# Supplementary material II: fermentation analysis

*Amino acid metabolism*

We have compared the amount of amino acids being taken up by cells during continuous fermentation, and the amount of amino acids found back in the biomass. **Table SII-1** shows the data for fermentations with 25 mM glucose in the feed. In **Table SII-2**, the data for the fermentations at 100 mM glucose are shown. Both sets of experiments showed that there is substantial excess of amino acid uptake over incorporation into biomass, i.e. active catabolism of amino acid. Subsequently, we have incorporated amino acid degradation pathways into the model. These pathways were primarily based on literature data, since not many genes are known for these pathways (1). We have, however, also performed an analysis of the head space of a fermentor by means of a purge and trap method (detailed in (2)). A sample of the head space during fermentation was trapped on an active carbon carrier. The volatiles were subsequently released by helium gas and analyzed by GC-MS. **Table SII-3** shows identified compounds that were included in the model.

| | *amino acid uptake (mmol/gDW)* | | | *biomass content (mmol/gDW)* | | | *up/needed* | | | p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| | average | ± | stdev | average | ± | stdev | average | ± | stdev | |
| **Alanine** | 0.10 | ± | 0.02 | 0.60 | ± | 0.01 | 0.2 | ± | 0.03 | 0.000 |
| **Arginine** | 0.12 | ± | 0.02 | 0.10 | ± | 0.01 | 1.2 | ± | 0.08 | 0.070 |
| **Aspartic acid** | 0.49 | ± | 0.07 | 0.29 | ± | 0.01 | 1.7 | ± | 0.24 | 0.013 |
| **Cystine** | 0.07 | ± | 0.03 | 0.01 | ± | 0.00 | 5.4 | ± | 2.00 | 0.030 |
| **Glutamic acid** | 0.32 | ± | 0.03 | 0.40 | ± | 0.02 | 0.8 | ± | 0.11 | 0.010 |
| **Glycine** | 0.26 | ± | 0.07 | 0.20 | ± | 0.01 | 1.2 | ± | 0.26 | 0.288 |
| **Histidine** | | | | 0.04 | ± | 0.00 | | | | |
| **Isoleucine** | 0.14 | ± | 0.01 | 0.10 | ± | 0.01 | 1.3 | ± | 0.15 | 0.004 |
| **Leucine** | 0.22 | ± | 0.09 | 0.19 | ± | 0.01 | 1.2 | ± | 0.49 | 0.572 |
| **Lysine** | 0.17 | ± | 0.02 | 0.16 | ± | 0.01 | 1.1 | ± | 0.07 | 0.327 |
| **Methionine** | | | | 0.05 | ± | 0.00 | | | | |
| **Phenylalanine** | 0.09 | ± | 0.01 | 0.08 | ± | 0.00 | 1.1 | ± | 0.14 | 0.276 |
| **Proline** | 0.18 | ± | 0.10 | 0.10 | ± | 0.00 | 1.9 | ± | 1.01 | 0.194 |
| **serine** | 1.58 | ± | 1.06 | 0.14 | ± | 0.00 | 11.7 | ± | 8.15 | 0.072 |
| **threonine** | 0.70 | ± | 0.18 | 0.16 | ± | 0.00 | 4.5 | ± | 1.10 | 0.009 |
| **Tryptophane** | | | | 0.01 | ± | 0.00 | | | | |
| **Tyrosine** | 0.23 | ± | 0.11 | 0.07 | ± | 0.00 | 3.5 | ± | 1.69 | 0.060 |
| **Valine** | 0.32 | ± | 0.05 | 0.15 | ± | 0.01 | 2.2 | ± | 0.24 | 0.005 |

**Table SII-1. uptake and incorporation of amino acids into biomass during continuous cultivation with 25 mM glucose in the feed.** The p-value is based on a student's t-test testing the null hypothesis that the amount of amino acid taken up and the amount of amino acid found back in the cell pellet are equal. Dilution rates ranged form 0.11 – 0.47 $h^{-1}$.

| | *amino acid uptake (mmol/gDW)* | | | *biomass content (mmol/gDW)* | | | *up/needed* | | | p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| | average | ± | stdev | average | ± | stdev | average | ± | stdev | |
| **Alanine** | 0.2 | ± | 0.09 | 0.52 | ± | 0.20 | 0.5 | ± | 0.17 | 0.053 |
| **Arginine** | 0.1 | ± | 0.02 | 0.07 | ± | 0.02 | 1.0 | ± | 0.23 | 0.991 |
| **Aspartic acid** | 0.4 | ± | 0.05 | 0.24 | ± | 0.07 | 1.5 | ± | 0.28 | 0.045 |
| **Cystine** | 0.0 | ± | 0.01 | 0.01 | ± | 0.00 | 5.0 | ± | 1.06 | 0.003 |
| **Glutamic acid** | 0.2 | ± | 0.07 | 0.36 | ± | 0.14 | 0.7 | ± | 0.16 | 0.171 |
| **Glycine** | 0.2 | ± | 0.05 | 0.16 | ± | 0.04 | 1.4 | ± | 0.16 | 0.105 |
| **Histidine** | 0.0 | | 0.02 | 0.03 | ± | 0.01 | 0.9 | | 0.33 | 0.882 |
| **Isoleucine** | 0.2 | ± | 0.04 | 0.08 | ± | 0.02 | 2.4 | ± | 0.09 | 0.010 |
| **Leucine** | 0.3 | ± | 0.10 | 0.15 | ± | 0.04 | 1.6 | ± | 0.43 | 0.135 |
| **Lysine** | 0.1 | ± | 0.04 | 0.12 | ± | 0.03 | 0.4 | ± | 0.21 | 0.231 |
| **Methionine** | 0.1 | | 0.02 | 0.04 | ± | 0.02 | 1.3 | | 0.25 | 0.276 |
| **Phenylalanine** | 0.2 | ± | 0.05 | 0.06 | ± | 0.02 | 2.5 | ± | 0.57 | 0.031 |
| **Proline** | 0.1 | ± | 0.07 | 0.09 | ± | 0.03 | 0.9 | ± | 0.56 | 0.908 |
| **serine** | 0.8 | ± | 0.13 | 0.12 | ± | 0.05 | 7.6 | ± | 1.88 | 0.001 |
| **threonine** | 0.5 | ± | 0.08 | 0.12 | ± | 0.04 | 4.1 | ± | 0.56 | 0.001 |
| **Tryptophane** | 0.0 | | 0.01 | 0.01 | ± | 0.00 | 0.8 | | 0.43 | 0.933 |
| **Tyrosine** | 0.2 | ± | 0.03 | 0.05 | ± | 0.01 | 3.9 | ± | 0.70 | 0.001 |
| **Valine** | 0.2 | ± | 0.10 | 0.11 | ± | 0.02 | 2.0 | ± | 0.55 | 0.092 |

**Table SII-2. uptake and incorporation of amino acids into biomass during continuous cultivation with 100 mM glucose in the feed.** The p-value is based on a student's t-test testing the null hypothesis that the amount of amino acid taken up and the amount of amino acid found back in the cell pellet are equal. Dilution rates ranged form 0.08 – 0.11 $h^{-1}$.

| **Component** | **origin** |
|---|---|
| 2-methyl butanoic acid | isoleucine |
| 2-methylbutanol | isoleucine |
| 2-methylpropanoic acid | valine |
| 2-methylpropanal | valine |
| 2-methylpropanol | valine |
| 3-methylbutanol | leucine |
| phenol | tyrosine |
| formate | glucose |
| ethanol | glucose |
| acetaldehyde | glucose/threonine |

**Table SII-3: qualitative detection of volatiles in the head space of a fermentation of *L. plantarum* in CDM at 25 mM (D=0.1 $h^{-1}$).** Compounds are shown that are included in the model.

*Analysis of valine catabolism as example for branched chain and aromatic amino acids*

This section will explain how the excess uptake and subsequent catabolism of amino acids, and valine in particular, could contribute to ATP formation under our conditions. The maximal amount of ATP that the network could generate was calculated by introducing an ATP-hydrolysis step that dissipated ATP into heat. Under fully coupled conditions, this rate of ATP dissipation reflects the rate of ATP production that would be needed for growth and maintenance purposes. We use the reduced cost of each exchange reaction for ATP dissipation to assess the extent to which extra availability or excretion of compounds would affect the ATP production rate. Exchange reactions are defined for each compound that can (potentially) be exchanged from the fermentor to the environment. Constraints were defined on the basis of measured fluxes ± standard deviation for each chemostat, and ATP dissipation was maximized. The results of this exercise were presented in **Table 3** of the main text.

Catabolism of certain amino acids is known to generate free-energy in lactic acid bacteria, where specific systems contribute to the proton motive force over the cytoplasmic membrane (3,4). Since a proton-pumping ATPase is used to maintain such a proton motive force, any positive charge that is net exported is equivalent to 1/*n* ATP, *n* being the stoichiometry of protons pumped per ATP hydrolyzed (*n*=3 is used in the model ). Also *L. plantarum* has some of these amino acid catabolic systems, such as glutamate decarboxylation, that results in net export of a proton (5,6), but it lacks others, such as the arginine deiminase pathway (7). The model indicated that catabolism of branched chain and aromatic amino acids had a positive effect on ATP production (**Table 3 of main text**). This is not immediately obvious upon inspection of the catabolic pathways. We will illustrate for valine how, in the model, ATP dissipation can be promoted by valine catabolism.

Degradation of valine into 2-methylpropanol occurs via the branch-chain amino-acid transferase, with preference for α-ketoglutarate as the ammonium-acceptor. Subsequent decarboxylation and reduction leads to the formation of 2-methylpropanol, which probably diffuses out of the cell **(Fig. SII-2)**. Taking transport into account, the overall reaction is:

*valine(e) + α-ketoglutarate$^-$(c) + nadh(c) + h$^+$(c) + h$^+$(e) + -> 2-methylpropanol(e) + nad$^+$(c) + glutamate(c) + co2(c).*

where (c) and (e) mean cytosolic and extracellular, respectively. However, α-ketoglutarate cannot be synthesized de novo in most lactic acid bacteria including *L. plantarum*, and it therefore needs to be regenerated by a catabolic glutamate dehydrogenase that takes NADP as cofactor (in *L. plantarum* (8)). Moreover, ammonium, a product of amino acid catabolism, is transported over the membrane as neutral ammonia (9), leaving the proton in the cytoplasm. The overall reaction of valine degradation then becomes (**Figure SII-1**):

*valine(e) + h2o(c) + nadh(c) + nadp$^+$(c) + 2h$^+$(e) -> 2-methylpropanol(e) + nh$_4^+$ (e) + nad$^+$(c) + nadph(c) + h$^+$(c)*

This overall reaction is equivalent to a proton-motive-force driven transhydrogenase, as one extracellular positive charge is translocated to the cytoplasm. However, the transhydrogenase is a highly favourable reaction, as it regenerates catabolic NAD and produces NADPH for biosynthesis. Anaerobic oxidation of NADH allows for less ethanol formation (normally the redox sink for mixed acid fermentation) and hence more acetate and concomitant ATP production (note that acetate formation also has a positive reduced cost towards ATP production). Moreover, the NADH-generating glycolytic pathway yields more ATP than glucose degradation via the NADPH-generating pentose phosphate pathway (difference of 0.5 ATP per glucose). Since the valine catabolic pathway itself does not generate ATP directly, this is a good illustration of how a

genome-scale model identifies a previously unrecognised energetic advantage for the degradation of valine (and the other branched chain and aromatic amino acids) via transamination.

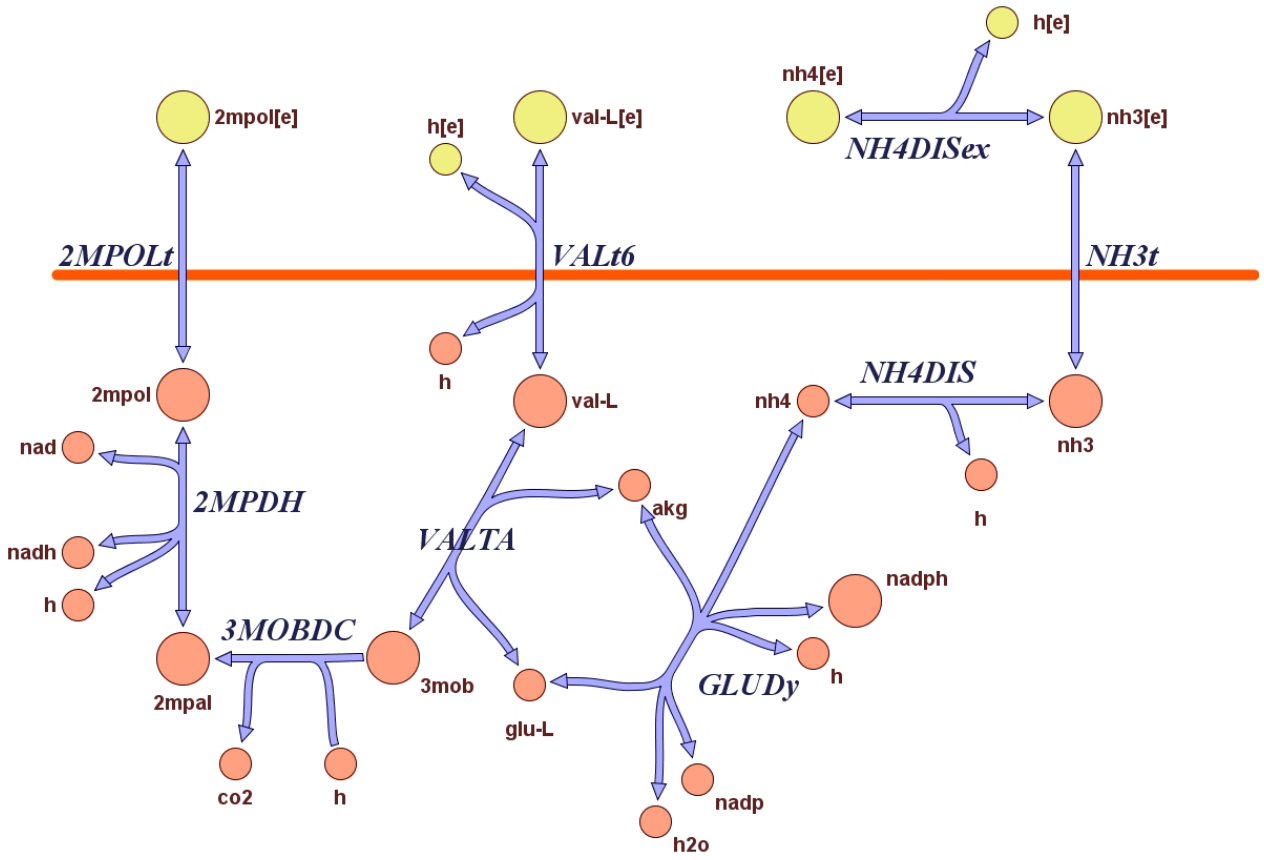


**Figure SII-1. Overall catabolic pathway of valine to 2-methylpropanol is a proton-motif force driven transhydrogenase.**

## References


1. Smit, G., Smit, B. A., and Engels, W. J. (2005) *FEMS Microbiol Rev* **29**(3), 591-610
2. Ayad, E. H. E., Awad, S., El Attar, A., de Jong, C., and El-Soda, M. (2004) Food Chem 86, 553-561
3. Konings, W. N. (2002) *Antonie Van Leeuwenhoek* **82**(1-4), 3-27
4. Poolman, B. (2002) *Antonie Van Leeuwenhoek* **82**(1-4), 147-164
5. Small, P. L., and Waterman, S. R. (1998) *Trends Microbiol* **6**(6), 214-216
6. Sanders, J. W., Leenhouts, K., Burghoorn, J., Brands, J. R., Venema, G., and Kok, J. (1998) *Mol Microbiol* **27**(2), 299-310
7. Christensen, J. E., Dudley, E. G., Pederson, J. A., and Steele, J. L. (1999) *Antonie Van Leeuwenhoek* **76**(1-4), 217-246
8. Tanous, C., Kieronczyk, A., Helinck, S., Chambellon, E., and Yvon, M. (2002) *Antonie Van Leeuwenhoek* **82**(1-4), 271-278
9. Khademi, S., O'Connell, J., 3rd, Remis, J., Robles-Colmenares, Y., Miercke, L. J., and Stroud, R. M. (2004) *Science* **305**(5690), 1587-1594