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## Closed carbon balance in calculation of metabolic fluxes – Application to the central metabolism of *Saccharomyces cerevisiae* in wine-making fermentation

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**Abstract:** This paper presents the metabolic-fluxes calculation of a metabolic network representing the central metabolism of the yeast *Saccharomyces cerevisiae* in the wine-making context. Two solution methods are compared: a metabolic flux analysis (MFA) using convex analysis and providing narrow intervals of variation for the fluxes, and a flux balance analysis (FBA) based on an objective function. The constraints allowing the solution of the underdetermined set of algebraic equations are typically originating from measurements of uptake and secretion rates of external metabolites, and/or the use of an objective function, and/or metabolic constraints. It is shown here that converting reactions schemes in Cmol unit combined with data reconciliation provides a convenient formulation of closed carbon balance. In this form, the constraint formulation is natural and facilitates the understanding of the carbon distribution within the yeast metabolism.

**Keywords:** metabolic flux analysis, *Saccharomyces cerevisiae*, carbon balance, wine-making fermentation.

### 1. INTRODUCTION

Metabolic Flux Analysis (MFA) is a mathematical method for analyzing metabolism of bacterial or eukaryotic cells. It is a powerful methodology for the determination of metabolic pathway fluxes. In this approach, the intracellular fluxes are calculated by using a stoichiometric matrix (metabolic pathway map) for the intracellular reactions and applying mass balances around intracellular metabolites. A set of measured extracellular fluxes, typically uptake rates of substrates and secretion rates of metabolites, is used as input to the calculations (Vallino and Stephanopoulos, 1993; Jorgensen et al., 1995).

This modelling approach has been used to study the metabolism of *Saccharomyces cerevisiae* for almost two decades (Nissen et al., 1997; Gombert et al., 2001, Forster et al., 2003). Nevertheless, studies concerning the modelling of the wine-making fermentation process have been performed only recently (Varela et al., 2004; Pizarro et al., 2007; Charnomordic et al., 2010; Celton et al., 2012).

The final objective of the EC CAFÉ project ([www.cafe-project.org](http://www.cafe-project.org)) concerning the wine-making process is to design control tools aimed at optimising the fermentation to obtain a well-defined aromatic profile. During the alcoholic fermentation, hexoses (glucose and fructose) are mainly converted to ethanol and carbon dioxide. A large set of other by-products (higher alcohols, esters, sulphur compounds) affecting the organoleptic properties of the wine are also formed during this process (Swiegers et al., 2005); but they

represent less than 1 % of the consumed sugar. Nevertheless, before considering these aroma compounds, a relevant description of the central carbon metabolism is needed. MFA appears to be an interesting tool to model this part of the yeast metabolism.

Based on the assumption that the internal metabolites are in steady state (no accumulation), the system of linear equations resulting from the stoichiometric model is usually underdetermined when only external measurements are used. In the literature, various suggestions are made to overcome this problem (Bonarius et al., 1997; Zamorano et al., 2010): (i) the introduction of additional metabolic theoretical constraints; (ii) the exploitation of linear optimization tools and the definition of suitable objective functions; or (iii) the use of isotopic tracer experiments to determine some intracellular fluxes. An objective function is usually used in metabolic models concerning the yeast *S. cerevisiae* (Forster et al., 2003; Celton et al., 2012). Recently, a method consisting in using no additional constraint and therefore solving the undetermined system of linear equations by obtaining intervals for the different metabolic fluxes was developed for CHO cells (Provost and Bastin, 2004; Zamorano et al., 2010).

In the present study, a novel aspect is the Cmol conversion of the reaction schemes (detailed in Section 2). This impacts the coefficients into the reaction schemes, i.e., those of the stoichiometric matrix, and allows a consistent carbon balance of the system through data reconciliation. We compare the results of two methods for calculating the intracellular fluxes

of the balanced carbon central metabolism of *S. cerevisiae*: (i) determination of intervals for the metabolic fluxes, (ii) use of an objective function.

The next two sections give a description of the metabolic network and of the experimental data respectively. MFA is presented in Section 4 and the objective function strategy (FBA) in Section 5. Discussion is provided in Section 6.

## 2. METABOLIC NETWORK DESCRIPTION

The central carbon metabolism of *S. cerevisiae* considered in this paper is represented by a set of 79 biochemical reactions listed in Table 1. The metabolic network involves the following pathways: glycolysis – pentose phosphate pathway (PPP) – TCA cycle – ethanol, glycerol and acetate synthesis – transport reactions (sugar uptake and product excretion) – amino acid synthesis – DNA and RNA synthesis – lipid synthesis – carbohydrate synthesis – biomass formation. It has to be stressed that this metabolic network corresponds to a metabolism of exponentially-growing cells in wine-making conditions. This network would be different if for example the stationary phase of the yeast was considered.

Knowing that the alcoholic fermentation is an anaerobic process, the TCA pathway does not operate as a cycle but as two branches (v15 and v16 in Table 1) leading both to succinate synthesis (Camarasa et al., 2003). The biomass composition in carbohydrates, lipids and proteins was experimentally measured (as described in Celton et al., 2012). The cellular contents of RNA and DNA were standard values available in the literature (Lange and Heijnen, 2001). It has to be noted that to simplify the network the energetic cofactors (NAD and NADP) were not taken into account. Nevertheless, the chosen reactions correctly described the central carbon metabolism of *S. cerevisiae*.

The metabolic network involves 70 internal metabolites and 9 external metabolites: glucose, ethanol, glycerol, acetate, succinate, CO<sub>2</sub>, biomass, exogenous fatty acids [A<sub>exo</sub>] and exogenous lipids [lipid<sub>exo</sub>]. A<sub>exo</sub> and lipid<sub>exo</sub> are not measured data, they correspond to nutrient requirements associated with growth and are thus proportional to the production of biomass (Celton et al., 2012).

The metabolic fluxes will be expressed in Cmol/L.h.g<sub>cell</sub>. The Cmol unit corresponds to a normalization of the elements contained in a molecule by the number of carbon atoms C present in the considered compound. For example, the formula of glucose in mol is C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> and turned into Cmol it becomes CH<sub>2</sub>O. When considering reaction schemes this normalization affects the stoichiometric coefficients as illustrated in the following example with the reaction v7:

- in mol: PYR → ACAL + CO<sub>2</sub>, i.e. '3 C → 2 C + 1 C';

- in Cmol: '1 C → 2/3 C + 1/3 C', see v7 in Table 1.

On the other hand, the sum of inlet fluxes must be equal to the sum of the outlet fluxes, as presented in equation (1):

$$\sum_i v_{in(i)} = \sum_j v_{out(j)} \quad \text{or} \quad \frac{\sum_j v_{out(j)}}{\sum_i v_{in(i)}} = 100\% \quad (1)$$

where  $v_{in(i)}$  corresponds to the inlet flux of substrate  $i$  and  $v_{out(j)}$  corresponds to the outlet flux of product  $j$ . If the equation (1) is satisfied, the "carbon balance" is closed.

Moreover, there is only one substrate: glucose. The rates of metabolite synthesis can be divided by the glucose consumption rate to be normalized. This fluxes expression in percent can be used within the metabolic-network model because the reaction schemes are defined in Cmol unit and not in mol unit.

Using Cmol instead of mol in addition with the calculation of carbon recovery in percentage provides a straightforward assessment of the use of carbon through the reactions network. It is also an easy and highlighting indicator of the precision of the measurements (as detailed in Section 3).

**Table 1. Metabolic reactions for the metabolism of *S. cerevisiae***

Flux	Reaction
<b>Glycolysis</b>	
v1	Glucose → G6P
v2	G6P → F6P
v3	F6P → G3P
v4	G3P → PEP
v5	PEP → PYR
<b>Ethanol, glycerol and acetate synthesis</b>	
v6	G3P → Glycerol
v7	PYR → 2/3 ACAL + 1/3 CO <sub>2</sub>
v8	ACAL → Ethanol
v9	ACAL → Acetate
v10	Acetate → ACCOA
<b>TCA cycle</b>	
v11	PYR → 2/3 ACCOA + 1/3 CO <sub>2</sub>
v12	3/4 PYR + 1/4 CO <sub>2</sub> → OAA
v13	4/6 OAA + 2/6 ACCOA → ICIT
v14	ICIT → 5/6 AKG + 1/6 CO <sub>2</sub>
v15	MAL → Succinate
v16	AKG → 4/5 Succinate + 1/5 CO <sub>2</sub>
v17	OAA → MAL
<b>Pentose phosphate pathway (PPP)</b>	
v18	G6P → 5/6 R5P + 1/6 CO <sub>2</sub>
v19	R5P → 4/10 E4P + 6/10 F6P
v20	5/9 R5P + 4/9 E4P → 6/9 F6P + 3/9 G3P
<b>Transport reactions</b>	
v21	Glucose ext → Glucose
v22	Glycerol → Glycerol ext
v23	Ethanol → Ethanol ext
v24	Acetate → Acetate ext
v25	Succinate → Succinate ext
v26	CO <sub>2</sub> → CO <sub>2</sub> ext
v27	A <sub>exo</sub> ext → A <sub>exo</sub>
v28	Lipid <sub>exo</sub> ext → Lipid <sub>exo</sub>

<b>Amino acid synthesis</b>	
v29	PYR → ALA
v30	5/6 AKG + 1/6 CO <sub>2</sub> → ARG
v31	OAA → ASN
v32	OAA → ASP
v33	G3P → CYS
v34	AKG → GLN
v35	5/6 R5P + 1/6 CO <sub>2</sub> → HIS
v36	4/7 OAA + 3/7 PYR → 6/7 ILE + 1/7 CO <sub>2</sub>
v37	6/8 PYR + 2/8 ACCOA → 6/8 LEU + 2/8 CO <sub>2</sub>
v38	5/7 AKG + 2/7 ACCOA → 6/7 LYS + 1/7 CO <sub>2</sub>
v39	4/5 OAA + 1/5 CO <sub>2</sub> → MET
v40	4/10 E4P + 6/10 PEP → 9/10 PHE + 1/10 CO <sub>2</sub>
v41	AKG → PRO
v42	G3P → SER
v43	OAA → THR
v44	4/12 E4P + 3/12 PEP + 5/12 R5P → 11/12 TRP + 1/12 CO <sub>2</sub>
v45	4/10 E4P + 6/10 PEP → 9/10 TYR + 1/10 CO <sub>2</sub>
v46	PYR → 5/6 VAL + 1/6 CO <sub>2</sub>
v47	G3P → 2/3 GLY + 1/3 CO <sub>2</sub>
v48	OAA → 1/2 GLY + 1/2 ACAL
v49	AKG → GLU
<b>DNA, RNA synthesis</b>	
v50	5/11 R5P + 3/11 G3P + 3/11 CO <sub>2</sub> → dATP
v51	5/10 R5P + 4/10 OAA + 1/10 CO <sub>2</sub> → dTTP
v52	5/11 R5P + 3/11 G3P + 3/11 CO <sub>2</sub> → dGTP
v53	5/10 R5P + 4/10 OAA + 1/10 CO <sub>2</sub> → dCTP
v54	5/11 R5P + 3/11 G3P + 3/11 CO <sub>2</sub> → ATP
v55	5/10 R5P + 4/10 OAA + 1/10 CO <sub>2</sub> → UTP
v56	5/11 R5P + 3/11 G3P + 3/11 CO <sub>2</sub> → GTP
v57	5/10 R5P + 4/10 OAA + 1/10 CO <sub>2</sub> → CTP
<b>Lipid synthesis</b>	
v58	ACCOA → A10
v59	ACCOA → A12
v60	ACCOA → A14
v61	ACCOA → A16
v62	ACCOA → A18
v63	0.00917 A10 + 0.0413 A12 + 0.0775 A14 + 0.24 A16 + 0.0554 + 0.577 <i>A<sub>exo</sub></i> → AG
v64	54/57 AG + 3/57 G3P → TRIGLY
v65	3/39 G3P + 36/39 AG → PA
v66	6/42 G3P + 36/42 AG → PC
v67	6/42 G3P + 36/42 AG → PS
v68	6/42 G3P + 36/42 AG → PE
v69	3/45 G3P + 6/45 G6P + 36/45 AG → PINS
<b>Carbohydrate synthesis</b>	
v70	F6P → MANNAN
v71	G6P → GLYCOGEN
v72	G6P → TREHALOSE
v73	G6P → GLUCAN
<b>Biomass formation</b>	
v74	0.04 GLYCOGEN + 0.0094 TREHALOSE + 0.32 MANNAN + 0.63 GLUCAN → CARBO
v75	0.216 TRIGLY + 0.0129 PA + 0.143 PC +

	0.0391 PS + 0.107 PE + 0.137 PINS + 0.345 <i>Lipid<sub>exo</sub></i> → LIPID
v76	0.0763 ALA + 0.0547 ARG + 0.0229 ASN + 0.0681 ASP + 0.00125 CYS + 0.0301 GLN + 0.0224 HIS + 0.0749 ILE + 0.0949 LEU + 0.0965 LYS + 0.0127 MET + 0.0673 PHE + 0.0459 PRO + 0.0309 SER + 0.0423 THR + 0.0176 TRP + 0.0501 TYR + 0.0720 VAL + 0.0315 GLY + 0.0847 GLU → PROT
v77	0.313 dATP + 0.192 dCTP + 0.211 dGTP + 0.284 dTTP → DNA
v78	0.277 ATP + 0.185 CTP + 0.285 GTP + 0.252 UTP → RNA
v79	0.416 CARBO + 0.0514 LIPIDS + 0.430 PROT + 0.0922 RNA + 0.00991 DNA → <i>Biomass</i>

### 3. EXPERIMENTAL DATA

The experimental data used in the current work correspond to the monitoring of the growth of the *Saccharomyces cerevisiae* commercial strain EC1118 (Lallemand SA) at 20°C on a synthetic medium (SM300) simulating the grape juice (Bely et al., 1990) where glucose was the sole carbon source.

The extracellular measurements of glucose, ethanol, glycerol, acetate, succinate, biomass and CO<sub>2</sub> used in this study are listed in Table 2. Using these values, the carbon balance is equal to 102%, meaning that the measurements are accurate. Nevertheless, these raw data cannot be used directly. Indeed, if the incoming and outgoing fluxes are not equal, the system of equations cannot be solved. So, it was decided to distribute the error on the ethanol and CO<sub>2</sub> (because they are the main produced metabolites) keeping the ratio ethanol/CO<sub>2</sub> constant. Finally, the rates of metabolite synthesis were divided by the glucose consumption rate to be normalized and ready to use for the model.

**Table 2. Extracellular measurements**

Compound	Experimental specific rate (Cmol/L.h.gX)	Specific rates modified to get a closed carbon balance (Cmol/L.h.gX)	Normalized specific rate (%)
Glucose	4.47E-02	4.47E-02	100
Glycerol	2.19E-03	2.19E-03	4.89
Ethanol	2.30E-02	2.25E-02	50.46
Acetate	1.36E-04	1.36E-04	0.30
Succinate	8.34E-05	8.34E-05	0.19
CO <sub>2</sub>	1.45E-02	1.42E-02	31.86
Biomass	5.70E-03	5.70E-03	12.75

### 4. METABOLIC FLUX ANALYSIS

#### 4.1 Principle of the method

The yeast metabolism considered in this analysis is described

by a set of  $n = 79$  biochemical pathways listed in Table 1. The metabolic network involves a group of  $m = 70$  internal balanced metabolites.

MFA is a methodology for the quantification of the pathway fluxes from limited experimental data. In the present work, we consider the special case where extracellular measurements in the culture medium are the only available data (using a method described in Provost and Bastin, 2004 and Zamorano et al., 2010). On the basis of the metabolic network, the flux distributions are found by applying steady-state mass balances around the internal balanced metabolites. Each admissible flux distribution is represented by a vector  $v = (v_1, v_2, \dots, v_m)^T$  whose entries are the rates (or fluxes) at which the reactions proceed. The steady-state balance around the internal metabolites is expressed by the algebraic set of equations:

$$N \bullet v = 0; v \geq 0 \quad (2)$$

where the  $(m \times n)$  matrix  $N$  is the stoichiometric matrix deduced from the metabolic network ( $m$  is the number of internal balanced metabolites and  $n$  is the number of fluxes). In our case, the stoichiometric matrix  $N$  has dimensions  $70 \times 79$ . It is a rather sparse matrix (4.4% of filling). An admissible flux distribution  $v$  must satisfy the steady state balance (2) and be compatible with the experimental measurements. The specific uptake and excretion rates of the measured external species are collected in a vector  $v_m$  and are by definition linear combinations of the unknown fluxes  $v_i$ . This is expressed as follows:

$$v_m = N_m \bullet v \quad (3)$$

Where  $N_m$  is a proper  $(p \times n)$  full-rank matrix with  $p$  the number of available measurements.

The aim of MFA is to compute the set of admissible flux distributions  $v$ , i.e., the set of non-negative vectors  $v$  that satisfies the equations (2) (3). The problem is said to be well-posed if the solution set is not empty and if all the solutions are bounded. Otherwise, the system is said to be ill-posed.

When the problem is well-posed, the solution set is a polytope in the positive orthant and each admissible flux distribution  $v$  can be expressed as a convex combination of a set of non-negative basis vectors  $f_i$  which are the vertices of this polytope and form therefore a unique convex basis of the solution space. In other words, the solution set of the MFA problem is the set of admissible flux vectors defined as:

$$v = \sum_i \alpha_i f_i \quad \text{with} \quad \alpha_i \geq 0 \quad \text{and} \quad \sum_i \alpha_i = 1 \quad (4)$$

The basis vectors  $f_i$  are obtained by applying the toolbox METATOOL (Pfeiffer et al., 1999; Schuster et al., 1999) to the matrix

$$\begin{pmatrix} N & 0 \\ N_m & -v_m \end{pmatrix}$$

Once the basis vectors are known, we can compute the limiting values of the flux interval for each metabolic flux (Provost and Bastin, 2004):

$$v_i^{\min} \leq v_i \leq v_i^{\max}$$

with

$$v_i^{\min} \equiv \min \{f_{ki}, k = 1, \dots, m\}, v_i^{\max} \equiv \max \{f_{ki}, k = 1, \dots, m\}$$

where  $f_{ki}$  denotes the  $i$ -th element of the basis vector  $f_k$ .

Calculating the range of possible values for each metabolic flux is relevant for underdetermined systems which do not have a unique solution.

## 4.2 Results

We perform a flux analysis for yeast cells on the basis of the underlying metabolic network presented in Table 1. The first purpose is to characterize the feasible set of solutions using extracellular measurements only. Our system is well-posed (i.e. the sets of equations (2) (3) are reliably satisfied), therefore the number of basis vectors  $f_i$  and the size of the flux intervals will depend on the extracellular measurements that are considered.

The size of the intracellular flux intervals is analyzed under different assumptions. In the first place the 7 experimental rates of the external metabolites are used to compute the flux intervals. In this case, most of the fluxes have fixed values; the values of the undetermined fluxes (internal fluxes) are within a fairly narrow interval. This observation makes sense because a system of 79 linear equations (metabolic reactions) constrained with 70+7 values (internal+external metabolites) is still underdetermined.

Figure 1 illustrates the distribution of G6P between the glycolysis pathway ( $v_2$ ) and the PPP ( $v_{18}$ ). It stresses solution intervals that are between 59 and 61% for  $v_2$  and between 35 and 38% for  $v_{18}$ . The reaction  $v_{20}$  corresponding to the outlet of the PPP is between 17 and 18%. These results are satisfactory because the narrow intervals of variation of the metabolic fluxes make possible a good understanding of the carbon distribution within the yeast metabolism.

If we use 6 rates of external metabolites, results are similar because the rate of the "missing" external compound is equal to the experimental value (see Figure 2 for example). This observation is not surprising: there is no carbon accumulation within the cell (the sum of the rates of external metabolites is equal to zero), therefore when only one external metabolite is not taken into account, its value must be equal to its experimental value (knowing that, for our experimental values, the carbon balance is kept).

If we use 5 rates of external metabolites, the intervals of variation that are defined for the two last measured rates are large and include the corresponding measurement value. The ranges of variation of some internal fluxes are consequently large too, making difficult the interpretation of the obtained results. In Figure 1, where  $\text{CO}_2$  and biomass fluxes are considered as unknown, the fluxes  $v_2$  and  $v_{18}$  corresponding

to the distribution of G6P between the glycolysis pathway and the PPP are almost undetermined as they nearly varies between 0 and 100%. The reaction  $v_{20}$  corresponding to the outlet of the PPP is between 0 and 50%. This is obviously a too large interval and the maximum value is far from a standard flux value for the considered strain of yeast (personal communication). Figure 2 also illustrates this result with the calculated fluxes of  $\text{CO}_2$  and biomass: the mean value of the interval of variation respectively deviates from 5 and 18 % compared to the experimental value. This is reasonable for  $\text{CO}_2$  but not for the biomass.

These last observations show that if the number of constraints is low, the span of solution highly increases and it becomes difficult to analyze and understand the results. On the contrary, using 6 or 7 measurements provides satisfactory results demonstrating the reliability of these measurements and the relevance of the data reconciliation.

## 5. USE OF AN OBJECTIVE FUNCTION

A FBA method (Lee et al., 2006) based on an objective function was tested on the metabolic network. The assumption considered for defining the objective function is the maximization of the biomass production as the measurements were taken during the growth phase of the yeast (see Section 3). This corresponds to the maximization of the reaction  $v_{79}$ .

If we use the rates of the 6 external metabolites except biomass, we observe that the calculated biomass production rate is equal to the experimental value (see Figure 2). Once again, this observation makes sense; it is due to the carbon balance. Using the objective function, a single value is obtained for each metabolic flux. An interesting observation is that this value is equal to the central value of the interval obtained with the previous solution method when considering internal fluxes (example in Figure 1). This shows that the methods consisting either in only solving the underdetermined system or in using an objective function give close results as long as sufficient external metabolites are measured.

When we use 5 rates of external compounds, several observations are made:

1) if glycerol, acetate or succinate are among the unused measurements their calculated values are equal to zero. Indeed, the objective function consists in maximizing the biomass production; so no carbon is sent or assigned to the production of a metabolite that is not directly involved in biomass synthesis such as the before-mentioned compounds.

2)  $\text{CO}_2$  is involved in different metabolic pathways (glycolysis, ethanol production, biomass synthesis) so its value cannot be equal to zero; nevertheless, when its rate is not fixed, the calculated value is underestimated by 20% compared to the experimental one (see Figure 2). The results in Figure 1 provide an explanation.  $v_2$  value is maximum whereas  $v_{18}$  and  $v_{20}$  values are close to 0 meaning that most

of the carbon is transferred to glycolysis to the detriment of PPP. As  $v_{18}$  produces  $\text{CO}_2$ , it can explain the underestimation of the  $\text{CO}_2$  flux.

Consequently the biomass synthesis is systematically overestimated. Figure 2 stresses an overestimation up to 47% for instance.

It can be concluded that the maximization of biomass production is a validated assumption as long as the needed measurements are available.

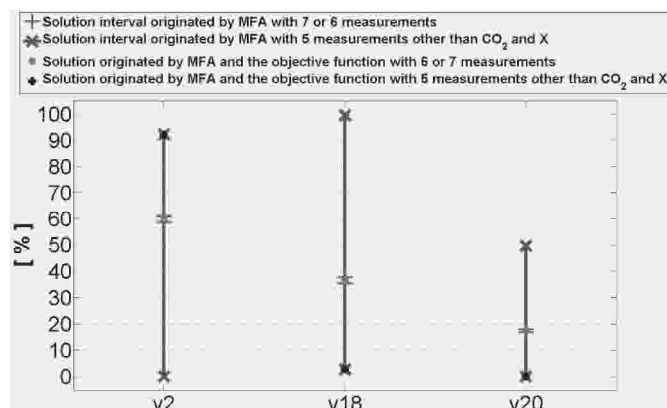


Fig. 1. Calculated fluxes for reactions  $v_2$ ,  $v_{18}$  and  $v_{20}$  depending on the available measurements and the solution method.

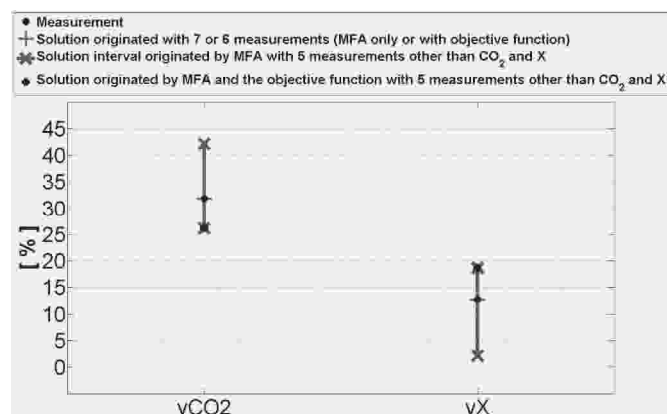


Fig. 2. Calculated fluxes for  $\text{CO}_2$  ( $v_{26}$ ) and biomass X ( $v_{79}$ ) depending on the available measurements and the solution method.

## 6. DISCUSSION

The Cmol conversion of the reaction schemes in addition with the calculation of carbon recovery in percentage provides a quite convenient overview of the use of carbon through the metabolic network. This formulation also eases data reconciliation prior to the use of MFA tools.

The two solution methods used in this work provide reliable and comparable results. As a matter of fact, when all the rates of external metabolites are used to solve the system of equations, the flux calculated for each reaction of the network using an objective function is equal to the central value of the calculated interval provided by the toolbox METATOOL. A good reliability is also obtained when only one external measurement is missing.

With less measurements the convex analysis also provides solutions but with larger intervals making difficult any metabolic interpretation. Results obtained with the objective function do not account for key metabolites (succinate, acetate, and glycerol) if they are not included into the measurements. Indeed, in the considered metabolic network, these compounds are not directly linked to the biomass production and consequently to the objective function. This last observation gives insight about the selection of the metabolic network: the production of these products is associated with the regeneration of energetic cofactors necessary to biomass synthesis, i.e. linking the production of succinate, acetate, glycerol, to the production of biomass. So it would be interesting to establish this link in the next version of the metabolic network so as to overcome this issue. Moreover, taking into account these metabolic intermediates is also of interest because the intensity of some internal fluxes involved in the regeneration of cofactors appears to be unappropriate (for example the flux values in PPP).

In the future, the conversion of reaction schemes into Cmol and Nmol will be considered so as to close carbon and nitrogen balances. This will allow studying an extended version of the yeast metabolism including the nitrogen metabolism and some volatile compounds having an organoleptic interest.

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