COMPUTATIONAL METHODS TO DETERMINE CONSERVED MOIETIES AND PARALLEL PATHWAYS IN METABOLIC NETWORK MODELS

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Abstract: In this article some techniques to analyse metabolic network models are presented. First a tool to make sure the metabolic model is stoichiometrically correct is explained. After that, a method to detect dead-ends is proposed. Dead-ends are reactions that cannot occur in pseudo steady state conditions. The third part deals with an algorithm to extract conserved moieties. In a fourth part the concept of nullcycles is introduced. A nullcycle is composed of two parallel pathways, running in opposite direction, so that the net result is zero.

Keywords: metabolic modelling, consistency, conserved moieties, parallel pathways

1. INTRODUCTION

According to Edwards *et al.* (2002), biological discovery will not be limited by the availability of data, but by the lack of available tools to analyse and interpret these data. One of those tools is metabolic modelling. Information about reactions is put into a network. Several mathematical tools can be used to extract useful information out of the model about the micro-organism under study (Klamt *et al.*, 2002; Schilling *et al.*, 2000, 1999; Noorman *et al.*, 1991).

In this contribution, four such techniques will be discussed. First the simple, but very useful elemental consistency test will be explained. In a second section the detection of dead-ends will be discussed. Then a method to extract conserved moieties will be presented and finally the concept of null cycles will be introduced.

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2. CONSTRUCTION OF THE METABOLIC MODEL

A set of reaction equations can be described by the following matrix equation:

$$\frac{\mathrm{d}\,\boldsymbol{r}}{\mathrm{d}\,t} = \mathsf{S} \cdot \boldsymbol{v} - \boldsymbol{r} \tag{1}$$

Herein matrix S is the stoichiometric matrix (constructed as explained in equation 2), vector $\boldsymbol{v}$ contains the reaction rates and vector $\boldsymbol{r}$ contains the exchange rates (i.e. the net production or consumption rates) of the different metabolites.

When pseudo steady state is assumed, there is no difference of the net production and consumption rates and the left side of equation 1 can be set to zero.

Equation 1 can then be rewritten more explicitly as:

$$\begin{bmatrix} S_{1,1} & S_{1,2} & \dots & S_{1,m} \\ S_{2,1} & S_{2,2} & \dots & S_{2,m} \\ \vdots & \vdots & \ddots & \vdots \\ S_{p,1} & S_{p,2} & \dots & S_{p,m} \\ S_{p+1,1} & S_{p+1,2} & \dots & S_{p+1,m} \\ \vdots & \vdots & \ddots & \vdots \\ S_{n,1} & S_{1,2} & \dots & S_{n,m} \end{bmatrix} \cdot \begin{bmatrix} v_1 \\ v_2 \\ \vdots \\ v_m \end{bmatrix} = \begin{bmatrix} r_1 \\ r_2 \\ \vdots \\ r_p \\ r_{p+1} \\ \vdots \\ r_n \end{bmatrix} \tag{2}$$

The m different columns of the stoichiometric matrix S represent the m different reactions with corresponding rates v_1 to v_m. Of the n metabolites involved in the network, there are p metabolites that are considered exchangeable with the environment (the first p rows of the stoichiometric matrix). The other $n-p$ metabolites occur only inside the cell and are not exchanged with the environment. Hence, the last $n-p$ exchange rates (r_{p+1} to r_n) are equal to zero.

Equation 2 can be split into two parts. One part that involves the metabolites that are exchangeable with the environment and another part that involves the metabolites of which there is no net production or consumption (because of the pseudo steady state assumption):

$$\begin{cases} \mathsf{S}_{\mathsf{acc}} \cdot \boldsymbol{v} = \boldsymbol{r_{acc}} \\ \mathsf{S}_{\mathsf{pss}} \cdot \boldsymbol{v} = \boldsymbol{r_{pss}} = \mathbf{0} \end{cases} \tag{3}$$

$\mathsf{S}_{\mathsf{acc}}$ contains the rows of S that correspond to the exchangeable metabolites (i.e. the first p rows of the stoichiometric matrix in equation 2). The corresponding exchange rates are summarised in $\boldsymbol{r_{acc}}$. Thus $\boldsymbol{r_{acc}}$ contains the p first r's of equation 2, i.e. all exchange rates that are not zero. $\mathsf{S}_{\mathsf{pss}}$ is constituted of those rows that agree with the non accumulating metabolites (i.e. those that are in pseudo steady state). Hence, the corresponding rates, summarised in $\boldsymbol{r_{pss}}$ are equal to zero.

3. ELEMENTAL CONSISTENCY CHECK

Because the stoichiometric matrix is the foundation of all techniques used in metabolic modelling, it is important to make sure this matrix is correct i.e. that each reaction obeys the chemical law of conservation. Therefore a second matrix is constructed, the elemental composition matrix E.

The atomic composition of each metabolite that occurs in the stoichiometric matrix is put in a column of the elemental composition matrix. The order in which the metabolites occur in the elemental composition matrix should be the same as in the stoichiometric matrix. So if a metabolite occurs in row i of the stoichiometric matrix, it should also appear in column i of the elemental composition matrix.

The different rows of the elemental composition matrix represent the different atoms that are used to build up the metabolites. When electrical charge is also accounted for, an extra row is added to the elemental composition matrix.

This gives a elemental composition matrix with dimensions $\langle q \times n \rangle$, with q the number of atoms considered to be relevant for the used metabolites and n the number of metabolites considered in the model, as seen in equation 2.

To test whether the stoichiometric matrix is correct, the following equation should hold:

$$\mathsf{E} \cdot \mathsf{S} = \mathsf{0} \tag{4}$$

This equation should give the zero matrix with dimension $\langle q \times m \rangle$. If, for example, element (i, j) of that matrix is not zero, it can be concluded that the balance of atom i in reaction j is not correct. Before doing further work with the metabolic model, elemental consistency errors should be corrected.

4. DEAD-ENDS

Not every set of individual reactions forms an acceptable and meaningful metabolic reaction system. Sometimes a set of reactions cannot function because one reaction is absent. For example, if one forgets to include the first reaction of the glycolysis, the other reactions have a steady state flux equal to zero. Those sets of reactions that surely have a flux equal to zero, are called dead-ends. Dead-ends can also be caused by accumulation instead of depletion, for example when a transport reaction for an intracellular metabolite to the environment is omitted.

Furthermore, it is desirable to know whether the metabolites that are assumed to be exchangeable, are really exchangeable. If not, they will be detected with the same methodology as the on with which dead-ends in reaction sets are detected.

To find dead-ends, the test proposed by van der Heijden and Heijnen (1995) can be used. Initially the test was developed to detect dead-ends in reaction fluxes, but here a version will be described that also detects metabolites that are initially set as exchangeable, but are not. To run this test, equation 2 should be rewritten to (using the fact that $r_{p+1}, \dots, r_n$ are equal to zero):

$$\begin{bmatrix} S_{1,1} & \dots & S_{1,m} & -1 & 0 & \dots & 0 \\ S_{2,1} & \dots & S_{2,m} & 0 & -1 & \dots & 0 \\ \vdots & \ddots & \vdots & \vdots & \vdots & \ddots & \vdots \\ S_{p,1} & \dots & S_{p,m} & 0 & 0 & \dots & -1 \\ S_{p+1,1} & \dots & S_{p+1,m} & 0 & 0 & \dots & 0 \\ \vdots & \ddots & \vdots & \vdots & \vdots & \ddots & \vdots \\ S_{n,1} & \dots & S_{n,m} & 0 & 0 & \dots & 0 \end{bmatrix} \cdot \begin{bmatrix} v_1 \\ \vdots \\ v_m \\ r_1 \\ \vdots \\ r_p \end{bmatrix} = \mathbf{0} \tag{5}$$

This equation can be summarised to (which contains the same information as equation 3):

$$\mathsf{W} \cdot \boldsymbol{a} = \mathbf{0} \tag{6}$$

W is called the extended stoichiometric matrix. Vector $\boldsymbol{a}$ is the combination of reaction rates and exchange rates.

The pseudo steady state assumption implies that the formation rate of each non-accumulating metabolite (corresponding to the rows $p+1$ to n from matrix W) must equal its consumption rate. If a metabolite is produced by a certain reaction and not consumed (by some other reactions), then that reaction will have a flux equal to zero. On the other hand, a reaction that consumes a metabolite that is not produced in any other reaction, will also have a zero flux.

For the exchangeable metabolites (rows 1 to p from matrix W) the same is true: there must be a reaction that produces or consumes a net amount of the exchangeable metabolite. Otherwise the exchange rate is zero. To detect the zero fluxes or exchange rates, equation 6 is solved (Golub and Loan, 1996):

$$\boldsymbol{a} = \mathsf{W}^{\#} \cdot \mathbf{0} + \text{null}\,(\mathsf{W}) \cdot \boldsymbol{F} = \text{null}\,(\mathsf{W}) \cdot \boldsymbol{F} \tag{7}$$

$\mathsf{W}^{\#}$ is the pseudo inverse of the matrix W and $\boldsymbol{F}$ is a column vector with as many elements as there are dimensions in the nullspace of W. Vector $\boldsymbol{F}$ represents the freedom of the system i.e. how much fluxes (v_i's) or exchange rates (r_i's) may be chosen before there is an unique solution. If a reaction or exchange rate is dead-ended, the flux is zero, whatever the elements of $\boldsymbol{F}$ are. This means that the fluxes v_i or exchange rate r_i corresponding to rows of the nullspace of W containing only zero's, are dead-ends. Dead-ends do not contribute to the final solution and can be removed from the model. But it should be investigated whether they are not caused by the omission of a reaction, in which case the model should be corrected.

5. CONSERVED MOIETIES

Conserved moieties are molecular structures in the metabolites for which the production and consumption reactions are not included in the metabolic network model (van der Heijden and Heijnen, 1995), for example, AMP in AMP, ADP and ATP or NAD in NAD^+ and NADH. The total amount of those conserved moieties does not change. The sum of the rates of the reactions that use compounds that all have a certain conserved moiety, should be zero. For example, the sum of the rates of all reactions that consume/produce NADH/NAD^+ should be zero:

$$\sum r(\text{NADH/NAD}^+) = 0 \tag{8}$$

Some conserved moieties are easily detected, but others are not. In van der Heijden and Heijnen (1995) a systematic algorithm is presented to detect the different sets of metabolites that have a common conserved moiety.

5.1 *Finding conserved moieties*

It is assumed that all conservation relations as exemplified in equation 8 are summarised in the following matrix equation:

$$\mathsf{G} \cdot \boldsymbol{r_{pss}} = \mathbf{0} \tag{9}$$

To identify the conserved moieties, matrix G should be identified. To this end, equation 9 is rewritten more explicitly (according to equation 3):

$$\mathsf{G} \cdot \mathsf{S}_{\mathsf{pss}} \cdot \boldsymbol{v} = \mathbf{0} \tag{10}$$

This equation must hold for any reaction rate vector $\boldsymbol{v}$ that is used to generate $\boldsymbol{r_{pss}}$ (with use of the relation found in equation 3), even in non-pseudo steady state conditions where intracellular metabolites accumulate and thus vector $\boldsymbol{r_{pss}}$ is not equal to zero. That means that equation 10 must be true for every possible $\boldsymbol{v}$ and thus can be simplified to:

$$\mathsf{G} \cdot \mathsf{S}_{\mathsf{pss}} = 0 \tag{11}$$

Transposing both matrices gives:

$$\mathsf{S}_{\mathsf{pss}}^{\mathsf{T}} \cdot \mathsf{G}^{\mathsf{T}} = 0 \tag{12}$$

This last equation is a common linear algebra problem. A solution for G can be found by taking the nullspace of $\mathsf{S}_{\mathsf{pss}}$ (Golub and Loan, 1996):

$$\mathsf{G}^{\mathsf{T}} = \text{null}\left(\mathsf{S}_{\mathsf{pss}}^{\mathsf{T}}\right) \tag{13}$$

The dimension of the nullspace (i.e. the number of columns of null $\left(\mathsf{S}_{\mathsf{pss}}^{\mathsf{T}}\right)$) is equal to the number of conserved moieties that occur in the metabolic model. The number of rows in G^{T} is equal to the number of rows of $\mathsf{S}_{\mathsf{pss}}$, this is the number of metabolites that are not exchanged with the environment. Thus, if an element (i, j) of matrix G^{T} is not zero, this means that conserved moiety j occurs in metabolite i of matrix $\mathsf{S}_{\mathsf{pss}}$. On the other hand, if an element (i, j) of G^{T} is zero, conserved moiety j does not occur in metabolite i.

Mathematically, conservation relations such as in equation 8, imply that several rows of the stoichiometric matrix are linearly dependent on each other (Schuster, 1999). Thus, equation 13 can be interpreted as seeking for non-trivial solutions to the homogeneous system of equations that are represented by the transposed stoichiometric matrix.

5.2 *Naming conserved moieties*

When G^{T} is found, it is possible to construct for each conserved moiety an elemental composition

matrix in which all metabolites that contain the conserved moiety under consideration, are summarised. For example, the following matrix could be obtained for G^T by taking the nullspace of a certain $\mathsf{S}^\mathsf{T}_\mathsf{pss}$:

$$\begin{bmatrix} & \boldsymbol{CM_1} & \boldsymbol{CM_2} & \boldsymbol{CM_3} \\ \text{T} & 1 & 0 & 0 \\ \text{TMP} & 1 & 0 & 0 \\ \text{NAD} & 0 & 0 & 1 \\ \text{NADH} & 0 & 1 & 0 \\ \text{NADPH} & 0 & 1 & -1 \\ \text{H} & 0 & -1 & 1 \\ \text{OH} & 0 & 1 & -1 \end{bmatrix} \tag{14}$$

For simplicity, metabolites that do not contain any conserved moiety, have no row entry in the equation above, i.e. the rows with only zeros are not shown.

With the metabolites that have a non zero entry in the first column of the matrix G^T in equation 14, the following matrix can be constructed.

$$\left[\begin{array}{cccc} & \text{T} & \text{TMP} & \text{MCC}_1 \\ \text{C} & 5 & 10 & 5 \\ \text{H} & 6 & 15 & 6 \\ \text{O} & 2 & 9 & 2 \\ \text{N} & 2 & 2 & 2 \\ \text{P} & 0 & 1 & 0 \end{array}\right] \tag{15}$$

Each row represents a chemical element. Each column, except the last, represents the elemental composition of a metabolite. The last column represents the minimal chemical composition (MCC) of the first conserved moiety. The minimal chemical composition is found by taking the minimum of the occurrence of an element in each metabolite. For example, carbon occurs five times in thymidine (T) and ten times in thymidine mono phosphate (TMP). Thus the number of carbon atoms in the first conserved moiety is five.

This procedure can be repeated for the other conserved moieties. Often, the name of that unknown compound can be looked up in the elemental composition matrix.

Sometimes two conserved moieties have the same chemical composition. For example the minimal chemical composition of $\boldsymbol{CM_2}$ and $\boldsymbol{CM_3}$ in equation 14 is equal to H.

Matrix G^T is the nullspace of $\mathsf{S}^\mathsf{T}_\mathsf{pss}$. But nullspace vectors are not unique. An infinite number of bases can be constructed, each being a linear combination of the other. The only condition is that each vector in a set of vectors that determines the nullspace, should be linearly independent of the other vectors in that set.

Thus, to avoid that twice the same conserved moiety is found, a new vector $\boldsymbol{CM_4}$ can be constructed by taking a linear combination of vector $\boldsymbol{CM_2}$ and $\boldsymbol{CM_3}$ (summing them up):

$$\begin{bmatrix} & \boldsymbol{CM_2} & \boldsymbol{CM_3} & \boldsymbol{CM_4} \\ \text{T} & 0 & 0 & 0 \\ \text{TMP} & 0 & 0 & 0 \\ \text{NAD} & 0 & 1 & 1 \\ \text{NADH} & 1 & 0 & 1 \\ \text{NADPH} & 1 & -1 & 0 \\ \text{H} & -1 & 1 & 0 \\ \text{OH} & 1 & -1 & 0 \end{bmatrix} \tag{16}$$

As new base of the nullspace of $\mathsf{S}^\mathsf{T}_\mathsf{pss}$, the vectors $\boldsymbol{CM_1}$, $\boldsymbol{CM_2}$ and $\boldsymbol{CM_4}$ are taken. They are all three linearly independent of each other but now each vector gives a different minimal chemical composition: $\boldsymbol{CM_2}$ gives H and $\boldsymbol{CM_4}$ gives NAD.

To determine the conserved moieties of a model, the following procedure can be applied. From each nullspace vector (i.e. column) of G^T, the minimal chemical composition is determined. If there are two vectors, $\boldsymbol{A}$ and $\boldsymbol{B}$, that generate the same minimal chemical composition, one is replaced by a linear combination of both vectors. This is a standard matrix operation and ensures that all the new vectors in the new set are linearly independent.

In our implementation, if two vectors of G^T, $\boldsymbol{A}$ and $\boldsymbol{B}$, have the same chemical composition, the second vector $\boldsymbol{B}$ is replaced according to following rules:

(1) If the minimal chemical structure of the conserved moiety described by vector $\boldsymbol{A}$ and $\boldsymbol{B}$ can be identified as a metabolite i of the stoichiometric model (for example by comparing it with the elemental composition matrix as defined in equation 4) and none of the two vectors has a zero in the row of the metabolite i, then the second vector $\boldsymbol{B}$ is replaced by the following linear combination of $\boldsymbol{A}$ and $\boldsymbol{B}$:

$$B[i] \cdot \boldsymbol{A} - A[i] \cdot \boldsymbol{B} \tag{17}$$

with $A[i]$ and $B[i]$ being element i of respectively $\boldsymbol{A}$ and $\boldsymbol{B}$.

(2) If the chemical composition of the conserved moiety cannot be determined as being a full metabolite or the chemical composition of the conserved moiety does occur as a metabolite but one of the two (or both) vectors $\boldsymbol{A}$ and $\boldsymbol{B}$ contains a zero on the row of that metabolite, then the first position j is sought on which both vectors $\boldsymbol{A}$ and $\boldsymbol{B}$ are different from zero. The second vector $\boldsymbol{B}$ is then replaced by:

$$B[j] \cdot \boldsymbol{A} - A[j] \cdot \boldsymbol{B} \tag{18}$$

(3) If there is no position where the elements of both vectors are different from zero, the solution is less trivial and more difficult to automate. But this should occur rarely. In fact,

we never detected this case and therefore it is not accounted for in our implementation.

Taking the linear combinations as explained in point 1 and 2, can result in overflow errors, but as the algorithm was implemented in a symbolic algebra environment where there was no limit on the size of numbers, overflow was avoided.

6. NULLCYCLES AND PARALLEL PATHWAYS

The number of parallel pathways in a metabolic network is a measure for the robustness of the network. The net production of two parallel routes should be strictly the same. Thus, futile cycles are not parallel pathways as they have ATP consumption as a net effect. Under thermodynamic considerations, each pair of parallel pathways should go in the same direction. To mathematically detect them, they are considered to run in opposite direction such that the net production is zero: the second parallel pathway of the pair consumes what the first pathway produces and vice versa. This gives for equation 2:

$$\mathsf{S} \cdot \boldsymbol{v} = \mathbf{0} \tag{19}$$

This represents a homogeneous system of equations. To find the non-trivial solutions, the nullspace of S has to be taken. By definition (Golub and Loan, 1996), each vector $\boldsymbol{k}$ (column) of the nullspace of a matrix M obeys the equation: $\mathsf{M} \cdot \boldsymbol{k} = \mathbf{0}$.

The dimension (number of columns) of the nullspace of S is equal to the number of pairs of parallel pathways. Each pair of parallel pathways defines a set of reactions that, if run in the stoichiometric quantities dictated by the coefficients of the nullspace vector, produces nothing. Therefore, we will call those pairs of parallel pathways nullcycles.

In figure 1 an example of a metabolic network with one nullcycle is given: $\{2v_1, -v_2, -v_3\}$. Running v_2 and v_3 in opposite direction cancels the effect of two times reaction v_1.

Several couples of parallel pathways can be extracted from a nullcycle, depending on where the cycle is broken. In the nullcycle of figure 1 ($\{2v_1, -v_2, -v_3\}$), a first couple of parallel pathways can be $\{-v_2, 2v_1\}$ and $\{v_3\}$ (breaking the nullcycle at metabolite B and C). Another couple of parallel pathways can be constructed if the nullcycle is broken at metabolite A and C: $\{2v_1\}$ and $\{v_2, v_3\}$.

It is desirable to choose parallel pathways that give insight into the metabolic model. One property of the chosen parallel pathways should be that they are thermodynamically feasible. But

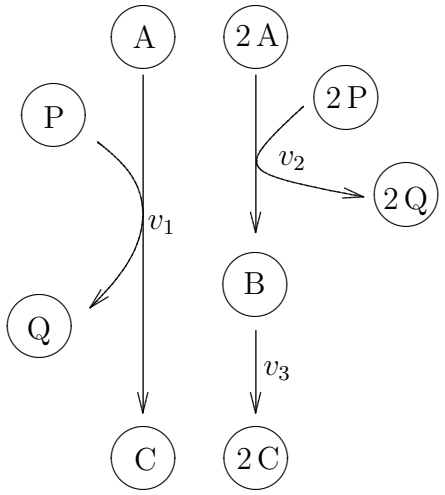

Figure 1. Metabolic network with one nullcycle.

even then, there can be more than one possible couple of parallel pathways in a nullcycle if some reactions are considered reversible. Therefore, the extra condition that all reactions are irreversible, can be used to unequivocally extract the parallel pathways from a nullcycle. Reactions with positive coefficients in the nullcycle make up the first parallel pathway and reactions with negative coefficients form the second parallel pathway. In the example of figure 1, the first parallel pathway is $\{2v_1\}$ and the second is $\{v_2, v_3\}$.

To know what the net reaction of the parallel pathways of a nullcycle is, the reactions of one of the parallel pathways are multiplied with the absolute values of their coefficients of the nullspace and the resulting net reaction is calculated. With the above example this can be summarised in the following matrix:

$$\begin{bmatrix} & v_2 & v_3 & \mathit{Net\ reaction} \\ \mathrm{A} & -2 & 0 & -2 \\ \mathrm{B} & 1 & -1 & 0 \\ \mathrm{C} & 0 & 2 & 2 \\ \mathrm{P} & -2 & 0 & -2 \\ \mathrm{Q} & 2 & 0 & 2 \end{bmatrix} \tag{20}$$

It can be seen that the net reaction is the same as the reaction of the first parallel pathway $\{2v_1\}$.

Finding nullcycles relies on finding a base for a nullspace. However, different bases can be constructed for the same nullspace. This corresponds to the fact that different nullcycles can be detected but not all are independent of each other. In figure 2 three nullcycles can be seen: $\{v_{xy1}, v_{xy2}\}$, $\{v_{yz1}, v_{yz2}\}$ and $\{v_{xy1}, v_{yz1}, v_{xy2}, v_{yz2}\}$. However, the last nullcycle is the sum of the two first or the second is equal to the last minus the first. In fact, there really are only two nullcycles. Al the others can be constructed from the two nullcycles that are selected.

The question remains which nullcycle to choose. In the example of figure 2 it seems logical to take

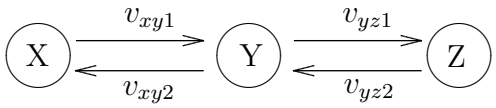

Figure 2. Network with two independent nullcycles but it seems as if there are three.

the first two, $\{v_{xy1}, v_{xy2}\}$ and $\{v_{yz1}, v_{yz2}\}$, as representation of the possible nullcycles. But it may be that it is easier to recognise the process of nullcycle three ($\{v_{xy1}, v_{yz1}, v_{xy2}, v_{yz2}\}$) than that of nullcycle two. Furthermore, a nullcycle with few reactions can generate a complicated net reaction of the corresponding pair of parallel pathways. Thus, even if all possible nullcycles (all possible vectors of all possible bases) could be determined, finding a criterion to select the simplest nullcycles is not a trivial task. Although the nullcycles cannot be determined unequivocally, the technique is useful to measure how much redundancy exists in the stoichiometric model. Redundancy is defined by Edwards and Palsson (1998) as the capability of the cell to redistribute its metabolic fluxes when faced with the loss of one or multiple enzymes; more nullcycles means more alternative pathways when one pathway is blocked. The selected nullcycles can be used to determine which internal reaction rates (the v_i's from equation 2) should be measured to solve equation 2, because net exchange rates (the p first r_i's of equation 2) do not give information about the partition workload between the different parallel pathways. To solve equation 2, at least one reaction rate of each nullcycle should be measured.

As can been seen in figure 2, if both a reaction and the reverse reaction are included in the stoichiometric matrix, this will give an extra nullcycle.

7. CONCLUSION

This article presented some techniques to analyse the structure of a metabolic network model. In a first section the elemental consistency test was presented. This test is used to check whether the stoichiometric matrix (the foundation of all techniques used in metabolic modelling) obeys the chemical law of conservation. The second part discussed a method to test whether all reactions included in the model can have a flux other than zero and whether the metabolites set as exchangeable are really exchangeable.

In a third section, a method was presented to extract conserved moieties out of a metabolic network model based on taking the nullspace of a submatrix of the stoichiometric matrix. Sometimes it seems that a conserved moiety occurs more than once. It was shown that if the base vector of one of the double conserved moieties is replaced by a linear combination of the other base vectors, another conserved moiety can be found.

In the fourth section, the concept of nullcycles was introduced. A nullcycle is composed of two parallel pathways, running in opposite direction. It is not possible to unequivocally determine the nullcycles: there are a lot of nullcycles that are linearly dependent. However, the number of independent nullcycles is a measure of the redundancy of the model. The determination of the nullcycles gives information about which internal reaction rates should be measured to fully solve the stoichiometric equation.

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