Additional file 2.

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

Methanol metabolism

1. Metoh => Form
2. Form => FOR + NADH
3. FOR + NAD^+ => NADH + CO_2
4. Xul5P + FOR + ATP => ADP + $GA3P_{per}$ + DHA
5. $GA3P_{per}$ => GA3P
6. DHA => GA3P

Glycolysis and gluconeogenesis pathways

7. Glc_{ext} + 2 ATP <=> Glc6P + 2 ADP
8. Glc6P <=> Fru6P
9. Fru6P + 1 ATP => FBP + 1ADP
10. FBP => Fru6P + Pi
11. FBP <=> DHAP + GA3P
12. GA3P + ADP + Pi + NAD^+ => PG3 + ATP + NADH
13. PG3 + ATP + NADH => GA3P + ADP + Pi + NAD^+
14. PG3 <=> Pep
15. Pep + ADP => Pyr + ATP
16. Pyr + NAD^+ => $ACCoA_{mit}$ + CO_2 + NADH
17. Pyr NAD^+ => $ACCoA_{cyt}$ + CO_2 + NADH

18. $Pyr + CO_2 + ADP \Rightarrow OAA + ATP$

19. $2\ Glc6P + UTP + H_2O \Rightarrow H^+ + 2\ Pi + UDP + T6P$

20. $T6P + H_2O \Rightarrow Pi + Treh$

21. $Treh + H_2O \Rightarrow 2\ Glc6P$

Pentose phosphate pathway

22. $Glc6P + 2\ NADP^+ \Rightarrow Rul5P + 2\ NADPH + CO_2$

23. $Rul5P \Leftrightarrow Rib5P$

24. $Rul5P \Leftrightarrow Xul5P$

25. $Rib5P + Xul5P \Leftrightarrow Sed7P + GA3P$

26. $Sed7P + GA3P \Leftrightarrow Fru6P + E4P$

27. $Xul5P + E4P \Leftrightarrow Fru6P + GA3P$

TCA cycle

28. $ACCoA_{mit} + OAA \Rightarrow CIT$

29. $CIT \Rightarrow ICIT$

30. $ICIT + NAD^+ \Rightarrow \alpha KG + CO_2 + NADH$

31. $\alpha KG + NAD^+ \Rightarrow SUCCoA + CO_2 + NADH$

32. $SUCCoA + Pi + ADP \Rightarrow SUCC + ATP$

33. $SUCC + ATP \Rightarrow SUCCoA + ADP + Pi$

34. $SUCC + NAD^+ \Rightarrow FUM + NADH$

35. $FUM + H_2O \Leftrightarrow MAL$

36. $MAL + NAD^+ \Leftrightarrow OAA + NADH$

37. $Asp + 2\ ATP + H_2O \Rightarrow FUM + 2\ ADP + 2Pi$

Biosynthesis of amino acids

Serine family

38. PG3 + Glut + NAD^+ => Ser + αKG + NADH +Pi
39. Ser + THF => Gly + MetTHF
40. Ser + ACCoA + H_2S => Cys

Alanine family

41. Pyr + NADPH => Ala + $NADP^+$

42. Pyr + Glut => αKG
43. 2 Pyr + NADPH => Kval + CO_2 + $NADP^+$
44. Kval + Glut => Val + αKG
45. Kval + $ACCoA_{mit}$ + Glut + NAD^+ => Leu + αKG + NADH + CO_2

Histidine family

46. Rib5P + ATP => PRPP + AMP
47. PRPP + ATP + Gln + 2 NAD^+ => His + αKG + Pi + 2 NADH

Aspartic family

48. OAA + Glut => Asp + αKG
49. Asp + Gln + ATP => Asn + Glut + AMP
50. Asp + ATP + 2 NADPH => Ser + ADP + Pi + 2 $NADP^+$
51. Thr + NADPH + Glut + Pyr => Ile + αKG + NH_4 + CO_2 + $NADP^+$
52. ACCoA + Ser + H_2S + MTHF => Met + THF

Aromatic family

53. 2 Pep + E4P + ATP + NADPH => CHOR + ADP + 4 Pi
54. CHOR + Glut => Phe + αKG + CO_2
55. CHOR + Glut => Tyr + αKG + NADH + CO_2
56. CHOR + Gln + PRPP + Ser => Trp + Glut + Pyr + GA3P + CO_2

Glutamic family

57. αKG +NH_4 + NADPH => Glut + NADP + H_2O
58. Glut + ATP + NH_4 => Gln + ADP + Pi
59. Glut + ATP + 2 NADPH => Pro + ADP + Pi
60. Gln + CO_2 + 2 ATP => CaP + Glut + 2 ADP + Pi
61. Glut + $ACCoA_{mit}$ + 4 ATP + NADPH + CaP + Asp => Arg + αKG + 4 ADP + FUM + 5 Pi
62. 2 Glut + $ACCoA_{mit}$+ 3 ATP + 2 NADPH + 2 NAD^+ => Lys + αKG + CO_2 + 2 NADH + 2 $NADP^+$

Biosynthesis and interconversion of one-carbon units

63. DHF + NADPH => THF + $NADP^+$
64. Gly + THF + NAD^+ => MTHF + NH_4^+ + NADH + CO_2
65. MTHF + NADH => THF + NAD^+

Transport reactions

66. $ACCoA_{cyt}$ => $ACCoA_{mit}$
67. $NH_4^+{}_{ext}$ + ATP => $NH_4^+{}_{cyt}$ + ADP + Pi

68. $SO_4^{-2}{}_{ext}$ + ATP => $SO_4^{-2}{}_{cyt}$ + ADP + Pi

69. $Metal_{ext}$ + ATP => $Metal_{cyt}$ + ADP + Pi

Respiratory chain

70. NADH + 0.5 O_2 => NAD^+ + H_2O

71. ADP + Pi => ATP

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])

72. 0.148 Pyr + 0.083 NAD^+ + 0.0423 NADPH + 1.21 ATP + 0.0072 Rib5P + 0.0147 E4P + 0.044 OAA+ 0.063 αKG_{mit} + 0.0137 $ACCoA_{cyt}$ => 1 C-mol Protein + 0.004 GA3P + 0.083 NADH + 0.0423 $NADP^+$ + 1.21 ADP + 1.21 Pi + 0.0448 CO_2

2. Carbohydrate synthesis (Composition derived [51]**)**

73. 0.113 Glc6P + 0.053 Fru6P + 0.167 ATP => 1 C-mol Carbohydrate + 0.167 ADP

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

74. 0.002 Glc6P + 0.0055 Pyr + 0.011 GA3P + 0.006 CO_2 + 0.039 $ACCoA_{mit}$ + 0.441 $ACCoA_{cyt}$ + 0.07 NADH + 0.599 NADPH + 0.42 ATP + 0.065 O_2 => 1 C-mol Lipid + 0.07 NAD^+ + 0.599 $NADP^+$ + 0.42 ADP + 0.42 Pi

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

75. 0.056 Pyr + 0.1136 CO_2 + 0.105 Rib5P + 0.104 NAD+ + 0.075 NADPH + 1.1 ATP + 0.0479 OAA=> 1 C-mol RNA + 1.1 Pi + 1.1 ADP + 0.075 $NADP^+$ + 0.104 NADH

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

76. 0.051 Pyr +0.102 NAD^+ + 0.273 NADPH + 1.146 ATP+ 0.132 CO_2 + 0.102 Rib5P + 0.051 OAA => 1 C-mol DNA + 1.146 Pi + 1.146 ADP + 0.102 NADH + 0.273 $NADP^+$

1.2 Reactions and atom transitions network used in ^{13}C-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}

#ABC > #ABC

emp11D: Pyr_{mit} > Pyr_{ext}

#ABC > #ABC

emp12: $ACCoA_{cyt}$ > $ACCoA_{mit}$

#AB > #AB

ppp1: Glc6P > CO2 + Rul5P

#abcdef > #a + #bcdef

ppp2: Rul5P <> Xul5P

#abcde > #abcde

ppp3: Rul5P <> Rib5P

#abcde > #abcde

ppp4: Xul5P + E4P <> GA3P + Fru6P

#ABCDE + #abcd > #CDE + #ABabcd

ppp5: Xul5P + 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} > $\alpha KG + CO_2$

#ABCDEF > #ABCEF + #D

TCA4: $\alpha KG > SUCC + CO_2$

#ABCDE > #BCDE + #A

TCA4B: $\alpha KG > SUCC + CO_2$

#ABCDE > #EDCB + #A

TCA5: SUCC <> FUM

#ABCD > #ABCD

TCA5B: SUCC <> FUM

#ABCD > #DCBA

TCA6: FUM <> MAL

#ABCD > #ABCD

TCA7: MAL <> OAA

#ABCD > #ABCD

TCA8: Asp > FUM

#ABCD > #ABCD

Met1: Form > CO_2

#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: αKG_{mit} > αKG_{bio}

#ABCDE > #ABCDE

BIO9: Pyrt > Pyr_{bio}

#ABC > #ABC

CO2out1: CO_2 > CO_2_ext

#A > #A

aa_ala: Pyr <> Ala

#ABC > #ABC

aa_glu: αKG_{mit} <> Glut

#ABCDE > #ABCDE

aa_asp: OAA <> Asp

#ABCD > #ABCD

1.3 Reaction network used for anaNET analysis

Abbreviation	Reaction
HXK	Glc_{int} + ATP > Glc6P + ADP
PGI	Glc6P <> Fru6P
PFK	ATP + Fru6P > ADP + FBP + h
FB	FBP + h2o > Fru6P + Pi
FBA	FBP <> DHAP + GA3P
TPI	DHAP <> GA3P
GAPDH	GA3P + NAD + pi <> h + NADH + 13dpg
PGK	13dpg + ADP <> PG3 + ATP
GPM	PG3 <> PG2
ENO	PG2 <> h2o + Pep
PYK	ADP + h + Pep > ATP + Pyr
G6PDH	Glc6P + NADP > PG6 + h + NADPH
6PGDH	PG6 + NADP + h2o > Rul5p + NADPH + h + CO_2tot
RPI	Rib5p <> Rul5p
RPE	Rul5p <> Xul5p
TK(1)+TA	Rib5p + Xul5p <> Fru6P + E4P
TK(3)	E4P + Sed7P <> Fru6P + Rib5p
TA	GA3P + Sed7P <> Fru6P + E4P
TK(1)	Rib5p+ Xul5p <> GA3P + Sed7P
G3PDH	DHAP + NADH <> glyc3p + NAD
PYRCK	Pyr + ATP + CO_2tot <> OAA + ADP + Pi + h2o
TPP	(2) Glc6P + utp + h2o > Treh + ppi + udp + Pi
TreP	Treh + h2o > (2) Glu_{int}

PDC	Pyr > ACALD + CO_2tot
DHAK	DHA + ATP <> DHAP + ADP
PMI	Fru6P <> Man6p
CAT	Metoh + (0.5) o2 + (2) NAD > CO2tot + (2) NADH
MET	Metoh + Xul5p + (0.5) o2 > DHA + $GA3P_{per}$