

# Methods in metabolic flux analysis for assessing the quality of measurement data



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## Introduction

Calculation of *in vivo* metabolic fluxes is an important tool for analysing metabolically engineered microorganisms. Part of such analysis is to assess the quality of the measured fluxes. Some statistical tests making use of a metabolic model are presented.

## Classical stoichiometric model:

$$S * v - r = 0$$

S Stoichiometric matrix  
v Reaction rate vector  
r Exchange rates of metabolites with outer cell environment

Can be rewritten as:

$$[S, -I] * [v^T, r^T]^T = 0 \quad \text{or} \quad W * a = 0$$

W Stoichiometric matrix extended with the identity matrix  
a Combination vector of the reaction rates and the exchange rates

Split equation in measured and calculated rates:

$$W_c * a_c + W_m * a_m = 0$$

This gives a common linear algebra problem:

$$a_c = -W_c^\# * W_m * a_m + \text{kernel}(W_c) * F$$

F Represents the degrees of freedom if the model is not fully solvable.  
 $W_c^\#$  Pseudo inverse of  $W_c$

## Good fit of the model to the data?

Substituting the solution back gives:

$$(W_m - W_c * W_c^\# * W_m) * a_m = e$$

Or shorter:

$$R * a_m = e$$

e Error of fit. Ideally zero  
R Redundancy matrix

If there are more measurements than degrees of freedom, they may contradict each other. This means that in general not an exact solution is obtained, but a least square one. In this case the error will not be zero. But can it be considered as zero, given the noise there is on the measurements?

A  $\chi^2$  test with as  $H_0$  hypothesis that the error  $e$  is zero, can be used. The test statistic  $h$  is calculated as:

$$h = e^T * (R * P_m * R^T)^{-1} * e$$

$P_m$  is the variance covariance matrix of the measured rates

## Vector comparison test

Assuming that the model is correct, and the variance-covariance matrix is correctly calculated, poor measurements can be detected with a vector comparison test. The error generating equation can be rewritten as:

$$R_1 * a_{m1} + R_2 * a_{m2} + \dots = e$$

$R_i$  The  $i$ -th column of  $R$ , which is always multiplied by the  $i$ -th element of  $a$ .

If an error is mainly caused by the  $i$ -th measurement,  $a_{mi}$ , the error vector  $e$  will resemble the  $i$ -th column of  $R$ . The same holds for linear combinations of columns of  $R$ .

## Conclusions

Metabolic models can be used to assess the quality of measured fluxes. The presented methods are general enough so that an extra measurement can easily be incorporated. This facilitates 'what if' analysis.

When doing such analysis, one should keep in mind that wrong fits can also be caused by a wrong metabolic model. Inconsistent results in the vector comparison test can point to such model errors.