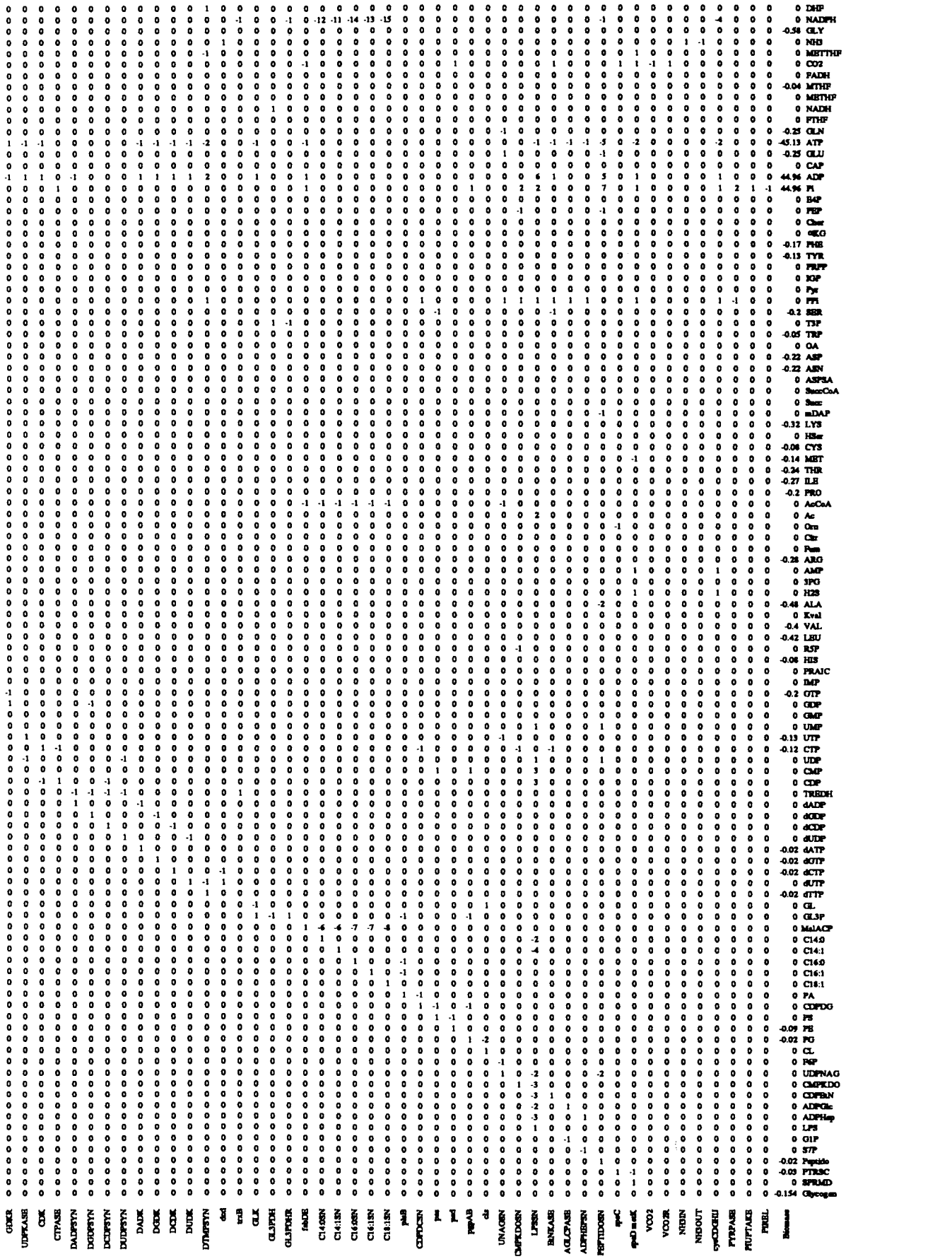


Figure 2. *(continued)*

Table I. Composition of *E. coli* B/r used to define biomass generation as a balanced drain on the metabolite pool, mmol/g biomass.

Metabolite	Demand	Metabolite	Demand	Metabolite	Demand
ALA	0.488	PHE	0.176	DGTP	0.0254
ARG	0.281	PRO	0.210	DCTP	0.0254
ASP	0.229	SER	0.205	DTTP	0.0247
ASN	0.229	THR	0.241	phosphatidyl serine	0.00258
CYS	0.087	TRP	0.054	phosphatidyl ethanolamine	0.09675
GLU	0.250	TYR	0.131	cardiolypin	0.00645
GLN	0.250	VAL	0.402	phosphatidyl glycerol	0.02322
GLY	0.582	~*P* (energy)	21.97	lipopolysaccharide	0.00785
HIS	0.090	ATP	0.165	peptiodoglycan	0.0276
ILE	0.276	GTP	0.203	glycogen	0.154
LEU	0.428	CTP	0.126	one carbon	0.0485
LYS	0.326	UTP	0.136	putrescine	0.0341
MET	0.146	DATP	0.0247	spermidine	0.007

Modified from refs. 6 and 14.

DEFINITION OF METABOLIC CONSTRAINTS

Optimization of an objective can be limited by metabolic constraints. The constraints identified here are stoichiometry, redox, energy, and combinations thereof. We need to establish a criteria for determining the constraints in a particular optimal solution. Stoichiometric constraints are defined as the use of necessary decarboxylaton steps resulting in a net CO_2 evolution. Redox and energy constraints are characterized by the utility of these cofactors in increasing production. Constraints and the criteria defining them are listed in Table II.

The presence of a constraint is indicated by a specific combination of the CO_2 production and the shadow prices of energy and biosynthetic redox. For example, a stoichiometric constraint is indicated by a net CO_2 evolution (i.e., less than 100% carbon conversion) and zero energy and redox shadow prices (see ref. 20). A redox constraint is indicated by a positive NADPH shadow price with a zero energy shadow price, indicating only the utility of redox for the objective of biochemical production. An energy constraint would result in a positive shadow price for both energy and redox due to the convertibility of redox to energy by the electron transfer system.

Combinations of constraints can be determined by decoupling the metabolic network from appropriate cofactors. Such decoupling implies not considering the particular cofactor in any of the reactions in the metabolic network. Decoupling can also be interpreted as providing an unlimited surplus of the cofactor to the metabolic network. For

Table II. Constraints faced in the production of amino acids and nucleotides along with their defining criteria.

		Shadow Prices		
Constraint	CO_2 Production	H_{exp}	NADPH	Decoupled
None	≤0	0	0	
Stoichiometry	>0	0	0	
Redox	—	0	>0	
Energy	—	>0	>0	
Redox +	—	>0	>0	
Energy	—	—	>0	energy
Redox +	—	0	>0	
Stochiometry	>0	0	—	redox
Energy +	>0	>0	>0	
Stoichiometry	>0	—	0	energy
Energy +	>0	>0	>0	
Redox +	>0	—	>0	energy
Stoichiometry	>0	—	—	energy + redox

Decoupling the metabolic network for a specific cofactor is essentially the same as externally supplying a surplus of the cofactor. The symbol "dashes" indicate any possible value. H_{exp} refers to the energy of the proton gradient.

example, the combined energy and redox constraint can be distinguished from the energy constraint by a positive shadow price for redox in the energy decoupled metabolic network. Other combinations of constraints are similarly determined by the appropriate decoupling of the metabolic network for energy and redox, as shown in Table II. Thus, the results from the flux balance based approach, such as the net CO_2 evolution and the utility of energy and biosynthetic redox, is interpreted in the form of metabolic constraints. The criteria for metabolic constraints given in Table II are hence in the nature of a logical truth table.

In addition, for the combined energy and redox constraint it is possible to determine the relative importance of the two constraints. Since the shadow prices of the proton gradient (H_{exp}) and NADPH represent the utility of energy and biosynthetic redox, respectively, the ratio of the two shadow prices would be indicative of the relative importance of redox compared to energy. A higher value would indicate a stronger redox constraint.

In comparison, the stoichiometric conversion of biosynthetic redox to energy has a ratio of 4 H_{exp}/NADPH. Since redox can be converted to energy, the ratio of the shadow prices must have a value above 4. The reverse conversion of energy to redox is not biochemically possible. However, by allowing a mathematical reversal of fluxes, we obtain a ratio of 6 H_{exp}/NADPH. Comparison of the ratio of the shadow prices to these numbers provides a good estimate of the importance of the energy and redox constraints.

RESULTS

Maximal Theoretical Performance

We have determined the production capabilities of the *E. coli* metabolic network by incorporating a drain for specific biochemicals in the metabolic network and maximizing them using linear programming. We compute maximal yields of amino acids and nucleotides from three substrates: glucose, glycerol, and acetate.

Glucose

The maximal conversion of glucose into amino acids and nucleotides is listed in Table III. In addition, the maximal biomass yield under fully aerobic conditions is shown. These values, determined without including the constant

Table III. Maximum theoretical yield of amino acids and nucleotides on a glucose substrate in absence of constant maintenance energy requirement[a].

Product	Maximum yield (mol/mol Glc)	CO_2 evolved[b] (mol/mol Glc)	Constraint[c]	Redox Energy
Biomass	0.097[d]	1.910	E + S	
ALA	2.000	0.000	N	
ARG	0.774	1.360	E	
ASN	1.560	−0.240	E	
ASP	1.820	−1.260	E	
CYS[f]	0.975	3.080	E + R	5.4
GLU	1.000	1.000	S	
GLN	1.000	1.000	S	
GLY[e]	2.000	0.000	N	
HIS	0.730	1.620	E + S	
ILE	0.734	1.600	E + R	6.0
LEU	0.667	2.000	S	
LYS	0.784	1.300	E + R	6.0
MET[f]	0.574	3.130	E + R	5.7
PHE	0.529	1.240	E + S	
PRO	1.000	1.000	S	
SER	2.000	0.000	N	
THR	1.230	1.090	E + R	5.7
TRP	0.414	1.450	E + S	
TYR	0.548	1.070	E + S	
VAL	1.000	1.000	S	
AMP	0.500	0.996	E	
CDP	0.540	1.140	E	
GMP	0.498	1.020	E	
UMP	0.600	0.600	E	

[a] Network constraints are determined according to the truth table shown in Table II. The redox-to-energy value represents the ratio of shadow price of the biosynthetic redox (NADPH) to that of the proton gradient.

[b] Carbon conversions can be calculated as $\frac{1}{6}$ (6 − CO_2 evolved).

[c] The constraints are indicated as E, energy; R, biosynthetic redox; S, stoichiometry; N, none.

[d] Biomass yield is in g DW/mmol Glc.

[e] A one-carbon drain has been included.

[f] Included are the redox requirements of sulfate reduction.

maintenance energy, represent the maximal theoretical stoichiometric production capability of the metabolic network. The goal of metabolic engineering is to move from the normal state of biomass production toward overproducing a specific biochemical. The net CO_2 production for the various conversions is also listed in Table III and is indicative of the carbon conversion. The carbon conversion is a function of the stoichiometry, the redox requirements, and the energy requirements of the specific product.

Applying the truth table shown in Table II to the maximal production of various amino acids and nucleotides from a glucose substrate, we observe the constraints listed in Table III. A variety of constraints are observed in the production of the different biochemicals depending on the biosynthetic requirements. In comparison, biomass generation is shown to have a combined stoichiometric and energy constraint. It is interesting to note that redox is always associated with energy as a constraint in the cases considered. The ratio of redox and energy shadow prices is also listed in Table III for the combined redox and energy constraints.

Glycerol

Glycerol can be used as a substrate for the production of biochemicals. The results from computations of the maximum theoretical yields and the corresponding constraints are shown in Table IV. In contrast to a glucose substrate we note that lysine production on glycerol does not have a redox constraint. Since glycerol is a more reduced substrate as compared to glucose, we would expect redox to be less of a constraint on glycerol. Also, since redox can also be converted to energy, we expect the energy constraints to be reduced as well. Thus, we note that aspartate, phenylalanine, and tyrosine do not have energy constraints on glycerol, in contrast to glucose.

The redox-to-energy ratio for the glycerol substrate is also observed to be higher than that for glucose, which is peculiar considering that glycerol is a more reduced substrate. The explanation for this anomaly lies in the stoichiometry of redox coupling. Glycerol incorporation into the central catabolic pathways produces redox in the form of NADH. NADH is easily converted to energy by the electron transfer system. More difficult is the conversion of NADH to biosynthetic redox (NADPH) by transhydrogenation, which requires a net input of energy. Thus the extra reducing power of glycerol is more easily converted to energy rather than biosynthetic redox. Therefore the redox-to-energy ratio when metabolizing glycerol is higher than for glucose metabolism.

Table IV. Maximum theoretical yield of amino acids and nucleotides on a glycerol substrate in absence of constant maintenance energy requirement.

Product	Maximum yield (mol/mol Glyc)	CO_2 evolved[a] (mol/mol Glyc)	Constraint[b]	Redox Energy
Biomass	0.054234[c]	0.711	E + S	
ALA	1.000	0.000	N	
ARG	0.430	0.419	E	
ASN	0.902	−0.610	E	
ASP	1.000	−1.000	N	
CYS[d]	0.852	1.340	E + R	6.0
GLU	0.500	0.500	S	
GLN	0.500	0.500	S	
GLY[e]	1.000	0.000	N	
HIS	0.413	0.520	E + S	
ILE	0.407	0.560	E + R	6.0
LEU	0.333	1.000	S	
LYS	0.435	0.388	E	
MET[d]	0.325	1.380	E + R	6.0
PHE	0.300	0.300	S	
PRO	0.500	0.500	S	
SER	1.000	0.000	N	
THR	0.698	0.207	E + R	6.0
TRP	0.237	0.391	E + S	
TYR	0.300	0.300	S	
VAL	0.500	0.500	S	
AMP	0.286	0.143	E	
CDP	0.304	0.262	E	
GMP	0.286	0.143	E	
UMP	0.342	−0.070	E	

[a] Carbon conversion can be calculated as $\frac{1}{3}$ (3 − CO_2 evolved).
[b] The constraints are indicated as E, energy; R, biosynthetic redox; S, stoichiometry; N, none.
[c] Biomasss yield is in g DW/mmol glycerol.
[d] Included are the redox requirements of sulfate reduction.
[e] A one-carbon drain has been included.

Acetate

Computations of the maximal theoretical yields and corresponding constraints using acetate as a substrate are presented in Table V. Energy is observed to be a constraint for all the products considered, which is indicative of the poor energetic value of acetate. Stoichiometry is also seen to be a constraint for many of the conversions of acetate into products. Acetate enters the catabolic network through the tricarboxylic acid (TCA) cycle and must therefore pass through several decarboxylation steps in order to produce the required biosynthetic precursors. The net CO_2 from these steps results in stoichiometric constraints during biochemical production.

Taken together, these results show that constraints on the production of biochemicals from a given substrate are a function of the energy and redox content of the substrate as well as its point of entry into the metabolic network.

Optimal Flux Distributions

The efficient production of biochemicals requires redirection of metabolic fluxes so that formation of the desired product is favored. In Figure 3 we present the optimal catabolic and biosynthetic flux distributions for maximal growth using a glucose supply of 10 mmol Glc/g DW h resulting in a growth rate of 0.94 h^{-1}. Under these conditions the energy yield is 70 mmol ATP/g biomass. The flux distribution shows the well-accepted use of metabolic pathways in *E. coli* during aerobic growth. An interesting feature of the catabolic flux distribution is the utilization of the acetate formed during the biosynthetic reactions.

Figure 4 displays the optimal catabolic flux distributions corresponding to the production of the various amino acids and nucleotides with a glucose supply of 10 mmol Glc/g DW h. The maximal yields are listed with the flux distributions. Note the dissipation of surplus energy shown as a drain of the proton gradient in some flux distributions. Surplus energy is observed in the flux distributions of ALA, GLU, GLN, and VAL, which have either no constraints or stoichiometric constraints (Table III). On the other hand, GLY, PRO, and SER, which also have no constraints or stoichiometric constraints (Table III), do not show a surplus of energy. The lack of surplus in these cases is due to the constant maintenance energy requirements being larger than the surpluses. The constant maintenance energy requirements are included in the computations for Figure 4.

Some interesting observations pertaining to the optimal utilization of pathways follow from the results given in

Table V. Maximum theoretical yield of amino acids and nucleotides on a acetate substrate in absence of constant maintenance energy requirement.

Product	Maximum yield (mol/mol Ac)	CO_2 evolved[b] (mol/mol Ac)	Constraint[c]	Redox Energy
Biomass	0.018438[d]	1.220	E + S	
ALA	0.393	0.821	E + S	
ARG	0.151	1.100	E	
ASN	0.324	0.706	E	
ASP	0.382	0.471	E	
CYS[e]	0.181	1.460	E + R + S	6.0
GLU	0.268	0.658	E + S	
GLN	0.250	0.750	E + S	
GLY[f]	0.394	0.818	E + S	
HIS	0.137	1.180	E + S	
ILE	0.144	1.130	E + R + S	6.0
LEU	0.159	1.040	E+ S	
LYS	0.155	1.070	E + S	
MET[e]	0.111	1.450	E + R + S	6.0
PHE	0.100	1.100	E + S	
PRO	0.210	0.952	E + S	
SER	0.394	0.818	E + S	
THR	0.250	1.000	E + R	6.0
TRP	0.076	1.160	E + S	
TYR	0.103	1.070	E + S	
VAL	0.196	1.020	E + S	
AMP	0.095	1.050	E + S	
CDP	0.100	1.100	E + S	
GMP	0.093	1.070	E + S	
UMP	0.113	0.986	E + S	

[a] Acetate uptake is assumed to utilize the scavenging AcCoA synthase pathway.
[b] Carbon conversion can be calculated as $\frac{1}{2}$ (2 − CO_2 evolved).
[c] The constraints are indicated as E, energy; R, biosynthetic redox; S, stoichiometry; N, none.
[d] Biomass yield is in g DW/ mmol acetate.
[e] Included are the redox requirements of sulfate reduction.
[f] A one-carbon drain has been included.

Figure 4. The optimal production of ALA and VAL does not consume any oxygen as indicated by the absence of a flux through the cytochromes. Thus these amino acids are optimally produced by fermentation. Utilization of the glyoxalate shunt is observed during the optimal production of ARG, ASN, MET, THR, CDP, and UMP. Since the glyoxalate shunt is not observed to be operative while glucose is a substrate, this shunt may represent a prime target for metabolic engineering for the production of these biochemicals. Similarly, the complete TCA cycle is seen to be operative during the optimal production of CYS, HIS, PHE, TRP, TYR, AMP, and GMP. Again, repression of the TCA cycle in the presence of glucose is a suitable area for study in these cases.

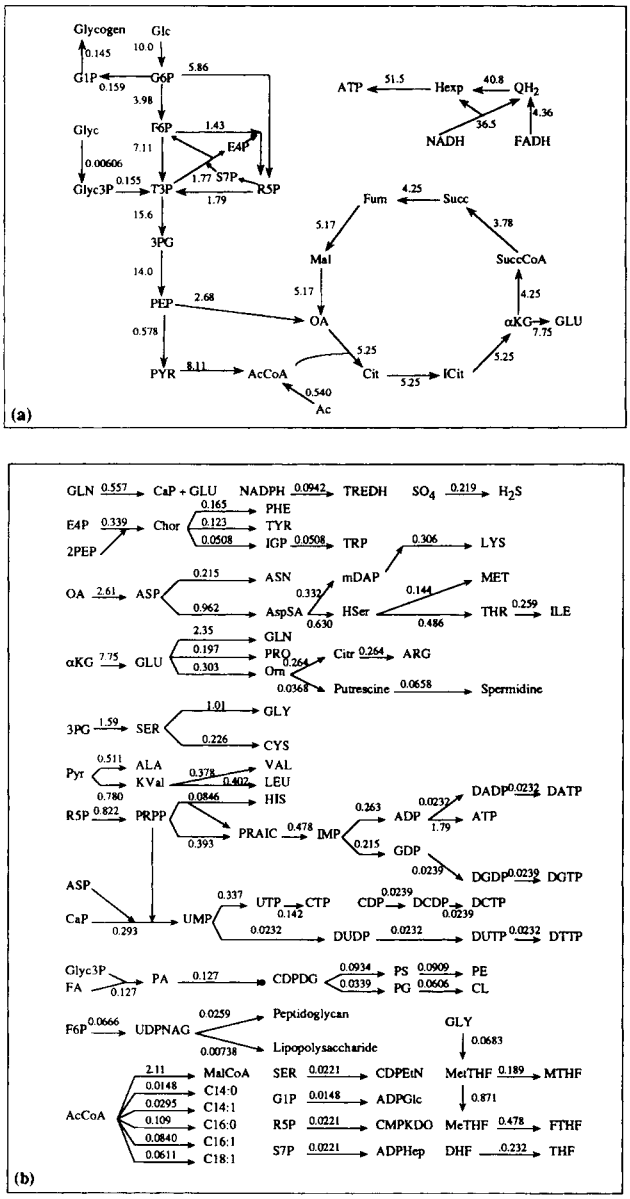


Figure 3. Flux distributions for maximal cell growth with an input of 10 mmol Glc/g DW-h: (a) catabolic flux distribution; (b) biosynthetic flux distribution.

In general, the flux distributions for maximal product formation should be compared to the flux distribution for maximal growth (Fig. 3). Efficient biochemical production requires the redistribution of metabolic fluxes from producing biomass toward producing the specific product.

Balanced Growth with Biochemical Production

It is often desirable to produce the product while simultaneously generating biomass. An optimal trade-off between growth and biochemical production can be assessed by choosing a production rate for a particular product between zero and the maximum production rate and then maximizing the growth rate. Figure 5 shows the optimal trade-off between growth and the production rate for a few select biochemicals. A negative correlation is observed between growth of the cell and biochemical production. The trade-off is piece-wise linear and encloses a convex space.

The production of leucine provides a good example of a piecewise linear relationship between biomass generation and product formation. The production of leucine results in a surplus of energy generation (Fig. 4k). Biomass generation, on the other hand, is constrained for energy (Table III). Thus, the combined biomass and leucine production is able to utilize the energy surplus of leucine production. The increase in efficiency of the combined solution as compared to the addition of the individual solutions is the reason for the nonlinear trade-off enclosing a convex space.

However, the deviation from absolute linearity is not significant for most of the metabolic products considered here. We have therefore tabulated the initial slopes of the trade-off lines for all the amino acids and nucleotides for maximal growth in Table VI. These slopes are indeed the shadow prices of the corresponding biochemicals computed from the dual solution. The shadow prices represent the marginal decrease in growth due to product formation. This tradeoff is an important determinant of balanced growth and product formation.

DISCUSSION AND CONCLUSIONS

The primary goal in producing metabolic products with microbial cells is to obtain a high conversion of the substrate into the desired product. Determination of the limits of substrate to product conversions is of key concern. The maximum theoretical yield is constrained by the stoichiometry of the reaction pathways in the metabolic network, which includes balancing the consumption and generation of metabolic cofactors. We have determined these theoretical limits on microbial performance by applying a flux balance based approach to the metabolic network of the bacterium *E coli*.

In the natural state, metabolism of microbes is directed toward growth. It has been suggested that metabolic regulation in microbial cells has evolved to maximize growth within stoichiometric constraints.[21] Overproduction of a desired product thus requires the redirection of metabolic

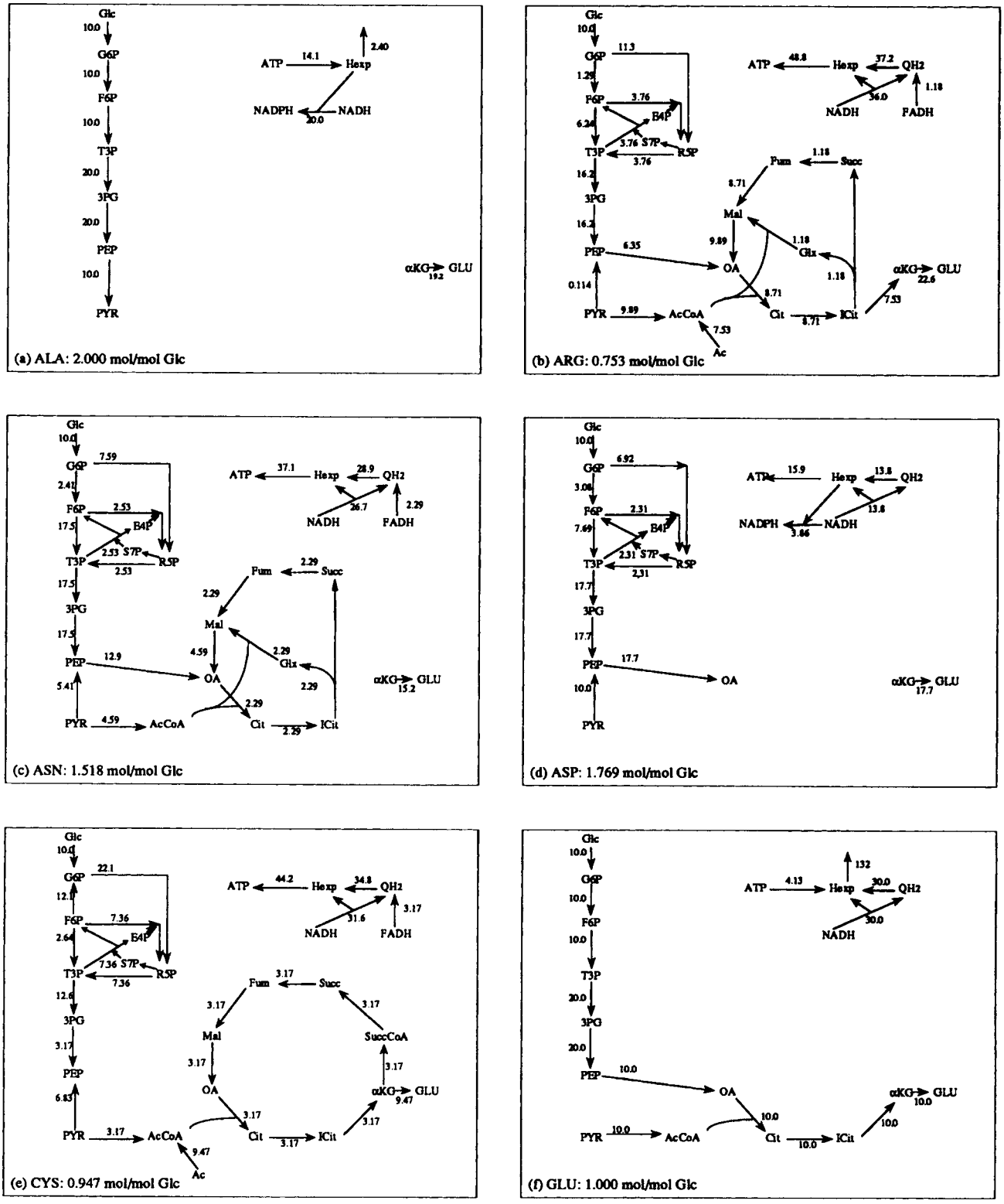
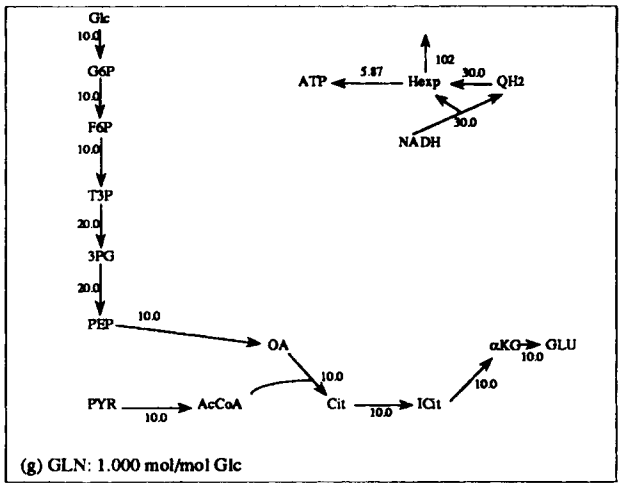


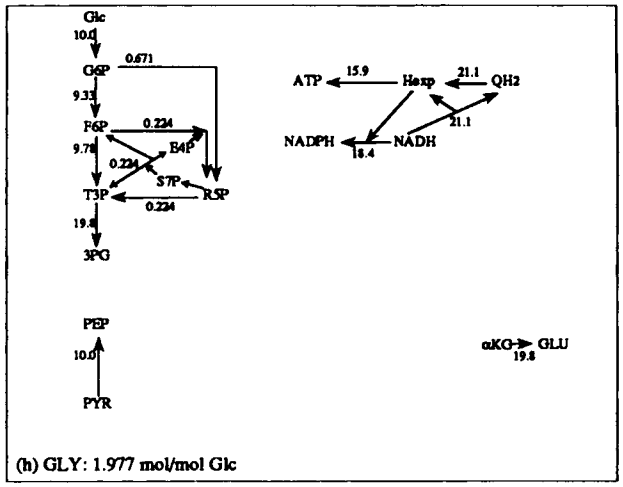
Figure 4. Optimal catabolic flux distributions for maximal biochemical productions. A substrate input of 10 mmol Glc/g DW-h has been provided and maintenance requirements have been included. The maximal productions are listed in the individual flux distributions in mmol product/g DW-h.

fluxes from generating biomass toward producing the desired biochemical product. We have determined the flux distributions that correspond to the maximal production of various amino acids and nucleotides as illustrative biochemical products. The goal of engineering the strain can therefore be defined as the redirection of metabolic fluxes from the optimal growth solution to the optimal biochemical solution.

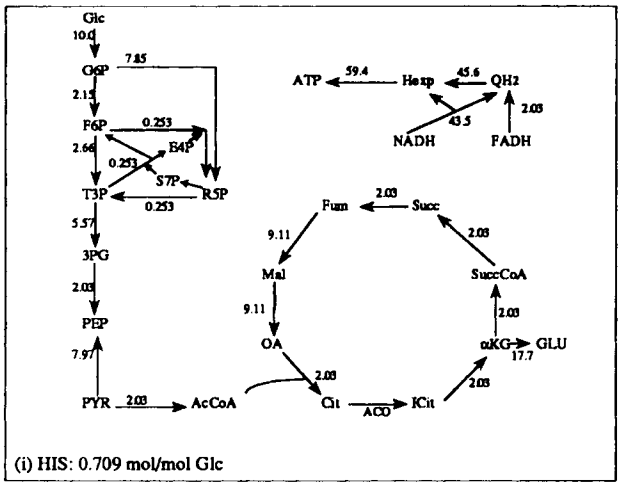
Thus, to produce a specific biochemical product, engineering metabolism raises two questions: what production



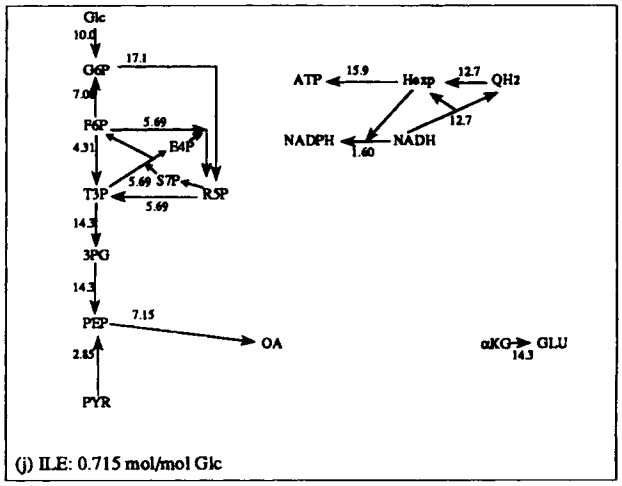
(g) GLN: 1.000 mol/mol Glc



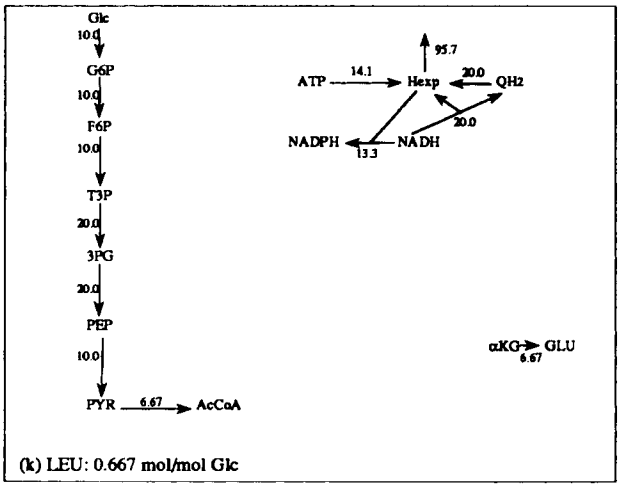
(h) GLY: 1.977 mol/mol Glc



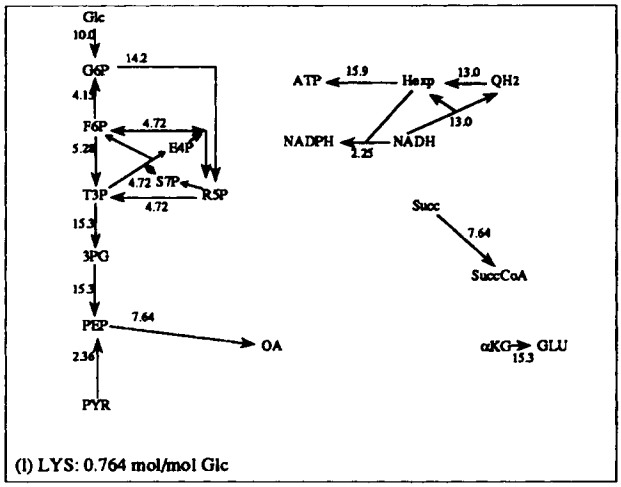
(i) HIS: 0.709 mol/mol Glc



(j) ILE: 0.715 mol/mol Glc



(k) LEU: 0.667 mol/mol Glc



(l) LYS: 0.764 mol/mol Glc

Figure 4. *(continued)*

level can be achieved, and how may it be attained? We choose lysine production as an illustrative example to answer these two questions. The maximal theoretical yield of lysine on a molar basis on various substrates is computed as glucose = 0.784, glycerol = 0.435, and acetate = 0.155. The corresponding carbon conversion is computed as glucose = 78%, glycerol = 87%, and acetate = 46%. Thus, it would appear that glycerol is the best substrate with the highest carbon conversion. However, we also note that although maximal lysine production has energy constraints

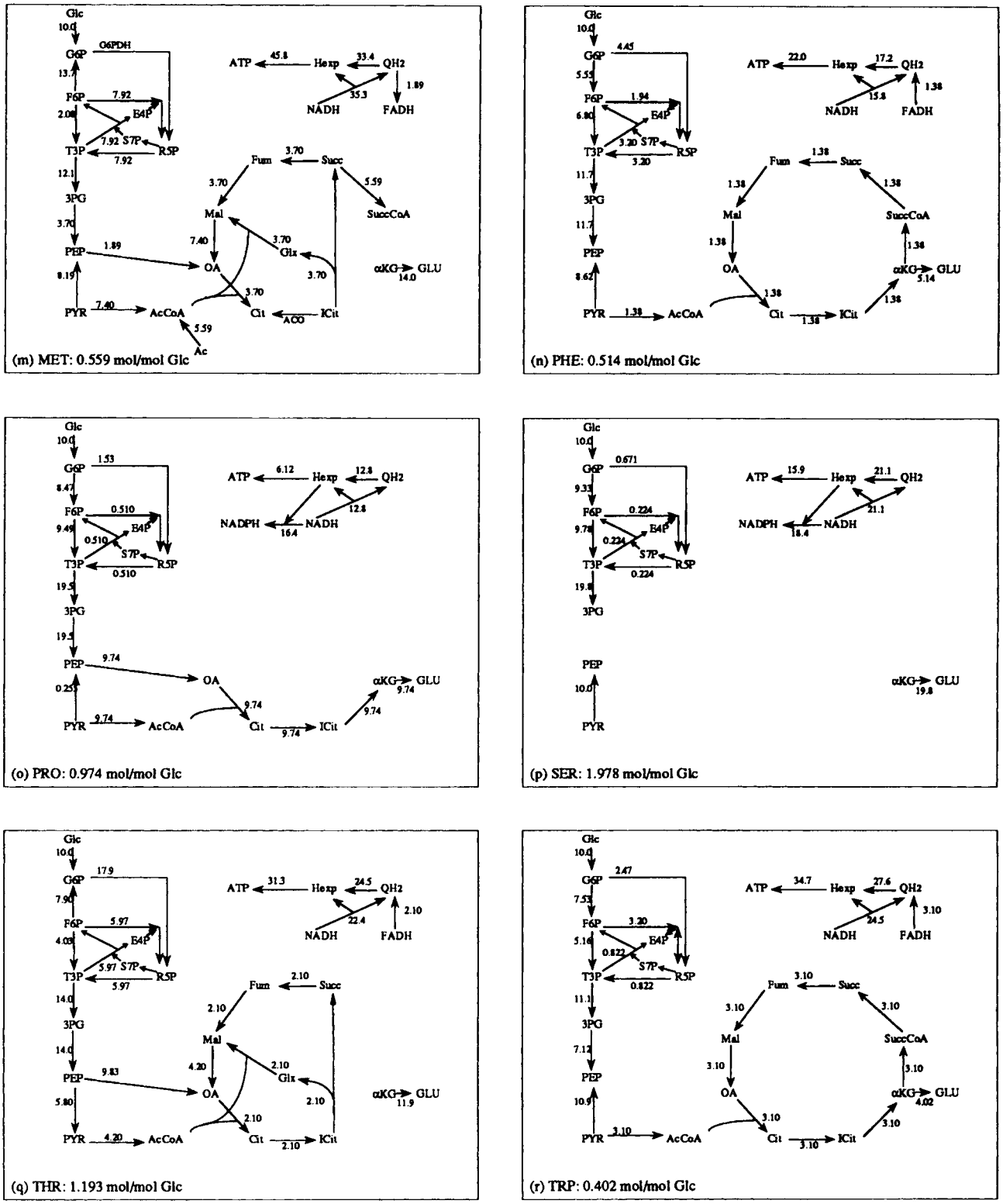
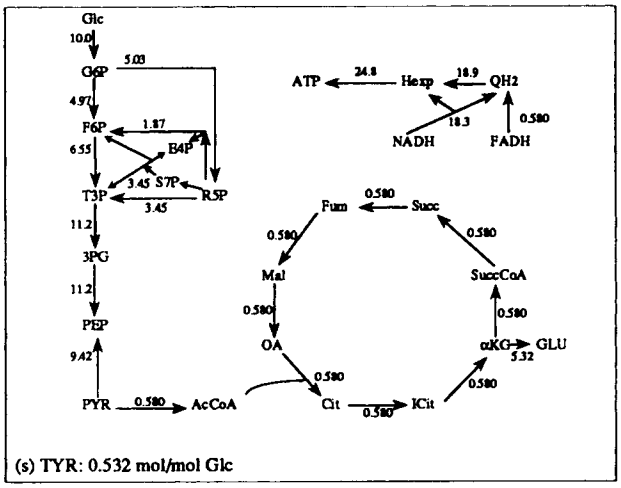


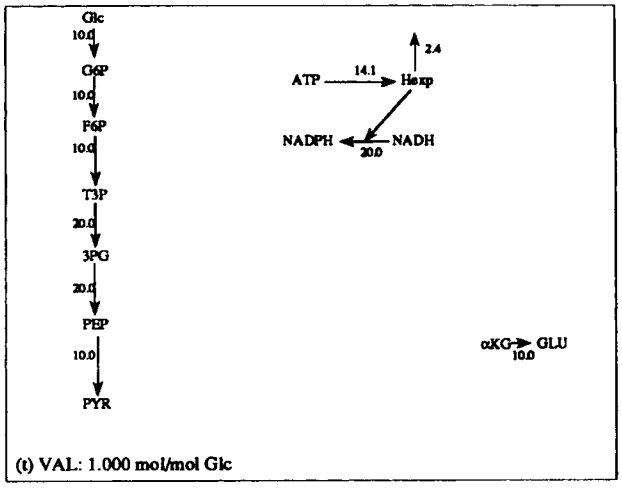
Figure 4. *(continued)*

with all the substrates considered, it has additional constraints of redox on glucose and stoichiometry on acetate. Therefore, there exists the potential for a syntergistic effect in the presence of multiple substrates that could further enhance the carbon conversion. Some precedent for the cometabolization of glucose and acetate at least during a transitional growth phase does exist.[22]

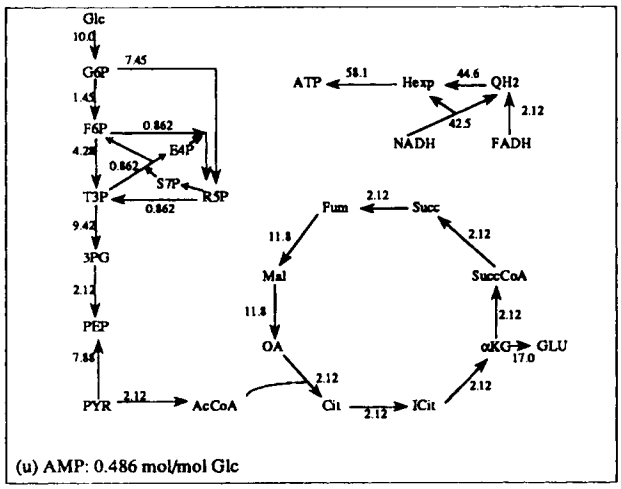
The actual optimal conversion of substrate into the desired product requires the manipulation of metabolic fluxes. For the case of lysine production the optimal flux



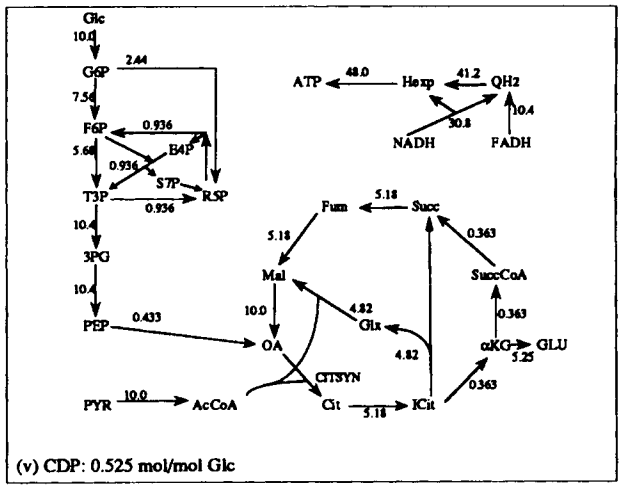
(s) TYR: 0.532 mol/mol Glc



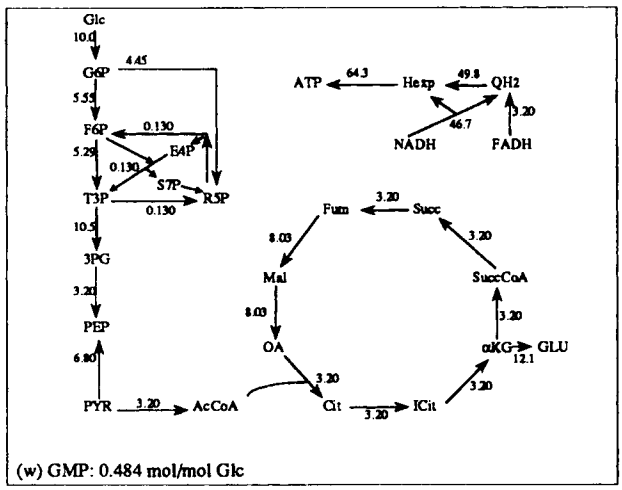
(t) VAL: 1.000 mol/mol Glc



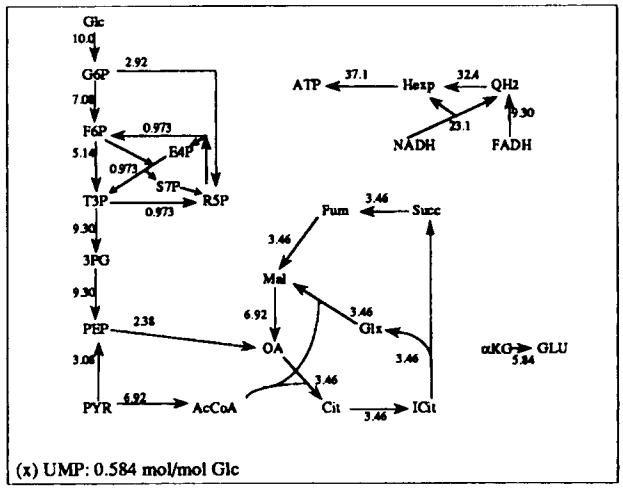
(u) AMP: 0.486 mol/mol Glc



(v) CDP: 0.525 mol/mol Glc



(w) GMP: 0.484 mol/mol Glc



(x) UMP: 0.584 mol/mol Glc

Figure 4. *(continued)*

distribution converting glucose into lysine is shown in Figure 4l. Achieving this flux distribution represents the goal of metabolic engineering, to engineer a strain for lysine production. The use of alternate or mixed substrates also needs investigation as they can potentially ease the task of flux redirection for optimal performance.

For a deeper understanding of optimal biochemical production we need to identify metabolic constraints in an

Table VI. Optimal trade-off between growth and biochemical production represented as shadow prices of biochemicals in maximal growth solution.

Product	$\partial\mu/\partial$ Product	Product	$\partial\mu/\partial$ Product	Product	$\partial\mu/\partial$ Product
ALA	4.48	HIS	12.7	THR	7.61
ARG	12.5	ILE	12.9	TRP	22
ASN	5.99	LEU	11.3	TYR	16.8
ASP	4.98	LYS	12.1	VAL	8.95
CYS	9.56	MET	16	AMP	8.95
GLU	7.17	PHE	17.4	CDP	17.3
GLN	7.68	PRO	9.29	GMP	18.7
GLY	2.15	SER	4.31	UMP	15.8

The shadow price units are g biomass/100 mmol product. An input supply of 10 mmol Glc/g DW h has been used for the computations resulting in a growth rate of 0.94 g biomass/g DW h.

optimal solution. Biosynthesis of the product requires inputs of redox, energy, carbon units, and minerals. Redox and energy can be produced as a by-product during substrate conversion to the carbon skeleton. An inadequate production of redox and energy presents a constraint that would require the oxidation of substrate in order to provide the required redox and energy. In addition, specific metabolic reactions can result in the release or uptake of CO_2, thus affecting the net carbon conversion.

In the presence of adequate minerals, metabolic constraints during the production of specific biochemicals can be categorized in terms of stoichiometry, redox, and energy. These constraints determined according to the criteria in the truth table shown in Table II represent the demands or stress placed on cellular metabolism during biochemical overproduction. Computations for glucose, glycerol, and acetate substrates demonstrate that constraints on metabolism depend on the nature of the substrate in terms of its redox and energy content as well as its point of entry into the metabolic network. Of the various combinations of constraints observed, it is interesting that redox is always associated with energy as a constraint. A large fraction of cellular metabolic energy requirements are met by oxidative phosphorylation, which essentially converts redox into energy. Therefore, generally, a redox constraint would result in a simultaneous energy constraint.

A balance between growth and biochemical production is often important for a successful bioprocess. The generation of biomass in the bioprocess is necessary to provide the backbone of metabolism used to achieve substrate conversion into the desired biochemical. Although the growth phase (trophophase) is often considered as separate form the product formation phase (idiophse), it has been demonstrated that simultaneous growth and product formation can indeed result in a trophophase–idiophase separation.[4]

We have computed the optimal trade-off between simultaneous growth and biochemical production. As expected, a negative correlation is observed in the trade-off. For most of the biochemicals considered here the trade-off is practically linear. However, some piece-wise linear solutions do exist that demonstrate a syntergistic effect between biomass and biochemical production. Thus, the balanced growth and biochemical solution shows a higher efficiency compared to a simple addition of the individual solutions. An appropriate balance between growth and biochemical production is particularly important for nonsecreted products, such as for polyhydroxybutyrate accumulation in cells.[16]

The present study demonstrates an application of the flux based analysis of *E. coli* metabolism toward the study of biochemical production. The limits of substrate conversion to biochemicals and the distribution of flux in the metabolic network represent the goals of metabolic engineering. Representing the limits of metabolic behavior, the analysis is useful for evaluating and analyzing performance. The general conceptual framework presented here can be used to obtain a detailed analysis for a particular product as a guide to the development of a bioprocess.

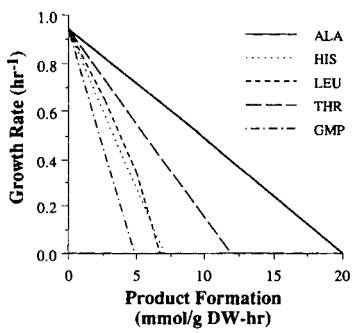


Figure 5. Simultaneous production of biomass and specific biochemicals. The optimal trade-off between biomass generation and biochemical production shows a piecewise linear negative correlation.

NOMENCLATURE

- **b** vector representing transport flux of metabolites out of cell
- d_M metabolic demands for growth, mmol metabolite/g biomass
- M any metabolite in the metabolic network
- **S** stoichiometric matrix for the metabolic network; an element S_{ij} represents the moles of metabolite i needed for reaction j
- **v** vector of reaction fluxes; fluxes are determined using linear optimization of an objective function
- Z denotes the linear optimization objective
- γ_i shadow price of ith metabolite

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