

Additional file 2.

1.1 Stoichiometric model for *P. pastoris* containing some additional reactions from the ^{13}C model (section 1.2)

Methanol metabolism

1. Metoh \Rightarrow Form
2. Form \Rightarrow FOR + NADH
3. FOR + NAD $^+$ \Rightarrow NADH + CO $_2$
4. Xu15P + FOR + ATP \Rightarrow ADP + GA3P_{per} + DHA
5. GA3P_{per} \Rightarrow GA3P
6. DHA \Rightarrow GA3P

Glycolysis and gluconeogenesis pathways

7. Glc_{ext} + 2 ATP \Leftrightarrow Glc6P + 2 ADP
8. Glc6P \Leftrightarrow Fru6P
9. Fru6P + 1 ATP \Rightarrow FBP + 1ADP
10. FBP \Rightarrow Fru6P + Pi
11. FBP \Leftrightarrow DHAP + GA3P
12. GA3P + ADP + Pi + NAD $^+$ \Rightarrow PG3 + ATP + NADH
13. PG3 + ATP + NADH \Rightarrow GA3P + ADP + Pi + NAD $^+$
14. PG3 \Leftrightarrow Pep
15. Pep + ADP \Rightarrow Pyr + ATP
16. Pyr + NAD $^+$ \Rightarrow ACCoA_{mit} + CO $_2$ + NADH
17. Pyr + NAD $^+$ \Rightarrow ACCoA_{cyt} + CO $_2$ + NADH

18. $\text{Pyr} + \text{CO}_2 + \text{ADP} \Rightarrow \text{OAA} + \text{ATP}$
19. $2 \text{Glc6P} + \text{UTP} + \text{H}_2\text{O} \Rightarrow \text{H}^+ + 2 \text{Pi} + \text{UDP} + \text{T6P}$
20. $\text{T6P} + \text{H}_2\text{O} \Rightarrow \text{Pi} + \text{Treh}$
21. $\text{Treh} + \text{H}_2\text{O} \Rightarrow 2 \text{Glc6P}$

Pentose phosphate pathway

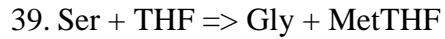
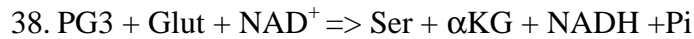
22. $\text{Glc6P} + 2 \text{NADP}^+ \Rightarrow \text{Rul5P} + 2 \text{NADPH} + \text{CO}_2$
23. $\text{Rul5P} \rightleftharpoons \text{Rib5P}$
24. $\text{Rul5P} \rightleftharpoons \text{Xul5P}$
25. $\text{Rib5P} + \text{Xul5P} \rightleftharpoons \text{Sed7P} + \text{GA3P}$
26. $\text{Sed7P} + \text{GA3P} \rightleftharpoons \text{Fru6P} + \text{E4P}$
27. $\text{Xul5P} + \text{E4P} \rightleftharpoons \text{Fru6P} + \text{GA3P}$

TCA cycle

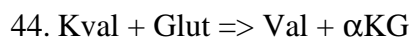
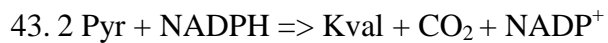
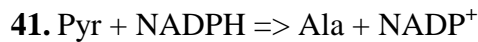
28. $\text{ACCoA}_{\text{mit}} + \text{OAA} \Rightarrow \text{CIT}$
29. $\text{CIT} \Rightarrow \text{ICIT}$
30. $\text{ICIT} + \text{NAD}^+ \Rightarrow \alpha\text{KG} + \text{CO}_2 + \text{NADH}$
31. $\alpha\text{KG} + \text{NAD}^+ \Rightarrow \text{SUCCoA} + \text{CO}_2 + \text{NADH}$
32. $\text{SUCCoA} + \text{Pi} + \text{ADP} \Rightarrow \text{SUCC} + \text{ATP}$
33. $\text{SUCC} + \text{ATP} \Rightarrow \text{SUCCoA} + \text{ADP} + \text{Pi}$
34. $\text{SUCC} + \text{NAD}^+ \Rightarrow \text{FUM} + \text{NADH}$
35. $\text{FUM} + \text{H}_2\text{O} \rightleftharpoons \text{MAL}$
36. $\text{MAL} + \text{NAD}^+ \rightleftharpoons \text{OAA} + \text{NADH}$
37. $\text{Asp} + 2 \text{ATP} + \text{H}_2\text{O} \Rightarrow \text{FUM} + 2 \text{ADP} + 2\text{Pi}$

Biosynthesis of amino acids

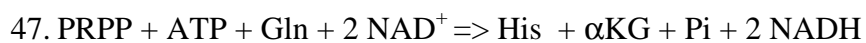
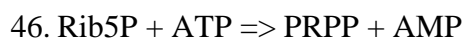
Serine family



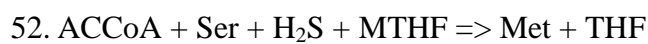
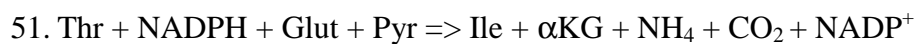
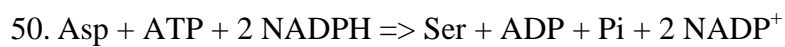
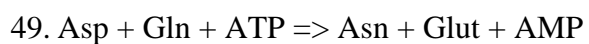
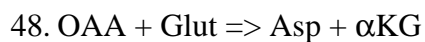
Alanine family



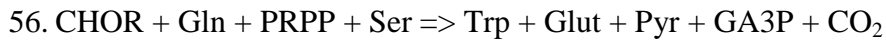
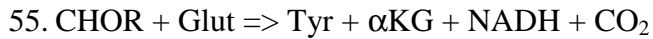
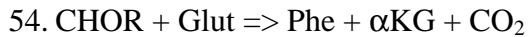
Histidine family



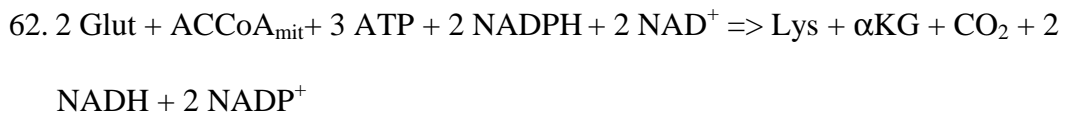
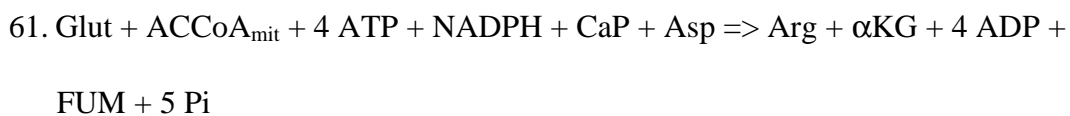
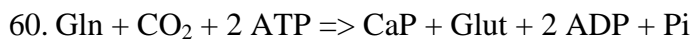
Aspartic family



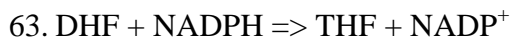
Aromatic family



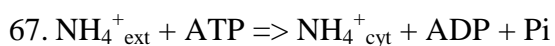
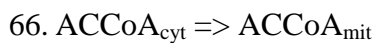
Glutamic family

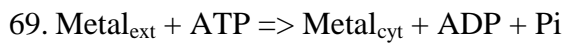
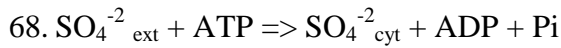


Biosynthesis and interconversion of one-carbon units

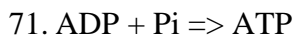
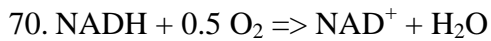


Transport reactions



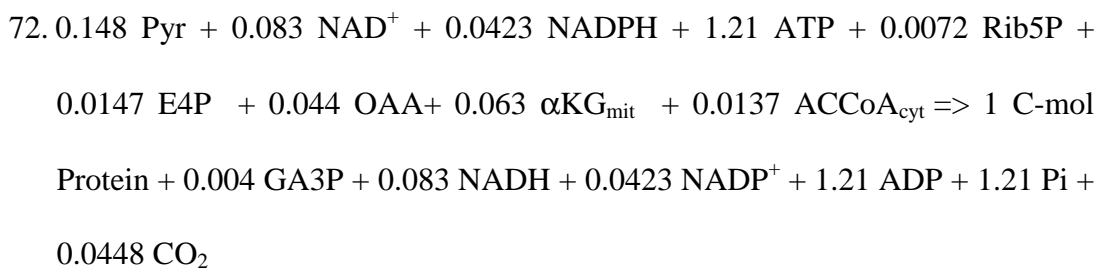


Respiratory chain

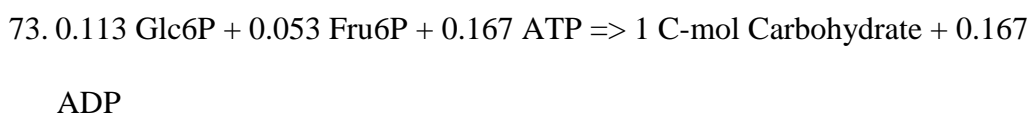


Biomass synthesis

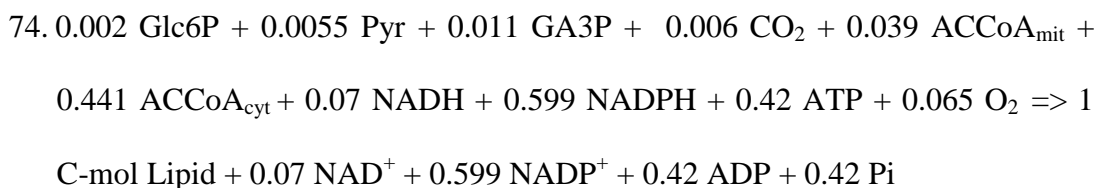
1. Protein synthesis (Composition derived from the measured amino acid composition [21]. The energy needed to biosynthesize 1 C-mol of protein was derived from the synthesis of each amino acid and the protein polymerization value taken from [50])



2. Carbohydrate synthesis (Composition derived [51])



3. Lipids synthesis (derived from the mean lipid composition from [30])



4. RNA synthesis (derived from the RNA composition [57])

75. $0.056 \text{ Pyr} + 0.1136 \text{ CO}_2 + 0.105 \text{ Rib5P} + 0.104 \text{ NAD}^+ + 0.075 \text{ NADPH} + 1.1 \text{ ATP} + 0.0479 \text{ OAA} \Rightarrow 1 \text{ C-mol RNA} + 1.1 \text{ Pi} + 1.1 \text{ ADP} + 0.075 \text{ NADP}^+ + 0.104 \text{ NADH}$

5. DNA synthesis (composition derived from [57] and the DNA polymerization [51])

76. $0.051 \text{ Pyr} + 0.102 \text{ NAD}^+ + 0.273 \text{ NADPH} + 1.146 \text{ ATP} + 0.132 \text{ CO}_2 + 0.102 \text{ Rib5P} + 0.051 \text{ OAA} \Rightarrow 1 \text{ C-mol DNA} + 1.146 \text{ Pi} + 1.146 \text{ ADP} + 0.102 \text{ NADH} + 0.273 \text{ NADP}^+$

1.2 Reactions and atom transitions network used in ¹³C-MFA, following the notation of [65].

Name	Reaction
feedGlcB:	FullyGlu > Glu _{ext} #abcdef > #abcdef
feedGlcC:	CGlu > Glu _{ext} #abcdef > #abcdef
uptGlc:	Glu _{ext} > Glu _{int} #abcdef > #abcdef
feedMeOHB:	MetohL > Metoh _{ext} #a > #a
uptMeOH:	Metoh _{ext} > Metoh _{int} #a > #a
upt1:	Glu _{int} > Glc6P #abcdef > #abcdef
upt2:	Metoh _{int} > Form #a > #a
TRE1:	Glc6P > T6P #abcdef > #abcdef
TRE2:	T6P > Treh #abcdef > #abcdef
TRE3:	Treh > Glu _{int} #abcdef > #abcdef
emp1:	Glc6P <> Fru6P

#abcdef > #abcdef
emp2: Fru6P > FBP
 #abcdef > #abcdef
emp2B FBP > Fru6P
 #abcdef > #abcdef
emp3: FBP <> DHAP + GA3P
 #abcdef > #cba + #def
emp4: DHAP <> GA3P
 #abc > #abc
emp5: GA3P <> PG3
 #abc > #abc
emp6: PG3 <> PG2
 #abc > #abc
emp7: PG2 <> Pep
 #abc > # abc
emp8: Pep > Pyr
 #abc > #abc
emp9: Pyr > ACCoA_{cyt} + CO2
 #abc > #bc + #a
emp10: Pyr + CO2 > OAA_{mit}
 #abc + #d > #abcd
emp11: Pyr > Pyrt
 #abc > #abc
emp11A: Pyr > Pyr_{mit}
 #abc > #abc
emp11B: Pyr_{mit} > Pyrt
 #abc > #abc
emp11C: NPyr > Pyr_{mit}

emp11D: #ABC > #ABC
 Pyr_{mit} > Pyr_{ext}
 #ABC > #ABC

emp12: ACCoA_{cyt} > ACCoA_{mit}
 #AB > #AB

ppp1: Glc6P > CO₂ + Ru5P
 #abcdef > #a + #bcdef

ppp2: Ru5P <> Xu5P
 #abcde > #abcde

ppp3: Ru5P <> Rib5P
 #abcde > #abcde

ppp4: Xu5P + E4P <> GA3P + Fru6P
 #ABCDE + #abcd > #CDE + #ABabcd

ppp5: Xu5P + Rib5P <> Sed7P + GA3P
 #abcde + #ABCDE > #ABabcde + #CDE

ppp6: GA3P + Sed7P <> E4P + Fru6P
 #ABC + #abcdefg > #defg + #abcABC

TCA1: Pyr_{mit} > ACCoA_{mit} + CO₂
 #ABC > #BC + #A

TCA2: ACCoA_{mit} + OAA > CIT_{mit}
 #AB + #abcd > #dcbaBA

TCA3: CIT_{mit} > αKG + CO₂
 #ABCDEF > #ABCEF + #D

TCA4: αKG > SUCC + CO₂
 #ABCDE > #BCDE + #A

TCA4B: αKG > SUCC + CO₂
 #ABCDE > #EDCB + #A

TCA5: SUCC <> FUM

TCA5B: #ABCD > #ABCD
 SUCC <> FUM
 #ABCD > #DCBA

TCA6: FUM <> MAL
 #ABCD > #ABCD

TCA7: MAL <> OAA
 #ABCD > #ABCD

TCA8: Asp > FUM
 #ABCD > #ABCD

Met1: Form > CO₂
 #A > #A

Met2: Xul5P + Form > DHA + GA3P_{per}
 #ABCDE + #F > #FAB + #CDE

Met2B: Xul5P + Form > DHA + GA3P_{per}
 #ABCDE + #F > #ABF + #CDE

Met3: DHA > DHAP
 #ABC > #ABC

Met4: GA3P_{per} > GA3P
 #ABC > #ABC

BIO1: Glc6P > Glc6P_{bio}
 #ABCDEF > #ABCDEF

BIO2: Fru6P > Fru6P_{bio}
 #ABCDEF > #ABCDEF

BIO3: ACCoA_{cyt} > ACCoA_{bio}
 #BC > #BC

BIO4: OAA > OAA_{bio}
 #ABCD > #ABCD

BIO5: E4P > E4P_{bio}

#ABCD > #ABCD

BIO6: Rib5P > Rib5P_{bio}

#ABCDE > #ABCDE

BIO7: GA3P > GA3P_{bio}

#ABC > #ABC

BIO8: α KG_{mit} > α KG_{bio}

#ABCDE > #ABCDE

BIO9: Pyrt > Pyr_{bio}

#ABC > #ABC

CO2out1: CO₂ > CO_{2_ext}

#A > #A

aa_ala: Pyr <> Ala

#ABC > #ABC

aa_glu: α KG_{mit} <> Glut

#ABCDE > #ABCDE

aa_asp: OAA <> Asp

#ABCD > #ABCD

1.3 Reaction network used for anaNET analysis

<u>Abbreviation</u>	<u>Reaction</u>
HXK	$\text{Glc}_{\text{int}} + \text{ATP} > \text{Glc6P} + \text{ADP}$
PGI	$\text{Glc6P} \leftrightarrow \text{Fru6P}$
PFK	$\text{ATP} + \text{Fru6P} > \text{ADP} + \text{FBP} + \text{h}$
FB	$\text{FBP} + \text{h}_2\text{o} > \text{Fru6P} + \text{Pi}$
FBA	$\text{FBP} \leftrightarrow \text{DHAP} + \text{GA3P}$
TPI	$\text{DHAP} \leftrightarrow \text{GA3P}$
GAPDH	$\text{GA3P} + \text{NAD} + \text{pi} \leftrightarrow \text{h} + \text{NADH} + 13\text{dpg}$
PGK	$13\text{dpg} + \text{ADP} \leftrightarrow \text{PG3} + \text{ATP}$
GPM	$\text{PG3} \leftrightarrow \text{PG2}$
ENO	$\text{PG2} \leftrightarrow \text{h}_2\text{o} + \text{Pep}$
PYK	$\text{ADP} + \text{h} + \text{Pep} > \text{ATP} + \text{Pyr}$
G6PDH	$\text{Glc6P} + \text{NADP} > \text{PG6} + \text{h} + \text{NADPH}$
6PGDH	$\text{PG6} + \text{NADP} + \text{h}_2\text{o} > \text{Rul5p} + \text{NADPH} + \text{h} + \text{CO}_2\text{tot}$
RPI	$\text{Rib5p} \leftrightarrow \text{Rul5p}$
RPE	$\text{Rul5p} \leftrightarrow \text{Xul5p}$
TK(1)+TA	$\text{Rib5p} + \text{Xul5p} \leftrightarrow \text{Fru6P} + \text{E4P}$
TK(3)	$\text{E4P} + \text{Sed7P} \leftrightarrow \text{Fru6P} + \text{Rib5p}$
TA	$\text{GA3P} + \text{Sed7P} \leftrightarrow \text{Fru6P} + \text{E4P}$
TK(1)	$\text{Rib5p} + \text{Xul5p} \leftrightarrow \text{GA3P} + \text{Sed7P}$
G3PDH	$\text{DHAP} + \text{NADH} \leftrightarrow \text{glyc3p} + \text{NAD}$
PYRCK	$\text{Pyr} + \text{ATP} + \text{CO}_2\text{tot} \leftrightarrow \text{OAA} + \text{ADP} + \text{Pi} + \text{h}_2\text{o}$
TPP	$(2) \text{Glc6P} + \text{utp} + \text{h}_2\text{o} > \text{Treh} + \text{ppi} + \text{udp} + \text{Pi}$
TreP	$\text{Treh} + \text{h}_2\text{o} > (2) \text{Glu}_{\text{int}}$

PDC	$\text{Pyr} > \text{ACALD} + \text{CO}_2^{\text{tot}}$
DHAK	$\text{DHA} + \text{ATP} \leftrightarrow \text{DHAP} + \text{ADP}$
PMI	$\text{Fru6P} \leftrightarrow \text{Man6p}$
CAT	$\text{Metoh} + (0.5) \text{o}_2 + (2) \text{NAD} > \text{CO}_2^{\text{tot}} + (2) \text{NADH}$
MET	$\text{Metoh} + \text{Xul5p} + (0.5) \text{o}_2 > \text{DHA} + \text{GA3P}_{\text{per}}$