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Metabolic Explosion in Yeast Glycolysis

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Abstract

To study metabolism in biology, budding yeast (*S. cerevisiae*) is often chosen as the subject of study. As such a model organism, its metabolic network is known in more detail than any other unicellular eukaryote. Therefore it is possible to model the dynamics of its metabolism rather precisely. A specific mutant of this species is known in some cases to fail to grow when glucose is abundantly present. Measurements then show that in the cell cytoplasm concentrations of intermediate metabolites, such as fructose-1,6-biphosphate, rise and keep rising beyond healthy levels yielding impeded growth and even death. This is called metabolic explosion.

Recently, Hulshof, Planqué, Bruggeman and Teusink [1] introduced a novel opproach to the analysis of a well known simple toy model, 'the Old Lady', that allowed to study its bifurcation behaviour in more detail. It is founded on smart monotonicity arguments to derive properties of implicitly defined functions to get at existence of (non-trivial) steady states. They were able to draw conclusions on the stability of these steady states too.

In this thesis a new model is presented for the pathway of glycolysis. It is introduced as an extention of the Old Lady and is meant to bridge the gap in complexity between this toy model, favored in mathematics [1] and a descriptive model favored in biology [2], such that the model is more descriptive than the Old Lady, but the mathematical framework used for the toy model is still applicable.

Some general theory of Metabolic Network Analysis is considered. The existence of trivial and non-trivial steady states is described, where the general theory is shown to be applicable. This is then formulated in a general proposition which can help to show existence also in larger models. The mathematical framework to describe metabolic explosion, as introduced in [1], is formulated for the new model. Existence of steady states proves to be a subtle exercise in mathematics as the five metabolite concentrations that are considered, in steady state, are implicit solutions to non-linear equations. The stability analysis is done only for the trivial steady states as computing eigenvalues of the Jacobian showed no meaningful results for the non-trivial steady states.

This work will be continued in a follow up PhD project in the group of Joost Hulshof at the University Amsterdam (VU).

1 Introduction

1.1 Metabolic Explosion

Yeast metabolism is a favorite model system. Its reaction network is known to have many metabolites and reactions. As shown in figure 1 it cannot be easily overseen. One very important pathway in this network is glycolysis, where intracellular glucose is converted by means of a series of reactions into ethanol.

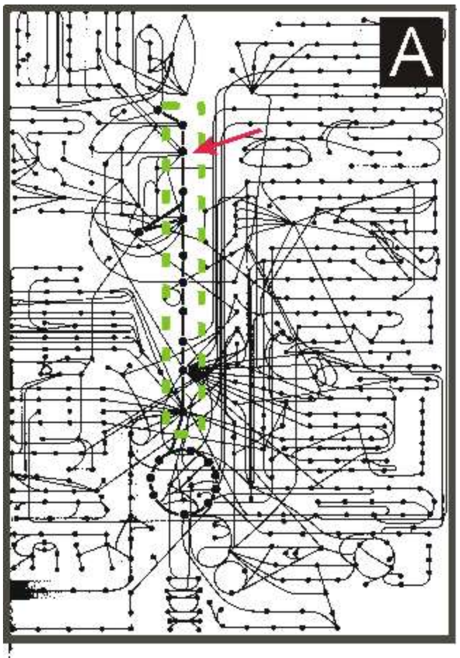


Figure 1: A reaction network model of yeast metabolism. Nodes are metabolites and vertices are enzymatic reactions. The pathway circled in green is glycolysis.

This pathway generates Adenosine Tri-Phosphate (ATP) out of Adenosine Di-Phosphate (ADP) and inorganic phosphate (p_i). The conversion of ADP and p_i to ATP is a biological means to store chemical energy. This can then be used in other biological processes which require energy, such as active transport of metabolites over a membrane. Glycolysis yields two molecules of ATP for each molecule of glucose, but two of the first reactions in this chain use an ATP molecule and the positive yield is only due to the production of four ATP molecules in subsequent reactions. In "*The danger of metabolic pathways with turbo design*", Teusink *et al.* [3] compare this to the turbo engine: exhaust gases are fed back to enhance the fuel-input step.

In [3] experiments were described with budding yeast, the yeast species *Saccharomyces cerevisiae*, see figure 2. They were placed in an environment with abundant glucose. Concentrations of several metabolites in the cytoplasm were measured. The tps1-Δ mutant was also studied in this way. This mutant is genetically manipulated such that a single gene was inactivated. The specific gene governs a seemingly inconsequential side branch of the yeast glycolysis: trehalose synthesis. The phenotype of some of the individual cells of this type of mutant was failing to grow on the glucose, while having intermediate metabolite concentrations that kept growing over time: a *metabolic explosion*.

The authors show by means of a mathematical model and numerical simulations that the turbo design is crucial to this behaviour of metabolic explosion and assert that trehalose synthesis yields some kind of feedback mechanism on the first steps of glycolysis that inhibits these first steps, yielding a balanced metabolism and no metabolic explosion.

It has to be noted that some of the mutant cells showed this behaviour, while others functioned properly.

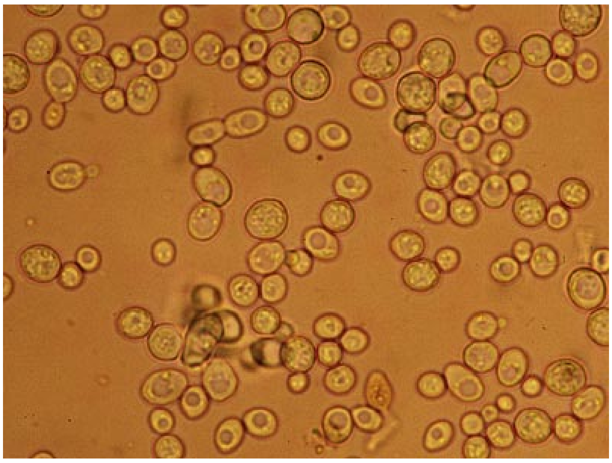


Figure 2: Budding Yeast cells. The dark spot which is visible in some cells is the cell vacuole.

1.2 A Descriptive Model

One way to describe glycolysis mathematically is to take the concentration of all metabolites involved as variables. Then describe a dynamical system where changes in metabolite concentrations due to reactions are defined through a system of ordinary differential equations. The reactions all correspond to specific enzymes which enable these reactions. The effect of a certain enzyme is considered with respect to consumption and production of metabolites, if an enzymatic reaction converts a into b, then it is said to consume a and to produce b. The reaction speed, reaction flux, is described through a function involving the concentrations of the metabolites. The specific form of the function can then be guessed by in vitro experiments, i.e. measuring the effect of an enzyme on metabolite concentrations in the controlled environment of a test tube.

Teusink *et al.* [2] describe how they modelled the pathway of glycolysis with a set of 14 differential equations. To set the parameters in the model realistically, they conducted most of the in vitro experiments themselves, thus assuring that the conditions for all experiments were comparable. The enzyme kinetics they use are highly non-linear, constructed with the aim to be as descriptive as possible.

With this model predictions were made for yeast glycolysis based on numerical simulations. The results were compared to in vivo measurements, i.e. measurements of live yeast cells.

With this comparison it was concluded that of the enzymes that were consideren, half of them matched their predicted flux in vivo within a factor of 2, which is considered reasonable, while the discrepancy of the other enzymes is explained by in vivo and in vitro kinetic characteristics.

This is an example of a very descriptive model of yeast glycolysis.

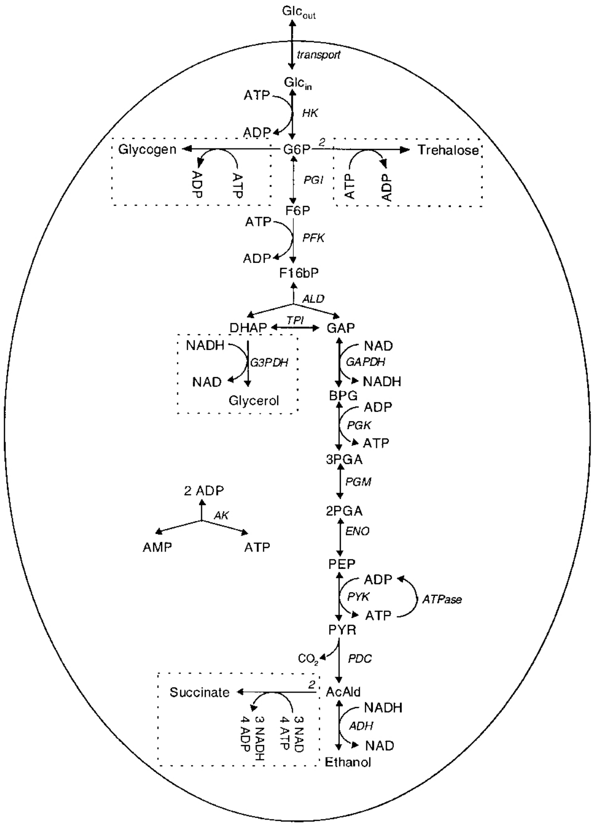


Figure 3: Reaction network model of [2]

1.3 'The Old Lady': A Popular Toy Model

A model with three metabolites and five reactions that can be mathematically analysed is introduced by Hulshof *et al.* [1]. Because this model has been analysed and researched often, it is commonly referred to as 'the Old Lady'.

The production and consumption of the metabolites ATP, p_i and fructose-1,6-biphosphate (fbp) is considered, see Figure 4. The glucose concentration is not considered. Like Teusink *et al.* [3], the biological situation is that yeast is placed in a glucose abundant environment.

It is assumed that the glucose uptake of the cell is not inhibited, hence glucose is present in abundance in the cell. Its intracellular concentration is assumed to

be approximately constant.
It is assumed that the total concentration of ATP and ADP together remains constant. Hence using this conservation law [ATP] + [ADP] $= A_T$, the ADP concentration is known when the ATP concentration is known.

The reactions that are considered are:

v_1 The production of fbp through consumption of intracellular glucose and ATP. Only the ATP concentration influences the reaction flux. This is a lumped reaction to several intermediate steps, namely *HK, PGI* and *PFK* in Figure 3, however, it is assumed that the PFK reaction flux is dominant.

v_2 The production of ethanol through consumption of fbp, ADP and p_i. The produced ethanol is assumed not to feedback onto the system.

v_3 The production of glycerol and p_i through consumption of fbp. The produced glycerol is assumed not to feedback onto the system.

v_4 The lumped reaction of all ATP consuming reactions in the cell to produce ADP and p_i other than fbp production. The constituent enzymes are called ATPases.

v_5 A reversible reaction that buffers the p_i concentration to a pool of inorganic phosphate in a vacuole, called $p_{i,vac}$. The buffer concentration is defined as $\Pi \in \mathbb{R}_+$.

All these reactions are lumped from several enzymatic reactions. The simplification of the system to this three-dimensional model cannot be justified in all conditions. As an example, v_2 consists of several reversible reactions of which the reverse is actually dominant for low concentrations of fbp. This is therefore a toy model and should not be considered as descriptive, but rather a model that is to be mathematically analysed to gain insights into the larger system.

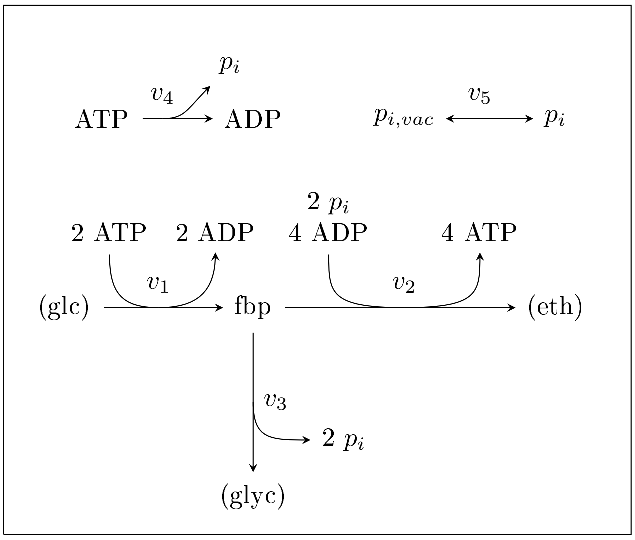


Figure 4: Reaction network model of 'The Old Lady'

This model is studied to analyse qualitatively the mechanism of yeast glycolysis. The found insights of Hulshof *et al.*[1] that are found are to be used for a biological article.

1.4 Thesis Goals

Note that there is a huge difference in complexity between the Old Lady and the descriptive model depicted in Figure 3. The first is simpler, non-descriptive and can be analysed mathematically. The second is far more complex, more descriptive, but is tractable only by using numerical simulations.

In this thesis, a new model is introduced. It is constructed personally by collaboration of Joost Hulshof, Frank Bruggeman and Bas Teusink, who are all authors of [1], to bridge the gap in complexity between the models described above. The modeling choices are elaborated upon in the first parts of Section 2. It is more complex and descriptive than than the Old Lady, but still has only five dynamic variables. This model will be analysed mathematically. The goals of this thesis can be arranged into mathematical goals and biological goals.
As noted in the abstract, this thesis is a prelude to a PhD project in the group of Joost Hulshof at the University Amsterdam (VU). An extra educational goal is therefore to become familiar with this field of research: applied mathematics in systems biology. For this reason the thesis is partially supervised by Bob Planqué of the University Amsterdam (VU).

1.4.1 Mathematical Goals

- Understanding the mathematical techniques used by Hulshof *et al.* [1] and explore if they can be applied to the new model.
- If a mathematical technique proves applicable, try to formulate a general result yielding that this technique is applicable to other, more complex, models as well.

1.4.2 Biological Goals

- Find all types of behaviour that are seen in the biological experiments of Teusink *et al.* [3], i.e. a stable non-trivial equilibrium and metabolic explosion.
- Find out which parameters are important in determining when metabolic explosion occurs.

2 A New Model

This model is an extended version of the well understood toy model [1], the 'Old Lady'. The aim is to have a more descriptive model in which metabolites and reactions that are important in the dynamics of glycolysis are now added to consideration.

The metabolites which are added are pyruvate and NADH. NAD^+ and NADH have a conservation law, yielding that only NADH needs to be considered.
The same reactions are used, which are all lumped combinations of several individual chemical reactions with corresponding enzymes. The ethanol-production is now split up into 2 reactions, the first of which produces pyruvate. An extra lumped reaction is added, which converts 2 pyruvate into succinate using 4 ATP to produce 3 NADH.

The choice to add NADH and NAD^+ to consideration comes natural, because it is an energy carrier that is connected to many of the enzymatic reactions of yeast glycolysis. They are the most connected metabolites after ATP and ADP. In the first lumped part of ethanol production (v_2 in the Old Lady) NADH is produced, but in the second part an equal amount is consumed. Therefore to truely add NADH to consideration, these two parts have to be split in two reactions.
The side branch of succinate production is a choice that follows the choice to consider NADH. Without some side branch, there is no way that the consumption of NADH for the production of glycerol (v_3 in the Old Lady) can be canceled by another reaction. This follows as the only NADH producing reaction, v_2 below, is followed by a NADH consuming reaction, v_4 below, yielding no NADH production. Thus some extra (lumped) NADH producing reaction is needed. Another NADH producing branch could have been chosen, but in this model it is succinate production.
The choice to split at pyruvate comes natural as well. Partly because puruvate is a vastly connected metabolite in yeast metabolism and therefore it is not realistic to consider pyruvate merely as an intermediate metabolite. Also because it is the metabolite where the succinate branch splits from yeast glycolysis.

2.1 Mathematical Modeling

This can be denoted in formulas. The stoichiometry is clear: of all reactions it is exactly known which metabolites it consumes, which it produces and in which proportion. The reaction flux as a function of the metabolite concentrations is not known and is usually a topic of much debate [4]. For instance, the specific form of the reaction fluxes could be based on mathematical reduction from a descriptive model. However, certain characteristics of these functions can be assumed, with one exception.

1. When the concentration is zero of a metabolite which is consumed in a reaction, the reaction flux will be zero. For example, if there is no fbp present, then no pyruvate will be produced.

2. The reaction flux will be monotone increasing in the concentrations of the metabolites it consumes. This then increases to an upper bound, because

of saturation. This is of course a relation that only holds if the other concentrations of metabolites it consumes are not zero.

3. For this model it is also assumed that there is *no product inhibition*: the reaction flux only depends on the concentrations of metabolites it consumes, not the concentrations of its products.

For this model, specific functions are chosen which have these properties, namely the products of Michaelis-Menten (MM) rates for the individual metabolites. Some functions are assumed to be linear.
The specific form of a MM rate is for example, for the metabolite f in v_2 below:

$$F_2(f) = F_2^\infty \cdot \frac{f}{k_{2,f} + f}, \text{ where } F_2^\infty, k_{2,f} \in \mathbb{R}_+. \tag{1}$$

Here F_2^∞ is the saturation constant with the property that $\lim_{f\to\infty} F_2(f) = F_2^\infty$ and $k_{2,f}$ is the constant of half-value, with the property that $F_2(k_{2,f}) = \frac{1}{2}F_2^\infty$.

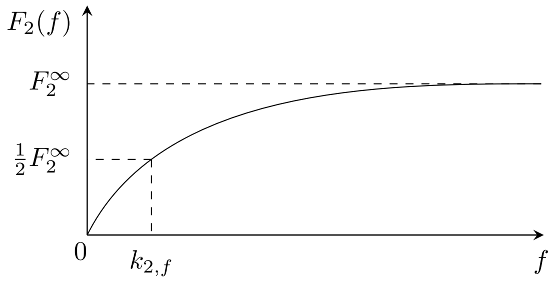


Figure 5: MM rate behaviour.

The exception is v_1, which is lumped from some reactions, including the so-called *PFK modeller's nightmare.* This enzyme is vastly complex with many binding sites for metabolites and feedback mechanisms. A specific function is assumed, derived by Teusink *et al.* [2], but now solely based on the ATP concentration a. This function *is not* monotone increasing in a:

$$\begin{aligned} H(a) &:= V_1 \frac{a(c_1 + a)}{(c_1 + a)^2 + L\left(\frac{K + c_2 a}{K + a}\right)^2 (K + c_3)^2} \\ &= V_1 h(a) \end{aligned} \tag{2}$$

It has a maximum and then decreases to the limit of H for large a: $H(\infty) := \frac{V_1}{1+LC_2^2C_3^2}$, where $C_2 = \frac{c_2}{K}, C_3 = c_3 + K$. V_1 is used for the Old Lady as the main bifurcation parameter and is important here also.

Furthermore it is assumed that the following conservation laws hold:

$$[\text{ATP}] + [\text{ADP}] = A_T, \qquad [\text{NADH}] + [\text{NAD}] = N_T \tag{3}$$

2.2 Model Description

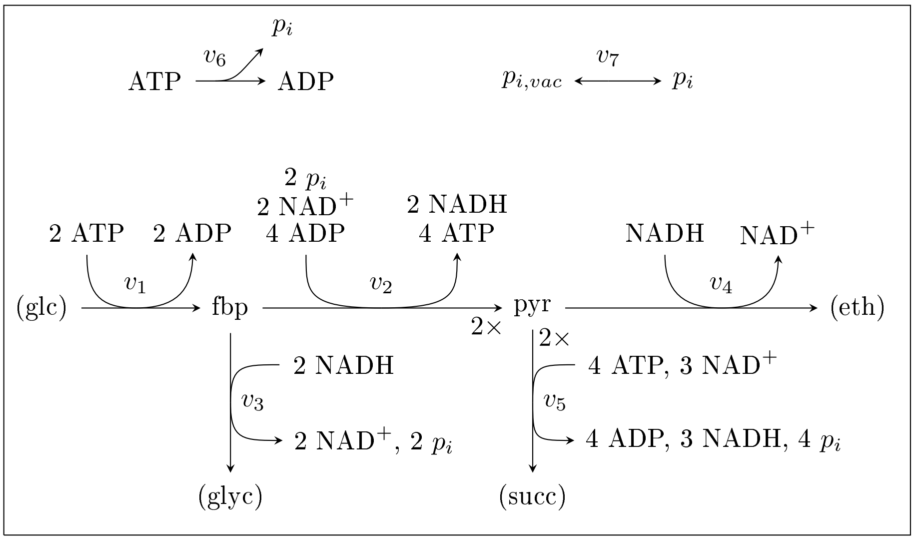


Figure 6: Reaction network model

The equations for fbp (f), phosphate ions (p), pyruvate (y), ATP (a) and NADH (n) are given below. Note that ADP and NAD^+ can be disregarded by the conservation laws (3). It is assumed that all values are in $\mathbb{R}_+$ and $a \leq A_T$, $n \leq N_T$. One exception is made for the value of f for the metabolic explosion and therefore $f \in \mathbb{R}_+ \cup \{\infty\}$ is assumed.
The system of ordinary differential equations (ODE) that describe the dynamics of these concentrations in time is now:

$$\begin{array}{lcllllllll} \dot{f} & = & v_1 & -v_2 & -v_3 & & & & \\ \dot{p} & = & & -2v_2 & +2v_3 & & +4v_5 & +v_6 & +v_7 \\ \dot{y} & = & & +2v_2 & & -v_4 & -2v_5 & & \\ \dot{a} & = & -2v_1 & +4v_2 & & & -4v_5 & -v_6 & \\ \dot{n} & = & & +2v_2 & -2v_3 & -v_4 & +3v_5 & & \end{array} \tag{4}$$

where:

$$\begin{array}{lcl} v_1 & = & H(a) \\ v_2 & = & F_2(f)B_2(A_T - a)P_2(p)D_2(N_T - n) \\ v_3 & = & F_3(f)N_3(n) \\ v_4 & = & Y_4(y)N_4(n) \\ v_5 & = & Y_5(y)A_5(a)D_5(N_T - n) \\ v_6 & = & r_6 a \\ v_7 & = & r_7(\Pi - p) \end{array} \tag{5}$$

Remember from the Old Lady that Π was the p_i concentration in the cell vacuole, which buffers the intracellular concentration of p_i. The values [ADP] and [NAD] are represented through $A_T - a$ and $N_T - n$ respectively. The following are MM rates: $F_2, B_2, P_2, D_2, F_3, N_3, Y_4, N_4, Y_5, A_5, D_5$.

2.3 Notation

Definition 2.1. *Some notation is introduced:*

Metabolite concentration space:

$$M = \left\{ \begin{pmatrix} f \\ p \\ y \\ a \\ n \end{pmatrix} : \begin{array}{rcl} f & \in & \mathbb{R}_+ \cup \{\infty\} \\ p, y & \in & \mathbb{R}_+ \\ a & \in & [0, A_T] \\ n & \in & [0, N_T] \end{array} \right\}$$

$$= (\mathbb{R}_+ \cup \{\infty\}) \times \mathbb{R}_+^2 \times [0, A_T] \times [0, N_T]$$

Reaction flux space:

$$V = \left\{ \begin{pmatrix} v_1 \\ \vdots \\ v_7 \end{pmatrix} \in \mathbb{R}^7 : v_1, \ldots, v_6 \geq 0 \right\}$$

$$= \mathbb{R}_+^6 \times \mathbb{R}.$$

Stoichiometric Matrix:

$$S = \begin{pmatrix} +1 & -1 & -1 & 0 & 0 & 0 & 0 \\ 0 & -2 & +2 & 0 & +4 & +1 & +1 \\ 0 & +2 & 0 & -1 & -2 & 0 & 0 \\ -2 & +4 & 0 & 0 & -4 & -1 & 0 \\ 0 & +2 & -2 & -1 & +3 & 0 & 0 \end{pmatrix}$$

Reaction fluxes: $\Phi : M \to V$

$$\vec{m} \mapsto \begin{pmatrix} H(a) \\ F_2(f)B_2(A_T - a)P_2(p)D_2(N_T - n) \\ F_3(f)N_3(n) \\ Y_4(y)N_4(n) \\ Y_5(y)A_5(a)D_5(N_T - n) \\ r_6 a \\ r_7(\Pi - p) \end{pmatrix}$$

Note that in the definition of V, the positivity constraints apply to those reactions that are considered irreversible only. The mathematical model (4), with given (5) can now be described in this notation. Let $\vec{m} \in M$, $\vec{v} \in V$.

$$\boxed{\frac{\mathrm{d}}{\mathrm{d}t}\vec{m} = S\vec{v} = S \cdot \Phi(\vec{m})} \tag{6}$$

3 Elementary Modes

For a general metabolic network with n metabolites and m reactions, the model can be described with the $n \times m$ stoichiometric matrix $S = (s_{i,j})$ in which $s_{i,j} \in \mathbb{Z}$ is an integer. It denotes the effect of reaction j on metabolite i, either positive for the number of molecules it produces or negative for the number of molecules it consumes.

$$\frac{\mathrm{d}}{\mathrm{d}t}\vec{m} = S\vec{v} \tag{7}$$

Metabolic network analysis can focus on the steady state behaviour, i.e. $S\vec{v} = 0$. This seems like a linear algebra problem, but there are constraints on the reaction fluxes yielding that the linear problem is not considered over a vector space. For example: often, some reaction fluxes can only be positive, like V in the new model, or they are bounded. The goal is to find all balanced configurations of *feasible* reaction fluxes such that the metabolic concentrations are constant, i.e. a configuration for which the reaction fluxes are within the given constraints and for all metabolites the rate of production equals the rate of consumption.

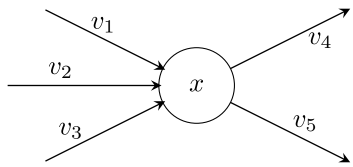


Figure 7: Example: metabolite x is in steady state when the production ($v_1 + v_2 + v_3$) equals the consumption ($v_4 + v_5$).

A lot of work has been done in this field and it is still developping. An overview of the research is given in the Bachelor's thesis of L.L. Diemer [5]. The work of Schuster *et al.* [6], [7] will be used here to compute the elementary modes. This aims to describe and compute within reasonable time, the steady state flux cone.

Definition 3.1. *For a reaction flux space V and stoichiometry S, one defines the* <u>*steady state flux cone*</u> *as follows.*

$$\mathcal{C} := \{\vec{v} \in V : S \cdot \vec{v} = 0\}$$

As one usually has $m > n$, there are multiple solutions to this problem. In fact, if two feasible solutions exist, any positive linear combination of them is also a solution, hence infinitely many configurations of reaction fluxes in $\mathcal{C}$. A way to represent the full space with finite elements is described below.

Definition 3.2. *For a vector space W over $\mathbb{R}, n \in \mathbb{N}, w_1, \ldots, w_n \in W$. The* <u>*positive linear span*</u> *is defined as*

$$\mathrm{sp}_+(w_1, \ldots, w_n) := \left\{ \sum_{i=1}^{n} \lambda_i w_i : \lambda_i \in \mathbb{R}_+ \right\} \tag{8}$$

In [6] it is shown that $\mathcal{C}$ is the positive span of a few elementary modes. In [7] an algorithm is provided to compute the specific vectors that represent $\mathcal{C}$.

3.1 Algorithm

To find elementary modes for a metabolic network with only positivity constraints, one can apply the following algorithm. This is a description to inspire insight, a more computational relevant description can be found in [7].

- For all reversible reaction fluxes, add its column times -1 to S to represent its reverse effect. This splits the reaction into two one-way reactions and increases m.
- Denote an identity matrix next to the transverse of S, such that the column lengths agree. This will be the first tableau $T^{(0)}$. All tableaus are $m \times (n+m)$ matrices.
- For all metabolites $i = 1, 2, \ldots, n$ use the tableau $T^{(i-1)}$ to create the next tableau. Its column $m+i$ represents the effects of the rows on metabolite i.
    1. Copy all rows of $T^{(i-1)}$ into $T^{(i)}$ which have a 0 in place $m+i$.
    2. If two rows are nonzero in place $m+i$, such that one is negative and one positive, denote in the new tableau a positive combination of the two rows such that this sum is 0 in place $m+i$. Thus all feasible combinations of reaction fluxes which are balanced for this metabolite are now found.
    3. Remove all rows which are in the positive linear span of other rows.
- Consider only the matrix A of the first m columns of the tableau as the right part is now a zero matrix. As reversible reactions were split, take the sum of both columns which represent the two one-way reactions. Add this column to A while removing the two columns of the one-way reactions.
- Remove all zero rows of A, which are still in there for the futile cycles (the two one way reactions balance each other).

The result is that the rows of the matrix A represent the Elementary Modes for the metabolic network. An example of an application of the algorithm can be found in Appendix A.

Remark 1. There are ways to use a similar algorithm for networks with various other constraints.

3.2 EMs for the Old Lady

The model is as described in Section 1.3.
The elementary modes are

$$OL_1 = \begin{pmatrix} 1 \\ 1 \\ 0 \\ 2 \\ 0 \end{pmatrix}, \qquad OL_2 = \begin{pmatrix} 2 \\ 1 \\ 1 \\ 0 \\ 0 \end{pmatrix}.$$

3.3 EMs for the New Model

The new model described in Section 2.2 has the following elementary modes:

$$EM_1 = \begin{pmatrix} 1 \\ 1 \\ 0 \\ 2 \\ 0 \\ 2 \\ 0 \end{pmatrix}, \qquad EM_2 = \begin{pmatrix} 14 \\ 9 \\ 5 \\ 14 \\ 2 \\ 0 \\ 0 \end{pmatrix} \tag{9}$$

These elementary modes are explicitely calculated in Appendix A.

Figure 8 shows the elementary modes as pathways in the model depicted in Figure 6.

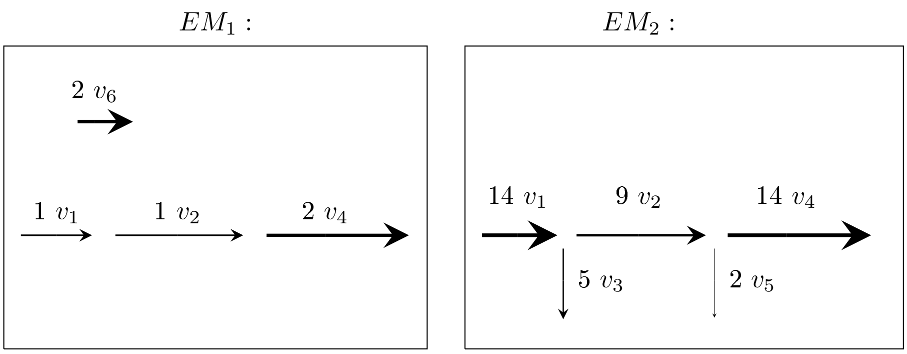


Figure 8: The elementary modes (9) of (6).

4 Existence of Steady States

Consider the new model described by (6). The notation follows Definition 2.1 and Definition 3.1.

At steady state $\frac{\mathrm{d}}{\mathrm{d}t}\vec{m} = S \cdot \Phi(\vec{m}) = 0$. There are two cases:

- $\Phi(\vec{m}) = 0$, the *trivial steady states.*
- $\Phi(\vec{m}) \neq 0$, but $S \cdot \Phi(\vec{m}) = 0$: the *non-trivial steady states.*

4.1 Trivial Steady States

If $\Phi(\vec{m}) = 0$, all reaction fluxes are zero and one has a trivial steady state. From the formulas (5), one can easily see that $p = \Pi$ and $a = 0$. If $f \neq 0$, then either v_2 or v_3 is nonzero, hence any trivial steady state has $f = 0$. Now only $v_4 = 0$ is not automatically satisfied. This yields two families of steady states:

- $\vec{m} = (0, \Pi, 0, 0, n)^T$, for any $n \in [0, N_T]$: the *n-family,*
- $\vec{m} = (0, \Pi, y, 0, 0)^T$, for any $y \in \mathbb{R}_+$: the *y-family.*

4.2 Non-Trivial Steady States

Remark 2. In this section many arguments are used where it is shown that two non-trivial functions have exactly one intersection. The n-coordinate or a-coordinate of this intersection is then considered as an implicitly defined function of some other variables. Continuity of these functions is not always shown, but often indirectly used further on. Showing continuity is not a great issue as the graphs of the reaction fluxes are continuously differentiable and usually it would be a rather straightforward application of an Implicit Function Theorem, Theorem 3.2.1, page 36 in [8], to show this continuity or even continuous differentiability.

4.2.1 Existence part 1

When $\Phi(\vec{m}) \neq 0$ one has a non-trivial steady state. By EM theory, the possibilities for $\Phi(\vec{m})$ are restricted to a convex cone $\mathcal{C} \subset V$, the steady state flux cone (see Definition 3.1).
As computed in Appendix A, $\mathcal{C} = \mathrm{sp}_+(EM_1, EM_2)$, with elementary modes EM_1 and EM_2 as in (9).
Thus $\vec{m} \in M$ is a non-trivial steady state if and only if

$$\Phi(\vec{m}) \in \mathrm{sp}_+(EM_1, EM_2) \backslash \{0\}.$$

Remark 3. Both EM_1 and EM_2 have $v_1 > 0, v_2 > 0, v_4 > 0$. $a = 0$ implies $v_1 = 0$. $f = 0$, $p = 0$, $a = A_T$ or $n = N_T$ implies $v_2 = 0$. $y = 0$ or $n = 0$ implies $v_4 = 0$. Therefore any non-trivial steady state value has $f, p, y, a, n > 0$ and $a < A_T, n < N_T$.

To find the non-trivial steady states, Hulshof *et al.* [1] consider equations that hold on all steady states and using monotone properties of the reaction fluxes, unique existence of the steady state value of [ATP] is found. Here one also has monotone properties of reaction fluxes as assumed in Section 2.1. To

find equations which yield unique existence for steady state values, one can use the structure of $\mathcal{C}$, i.e. the elementary modes.
Equations that hold for all $v \in \mathcal{C}$ can be found in a generic way.

Definition 4.1. *The solution matrix for a steady state flux cone is the matrix with the elementary modes as rows.*
For $\mathcal{C}$ one has

$$A = \begin{pmatrix} 1 & 1 & 0 & 2 & 0 & 2 & 0 \\ 14 & 9 & 5 & 14 & 2 & 0 & 0 \end{pmatrix} \tag{10}$$

Proposition 4.2. *Let W be an m-dimensional vector space of reaction fluxes, $\mathcal{D} \subset W$ be a steady state flux cone spanned by n elementary currents, A its solution matrix, $x \in \mathbb{R}^m$, then*

$$\text{For all } \vec{v} \in \mathcal{D}, \quad x \cdot \vec{v} = 0 \iff Ax = 0$$

Proof. Let $i = 1, \ldots, n$. Denote the rows of A as A_i.

- $\Rightarrow$ Consider the i^{th} element of Ax: $A_i \cdot x$. The rows of A are the elementary currents, so $A_i \in \mathcal{D}$. Thus, from the given property, we find $A_i \cdot x = 0$. i was chosen arbitrarily, so $Ax = 0$.
- $\Leftarrow$ Let $\vec{v} \in \mathcal{D}$. Note that $\mathcal{D} = \mathrm{sp}_+(A_i)$, hence there exist $\lambda_i \in \mathbb{R}_+$ such that $\vec{v} = \sum_{i=1}^n \lambda_i A_i$. Also $Ax = 0$ implies $A_i \cdot x = 0$.

$$x \cdot \vec{v} = x \cdot \sum_{i=1}^{n} \lambda_i A_i = \sum_{i=1}^{n} \lambda_i (x \cdot A_i) = 0$$

□

From this it is found for $\mathcal{C}$ that if $x \in \ker(A)$, then $x \cdot \vec{v} = 0$ is an equation that holds for any steady state flux distribution $\vec{v}$. So any relationship satisfied by some specific flux v_j, $j = 1, \ldots, 7$, is obtained by setting $x_j \neq 0$ and trying any configuration such that $Ax = 0$. Then $x \cdot \vec{v} = 0$ yields such a relationship.

It turns out that it is useful to have a description of $\ker(A)$ available in terms of basis vectors, such a set is not unique of course, but provides a good overview over all possible relationships for a particular v_j. In the 'New Model', $\ker(A)$ can be specified directly:

Corollary 4.3. *One basis B that can be chosen such that $\ker(A) = \mathrm{sp}(B)$ is:*

$$B = \left\{ \begin{pmatrix} 0 \\ 0 \\ 2 \\ 0 \\ -5 \\ 0 \\ 0 \end{pmatrix}, \begin{pmatrix} 0 \\ 10 \\ -4 \\ -5 \\ 0 \\ 0 \\ 0 \end{pmatrix}, \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \end{pmatrix}, \begin{pmatrix} 1 \\ -1 \\ -1 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}, \begin{pmatrix} 0 \\ 0 \\ 0 \\ -1 \\ 7 \\ 1 \\ 0 \end{pmatrix} \right\} \tag{11}$$

The basis vectors correspond to the following relationships that hold on $\mathcal{C}$:

$$2v_3 = 5v_5 \tag{12}$$
$$10v_2 = 4v_3 + 5v_4 \tag{13}$$
$$v_7 = 0 \tag{14}$$
$$v_1 = v_2 + v_3 \tag{15}$$
$$v_6 = v_4 - 7v_5. \tag{16}$$

Proof. It is clear that for all $x \in B$, $A \cdot x = 0$, also the vectors are linearly independent, so it follows from Proposition 4.2 □

Why these specific vectors were chosen to represent $\ker(A)$ is explained in the following remark.

Remark 4. When trying to find a unique value for n, one wants to use monotonicity. The reason is that an equation with monotone increasing left hand side (lhs) and monotone decreasing right hand side (rhs) often yields unique solutions.

Thus one tries to equate a function which is monotone increasing in n to one that is monotone decreasing in n. There are two fluxes that are monotone decreasing in n: v_2 and v_5. There are two fluxes that are monotone decreasing in n: v_3 and v_4.

- Set $x_1 = x_2 = x_6 = x_7 = 0$ to find an equation for v_5 in v_3 and v_4. Then $Ax = \begin{pmatrix} 2x_4 \\ 5x_3 + 14x_4 + 2x_5 \end{pmatrix}$. We find the unique solutions $x_4 = 0$, $x_3 = -\frac{2}{5}x_5$. Set $x_5 = 5$ to get integers. This is the first element of B. We find for $x \cdot \vec{v} = 0$:
$$5v_5 = 2v_3$$
- Set $x_1 = x_5 = x_6 = x_7 = 0$ to find an equation for v_2 in v_3 and v_4. Then $Ax = \begin{pmatrix} x_2 + 2x_4 \\ 9x_2 + 5x_3 + 14x_4 \end{pmatrix}$. We find the unique solutions $x_4 = -\frac{1}{2}x_2$, $x_3 = -\frac{2}{5}x_2$. Set $x_2 = 10$ to get integers. This is the second element of B. We find for $x \cdot \vec{v} = 0$:
$$10v_2 = 4v_3 + 5v_4.$$

And so all ways to describe the unique value n has in steady state have been found.

Another way to find these equations is:

$$\begin{aligned}
\dot{p} + \dot{a} = 0: \quad & v_7 = 2(v_1 - v_2 - v_3) \\
& v_7 = 2\dot{f} = 0 \\
\dot{n} = \dot{y}: \quad & 2v_2 - 2v_3 - v_4 + 3v_5 \\
& = 2v_2 - v_4 - 2v_5 \\
& 2v_3 = 5v_5 \\
3\dot{y} + 2\dot{n} = 0: \quad & 10v_1 - 4v_3 - 5v_4 = 0 \\
& 10v_2 = 4v_3 + 5v_4
\end{aligned}$$

Theorem 4.4. *Let $\vec{m} \in M$,then the following statements hold:*

1. *For all $f, y > 0$, $a \in (0, A_T]$, there exists a unique n, $n^{*1}(f, y, a)$, such that (12) holds.*

2. *For all $f, p, y > 0$, $a \in [0, A_T)$ there exists a unique value of n, $n^{*2}(f, p, y, a)$, such that (13) holds.*

3. *For all $f, y, p > 0$, there exist unique values of n, $n^*(f, p, y)$, and a, $a^*(f, p, y)$, such that (12) and (13) are satisfied.*

4. *$\vec{m}$ can only be a non-trivial steady state when $p = \Pi$.*

Proof. Note that Corollary 4.3 yields that $\vec{m}$ is only a non-trivial steady state if (12), (13) and (14) hold. Use (5) to rewrite them to

$$2F_3N_3 = 5Y_5A_5D_5 \tag{17}$$
$$10F_2B_2D_2P_2 = 4F_3N_3 + 5Y_4N_4. \tag{18}$$
$$r_7(\Pi - p) = 0 \tag{19}$$

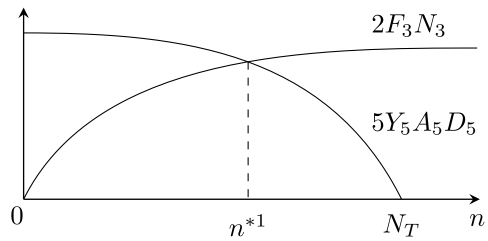


Figure 9: Graphical representation of argument for n^{*1}.

1. Let $f, y > 0$, $a \in (0, A_T]$. Considering (17), note that

   - $2F_3(f)N_3(0) = 0$ and $2F_3(f)N_3(N_T) > 0$.
   - $5Y_5(y)A_5(a)D_5(0) = 0$ and $5Y_5(y)A_5(a)D_5(N_T) > 0$.

   By assumption, v_3 and v_5 are monotone increasing in the concentrations of metabolites they consume when the other metabolites are present. Thus v_3 is monotone increasing in n and v_5 monotone increasing in $d = N_T - n$, the concentration of NAD$^+$. v_5 is therefore monotone decreasing in n. Hence (12) holds for a unique value $n^{*1}(f, y, a) \in (0, N_T)$ for n.

2. Consider (18) and use the same monotonicity argument as before: assume $f, p, y > 0$ and assume $a < A_T$ to find the non-trivial value of n, then note that

   - $10F_2(f)B_2(A_T - a)D_2(N_T - 0)P_2(p) > 0$ for $n = 0$ and $10F_2(f)B_2(A_T - a)D_2(N_T - N_T)P_2(p) = 0$ for $n = N_T$.
   - $4F_3(f)N_3(0) + 5Y_4(y)N_4(0) = 0$ for $n = 0$ and $4F_3(f)N_3(N_T) + 5Y_4(y)N_4(N_T) > 0$ for $n = N_T$.

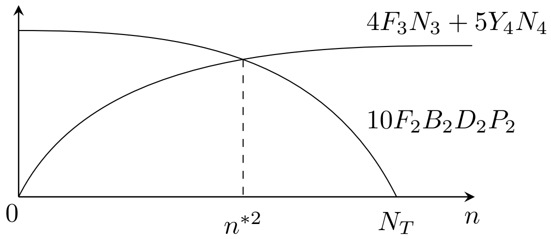


Figure 10: Graphical representation of argument for n^{*2}

v_2 is monotone increasing in $d = N_T - n$, thus monotone decreasing in n. v_3 and v_4 are monotone increasing in n. These monotone behaviours together with the lhs and rhs of (13) at $n = 0$ and $n = N_T$ given above yield that (13) holds for a unique value $n^{*2}(f, p, y, a) \in (0, N_T)$ for n.

3. For both (12) and (13) to hold, which is the case in a non-trivial steady state, it is needed that $n^{*1} = n^{*2}$. It will be shown that this restriction $n^{*1} = n^{*2}$ defines the non-trivial steady state value of a.

   n^{*1} is monotone increasing from 0 in a and n^{*2} is monotone decreasing to 0 in a. This will be shown rigorously. For graphic explanation, consider the figures above.

   - When $f, y > 0$, $a \in (0, A_T]$ there is a unique value of n^{*1}. If now f and y remain the same while a increases, then the defining equation for n^{*1}, (17) changes. $2v_3$ is independent of a, so the lhs will remain the same. $5v_5$ increases with increasing a as it consumes ATP. As a function of n it increases when a increases for every $n \in [0, N_T)$, but remains 0 for $n = N_T$. Hence the intersection of the two graphs will be at a higher value n^{*1} for a higher value of a.
     Thus n^{*1} is monotone increasing in a.
   - When $f, p, y > 0$, $a \in [0, A_T)$, there is a unique value of n^{*2}. If now f, p and y remain the same while a increases (but remains smaller than A_T), then the defining equation for n^{*2}, (18), changes. $4v_4$ is independent of a, so the rhs will remain the same. $10v_2$ decreases with increasing a as v_2 consumes ADP. As a function of n, it decreases when a increases for every $n \in [0, N_T)$, but remains 0 for $n = N_T$. Hence the intersection of the two graphs will be at a lower value n^{*2} for a higher value of a.
     Thus n^{*2} is monotone decreasing in a.

   Now consider the boundary values of n^{*1} and n^{*2} at $a = 0$ and $a = A_T$:

   If $a = 0$ (17) yields that $F_3(f)N_3(n) = 0$ and it is assumed that $f > 0$, so $F_3(f) > 0$, hence $N_3(n) = 0 \Leftrightarrow n^{*1} = 0$.
   (18) still yields a non-trivial unique intersection point by the above reasoning for n^{*2}, so $n^{*2} > 0$.

   If $a = A_T$ (17) still yields a non-trivial unique intersection point by the above reasoning for n^{*1}, so $n^{*1} > 0$.

(18) yields that $4F_3(f)N_3(n) + 5Y_4(y)N_4(n) = 0$ and it is assumed that $f, y > 0$, so $F_3(f), Y_4(y) > 0$, hence $N_3(n) = N_4(n) = 0 \Leftrightarrow n^{*2} = 0$.

Thus for all $f, p, y > 0$, $n^{*1}(f, y, a) = n^{*2}(f, p, y, a)$ yields a unique solution $a^*(f, p, y) \in (0, A_T)$. This is the non-trivial steady state value of a.

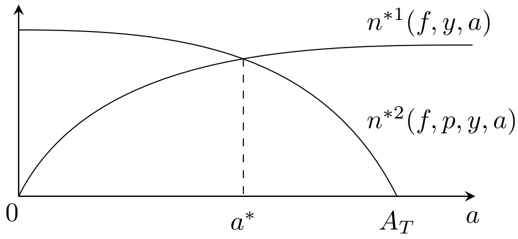


Figure 11: Graphical representation of argument for a^*.

Denote $n^*(f, p, y) := n^{*1}(f, y, a^*(f, p, y))$, in this way, $n^{*1} = n^{*2}$ always holds.

4. $r_7 > 0$, so it follows directly from (19) that $p = \Pi$.

□

Remark 5. Biologists can grow yeast cells in such a way that certain processes, reaction fluxes, of the glycolysis can be altered. For instance the ATPase can be increased. In mathematical terms this means that certain parameters can be altered in the model, the example leads to a higher r_6. The goal of this thesis is to find parameters which for different values lead to all types of behaviour we are interested in. Therefore we focus on V_1, which for increased values speeds up the fbp production, and r_6, the ATPase parameter.

So far it is known that: if one has a non-trivial steady state $\vec{m}$ for (6), then $p = \Pi$, $n = n^*(f, \Pi, y)$ and $a = a^*(f, \Pi, y)$. It is probably impossible to have a non-trivial steady state for every parameter setting, but one can set f and y and find unique parameter settings for which they yield a non-trivial steady state.

Definition 4.5. *For $f, y \in \mathbb{R}_+$, define*

$$\vec{m}^*(f, y) = (f, \Pi, y, a^*(f, \Pi, y), n^*(f, \Pi, y))^T \tag{20}$$

and put

$$\begin{aligned} \mathrm{Int}(\mathbb{R}^2_+) &= \{(f, y) \in \mathbb{R}_+ : f, y > 0\} \\ \Omega &= \left\{(f, y) \in \mathrm{Int}(\mathbb{R}^2_+) : \Psi(f, y) > 0\right\} & (21) \\ \textit{with } \Psi(f, y) &= Y_4(y)N_4(n^*) - 7Y_5(y)A_5(a^*)D_5(N_T - n^*) & (22) \\ \textit{where } n^* &= n^*(f, \Pi, y), \\ a^* &= a^*(f, \Pi, y) \end{aligned}$$

As a^* and n^* are continuous on $\mathrm{Int}(\mathbb{R}^2_+)$ and the Michaelis-Menten functions Y_4, N_4, Y_5, A_5, D_5 are continuous, it is readily seen that Ω is open. Below the question when $\Omega \neq \emptyset$ will be considered. Note that both $\vec{m}^*$ and Ω are independent of the parameters V_1 and r_6. These two objects are interesting because of

Theorem 4.6 (Existence part 1)**.** *The following hold:*

(i) If $f, y, V_1, r_6 > 0$ are such that $\vec{m}^*(f, y)$ is a non-trivial steady state, then $(f, y) \in \Omega$.

(ii) If $\Omega \neq \emptyset$, then for each $(f, y) \in \Omega$, there exist unique $V_1, r_6 > 0$ such that $\vec{m}^*(f, y)$ is a non-trivial steady state.

Proof. (i) If $f, y, V_1, r_6 > 0$ are such that $\vec{m}^*(f, y)$ is a non-trivial steady state, then $\Phi(\vec{m}^*) \in \mathcal{C}$. Thus (16) holds and it follows directly that

$$0 < r_6 a^*(f, \Pi, y) = v_4(\vec{m}^*) - 7v_5(\vec{m}^*) = \Psi(f, y).$$

Hence, $(f, y) \in \Omega$.

(ii) If $\Omega \neq \emptyset$, then there exist $(f, y) \in \Omega$. For these values of f and y, $\Psi(f, y) > 0$. Hence for $\vec{m}^*(f, y)$

- (16) holds if $v_6(\vec{m}^*) = \Psi(f, y)$. $\Psi(f, y) > 0$ is set and $a^*(f, \Pi, y) > 0$ is set. Hence as $r_6 = 0$ yields $v_6 = 0$, there is a unique value for r_6 such that (16) holds.
- Recall that $H(a) = V_1 \cdot h(a)$ for some function h. $v_2(\vec{m}^*) + v_3(\vec{m}^*) > 0$ is set and $h(a^*(f, \Pi, y)) > 0$ is set. Hence as $V_1 = 0$ yields $v_1 = 0$, there is a unique value for $V_1 > 0$ such that (15) holds.

□

Denote these values $V_1(f, y)$ and $r_6(f, y)$. Note that by the above proof, the values are given explicitly, but using the implicit functions n^* and a^*.

$$V_1(f, y) = \frac{F_2(f)B_2(A_T - a^*(f,\Pi,y))P_2(\Pi)D_2(N_T - n^*(f,\Pi,y)) + F_3(f)N_3(n^*(f,\Pi,y))}{h(a^*(f,\Pi,y))}$$

$$r_6(f, y) = \frac{Y_4(y)N_4(n^*(f,\Pi,y)) - 7Y_5(y)A_5(a^*(f,\Pi,y))D_5(N_T - n^*(f,\Pi,y))}{a^*(f,\Pi,y)}$$

The found non-trivial steady states exist only for $(f, y) \in \Omega$. It turned out to be hard to characterize when $\Omega \neq \emptyset$. The following observation provides a sufficient condition:

Lemma 4.7. *If*

$$\sup_{(f,y)\in\mathrm{Int}(\mathbb{R}^2_+)} v_4(\vec{m}^*(f, y)) > 7 \sup_{(f,y)\in\mathrm{Int}(\mathbb{R}^2_+)} v_5(\vec{m}^*(f, y)), \tag{23}$$

then $\Omega \neq \emptyset$.

Proof. Assume that (23) holds. Note that v_4 is bounded by definition: for all $(f, y) \in \mathrm{Int}(\mathbb{R}^2_+)$, $v_4 < Y_4^\infty N_4^\infty$, with Y_4^∞, N_4^∞ the saturation constants of the MM kinetics, so the supremum of v_4 is approached. Thus there exist $(f_1, y_1) \in \mathrm{Int}(\mathbb{R}_+^2)$ such that

$$\begin{aligned} v_4(\vec{m}^*(f_1, y_1)) &> 7 \sup_{(f,y)\in\mathrm{Int}(\mathbb{R}_+^2)} v_5(\vec{m}^*(f,y)) \\ &\geq 7 v_5(\vec{m}^*(f_1, y_1)) \end{aligned}$$

Thus $\Psi(f_1, y_1) > 0$ and by Definition 4.5 it holds that $(f_1, y_1) \in \Omega$. Thus $\Omega \neq \emptyset$. □

In order to establish what restrictions on the parameters yield $\Omega \neq \emptyset$, further details are needed on the implicit functions a^* and n^* that occur in the Definition 4.5.

4.2.2 Properties of implicit solutions

The steady state solutions a^* and n^* have been proven to exist. They are defined as functions of $f, p, y \in \mathbb{R}_+$. As such they have certain properties.
For convenience some abreviations are used. The defining equations (12) and (13) for n^* and a^* follow in this notation:

$$\begin{aligned} \alpha(f,n) &:= 2F_3N_3 & \qquad (12): \quad \alpha = \beta \\ \beta(y,a,n) &:= 5Y_5A_5D_5 & \qquad (13): \quad \gamma = \delta \\ \gamma(f,p,a,n) &:= 10F_2B_2D_2P_2 \\ \delta(f,y,n) &:= 4F_3N_3 + 5Y_4N_4 \end{aligned}$$

Corollary 4.8. *In the proof of Theorem 4.4, some properties of n^{*1} and n^{*2} were already formulated and proved in order to prove the unique existence of a^*. Here they are reformulated.*

- *If $f, y > 0$, then n^{*1} is monotone increasing in a, from $n^{*1}(f, y, 0) = 0$ to $n^{*1}(f, y, A_T) > 0$.*
- *If $f, p, y > 0$, then n^{*2} is monotone decreasing in a, from $n^{*2}(f, p, y, 0) > 0$ to $n^{*2}(f, p, y, A_T) = 0$.*

Proof. See the proof of Theorem 4.4. □

Lemma 4.9. *The following monotonicity arguments hold:*

1. *For $a \in (0, A_T)$, n^{*1} is monotone decreasing in f for $y > 0$ and monotone increasing in y for $f > 0$.*
2. *For $a \in (0, A_T)$, $f > 0$, n^{*2} is monotone decreasing in y for $p > 0$ and monotone increasing in p for $y > 0$.*
3. *For $f > 0$, a^* is monotone decreasing in y for $p > 0$ and monotone increasing in p for $y > 0$.*
4. *For $f, y > 0$ n^* is monotone increasing in p.*

Proof. 1. n^{*1} is when (12) holds. α is increasing in f for all $n > 0$, β is not affected by f. Hence when f is increased, the intersection will be for a lower n.
α is not affected by y, β is increasing in y, hence when y is increased, the intersection will be for a higher n.

2. n^{*2} is when (13) holds. γ is unaffected by y and δ is monotone increasing in y for all $n > 0$. Hence when y is increased, the intersection will be for a lower n.
γ is increasing in p for all $n < N_T$ and δ is not affected by p. Hence when p is increased, the intersection will be for a higher n.

3. n^{*1} is monotone increasing in y and n^{*2} is monotone decreasing in y. Both a higher n^{*1} and a lower n^{*2} have the effect that the intersection of n^{*1} and n^{*2} will be for a lower a^* hence a^* is monotone decreasing in y.
Note that both α and β and therefore n^{*1} are not affected by p. By the previous assertion, n^{*2} is increasing in p, hence as n^{*1} is increasing in a, the intersection a^*, where $n^{*1} = n^{*2}$ is for a higher a.

4. n^{*1} is uninfluenced by p. Hence by the definition

$$n^*(f, p, y) := n^{*1}(f, y, a^*(f, p, y)),$$

n^* is influenced by p only through a^*. n^{*1} is increasing in a by Corollary 4.8. By the previous assertion a^* is increasing in p, hence n^* is monotone increasing in p. □

For graphic representation, consider the figures in the proof of Theorem 4.4. For example: n^{*2} is monotone decreasing in y, because for $\varepsilon > 0$ and y goes to $y + \varepsilon$, the graph of (13) changes as follows:

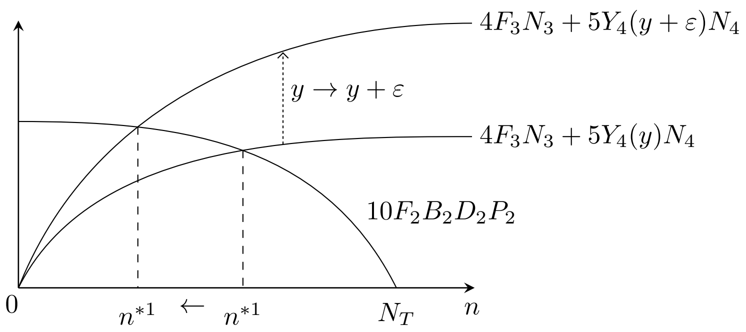


Figure 12: Graphical representation of monotonicity property of n^{*1}.

Because of these monotonicity properties in f and y, it is natural to consider the limits $f \downarrow 0$, $f \to \infty$ and $y \downarrow 0$, $y \to \infty$.

Lemma 4.10. *The following two assertions hold:*

1. *If* $p, y > 0$, *then* $\begin{cases} \lim_{f\to 0} n^*(f, p, y) = 0 \\ \lim_{f\to 0} a^*(f, p, y) = 0 \end{cases}$

2. If $f, p > 0$*, then* $\begin{cases} \lim_{y\to 0} n^*(f,p,y) = 0 \\ \lim_{y\to 0} a^*(f,p,y) = A_T \end{cases}$

Proof. 1. Let $p, y > 0$.

Claim: n^{*2}, the solution of (13), has an upper bound $\bar{n}$, such that $\bar{n} \to 0$ as $f \to 0$ for all a.

Proof of claim For $f \in \mathbb{R}_+$, γ has range:

$$[0, \bar{\gamma}], \qquad \bar{\gamma}(f,p) = 10F_2(f)B_2(A_T)D_2(N_T)P_2(p).$$

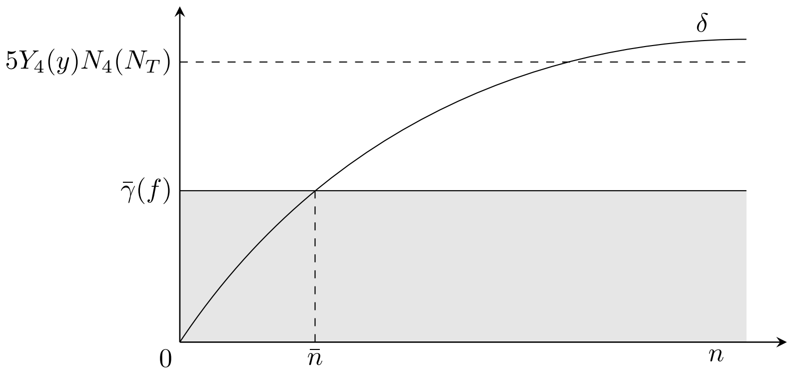
Its upper bound $\bar{\gamma}(f,p)$ will become small as f becomes small, because $F_2(0) = 0$.
For $f \in \mathbb{R}_+$, δ has range

$$[0, 4F_3(f)N_3(N_T) + 5Y_4(y)N_4(N_T)]$$

which is independent of a. As f becomes small, its upper bound will still be at least $5Y_4(y)N_4(N_T) > 0$.
As $f \to 0$, $\bar{\gamma}(f,p)$ will go to 0, in particular $\bar{\gamma}(f,p) < 5Y_4(y)N_4(N_T)$.
The part of the domain of δ which is mapped below $\bar{\gamma}$ is bounded by $\bar{n}$, if for $f \in \mathbb{R}_+$ $\bar{n}$ is such that

$$\delta(f, y, \bar{n}) = \bar{\gamma}(f,p).$$

δ is monotone increasing in n to at least $5Y_4(y)N_4(N_T)$, hence $\bar{n}$ becomes smaller as $\bar{\gamma}$ becomes smaller with decreasing f. The equality (13) can only occur in $[0, \bar{n}]$, hence the solution n^{*2} is below an upper bound $\bar{n}$, such that $\bar{n} \to 0$ as $f \to 0$ for all a.



The first part of this assertion now follows from the claim:
By definition one has $n^*(f,p,y) = n^{*2}(f,p,y,a^*)$, but $n^{*2} < \bar{n}$ for all $a \in [0, A_T]$, hence $n^* \to 0$ as $f \to 0$.

α is independent of a and, for $f \in \mathbb{R}_+$, it has range

$$[0, \bar{\alpha}(f)], \qquad \bar{\alpha}(f) = 2F_3(f)N_3(N_T)$$

So $\bar{\alpha}(f) \to 0$ as $f \to 0$.

Remember that a^* is such that $n^{*1}(f,y,a^*) = n^{*2}(f,p,y,a^*)$, hence for small f it follows from the claim that $n^{*1}(f,p,y,a^*) \in [0, \bar{n}]$.

β is monotone decreasing in n, thus for all $a \in [0, A_T]$ it has lower bound

$$\bar{\beta}(a) = 5Y_5(y)D_5(N_T - \bar{n})A_5(a)$$

on the domain $[0, \bar{n}]$.

Remember that n^{*1} is the intersection of α and β. For α and β to have an intersection on the domain $[0, \bar{n}]$

$$\bar{\alpha}(f) > \bar{\beta}(a)$$

has to hold. Hence a^* is such that this inequality will hold for all f.

If $f \to 0$, then $\bar{\alpha} \to 0$ and it follows that $\bar{\beta}(a) \to 0$. Also $Y_5(y) > 0$, $D_5(N_T - \bar{n}) \to D_5(N_T) > 0$ as $\bar{n} \to 0$, hence $A_5(a) \to 0$ follows. $A_5(a) = 0 \Leftrightarrow a = 0$, thus $a^* \to 0$ as $f \to 0$.

2. Let $f > 0$.

   Claim: n^{*1}, the solution of (12), has an upper bound $\bar{n}$, such that $\bar{n} \to 0$ as $y \to 0$ for all a.

   Proof of claim For $y \in \mathbb{R}_+$, β has range:

$$[0, \bar{\beta}], \qquad \bar{\beta}(y) = 5Y_5(y)A_5(A_T)N_5(N_T).$$

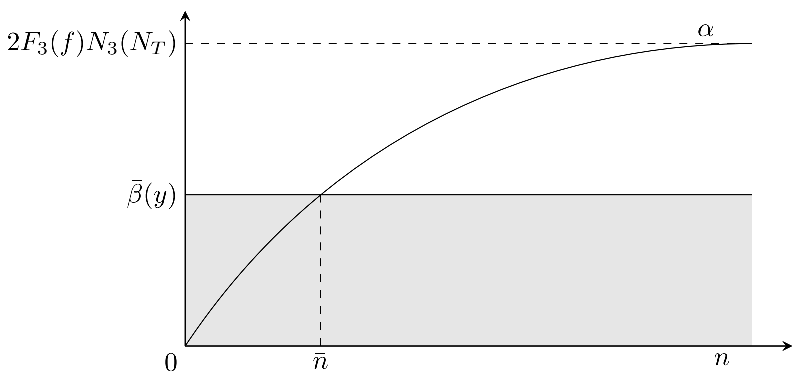
Its upper bound $\bar{\beta}(y)$ will become small as y becomes small, because $Y_5(0) = 0$.
α has range

$$[0, 2F_3(f)N_3(N_T)]$$

which is independent of a and y. For all y its upper bound will be $2F_3(f)N_3(N_T) > 0$.
As $y \to 0$, $\bar{\beta}(y)$ will go to 0, in particular $\bar{\beta}(y) < 2F_3(f)N_3(N_T)$. The part of the domain of α which is mapped below $\bar{\beta}$ is bounded by $\bar{n}$, if for $y \in \mathbb{R}_+$ $\bar{n}$ is such that

$$\alpha(f, \bar{n}) = \bar{\beta}(y).$$

α is monotone increasing in n to $2F_3(f)N_3(N_T)$, hence $\bar{n}$ becomes smaller as $\bar{\beta}(y)$ becomes smaller with decreasing y. The equality (17) can only occur in $[0, \bar{n}]$, hence the solution n^{*1} is below an upper bound $\bar{n}$, such that $\bar{n} \to 0$ as $y \to 0$ for all a.



The first part of this assertion now follows from the claim:
By definition one has $n^*(f,\Pi,y) = n^{*1}(f,y,a^*)$, but $n^{*1} < \bar{n}$ for all $a \in [0, A_T]$, hence $n^* \to 0$ as $f \to 0$.

Remember that a^* is such that $n^{*1}(f,a^*,y) = n^{*2}(f,\Pi,y,a^*)$, hence for small y it follows from the claim that $n^{*2}(f,\Pi,y,a^*) \in [0,\bar{n}]$.

δ is monotone increasing in n and independent of a, thus for small y it has upper bound

$$\bar{\delta}(y) = 4F_3(f)N_3(\bar{n}) + 5Y_4(y)N_4(\bar{n})$$

on the domain $[0,\bar{n}]$.

γ is monotone decreasing in n, thus for all $a \in [0, A_T]$ it has lower bound

$$\bar{\gamma}(a) = 10F_2(f)B_2(A_T - a)D_2(N_T - \bar{n}P_2(\Pi)$$

on the domain $[0,\bar{n}]$.

Remember that n^{*2} is the intersection of γ and δ. For γ and δ to have an intersection on the domain $[0,\bar{n}]$

$$\bar{\delta}(y) > \bar{\gamma}(a)$$

has to hold. Hence a^* is such that this inequality will hold for all y.

If $y \to 0$, then $\bar{\delta} \to 0$ and it follows that $\bar{\gamma}(a) \to 0$. Also $F_2(f) > 0$, $D_2(N_T - \bar{n}) \to D_5(N_T) > 0$ as $\bar{n} \to 0$, hence $B_2(A_T - a) \to 0$. $B_2(A_T - a) = 0 \Leftrightarrow a = A_T$, hence $a^* \to A_T$ as $f \to 0$.

□

Remark 6. With these results, one can extend the functions a^* and n^* continuously to where $f = 0$ or $y = 0$, but not both. Note that there is a discontinuity in a^* for all p when $f = y = 0$:

$$\begin{aligned} \lim_{y\to 0}\lim_{f\to 0} a^*(f,p,y) &= \lim_{y\to 0} 0 \\ &= 0 \end{aligned} \qquad \begin{aligned} \lim_{f\to 0}\lim_{y\to 0} a^*(f,p,y) &= \lim_{f\to 0} A_T \\ &= A_T. \end{aligned}$$

Hence there is no continuous extension to $f = y = 0$ for a^*.

In view of the monotonicity properties stated in Corollary 4.8 and Lemma 4.9 it is natural to consider the infimum and supremum of the various functions over f and/or y (and p for metabolic explosion), when these functions are monotone. To start with, put

$$\underline{n}^{*1}(y,a) := \inf_{f>0} n^{*1}(f,y,a). \tag{24}$$

Lemma 4.11. *For all $y, a > 0$, $\underline{n}^{*1}(y,a) > 0$ and*

$$\lim_{a\downarrow 0}\sup_{y>0} \underline{n}^{*1}(y,a) = 0$$

Proof. If $y, a > 0$, then n^{*1} is monotone decreasing in f by Lemma 4.9. So $\underline{n}^{*1}(y,a)$ is approached for increasing f and attained for $f = \infty$. Thus it

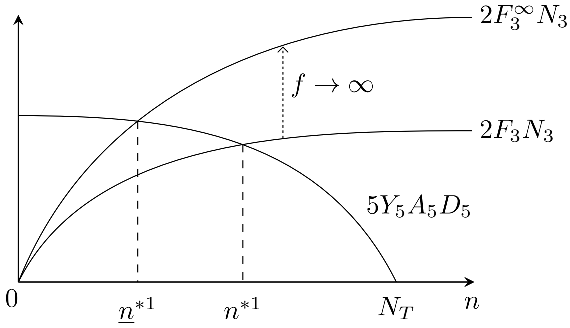


Figure 13: Graphical representation of $\underline{n}^{*1}$.

is the intersection of $\beta(y,a,n) = 5Y_5(y)A_5(a)D_5(N_T - n)$ and $\alpha(\infty,n) = 2F_3(\infty)N_3(n)$, $2F_3^\infty N_3$ in the notation suggested in (1).

$a > 0$, hence β is monotone increasing in y for all $n < N_T$, thus considering the intersection as above, one finds that $\underline{n}^{*1}$ is monotone increasing in y. Thus

$$\sup_{y>0} \underline{n}^{*1}(y,a) = \lim_{y\to\infty} \underline{n}^{*1}(y,a) = \underline{n}^{*1}(\infty,a)$$

Thus it is the solution for n to

$$\begin{aligned} \alpha(\infty,n) &= \beta(\infty,a,n) \\ 2F_3^\infty N_3(n) &= 5Y_5^\infty A_5D_5(N_T - n) \end{aligned}$$

If now $a \downarrow 0$, then $\beta(\infty,a,n)$ would have an upper bound that would go to zero, hence the intersection will shift to $n = 0$:

$$\lim_{a\downarrow 0} \sup_{y>0} \underline{n}^{*1}(y,a) = \lim_{a\downarrow 0} \underline{n}^{*1}(\infty,a) = 0$$

□

Recall that n^{*1} is increasing in y and bounded by N_T. Thus put

$$\bar{n}^{*1}(f,a) := \sup_{y>0} n^{*1}(f,y,a). \tag{25}$$

Lemma 4.12. *For $f > 0$, $a \in (0,A_T)$ $\bar{n}^{*1}(f,a) = \lim_{y\to\infty} n^{*1}(f,y,a) < N_T$.*

- *For $f > 0$, $\bar{n}^{*1}(f,a)$ is monotone increasing in a.*
- *For $a \in (0,A_T)$, $\bar{n}^{*1}(f,a)$ is monotone decreasing in f.*

Proof. If $f > 0$, $a \in (0,A_T)$, then n^{*1} is monotone increasing in y by Lemma 4.9. So $\bar{n}^{*1}(f,a)$ is approached for increasing y and attained for $y = \infty$. Thus it is the n-coordinate of the intersection of the graphs of $2F_3(f)N_3(n)$ and $5Y_5(\infty)A_5(a)D_5(N_T - n)$ as functions of n, i.e. $n^{*1}(f,\infty,a)$.

The two assertions follow from a special case of Corollary 4.8 and Lemma 4.9 for $y = \infty$. Y_5 is a saturating function, i.e. $Y_5(\infty) = Y_5^\infty < \infty$, thus these monotone properties still hold for $y = \infty$. □

Note: $\lim_{a\downarrow 0} \bar{n}^{*1}(f,a) = 0$, $\lim_{f\downarrow 0} \bar{n}^{*1}(f,a) = N_T$.
Consequently: $(f,a) \mapsto \bar{n}^{*1}(f,a)$ is discontinuous at $(0,0)$.

$$\text{Put } \underline{n}^{*2}(f,p,a) := \inf_{y>0} n^{*2}(f,p,y,a) \tag{26}$$

$$\text{and put } \bar{n}^{*2}(f,p,a) := \sup_{y>0} n^{*2}(f,p,y,a). \tag{27}$$

Lemma 4.13. *For all $f,p,y>0$, $a \in (0,A_T)$ it holds that*

$$0 < \underline{n}^{*2}(f,p,a) < n^{*2}(f,p,y,a) < \bar{n}^{*2}(f,p,a) < N_T.$$

For all $f,p>0$, $\underline{n}^{*2}(f,p,a)$ and $\bar{n}^{*2}(f,p,a)$ are monotone decreasing in a and

$$\sup_{a\in[0,A_T]} \bar{n}^{*2}(f,p) = \lim_{a\downarrow 0} \bar{n}^{*2}(f,p) < N_T.$$

Proof. Let $f,p>0$, $a \in (0,A_T)$. n^{*2} is monotone decreasing in y, hence $\underline{n}^{*2}$ and $\bar{n}^{*2}$ are approached and attained for the extreme values of y. Furthermore $\gamma(f,p,a,n)$ is a non-trivial function of n, because $f,p>0, a<A_T$. Also for all $y \in \mathbb{R}\cup\{\infty\}$, $\delta(f,y,n) \geq \delta(f,0,n) = 4F_3N_3$. $4F_3N_3$ is a non-trivial function of n. Hence

$$\underline{n}^{*2}(f,p,a) = n^{*2}(f,p,\infty,a) > 0 \qquad \bar{n}^{*2}(f,p,a) = n^{*2}(f,p,0,a) < N_T$$

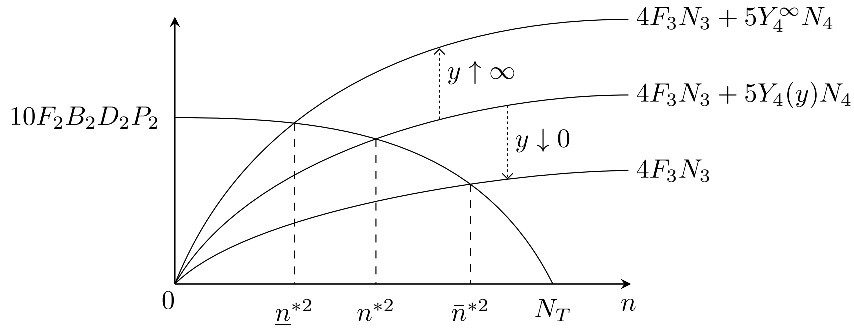


Figure 14: Graphical representation of $\underline{n}^{*2}$ and $\bar{n}^{*2}$.

$\underline{n}^{*2}$ and $\bar{n}^{*2}$ are special cases of n^{*2} for $y=\infty$ and $y=0$ respectively, hence the monotone properties follow from Corollary 4.8. As $\bar{n}^{*2}$ is monotone decreasing in a, it follows that $\sup_{a\in[0,A_T]} \bar{n}^{*2}(f,p) = \lim_{a\downarrow 0} \bar{n}^{*2}(f,p)$ holds. $\delta(f,0,n)$ and $\gamma(f,p,0,n)$ are non-trivial functions of n, hence their intersection is still nontrivial, i.e. $\lim_{a\downarrow 0} \bar{n}^{*2}(f,p) < N_T$. □

$$\text{Put } \underline{a}^{*}(f,p) := \inf_{y>0} a^{*}(f,p,y) \tag{28}$$

Proposition 4.14. *For all $f, p > 0$, it holds that*

$$\underline{a}^*(f,p) = \lim_{y\uparrow\infty} a^*(f,p,y) > 0.$$

Furthermore $\underline{a}^*(f,p)$ is the unique $x \in (0, A_T)$ such that $\bar{n}^{*1}(f,x) = \underline{n}^{*2}(f,p,x)$.

Proof. Let $f, p > 0$. By Lemma 4.9, a^* is monotone decreasing in y, hence $\inf_{y>0} a^*(f,y) = \lim_{y\uparrow\infty} a^*(f,y)$. From the previous lemmas, it is known that for all $x \in (0, A_T)$, if $y \uparrow \infty$, $n^{*1} \to \bar{n}^{*1}(f,x)$ and $n^{*2} \to \underline{n}^{*2}(f,p,x)$, which are non-trivial functions of x. Thus they intersect at a non-trivial value of x. Remember that a^* is the value of a where n^{*1} and n^{*2} intersect. Thus $\lim_{y\to\infty} a^*(f,p,y) = \underline{a}^*(f,p)$ is the intersection of these two functions. They are non-trivial, so $\underline{a}^*(f,p) > 0$. □

Remark 7. Note that for fixed $p, y > 0$, $a^*(f,p,y) \in (\underline{a}^*(f,p), A_T)$. As Lemma 4.10 dictates that $\lim_{f\downarrow 0} a^*(f,p,y) = 0$, it follows that also $\lim_{f\downarrow 0} \underline{a}^*(f,p) = 0$.

Observe that for fixed $f, p > 0$,

$$\{a^*(f,p,y) | y > 0\} = (\underline{a}^*(f,p), A_T) \tag{29}$$

$$\text{Put } \bar{n}^{*1}_\infty(a) := \inf_{f>0} \bar{n}^{*1}(f,a) \tag{30}$$

$$= \lim_{f\to\infty} \bar{n}^{*1}(f,a). \tag{31}$$

$$\text{Put } \bar{N}^{*1}_\infty := \bar{n}^{*1}_\infty(A_T) \tag{32}$$

(31) follows as $\bar{n}^{*1}$ is monotone decreasing in f by Lemma 4.12.

Lemma 4.15. *$\bar{n}^{*1}_\infty > 0$ for all $a \in (0, A_T]$, $a \mapsto \bar{n}^{*1}_\infty(a)$ is monotone increasing and*

$$\{\bar{n}^{*1}_\infty(a) | a \in [0, A_T]\} = \left[0, \bar{N}^{*1}_\infty\right]. \tag{33}$$

Proof. Let $a \in (0, A_T]$. By the proof of Lemma 4.12, for $f > 0$, $\bar{n}^{*1}$ is the intersection in n of $2F_3(f)N_3(n)$ and $5Y_5^\infty A_5(a) D_5(N_T - n)$. From (31) it follows that $\bar{n}^{*1}_\infty$ is the intersection in n of $2F_3^\infty N_3(n)$ and $5Y_5^\infty A_5(a) D_5(N_T - n)$. These are both non-trivial functions of n, hence $\bar{n}^{*1}_\infty(a) > 0$.
Thus it is the value of n^{*1} when $f = y = \infty$: $\bar{n}^{*1}_\infty(a) = n^{*1}(\infty, \infty, a)$ and therefore as a special case of Corollary 4.8, it follows that $\bar{n}^{*1}_\infty(a)$ is monotone increasing in a. This monotone behaviour dictates that

$$\bar{n}^{*1}_\infty([0, A_T]) = [\bar{n}^{*1}_\infty(0), \bar{n}^{*1}_\infty(A_T)]$$

$\bar{n}^{*1}_\infty(0) = 0$ by Corollary 4.8. $2F_3^\infty N_3(n)$ and $5Y_5^\infty A_5(A_T) D_5(N_T - n)$ are non-trivial functions of n, thus their intersection is non-trivial: $\bar{N}^{*1}_\infty < N_T$.

□

$$\text{Put } \underline{n}^{*2}_\infty(p,a) := \lim_{f\to\infty} \underline{n}^{*2}(f,p,a) \tag{34}$$

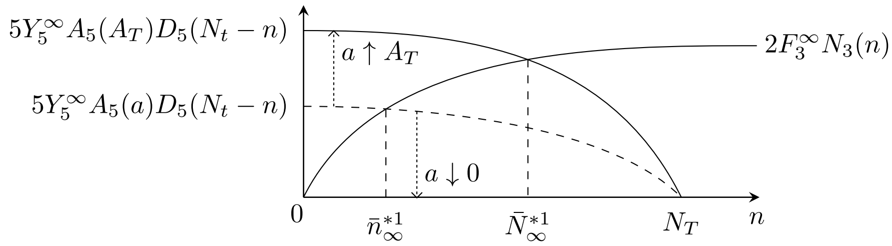


Figure 15: Graphical representation of $\{\bar{n}_\infty^{*1}(a)|a \in [0, A_T]\} = \big[0, \bar{N}_\infty^{*1}\big]$.

Lemma 4.16. *For all* $p > 0$, $a \in [0, A_T)$, $\underline{n}_\infty^{*2}(p,a)$ *exists and* $\underline{n}_\infty^{*2}(p,a) > 0$. *For all* $p > 0$, $a \mapsto \underline{n}_\infty^{*2}(p,a)$ *is monotone decreasing.*

Proof. Let $p > 0$, $a \in [0, A_T)$. For $f > 0$, $\underline{n}^{*2}$ is the intersection in n of $\gamma(f,p,a,n)$ and $\delta(f,\infty,n)$, which are non-trivial functions for these values of f,p,a. $\gamma(f,p,a,n)$ and $\delta(f,\infty,n)$ are monotone increasing and saturating in f. Hence for γ and δ the limit for $f \to \infty$ exists and is finite. Thus for $f \to \infty$, $\underline{n}^{*2}$ approaches the intersection of these two functions:

$$\underline{n}_\infty^{*2}(p,a) \text{ is such that } \gamma = 10F_2^\infty P_2 B_2 D_2 = 4F_3^\infty N_3 + 5Y_4^\infty N_4 = \delta.$$

For all $p > 0$, $\gamma(\infty,p,a,n)$ is monotone decreasing in a, if $n \in (0, N_T]$, hence $a \mapsto \underline{n}_\infty^{*2}(p,a)$ is monotone decreasing. □

$$\text{Put } \underline{a}_\infty^*(p) = \lim_{f\to\infty} \underline{a}^*(f,p) \tag{35}$$

Corollary 4.17. *For all* $p > 0$, $\underline{a}_\infty^*(p)$ *exists and* $0 < \underline{a}_\infty^*(p) < A_T$

Proof. Let $p > 0$. For all $f > 0$, $\underline{a}^*(f,p)$ is the intersection of $\bar{n}^{*1}$ and $\underline{n}^{*2}$ (Proposition 4.14).

- By Lemma 4.16, for all $a \in [0, A_T)$, $\underline{n}^{*2} = \lim_{f\to\infty} \underline{n}^{*2} > 0$ exists and it is monotone decreasing in a to 0.
- By Lemma 4.15, for all $a \in (0, A_T]$, $\bar{n}_\infty^{*1} = \lim_{f\to\infty} \bar{n}^{*1} > 0$ exists and it is monotone increasing in a from 0.

Hence $\underline{a}_\infty^*(p)$ exists and it is the intersection of the non-trivial functions of n: $\underline{n}^{*2}$ and $\bar{n}_\infty^{*1}$, see Figure 16. □

4.2.3 Existence part 2

With these properties of a^* and n^*, more can be discussed on the existence of non-trivial steady states, i.e. $\Omega \neq \emptyset$.

Remark 8. The estimations that we will show carry a stronger condition on the parameters then (23). On the other hand, (23) is a technical condition using the implicit functions n^* and a^*, while the final condition that is presented for the existence of non-trivial steady states (40) can be directly linked to the parameters and therefore it can be interpreted in a biological sense.

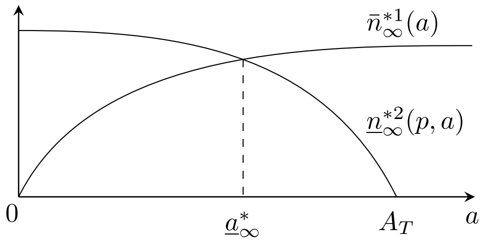


Figure 16: Graphical representation of argument for $\underline{a}^*_{\infty}$.

The following inequality can now be argued.

$$\begin{aligned}
\sup_{f,y>0} v_4\left(\vec{m}^*(f,y)\right) &= \sup_{f,y>0} Y_4(y) N_4\left(n^*(f,\Pi,y)\right) \\
&= \sup_{f,y>0} Y_4(y) N_4(n^{*1}(f,y,a^*(f,\Pi,y))) \\
&\geq \sup_{f,y>0} Y_4(y) N_4(n^{*1}(f,y,\underline{a}^*(f,\Pi)) && (36) \\
&= \sup_{f>0}\left(\lim_{y\uparrow\infty} Y_4(y)\cdot \lim_{y\uparrow\infty} N_4\left(n^{*1}(f,y,\underline{a}^*(f,\Pi))\right)\right) && (37) \\
&= \sup_{f>0} Y_4^{\infty} N_4\left(\bar{n}^{*1}(f,\underline{a}^*(f,\Pi))\right) && (38)
\end{aligned}$$

- Corollary 4.8 shows that n^{*1} is monotone increasing in a and $\underline{a}^*(f,\Pi)$ is the infimum over all y of a^* (28), hence

$$n^{*1}(f,y,a^*(f,\Pi,y)) \geq n^{*1}(f,y,\underline{a}^*(f,\Pi))$$

  N_4 is monotone increasing in n, so (36) holds.

- By Lemma 4.9 and the fact that N_4 is monotone increasing in n^{*1}, $y \mapsto N_4(n^{*1}(f,y,\underline{a}^*(f,\Pi)))$ is monotone increasing in y. Now both Y_4 and $y \mapsto N_4(n^{*1}(f,y,\underline{a}^*(f,\Pi)))$ are monotone increasing in y. Then their product is monotone increasing in y, hence the supremum over y is the limit for $y\uparrow\infty$. The limit of a product is the product of the limits, hence (37) holds.

- By Lemma 4.12, $\lim_{y\uparrow\infty} n^{*1}(f,y,\underline{a}^*) = \bar{n}^{*1}(f,\underline{a}^*)$. Thus (38) holds.

Corollary 4.18. *The following limit holds:*

$$\lim_{f\to\infty} \bar{n}^{*1}(f,\underline{a}^*(f,\Pi)) = \bar{n}^{*1}_{\infty}(\underline{a}^*_{\infty}(\Pi)) \tag{39}$$

Proof. By Lemma 4.15, if $f\uparrow\infty$, the function $\bar{n}^{*1}(f,a)$ will approach the function $\bar{n}^{*1}_{\infty}(a)$, which is monotone increasing in a. Also by Corollary 4.17, if $f\uparrow\infty$, $\underline{a}^*(f,\Pi)\to\underline{a}^*_{\infty}(\Pi)$. Hence the limit holds. □

The following lemma shows that there exist values for the parameters involved in v_2, v_3, v_4 and v_5, but *not* V_1 or r_6, that ensure that $\Omega \neq \emptyset$:

Lemma 4.19. *If the parameters in v_2, v_3, v_4 and v_5 are such that*

$$N_4(n^{*1}_{\infty}(\underline{a}^*_{\infty}(\Pi))) > 7\frac{Y_5^{\infty}A_5(A_T)D_5(N_T)}{Y_4^{\infty}}, \tag{40}$$

then $\Omega \neq \emptyset$.

Proof. $\bar{n}^{*1}(f,a)$ is continuous on $(0,\infty)\times(0,A_T)$:

Proof of continuity: $\bar{n}^{*1}(f,a)$ is tje n-coordinate of the intersection of the graphs of $2F_3(f)N_3(n)$ and $5Y_5^{\infty}A_5(a)D_5(N_T-n)$ as functions of n (proof of Lemma 4.12).
An Implicit Function Theorem, Theorem 3.2.1, page 36 in [8], yields that $\bar{n}^{*1}$ is continuously differentiable on $(0,\infty)\times(0,A_T)$ as the two graphs are.

Recall (30). By this continuity, Corollary 4.18 and (38), it holds that

$$\begin{aligned}(38):\sup_{(f,y)\in\mathrm{Int}(\mathbb{R}^2_+)} v_4(\vec{m}^*(f,y)) &\geq \sup_{f>0} Y_4^{\infty}N_4\left(\bar{n}^{*1}(f,\underline{a}^*(f,\Pi))\right)\\ &\geq \lim_{f\to\infty} Y_4^{\infty}N_4\left(\bar{n}^{*1}(f,\underline{a}^*(f,\Pi))\right)\\ &= Y_4^{\infty}N_4(\bar{n}^{*1}_{\infty}(\underline{a}^*_{\infty}(\Pi))>0\end{aligned}$$

On the other hand,

$$\sup_{(f,y)\in\mathrm{Int}(\mathbb{R}^2_+} v_5(\vec{m}^*(f,y)) \leq Y_5^{\infty}A_5(A_T)D_5(N_T).$$

According to Lemma 4.7, if (40) holds, then $\Omega\neq\emptyset$. □

Theorem 4.20 (Existence part 2)**.** *If* (40) *holds, then there is some $f_0>0$, such that for each $f>f_0$ there exists $y>0$ such that $(f,y)\in\Omega$.*

Proof. Assume that (40) holds. By Corollary 4.18 and continuity of N_4, it holds that $N_4(\bar{n}^{*1}(f,\underline{a}^*(f,\Pi))$ will approach $N_4(\bar{n}^{*1}_{\infty}(\underline{a}^*_{\infty}(\Pi)))$. Thus, by the assumption, for some $f_0>0$, it will hold that for all $f>f_0$, $N_4(\bar{n}^{*1}(f,\underline{a}^*(f,\Pi))$ is sufficiently close to $N_4(\bar{n}^{*1}_{\infty}(\underline{a}^*_{\infty}(\Pi)))$ that the following inequality holds:

$$N_4(\bar{n}^{*1}(f,\underline{a}^*(f,\Pi))>7\frac{Y_5^{\infty}A_5(A_T)D_5(N_T)}{Y_4^{\infty}}.$$

For such an f, there is a y sufficiently large for which it follows that

$$N_4(n^{*1}(f,y,\underline{a}^*(f,\Pi)))>7\frac{Y_5^{\infty}A_5(A_T)D_5(N_T)}{Y_4^{\infty}}.$$

Because this is a strict inequality and because $Y_4(y)\to Y_4^{\infty}$ for $y\to\infty$, it holds that we can increase y to y' such that $Y_4(y')$ is sufficiently close to Y_4^{∞} that the following holds:

$$\begin{aligned}N_4(n^{*1}(f,y',\underline{a}^*(f,\Pi))) &> 7\frac{Y_5^{\infty}A_5(A_T)D_5(N_T)}{Y_4(y')}\\ Y_4(y')N_4(n^{*1}(f,y',\underline{a}^*(f,\Pi))) &> 7Y_5^{\infty}A_5(A_T)D_5(N_T)\end{aligned}$$

So $(f,y')\in\Omega$. □

4.3 Infinite Steady States

In this section we investigate the system that is obtained from (4) and (5) in the limit $f \to \infty$. This corresponds to metabolic explosion, ultimately. Consider the following definitions to analyse this system.

Definition 4.21. *The metabolite concentration space is smaller, but the stoichiometry does not demand $\dot{f} = 0$.*

$$\begin{aligned} M_\infty &= \{\vec{m} \in M : f = \infty\} \\ &\subsetneq M \\ S_f &= \begin{pmatrix} 0 & -2 & +2 & 0 & +4 & +1 & +1 \\ 0 & +2 & 0 & -1 & -2 & 0 & 0 \\ -2 & +4 & 0 & 0 & -4 & -1 & 0 \\ 0 & +2 & -2 & -1 & +3 & 0 & 0 \end{pmatrix} \quad (41) \\ \mathcal{C}_f &:= \{\vec{v} \in V : S_f \cdot \vec{v} = 0\} \quad (42) \end{aligned}$$

Like $\mathcal{C}$, one can find elementary modes for $\mathcal{C}_f$. These are:

$$EM_3 = \begin{pmatrix} 2 \\ 1 \\ 0 \\ 2 \\ 0 \\ 0 \\ 2 \end{pmatrix}, EM_4 = \begin{pmatrix} 0 \\ 1 \\ 0 \\ 2 \\ 0 \\ 4 \\ -2 \end{pmatrix}, EM_5 = \begin{pmatrix} 0 \\ 2 \\ 5 \\ 0 \\ 2 \\ 0 \\ -14 \end{pmatrix}.$$

It follows that $\mathcal{C}_f = \mathrm{sp}_+(EM_3, EM_4, EM_5)$.

The steady state flux cone $\mathcal{C}$ is a subset of $\mathcal{C}_f$. This follows easily as the definition of S_f yields that

$$\begin{aligned} S\vec{v} = 0 &\Leftrightarrow S_f\vec{v} = 0 \text{ and } v_1 - v_2 - v_3 = 0 \quad (\dot{f} = 0). \\ S\vec{v} = 0 &\Rightarrow S_f\vec{v} = 0 \end{aligned}$$

Note that $v_7 = 0$ for EM_1 and EM_2, this leads to the following lemma:

Lemma 4.22. $\mathcal{C} = \mathcal{C}_f \cap \{\vec{v} \in V : v_7 = 0\}$

Proof. We prove both inclusions to prove equality.

- It is already shown that $\mathcal{C} \subset \mathcal{C}_f$. If $\vec{v} \in \mathcal{C}$, then $\vec{v} = \lambda_1 EM_1 + \lambda_2 EM_2$ for some $\lambda_1, \lambda_2 \in \mathbb{R}_+$. From EM_1 and EM_2 it follows that then $v_7 = 0$.
- If $\vec{v} \in \mathcal{C}_f$ and $v_7 = 0$, then $\vec{v} = \lambda_3 EM_3 + \lambda_4 EM_4 + \lambda_5 EM_5$, for some

$\lambda_3, \lambda_4, \lambda_5 \in \mathbb{R}_+$. Hence $v_7 = 0 = 2\lambda_3 - 2\lambda_4 - 14\lambda_5$. So $\lambda_3 = \lambda_4 + 7\lambda_5$.

$$\begin{aligned}\vec{v} &= (\lambda_4 + 7\lambda_5)\begin{pmatrix}2\\1\\0\\2\\0\\0\\2\end{pmatrix} + \lambda_4\begin{pmatrix}0\\1\\0\\2\\0\\4\\-2\end{pmatrix} + \lambda_5\begin{pmatrix}0\\2\\5\\0\\2\\0\\-14\end{pmatrix}\\ &= 2\lambda_4\begin{pmatrix}1\\1\\0\\2\\0\\2\\0\end{pmatrix} + \lambda_5\begin{pmatrix}14\\9\\5\\14\\2\\0\\0\end{pmatrix}\\ &= 2\lambda_4 EM_1 + \lambda_5 EM_2 \in \mathcal{C}.\end{aligned}$$

□

Hence considering the defining properties of $\mathcal{C}$, one finds that omitting $\dot{f} = 0$, but demanding $v_7 = 0$ still yields $\mathcal{C}$.

Lemma 4.22 proves that the only part of $\mathcal{C}_f$ where $\dot{f} = 0$ is where $v_7 = 0$. This is all defined by linear equations, so it can be argued that the sections of $\mathcal{C}_f$ where $\dot{f} < 0$ and $\dot{f} > 0$ are separated by $\mathcal{C}$. We also find out to which section $\dot{f} > 0$ corresponds. This is the only part that has to be studied for metabolic explosion.

Lemma 4.23. *In $\mathcal{C}_f$ the following holds:*

$$v_7 = 2\dot{f}$$

Proof.

$$\begin{aligned}0 = \dot{a} + \dot{p} &= -2v_1 + 4v_2 - 4v_5 - v_6 - 2v_2 + 2v_3 + 4v_5 + v_6 + v_7\\ &= 2v_1 - 2v_2 - 2v_3 - v_7\\ v_7 &= 2\dot{f}\end{aligned}$$

□

Now note that $v_7 = 0$ is actually equivalent to $p = \Pi$.

Hence we can consider the following equavalences for $\Phi(M) \cap \mathcal{C}_f$:

$$\begin{array}{ccc} p > \Pi & p = \Pi & p < \Pi \\ \Updownarrow & \Updownarrow & \Updownarrow \\ v_7 < 0 & v_7 = 0 & v_7 > 0 \\ \Updownarrow & \Updownarrow & \Updownarrow \\ \dot{f} < 0 & \dot{f} = 0 & \dot{f} > 0 \end{array}$$

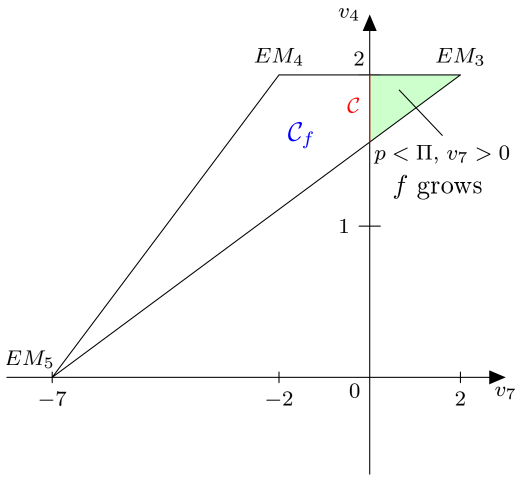


Figure 17: Intersect $\mathcal{C}$ and $\mathcal{C}_f$ with $v_2 = 1$ and represent them for the values of v_7 and v_4 on a plane (EM_5 has $v_2 = 2$, hence in this figure it is represented as $v_7 = -\frac{14}{2} = -7$ and $v_4 = 0$).

Remark 9. Biologically, this can be argumented. The interesting part of $\mathcal{C}_f$ for metabolic explosion is where the reaction fluxes are balanced in all metabolites except for the fbp production which grows. This interesting part is exactly where the inorganic phosphate concentration is lower, which is needed for v_2 yielding a rate of consumption of fbp $(v_2 + v_3)$ which is lower than the production v_1.

Hence to study metabolic explosion, one only has to consider the following:

Definition 4.24. $\mathcal{C}_\infty := \{\vec{v} \in \mathcal{C}_f : v_7 \geq 0\}$*, the section of* $\mathcal{C}_f$ *where* $v_7 \geq 0$.

From lemmas 4.22 and 4.23 it follows that $\mathcal{C}_\infty$ is $\mathcal{C}_f$ where $\dot{f} \geq 0$.

Lemma 4.25. $\mathcal{C}_\infty = \mathrm{sp}_+(EM_1, EM_2, EM_3)$.

Proof. Note that $EM_1, EM_2, EM_3 \in \mathcal{C}_f$ and all have $v_7 \geq 0$.

Conversely if $\vec{v} \in \mathcal{C}_\infty$, it follows that there exist $c_3, c_4, c_5 \in \mathbb{R}_+$ such that

$$\vec{v} = \sum_{i=3}^{5} c_i EM_i = \begin{pmatrix} 2c_3 \\ c_3 + c_4 + 2c_5 \\ 5c_5 \\ 2c_3 + 2c_4 \\ 2c_5 \\ 4c_4 \\ 2c_3 - 2c_4 - 14c_5 \end{pmatrix} \text{where } 2c_3 - 2c_4 - 14c_5 \geq 0$$

$$\text{Let } k = c_3 - c_4 : \vec{v} = \begin{pmatrix} 2k + 2c_4 \\ k + 2c_4 + 2c_5 \\ 5c_5 \\ 2k + 4c_4 \\ 2c_5 \\ 4c_4 \\ 2k - 14c_5 \end{pmatrix} \text{where } 2k - 14c_5 \geq 0$$

$$\begin{aligned} \text{Let } l = k - 7c_5 : \vec{v} &= \begin{pmatrix} 2l & +2c_4 & +14c_5 \\ l & +2c_4 & +9c_5 \\ & & 5c_5 \\ 2l & +4c_4 & +14c_5 \\ & & 2c_5 \\ & 4c_4 & \\ 2l & & \end{pmatrix} \text{where } l \geq 0 \\ &= lEM_3 + 2c_4EM_1 + c_5EM_2 \\ &\in \mathrm{sp}_+(EM_1, EM_2, EM_3) \end{aligned}$$

□

$$\text{For } \mathcal{C}_\infty : A_\infty = \begin{pmatrix} 1 & 1 & 0 & 2 & 0 & 2 & 0 \\ 14 & 9 & 5 & 14 & 2 & 0 & 0 \\ 2 & 1 & 0 & 2 & 0 & 0 & 2 \end{pmatrix} \text{ is the solution matrix.} \tag{43}$$

Like for A, a basis has to be found of $\ker(A_\infty)$.

- The basis should be chosen such that these equations will define $\vec{m} \in M_\infty$.
- This will yield equations that have to hold on all $v \in \mathcal{C}_\infty$ by theorem 4.2.

Corollary 4.26. *A basis B_∞ that can be chosen such that $\ker(A_\infty) = \mathrm{sp}(B_\infty)$ is*

$$B_\infty = \left\{ \begin{pmatrix} 0 \\ 0 \\ 2 \\ 0 \\ -5 \\ 0 \\ 0 \end{pmatrix}, \begin{pmatrix} 0 \\ 10 \\ -4 \\ -5 \\ 0 \\ 0 \\ 0 \end{pmatrix}, \begin{pmatrix} 0 \\ 10 \\ -18 \\ 0 \\ 0 \\ -5 \\ -5 \end{pmatrix}, \begin{pmatrix} 2 \\ 0 \\ 0 \\ -2 \\ 0 \\ 1 \\ 0 \end{pmatrix} \right\}. \tag{44}$$

These basis vectors correspond to the following realtionships holding on $v \in \mathcal{C}_\infty$:

$$2v_3 = 5v_5 \tag{45}$$
$$10v_2 = 4v_3 + 5v_4 \tag{46}$$
$$10v_2 = 18v_3 + 5v_6 + 6v_7 \tag{47}$$
$$v_6 = 2(v_4 - v_1) \tag{48}$$

Proof. It is clear that for all $x \in B_\infty$, $A_\infty \cdot x = 0$, also the vectors are linearly independent, so it follows from Proposition 4.2 □

Remark 10. Note that the equations described above work for all $\vec{v} \in \mathcal{C}_f$, because $v_7 \geq 0$ isn't demanded by them. Therefore the steady states that are found should be considered especially in the p-coordinate. For if $p > \Pi$ of such a steady state it cannot be stable, for then $\dot{f} < 0$ by Lemma 4.23.

Lemma 4.27. *For all $a \in [0, A_T]$ and $n \in [0, N_T]$ there is a unique value of $p \in \mathbb{R}_+$ such that* (47) *holds. Denote this value by $p^*(a, n)$.*

Proof. (47) as a function of $\vec{m} \in M_\infty$ is

$$10F_2^\infty P_2(p)B_2(A_T - a)D_2(N_T - n) = 18F_3^\infty N_3(n) + 5r_6a + 5r_7(\Pi - p). \tag{49}$$

Note that for $p = 0$, the lhs of (49) is 0, while the rhs is clearly non-zero. Conversely, the lhs is monotone increasing in p while the rhs is linearly decreasing in p. Therefore the rhs will be zero for some $p > 0$.

Inbetween these two values of p there is a unique intersection, thus there is a unique solution $p^*(a, n) \in \mathbb{R}_+$ such that (47) is satisfied. □

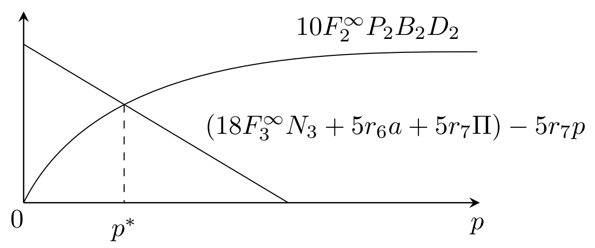


Figure 18: Graphical representation of argument for p^*

The image of $p^* : [0, A_T] \times [0, N_T]$ has non-trivial bounds, so put

$$\bar{p}^* := \sup_{a\in[0,A_T],n\in[0,N_T]} p^*(a, n), \tag{50}$$
$$\underline{p}^* := \inf_{a\in[0,A_T],n\in[0,N_T]} p^*(a, n). \tag{51}$$

Lemma 4.28. *p^* as a function of a and n has the following properties:*

- *For all $n \in [0, N_T]$, p^* is monotone increasing in a.*
- *For all $a \in [0, A_T]$, p^* is monotone increasing in n.*
- *$\bar{p}^* = p^*(A_T, N_T) < \infty$ and $\underline{p}^* = p^*(0, 0) > 0$.*

Proof.
- Let $n \in [0, N_T]$. With increasing n the lhs of (49) decreases for all p while the rhs increases for all p. Hence p^* is monotone increasing in n.
- Let $a \in [0, A_T]$. Also with increasing a the lhs of (49) decreases for all p while the rhs increases for all p. Hence p^* is monotone increasing in a.
- By the above properties one can see that the value $\bar{p}^*$ is approached for $a \uparrow A_T$ and $n \uparrow N_T$, while $\underline{p}^*$ is appoached for $a, n \downarrow 0$. So $\bar{p}^* = p^*(A_T, N_T)$ and $\underline{p}^* = p^*(0,0)$ hold.
  When $a = A_T$, $n = N_T$, the lhs of (49) is the zero function of p while the rhs known and thus $\bar{p}^*$ can be explicitely computed:

  $$\text{rhs of (49):} \quad (18F_3^\infty N_3(N_T) + 5r_6A_T + 5r_7\Pi) - 5r_7p$$
  $$\bar{p}^* = \frac{18F_3^\infty N_3(N_T) + 5r_6A_T + 5r_7\Pi}{5r_7} < \infty$$

  When $a = n = 0$, the lhs and rhs of (49) are the following functions of p:

  $$\text{lhs of (49):} \quad 10F_2^\infty P_2(p)B_2(A_T)D_2(N_T)$$
  $$\text{rhs of (49):} \quad 5r_7(\Pi - p)$$

  Hence as these are still non-trivial functions of p with montone properties, it is easily seen that their intersection, $\underline{p}^*$, is non-trivial. Thus $\underline{p}^* > 0$.

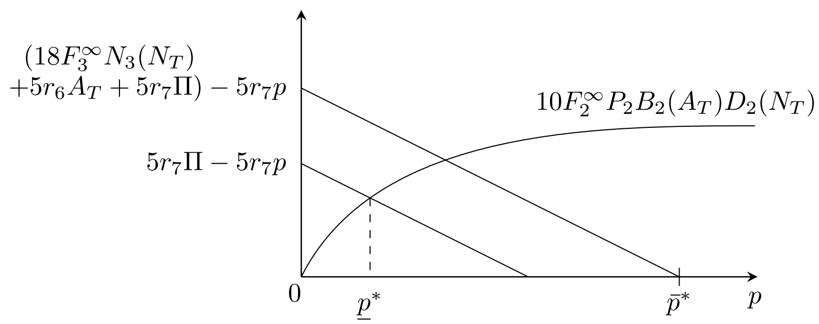


Figure 19: Graphical representation of argument for $\bar{p}^*$ and $\underline{p}^*$

□

Corollary 4.29. *For all $p, y \in \mathbb{R}_+$ there are unique values of $a \in [0, A_T]$ and $n \in [0, N_T]$ such that (45) and (46) hold.*

Proof. This is a specific case of the third part of theorem 4.4 as equations (45) and (46) are the same as equations (12) and (13) respectively. Set $f = \infty$. These unique values are $n = n^*(\infty, p, y)$, $a = a^*(\infty, p, y)$. Note that by Lemma 4.9, a^* and n^* are monotone increasing in p and a^* is monotone decreasing in y □

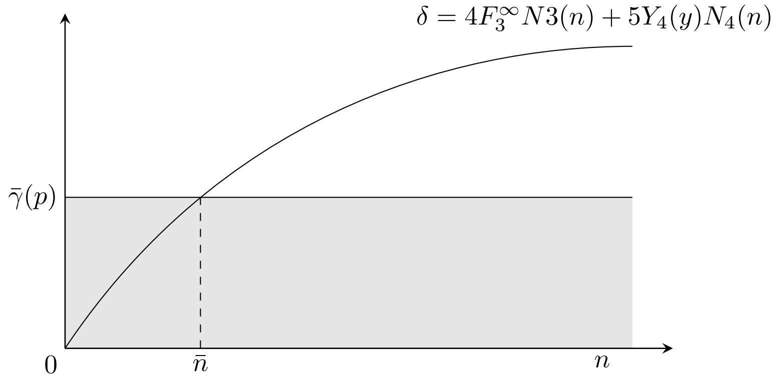
The following lemma and Lemma 4.10 are much alike. Now $p \downarrow 0$ is considered.

Lemma 4.30. *For all $y > 0$, then* $\begin{cases} \lim_{p\to 0} n^*(\infty, p, y) = 0 \\ \lim_{p\to 0} a^*(\infty, p, y) = 0 \end{cases}$

Proof. Let $y > 0$.
Recall the abreviations α, β, γ and δ as introduced at the start of Section 4.2.2, such that the defining equations of a^* and n^* like above, (45) and (46) become $\alpha = \beta$ and $\gamma = \delta$ respectively. Only γ is a function of p, the rest is uninfluenced by p. γ is monotone decreasing in a and n. From this monotone behaviour it follws that for all $p > 0$, $a \in [0, A_T]$, $n \in [0, N_T]$ it holds that

$$\gamma(\infty, p, a, n) \leq \gamma(\infty, p, 0, 0) =: \bar{\gamma}(p).$$

$\bar{\gamma}(p) = 10F_2^\infty P_2(p)B_2(A_T)D_2(N_T) \downarrow 0$ as $p \downarrow 0$, because $P_2(p) \downarrow 0$ as $p \downarrow 0$. Let $\bar{n}$ denote the intersection of $\bar{\gamma}$ and $\delta(\infty, y, n)$. $\delta(\infty, y, n)$ is monotone increasing in n and uninfluenced by p, it can be concluded that when $p \downarrow 0$, n^{*2}, the n-coordinate of the intersection $\gamma = \delta$, will be in $[0, \bar{n}]$ and $\bar{n} \downarrow 0$. Hence for all a, $\lim_{p\downarrow 0} n^{*2}(\infty, p, y, a) \downarrow 0$.



Remember that by definition $n^*(\infty, p, y) = n^{*2}(\infty, p, y, a^*)$. Thus as a special case for $a = a^*$, it follows that $\lim_{p\to 0} n^*(\infty, p, y) = 0$.

Recall that $n^{*1}(\infty, y, a)$ is the n-coordinate of the intersection $\alpha = \beta$. It is uninfluenced by p. By definition of n^* it holds that

$$n^{*1}(\infty, y, a^*(\infty, p, y)) = n^{*2}(\infty, p, y, a^*(\infty, p, y)).$$

$$\text{Let } p \downarrow 0: \quad n^{*1}(\infty, y, \lim_{p\downarrow 0} a^*(\infty, p, y)) = 0$$

By Corollary 4.8 $n^{*1}(\infty, y, a)$ is monotone increasing in a from 0, hence

$$\lim_{p\to 0} a^*(\infty, p, y) = 0.$$ □

Lemma 4.31. *For all $y \in \mathbb{R}_+$ there exists at least one configuration of $\vec{m} \in M_\infty$ such that* (45)*,* (46) *and* (47) *hold. Call the configuration with the smallest p-coordinate*

$$\vec{m}^*_\infty(y) := (\infty, p^*_\infty(y), y, a^*_\infty(y), n^*_\infty(y))$$

Proof. Consider the map

$$\begin{array}{llll} \varphi: & \mathbb{R}_+^2 & \to & \mathbb{R}_+ \\ & (p, y) & \mapsto & p^*(a^*(\infty, p, y), n^*(\infty, p, y)). \end{array} \tag{52}$$

Let $y > 0$. The result of this Corollary follows from the equation $p = \varphi(p, y)$ and the following properties of φ.

- By Lemma 4.30 there is a continuous extention at $p = 0$: $a^*(\infty, 0, y) = 0$, $n^*(\infty, 0, y) = 0$. Thus by Lemma 4.28 $\varphi(0) = p^*(0, 0) = \underline{p}^* > 0$.
- By Lemma 4.28, $\varphi(p, y) \leq \bar{p}^*$.
- By Corrolary 4.29, a^* and n^* are monotone increasing in p. Hence as $p^*(a, n)$ is monotone increasing in both a and n, $p \mapsto \varphi(p, y)$ is monotone increasing.

Hence the equation $p = \varphi(p, y)$ has *at least* one solution for $p \in [0, \bar{p}^*]$. □

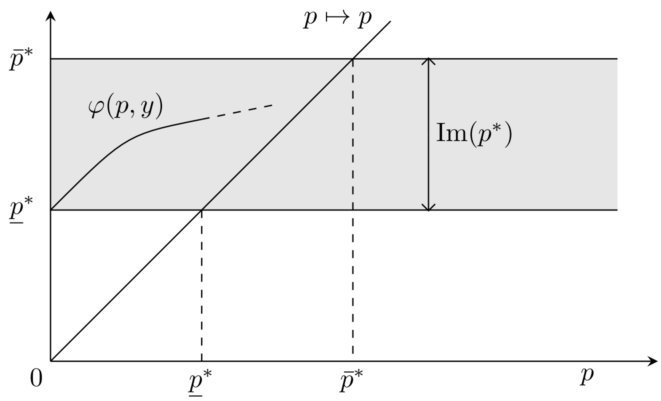


Figure 20: Graphical argument for $\vec{m}^*_\infty$.

In the following theorem, recall (2) and its notation.

Theorem 4.32 (Existence of Metabolic Explosion). *For all $y > 0$, with $\vec{m} = (f, p, y, a, n) = \vec{m}^*_\infty$ the following holds:*
For any $V_1 < \frac{v_4(\vec{m}^*_\infty)}{h(a^*_\infty)}$, there is a unique $r_6 > 0$ such that $\Phi(\vec{m}^*_\infty) \in \mathcal{C}_f$.

Proof. Let $y > 0$, then $\vec{m}^*_\infty$ is set. By Lemma 4.31 (45), (46) and (47) hold. Also $v_4(\vec{m}^*_\infty = Y_4(y)N_4(n^*_\infty(y))$ is set. Recall that $v_1 = H(a^*_\infty) = V_1 \cdot h(a^*_\infty)$ which is also set. Assume

$$V_1 < \frac{v_4(\vec{m}^*_\infty)}{h(a^*_\infty)}.$$

Then $0 < v_4(\vec{m}^*_\infty) - V_1 h(a^*_\infty)$

This last expression is the rhs of (48). Now because $a^*_\infty(y) > 0$ is set, there is a unique $r_6 > 0$ such that (48) holds.

$$r_6 a^*_\infty = 2(Y_4(y)N_4(n^*_\infty(y)) - V_1 h(a^*_\infty))$$

All defining equations (45), (46), (47) and (48) hold, hence $\Phi(\vec{m}^*_\infty) \in \mathcal{C}_f$. □

These combinations of V_1 and r_6 form a line segment in the parameter space.

5 Stability of Steady States

To analyse linearised stability of all steady states that will follow, compute the Jacobian. First compute $D\Phi = \left(\frac{\partial v_i}{\partial m_j}\right)_{i,j}$, $1 \leq i \leq 7$, $1 \leq j \leq 5$, $m_j \in \{f, p, y, a, n\}$. One can reason which terms are non-zero.

$$D\Phi(\vec{m}) = \begin{pmatrix} 0 & 0 & 0 & \frac{\partial v_1}{\partial a} & 0 \\ \frac{\partial v_2}{\partial f} & \frac{\partial v_2}{\partial p} & 0 & \frac{\partial v_2}{\partial a} & \frac{\partial v_2}{\partial n} \\ \frac{\partial v_3}{\partial f} & 0 & 0 & 0 & \frac{\partial v_3}{\partial n} \\ 0 & 0 & \frac{\partial v_4}{\partial y} & 0 & \frac{\partial v_4}{\partial n} \\ 0 & 0 & \frac{\partial v_5}{\partial y} & \frac{\partial v_5}{\partial a} & \frac{\partial v_5}{\partial n} \\ 0 & 0 & 0 & r_6 & 0 \\ 0 & -r_7 & 0 & 0 & 0 \end{pmatrix}$$

$$\text{where } \frac{\partial v_1}{\partial a} = H'(a) \text{ as in (2)}$$
$$\frac{\partial v_2}{\partial f} = F_2'(f)P_2(p)B_2(A_T - a)D_2(N_T - n)$$
$$\frac{\partial v_2}{\partial p} = F_2(f)P_2'(p)B_2(A_T - a)D_2(N_T - n)$$
$$\frac{\partial v_2}{\partial a} = -F_2(f)P_2(p)B_2'(A_T - a)D_2(N_T - n)$$
$$\frac{\partial v_2}{\partial n} = -F_2(f)P_2(p)B_2(A_T - a)D_2'(N_T - n)$$
$$\frac{\partial v_3}{\partial f} = F_3'(f)N_3(n)$$
$$\frac{\partial v_3}{\partial n} = F_3(f)N_3'(n)$$
$$\frac{\partial v_4}{\partial y} = Y_4'(y)N_4(n)$$
$$\frac{\partial v_4}{\partial n} = Y_4(y)N_4'(n)$$
$$\frac{\partial v_5}{\partial y} = Y_5'(y)A_5(a)D_5(N_T - n)$$
$$\frac{\partial v_5}{\partial a} = Y_5(y)A_5'(a)D_5(N_T - n)$$
$$\frac{\partial v_5}{\partial n} = -Y_5(y)A_5(a)D_5'(N_T - n)$$

This yields the Jacobian as follows:

$$J(\vec{m}) = S \cdot D\Phi(\vec{m}) =$$

$$\begin{pmatrix} -\frac{\partial v_2}{\partial f}-\frac{\partial v_3}{\partial f} & -\frac{\partial v_2}{\partial p} & 0 & \frac{\partial v_1}{\partial a}-\frac{\partial v_2}{\partial a} & -\frac{\partial v_2}{\partial n}-\frac{\partial v_3}{\partial n} \\ -2\frac{\partial v_2}{\partial f}+2\frac{\partial v_3}{\partial f} & -2\frac{\partial v_2}{\partial p}-r_7 & 4\frac{\partial v_5}{\partial y} & A & C \\ 2\frac{\partial v_2}{\partial f} & 2\frac{\partial v_2}{\partial p} & -\frac{\partial v_4}{\partial y}-2\frac{\partial v_5}{\partial y} & 2\frac{\partial v_2}{\partial a}-2\frac{\partial v_5}{\partial a} & D \\ 4\frac{\partial v_2}{\partial f} & 4\frac{\partial v_2}{\partial p} & -4\frac{\partial v_5}{\partial y} & B & 4\frac{\partial v_2}{\partial n}-4\frac{\partial v_5}{\partial n} \\ 2\frac{\partial v_2}{\partial f}-2\frac{\partial v_3}{\partial n} & 2\frac{\partial v_2}{\partial p} & -\frac{\partial v_4}{\partial y}+3\frac{\partial v_5}{\partial y} & 2\frac{\partial v_2}{\partial a}+3\frac{\partial v_5}{\partial a} & E \end{pmatrix} \tag{53}$$

$$\begin{aligned} \text{where } A &= -2\frac{\partial v_2}{\partial a}+4\frac{\partial v_5}{\partial a}+r_6 \\ B &= -2\frac{\partial v_1}{\partial a}+4\frac{\partial v_2}{\partial a}-4\frac{\partial v_5}{\partial a}-r_6 \\ C &= -2\frac{\partial v_2}{\partial n}+2\frac{\partial v_3}{\partial n}+4\frac{\partial v_5}{\partial n} \\ D &= 2\frac{\partial v_2}{\partial n}-\frac{\partial v_4}{\partial n}-2\frac{\partial v_5}{\partial n} \\ E &= 2\frac{\partial v_2}{\partial n}-2\frac{\partial v_3}{\partial n}-\frac{\partial v_4}{\partial n}+3\frac{\partial v_5}{\partial n} \end{aligned}$$

5.1 Trivial Steady States

To study the local stability behaviour of the trivial steady states, consider the eigenvalues of the Jacobian there.

Lemma 5.1. *All trivial steady states, with one exception, have an eigenvalue zero, three negative eigenvalues and one real eigenvalue λ_+ which has the property that its sign is equal to the sign of*

$$2H'(0) - r_6 - 4Y_5(y)A_5'(0)D_5(N_T) \tag{54}$$

for $\vec{m} = (0, \Pi, y, 0, 0)^T, y \in \mathbb{R}_+$, *and*

$$\begin{aligned} &F_2'(0)P_2(\Pi)B_2(A_T)D_2(N_T - n) \cdot (2H'(0) - r_6) \\ &\qquad - F_3'(0)N_3(n) \cdot (r_6 + 2H'(0)) \end{aligned} \tag{55}$$

for $\vec{m} = (0, \Pi, 0, 0, n), n \in [0, N_T]$.
The exception is $(0, \Pi, 0, 0, 0)$ *which has two zero eigenvalues, two negative eigenvalues and one eigenvalue with the property that its sign is equal to the sign of*

$$2H'(0) - r_6. \tag{56}$$

Proof. As shown, there are two families of steady states:

1. $\vec{m} = (0, \Pi, y, 0, 0)^T$, for $y \in \mathbb{R}_+$, $y > 0$. This yields

$$\frac{\partial v_2}{\partial p} = \frac{\partial v_2}{\partial a} = \frac{\partial v_2}{\partial n} = \frac{\partial v_3}{\partial f} = \frac{\partial v_3}{\partial n} = \frac{\partial v_4}{\partial y} = \frac{\partial v_5}{\partial y} = \frac{\partial v_5}{\partial n} = 0$$

The Jacobian then has the following form:

$$J(\vec{m}) = \left(\begin{array}{c|c|c|c|c} -\frac{\partial v_2}{\partial f} & 0 & 0 & H'(0) & 0 \\ -2\frac{\partial v_2}{\partial f} & -r_7 & 0 & r_6 + 4\frac{\partial v_5}{\partial a} & 0 \\ 2\frac{\partial v_2}{\partial f} & 0 & 0 & -2\frac{\partial v_5}{\partial a} & -\frac{\partial v_4}{\partial n} \\ 4\frac{\partial v_2}{\partial f} & 0 & 0 & -2H'(0) - 4\frac{\partial v_5}{\partial a} - r_6 & 0 \\ 2\frac{\partial v_2}{\partial f} & 0 & 0 & 3\frac{\partial v_5}{\partial a} & -\frac{\partial v_4}{\partial n} \end{array}\right)$$

Thus the eigenvalues can be computed. In particular, it can be computed if the eigenvalues are positive, negative or zero. Let $\lambda \in \mathbb{R}$,

$$\begin{aligned} 0 &= |J - \lambda I| \\ &= -\lambda(\lambda + r_7)\left(\lambda + \frac{\partial v_4}{\partial n}\right)\left|\begin{array}{cc} -\frac{\partial v_2}{\partial f} - \lambda & H'(0) \\ 4\frac{\partial v_2}{\partial f} & -2H'(0) - 4\frac{\partial v_5}{\partial a} - r_6 - \lambda \end{array}\right| \\ \lambda &= 0, \lambda = -r_7 < 0, \lambda = -Y_4(y)N_4'(0) < 0 \end{aligned}$$

$$\begin{aligned} \text{or } \lambda^2 + b\lambda + c &= 0, \text{ where} \\ b &= \tfrac{\partial v_2}{\partial f} + 2H'(0) + 4\tfrac{\partial v_5}{\partial a} + r_6 > 0 \\ c &= \tfrac{\partial v_2}{\partial f}\left(2H'(0) + 4\tfrac{\partial v_5}{\partial a} + r_6 - 4H'(0)\right) \\ &= \tfrac{\partial v_2}{\partial f}\left(-2H'(0) + 4\tfrac{\partial v_5}{\partial a} + r_6\right). \\ \text{Hence } \lambda_\pm &= \frac{-b \pm \sqrt{b^2 - 4c}}{2} \end{aligned}$$

This yields real valued solutions. To show this, use $H'(0) > 0$:

$$\begin{aligned} b^2 - 4c &> \left(\tfrac{\partial v_2}{\partial f} + 4\tfrac{\partial v_5}{\partial a} + r_6\right)^2 - 4\tfrac{\partial v_2}{\partial f}\left(4\tfrac{\partial v_5}{\partial a} + r_6\right) \\ &= \left(\tfrac{\partial v_2}{\partial f}\right)^2 + (2-4)\tfrac{\partial v_2}{\partial f}\left(4\tfrac{\partial v_5}{\partial a} + r_6\right) + \left(4\tfrac{\partial v_5}{\partial a} + r_6\right)^2 \\ &= \left(\tfrac{\partial v_2}{\partial f} - 4\tfrac{\partial v_5}{\partial a} - r_6\right)^2 \geq 0 \end{aligned}$$

Thus $\lambda_- < 0$.
Furthermore $\lambda_- \cdot \lambda_+ = \frac{b^2 - b^2 + 4c}{2^2} = c$, so the sign of λ_+ equals minus the sign of c. Thus,

$$\text{sign}(\lambda_+) = \text{sign}\left(-4Y_5(y)A_5'(0)D_5(N_T) - r_6 + 2H'(0)\right),$$

because $\frac{\partial v_2}{\partial f} > 0$.

2. $\vec{m} = (0, \Pi, 0, 0, n)^T$, for $n \in (0, N_T]$. This yields

$$\frac{\partial v_2}{\partial p} = \frac{\partial v_2}{\partial a} = \frac{\partial v_2}{\partial n} = \frac{\partial v_3}{\partial n} = \frac{\partial v_4}{\partial n} = \frac{\partial v_5}{\partial y} = \frac{\partial v_5}{\partial a} = \frac{\partial v_5}{\partial n} = 0$$

The Jacobian then has the following form:

$$J(\vec{m}) = \left(\begin{array}{c|c|c|c|c} -\frac{\partial v_2}{\partial f} - \frac{\partial v_3}{\partial f} & 0 & 0 & H'(0) & 0 \\ -2\frac{\partial v_2}{\partial f} + 2\frac{\partial v_3}{\partial f} & -r_7 & 0 & r_6 & 0 \\ 2\frac{\partial v_2}{\partial f} & 0 & -\frac{\partial v_4}{\partial y} & 0 & 0 \\ 4\frac{\partial v_2}{\partial f} & 0 & 0 & -2H'(0) - r_6 & 0 \\ 2\frac{\partial v_2}{\partial f} - 2\frac{\partial v_3}{\partial f} & 0 & -\frac{\partial v_4}{\partial y} & 0 & 0 \end{array}\right)$$

The eigenvalues:

$$\begin{aligned} 0 &= |J - \lambda I| \\ &= -\lambda(\lambda + r_7)\left(\lambda + \frac{\partial v_4}{\partial y}\right) \begin{vmatrix} -\frac{\partial v_2}{\partial f} - \frac{\partial v_3}{\partial f} - \lambda & H'(0) \\ 4\frac{\partial v_2}{\partial f} & -2H'(0) - r_6 - \lambda \end{vmatrix} \\ \lambda &= 0, \lambda = -r_7 < 0, \lambda = -Y_4'(0)N_4(n) < 0 \end{aligned}$$

$$\begin{aligned} \text{or } \lambda^2 + b\lambda + c &= 0, \text{ where} \\ b &= \tfrac{\partial v_2}{\partial f} + \tfrac{\partial v_3}{\partial f} + 2H'(0) + r_6 > 0 \\ c &= \left(\tfrac{\partial v_2}{\partial f} + \tfrac{\partial v_3}{\partial f}\right)(2H'(0) + r_6) - 4\tfrac{\partial v_2}{\partial f}H'(0) \\ &= \tfrac{\partial v_2}{\partial f}(r_6 - 2H'(0)) + \tfrac{\partial v_3}{\partial f}(r_6 + 2H'(0)) \\ \text{Hence } \lambda_\pm &= \frac{-b \pm \sqrt{b^2 - 4c}}{2} \end{aligned}$$

This yields real valued solutions. To show this, use $4\frac{\partial v_2}{\partial f}H'(0) > 0$:

$$\begin{aligned} b^2 - 4c &> \left(\tfrac{\partial v_2}{\partial f} + \tfrac{\partial v_3}{\partial f} + 2H'(0) + r_6\right)^2 - 4\left(\tfrac{\partial v_2}{\partial f} + \tfrac{\partial v_3}{\partial f}\right)(2H'(0) + r_6) \\ &= \left(\tfrac{\partial v_2}{\partial f} + \tfrac{\partial v_3}{\partial f} - 2H'(0) - r_6\right)^2 \geq 0 \end{aligned}$$

Thus $\lambda_- < 0$.
Again, the sign of λ_+ equals minus the sign of c, hence the sign of λ_+ equals the sign of

$$F_2'(0)P_2(\Pi)B_2(A_T)D_2(N_T - n)(2H'(0) - r_6) - F_3'(0)N_3(n)(r_6 + 2H'(0))$$

3. $\vec{m} = (0, \Pi, 0, 0, 0)$ yields the Jacobian:

$$J(\vec{m}) = \left(\begin{array}{c|c|c|c|c} -\frac{\partial v_2}{\partial f} & 0 & 0 & H'(0) & 0 \\ -2\frac{\partial v_2}{\partial f} & -r_7 & 0 & r_6 & 0 \\ 2\frac{\partial v_2}{\partial f} & 0 & 0 & 0 & 0 \\ 4\frac{\partial v_2}{\partial f} & 0 & 0 & -2H'(0) - r_6 & 0 \\ 2\frac{\partial v_2}{\partial f} & 0 & 0 & 0 & 0 \end{array}\right)$$

The eigenvalues:

$$
\begin{aligned}
0 &= |J - \lambda I| \\
&= -\lambda^2(\lambda + r_7) \cdot \begin{vmatrix} -\frac{\partial v_2}{\partial f} - \lambda & H'(0) \\ 4\frac{\partial v_2}{\partial f} & -2H'(0) - r_6 - \lambda \end{vmatrix} \\
\lambda &= 0, \lambda = r_7,
\end{aligned}
$$

$$
\begin{aligned}
\text{or } \lambda^2 + b\lambda + c &= 0, \text{ where} \\
b = &= \tfrac{\partial v_2}{\partial f} + 2H'(0) + r_6 > 0 \\
c &= \tfrac{\partial v_2}{\partial f}\left(2H'(0) + r_6\right) - 4\tfrac{\partial v_2}{\partial f}H'(0) \\
&= \tfrac{\partial v_2}{\partial f}\left(r_6 - 2H'(0)\right) \\
\text{Hence } \lambda_\pm &= \frac{-b \pm \sqrt{b^2 - 4c}}{2}
\end{aligned}
$$

By the same arguments as before, $b^2 - 4c > 0$, hence $\lambda_\pm$ are real-valued and $\lambda_- < 0$. Also the sign of λ_+ is equal to the sign of $-c$. Hence, because $\frac{\partial v_2}{\partial f} > 0$,

$$
\operatorname{sign}(\lambda_+) = \operatorname{sign}\left(2H'(0) - r_6\right).
$$

□

Remark 11. The zero eigenvalue is due to the nature of the steady states: each trivial steady state is part of a family along the axis, therefore in this direction there can be neither stability nor instability. In this direction there is a continuous line of steady states, where the dynamics do not move towards the steady state and do not move away either, because *they are at steady state.*
The special case is where $n = y = 0$, this is exactly the intersection of these two families and hence there are *two directions* in which there is neither stability nor instability, hence it has two zero eigenvalues.

Lemma 5.1 also yields that there are three eigenvalues that are *always* negative, hence the possible instability of the trivial steady states lies only in the sign of one eigenvalue. This leads to some corollaries.

Corollary 5.2. *If* $2H'(0) < r_6$*, then* all *trivial steady states have non-positive eigenvalues.*

Proof. If one states that $2H'(0) < r_6$, immediately from Lemma 5.1 in (54), (55) and (56)it follows. □

Corollary 5.3. *The trivial steady state* $(0, \Pi, 0, 0, N_T)$ *always has non-positive eigenvalues.*

Proof. From Lemma 5.1 it follows that there is only one eigenvalue λ_+ which could possibly counter this statement, for the other four eigenvalues are already non-positive. The sign of this eigenvalue is equal to the sign of the following for $n = N_T$:

$$\begin{aligned}
&F_2'(0)P_2(\Pi)B_2(A_T)D_2(N_T - N_T) \cdot (2H'(0) - r_6) \\
&\quad - F_3'(0)N_3(N_T) \cdot (r_6 + 2H'(0)) \\
&= -F_3(0)N_3(N_T) \cdot (r_6 + 2H'(0))
\end{aligned}$$

Now $F_3'(0), N_3(N_T), r_6, 2H'(0) > 0$ by assumption, thus $\text{sign}(\lambda_+) < 0$. □

Corollary 5.4. *If $2H'(0) > r_6$, then one has the following*

1. *The trivial steady state $(f, p, y, a, n,) = (0, \Pi, 0, 0, 0)$ is locally unstable.*
2. *There is a transcritical bifurcation in the n-family of trivial steady states: if n is regarded as a parameter on $[0, N_T]$, then for some $n_b \in (0, N_T)$, λ_+ changes sign from positive to negative and the trivial steady state $(0, \Pi, 0, 0, n_b)$ will have $\lambda_+ = 0$.*
3. *If and only if also $2H'(0) < r_6 + 4Y_5^\infty A_5'(0)D_5(N_T)$, there is a transcritical bifurcation in the y-family of steady states.*

Proof. Assume $2H'(0) > r_6$.

1. This steady state has eigenvalue $\lambda_+ = 2H'(0) - r_6$ by Lemma 5.1, which is positive by the assumption, so as it has a positive eigenvalue it is unstable.
2. By Corollary 5.3, $(0, \Pi, 0, 0, N_T)$ has only non-positive eigenvalues, with $\lambda_+ < 0$ while by the above $(0, \Pi, 0, 0, 0)$ has a positive eigenvalue $\lambda_+ > 0$. Thus consider n as a parameter of the function λ_+. Remark that the function that controls the sign of λ_+ (55) is a monotone decreasing function in n. Thus by the Intermediate Value Theorem there is a unique point for the n-family between $n = 0$ and $n = N_T$, n_b, where $\lambda_+ = 0$.
3. Note that the function that controls the sign of λ_+ (54) is monotone decreasing in y.

   If the inequality does not hold, then for all $y \in \mathbb{R}_+$, the trivial steady state $(0, \Pi, y, 0, 0)$ has a positive eigenvalue λ_+:

   $$\text{sign}(\lambda_+) = \text{sign}(2H'(0) - r_6 - 4Y_5(y)A_5'(0)D_5(N_T))$$
   $$4Y_5(y)A_5'(0)D_5(N_T) < 4Y_5^\infty A_5'(0)D_5(N_T),$$
   $$\begin{aligned}
   2H'(0) \quad & -r_6 - 4Y_5(y)A_5'(0)D_5(N_T) \\
   & > 2H'(0) - r_6 - 4Y_5^\infty A_5'(0)D_5(N_T) \\
   & \geq 0 \text{ by assumption.}
   \end{aligned}$$

   Thus $\lambda_+ > 0$.

   Conversely, assuming $2H'(0) < r_6 + 4Y_5^\infty A_5'(0)D_5(N_T)$, then when $y \to \infty$, λ_+ will become negative. Thus by the intermediate value theorem there is a value $y_b \in \mathbb{R}_+$, for which the corresponding trivial steady state in the y-family has eigenvalue $\lambda_+ = 0$.

□

6 Discussion

Recall that this thesis is a prelude to a PhD position in the same field: mathematics applied to systems biology, specifically with yeast. Therefore suggestions for further reasearch will very likely be carried out by myself.

6.1 Existence

On Non-Trivial Steady States

In theorem 4.6 it is shown that all values of $f, y > 0$ give rise to a non-trivial steady state value, given that $(f, y) \in \Omega$.

In Remark 5 the choice to consider V_1 and r_6 as main parameters is made clear. Although this yields mathematical results, Theorem 4.6, there is another reason these paramters were chosen:
the reason that the first three basis vectors with corresponding relations of B in (11) were chosen are explained as to be natural choices that yield the implicit solutions n^* and a^* in Remark 4. Only v_1 and v_6 are zero in the three vectors. Thus their value does not influence the found steady state values a^*, n^* and Π. This is another reason to consider V_1 and r_6 as main parameters.

In Theorem 4.20 a condition has to hold, but when this condition holds it follows there is existence of the non-trivial steady state for a half open range $f \in (f_0, \infty)$. Hence it should be possible to connect this to the metabolic explosion for $f \to \infty$.

On Infinite Steady States

The existence of infinite steady states is shown unconditionally. However, as noted in Lemma 4.31, this existence is not shown to be unique. This existence might still be unique, but is not shown to be so. Hence it should still be researched wether the unique existence of infinite steady states can be shown.

6.2 Stability

On the Found Stability Properties

Note that the Corollaries 5.2 and 5.3 do not claim anything about stability. This is because these trivial steady states are not locally stable in the traditional sense, due to the zero eigenvalue:
consider Corollary 5.2. The nature of the zero eigenvalue is discussed in Remark 11. There will be no instability due to some non-linear effect, but solutions near a certain trivial steady state most likely converge to another nearby trivial steady state because the local stable manifold is only four-dimensional in the five-dimensional space. However there is a whole axis of steady states, so one could assert that the trivial steady states as a whole are locally stable in this case. There are two problems with this:

1. One needs to define what stability of a family of steady states means, which is a technical exercise in Dynamical System Theory. It can perhaps be proved that the trivial steady states in this case form an Omega-limit set.

2. The concept of whole families of trivial steady states is biologically irrelevant as described below at the modeling errors.

Instability of Trivial and Existence of Non-Trivial Steady States

It is interesting to see if there is a connection between the instability of trivial steady states and the existence of non-trivial steady states. Hulshof *et al.* [1] find that these two conditions are actually the same and therefore assert the connection that a transcritical bifurcation occurs in the system that transfers stability of the trivial steady state to the non-trivial steady state.

In the case of the new model defined in Section 2, it is clear what parameter conditions are to be imposed for the instability of the trivial steady states, see Corollaries 5.2, 5.3 and 5.4. This is connected to the parameters V_1 (in $H'(0)$) and r_6, but the condition for the existence of steady states as described in (21), (22) and Theorem 4.6 is completely independent of V_1 and r_6. Therefore the assertion that the conditions are connected or even the same is not true for this model.

On the Stability of Non-Trivial Steady States

Consider (53), 24 out of 25 entries of the Jacobian are non-trivial, non-linear functions of several variables. The found non-trivial steady states are implicit solutions to equations, see Theorem 4.4, and have a complicated condition that has to be satisfied, see Theorem 4.6. It has been attempted to look for meaning in the Jacobian for these non-trivial steady states. Through the equations that define the non-trivial steady states and implicit differentiation it might be possible to find meaning, but I was unsuccesful.

On the Stability of the Infinite Steady States

The stability analysis of the infinite steady states by means of the Jacobian was not attempted, therefore this analysis is also a suggestion for further research.

Recall Remark 10. The found infinite steady states described in Theorem 4.32 should be considered with respect to the p-coordinate especially. From this it would follow if $v_7 < 0$ as would be necessary for stability. However, the value p^*_∞ is an implicit solution dependent on many parameters and only unique because it is defined as the infinite steady state with the lowest p-coordinate, Lemma 4.31. Hence it should still be researched if $p^*_\infty < \Pi$ holds.

6.3 Modeling Errors

On the Excess of Trivial Steady States

In Section 4.1 it was shown that the New Model has two families of steady states, because through reasoning it could be shown that for a trivial steady state, ($\Phi(\vec{m}) = 0$ as in Definition 2.1, there was no need for both n and y to be zero.

Biologically this means the following in both cases:

y-family: for any concentration of pyruvate, the concentrations of the other metabolites that are considered can be such that the pyruvate concentration is not influenced, because the considered reaction fluxes are all zero.

n-family: for any concentration of NADH, the concentrations of other metabolites that are considered can be such that the NADH concentration is not influenced, because the considered reaction fluxes are all zero.

But there are many other reactions that consume pyruvate and many unconsidered reactions that consume NADH. The choice to only consider these reactions is made, because these are dominant in normal cell behaviour. But when their fluxes are zero other reactions must be dominant.

A correction one might choose is to model the effect of unconsidered reactions on NADH and pyruvate like they are modeled for ATP or p_i, by introducing for each NADH and pyruvate a function which over time degrades or buffers the metabolite and that metabolite alone. For ATP and p_i, v_6 and v_7 are have this effect. Then the model is such that for each metabolite a unique trivial steady state value is found.

On the Difficulty of Existence

It is interesting that the infinite steady states have no condition for existence and Theorem 4.32 even yields full line segments of parameter settings. On the other hand a lot of work had to be done to show existence of the non-trivial steady states and even after demanding a condition on the parameters there was for each $(f, y) \in \Omega$ only one unique setting of V_1 and r_6 that would yield a steady state.
In healthy biological systems there should a stable non-trivial steady state, because living cells usually have all kinds of feedback mechanisms to regulate that the cell doesn't die, either by malproduction (trivial steady state) or overproduction (infinite steady state/metabolic explosion).
Hence the mathematical problem as described suggests a modeling error and should be researched further.

6.4 Thesis Goals

On the Mathematical Goals

It was difficult at first to find equations on the reaction fluxes that would yield steady state values, as was done in [1]. By means of the work of Schuster *et al.* [6], [7], it was possible to find these equations in a more general way, focussing on which reaction fluxes were monotone in which variable. Proposition 4.2 is a generalisation of the framework provided in [1].

The fact that both a and n were implicitly calculated created a lot more difficulty in using implicit differentiation than was encountered by Hulshof *et al.* This was partially solved by the results of Section 4.2.2, but still significantly more complex than the toy model.

On the Biological Goals

Existence was shown for both non-trivial and infinite steady states, in that sense the behaviour as noted by Teusink *et al.* was shown. Recall that of the mutant population only a segment would show the behaviour of metabolic explosion, while other individuals would be in the regular steady state. This behaviour suggests bistability of the system for the non-trivial and infinite steady state, because these individuals have the same genotype (parameters in the model), but might have different internal conditions when they are put into the glucose abundant environment (different initial conditions). This bistability remains to be shown, hence it is a suggestion for further research.

In many ways it has been elaborated upon why V_1 and r_6 are the parameters to consider. However, as shown, for the stability of the infinite steady state and therefore the occurence of metabolic explosion, it is interesting to also consider r_7.

In Remark 8, it was asserted that the condition (40) would have a biological interpretation. Rewriting (40) yields:

$$Y_4^{\infty} N_4(n_{\infty}^{*1}(\underline{a}_{\infty}^{*}(\Pi))) > 7Y_5^{\infty} A_5(A_T) D_5(N_T)$$

The lhs has the following mathematical interpretation: $n_{\infty}^{*1}(\underline{a}_{\infty}^{*}(\Pi))$ is the configuration such that if the system is in a non-trivial steady state with $f = y = \infty$, then this is the value n would have, thus the whole lhs is the reaction flux of v_4 in that case.

Biologically $n_{\infty}^{*1}(\underline{a}_{\infty}^{*}(\Pi))$ is what the concentration of NADH would be in chemical equilibrium if fbp and pyruvate would be present in abundance and hence the whole lhs would be the reaction flux of v_4 in that situation.

The rhs is simply seven times the absolute maximum reaction flux of v_5, so the speed of succinate production.

A EM calculation for the new Model

Consider the model of glycolysis with 7 reactions, 5 metabolites as given in Section 2. Use the given notation in Definition 2.1 to compute $\mathcal{C} = \{\vec{v} \in V : S\vec{v} = 0\}$. Recall:

$$S = \left(\begin{array}{c|cccccccc} & v_1 & v_2 & v_3 & v_4 & v_5 & v_6 & v_{7+} & v_{7-} \\ \hline f & +1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 \\ p & 0 & -2 & +2 & 0 & +4 & +1 & +1 & -1 \\ y & 0 & +2 & 0 & -1 & -2 & 0 & 0 & 0 \\ a & -2 & +4 & 0 & 0 & -4 & -1 & 0 & 0 \\ n & 0 & +2 & -2 & -1 & +3 & 0 & 0 & 0 \end{array}\right)$$

Then the first tableau is given, the other tableaus can be computed using the algorithm given in Section 3.1. As in the algorithm, rows that are positive linear combinations of other rows are removed from consideration, these are denoted with $*$ in front.

$$T^{(0)} = (I|S^{-1})$$

$$= \left(\begin{array}{cccccccc|ccccc} v_1 & v_2 & v_3 & v_4 & v_5 & v_6 & v_{7+} & v_{7-} & f & p & y & a & n \\ \hline 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & +1 & 0 & 0 & -2 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & -2 & +2 & +4 & +2 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & -1 & +2 & 0 & 0 & -2 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & -1 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & +4 & -2 & -4 & +3 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & +1 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & +1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 \end{array}\right)$$

$$T^{(1)} = \left(\begin{array}{cccccccc|ccccc} 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & -1 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & +4 & -2 & -4 & +3 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & +1 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & +1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -2 & +2 & +2 & +2 \\ 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & +2 & 0 & -2 & -2 \end{array}\right)$$

$$T^{(2)} = \left(\begin{array}{cccccccc|ccccc} 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & -1 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 4 & 0 & 0 & -2 & -4 & +3 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 & 0 & 0 & 2 & 0 & 0 & 0 & -2 & -2 \\ 2 & 2 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & +2 & 0 & +7 \\ 1 & 1 & 0 & 0 & 0 & 2 & 0 & 0 & 0 & 0 & +2 & 0 & +2 \\ 1 & 1 & 0 & 0 & 0 & 0 & 2 & 0 & 0 & 0 & +2 & +2 & +2 \\ 2 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & +2 & 0 & 0 \end{array}\right)$$

$$T^{(3)} = \left(\begin{array}{cccccccc|ccccc}
0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 & -1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 \\
1 & 0 & 1 & 0 & 0 & 0 & 0 & 2 & 0 & 0 & 0 & -2 & -2 \\
2 & 2 & 0 & 2 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & +5 \\
1 & 1 & 0 & 2 & 0 & 2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & 1 & 0 & 2 & 0 & 0 & 2 & 0 & 0 & 0 & 0 & +2 & 0 \\
2 & 1 & 1 & 2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -2 \\
2 & 2 & 0 & 0 & 2 & 0 & 0 & 4 & 0 & 0 & 0 & -4 & +10 \\
1 & 1 & 0 & 0 & 1 & 2 & 0 & 4 & 0 & 0 & 0 & -4 & +5 \\
1 & 1 & 0 & 0 & 1 & 0 & 2 & 4 & 0 & 0 & 0 & -2 & +5 \\
2 & 1 & 1 & 0 & 1 & 0 & 0 & 4 & 0 & 0 & 0 & -4 & +3
\end{array}\right)$$

$$T^{(4)} = \left(\begin{array}{ccccccccc|ccccc}
 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 \\
 & 2 & 2 & 0 & 2 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & +5 \\
 & 1 & 1 & 0 & 2 & 0 & 2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
 & 2 & 1 & 1 & 2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -2 \\
* & 1 & 1 & 0 & 2 & 0 & 2 & 2 & 2 & 0 & 0 & 0 & 0 & 0 \\
* & 2 & 1 & 1 & 2 & 0 & 0 & 2 & 2 & 0 & 0 & 0 & 0 & -2 \\
* & 4 & 4 & 0 & 4 & 2 & 0 & 4 & 4 & 0 & 0 & 0 & 0 & +10 \\
* & 3 & 3 & 0 & 4 & 1 & 2 & 4 & 4 & 0 & 0 & 0 & 0 & +5 \\
* & 2 & 2 & 0 & 2 & 1 & 0 & 4 & 4 & 0 & 0 & 0 & 0 & +5 \\
* & 4 & 3 & 1 & 4 & 1 & 0 & 4 & 4 & 0 & 0 & 0 & 0 & +3
\end{array}\right)$$

$$T^{(5)} = \left(\begin{array}{cccccccc|ccccc}
v_1 & v_2 & v_3 & v_4 & v_5 & v_6 & v_{7+} & v_{7-} & f & p & y & a & n \\
\hline
0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 \\
1 & 1 & 0 & 2 & 0 & 2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
14 & 9 & 5 & 14 & 2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{array}\right) \tag{57}$$

The first row of (57) yields a futile cycle. The elementary modes are given as follows:

$$EM_1 = \begin{pmatrix} 1 \\ 1 \\ 0 \\ 2 \\ 0 \\ 2 \\ 0 \end{pmatrix}, \qquad EM_2 = \begin{pmatrix} 14 \\ 9 \\ 5 \\ 14 \\ 2 \\ 0 \\ 0 \end{pmatrix}$$

References

[1] J. Hulshof, R. Planqué, F. Bruggeman, and B. Teusink. Metabolic explosion and oscillations in a model for yeast glycolysis. In preparation, 2013.

[2] B. Teusink, J. Passarge, C. A. Reijenga, E. Esgalhado, C. C. van der Weijden, M. Schepper, M. C. Walsh, B. M. Bakker, K. van Dam, H. V. Westerhoff, and J. L. Snoep. Can yeast glycolysis be understood in terms of in vitro kinetics of the constituent enzymes? testing biochemistry. *European Journal of Biochemistry*, 267:5313–5329, 2000.

[3] B. Teusink, M. C. Walsh, K. van Dam, and H. V. Westerhoff. The danger of metabolic pathways with turbo design. *Trends in Biochemical Sciences*, 23:162–169, may 1998.

[4] H. M. Sauro. *Enzyme Kinetics for Systems Biology.* Ambrosius Publishing, 2011.

[5] L.L. Diemer. Pathway analysis of metabolic networks. Bachelor's thesis, Leiden University, 2012. http://www.math.leidenuniv.nl/nl/theses/322/.

[6] S. Schuster and C. Hilgetag. On elementary flux modes in biochemical reaction systems at steady state. *Journal of Biological Systems*, 2:165–185, 1994.

[7] S. Schuster, C. Hilgetag, J.H. Woods, and D.A. Fell. Reaction routes in biochemical reaction systems: algebraic properties, validated calculation procedure and example from nucleotide metabolism. *Journal of Mathematical Biology*, 45:153–181, 2002. http://dx.doi.org/101007/s002850200143.

[8] S.G. Krantz and H.R. Parles. *The Implicit Function Theorem; History, Theory and Applications.* Birkhauser, Boston, 2003.