Supplementary file 4. Stochiometric model for *P. pastoris*

Methanol Metabolism

1. MET => FORM
2. FORM => FOR + NADH
3. FOR => NADH + CO_2
4. XU5P + FORM + ATP => ADP + GAP + DHA
5. GAP => G3P
6. DHA => G3P

Glycolysis and Gluconeogenesis Pathways

7. GLC + ATP => G6P + ADP
8. G6P <=> F6P
9. F6P + ATP => 2G3P + ADP
10. 2 G3P => F6P +Pi
11. G3P + ADP + Pi => 3PG + ATP + NADH
12. 3PG + ATP + NADH => G3P + ADP + Pi
13. 3PG <=> Pep
14. Pep + ADP => Pyr + ATP
15. Pyr => $ACCoA_{mit}$ + CO_2 + $NADH_{mit}$
16. Pyr => $ACCoA_{cyt}$ +CO_2 + $NADH_{cyt}$
17. Pyr + CO_2 + ADP => Oaa + ATP

Pentose Phosphate Pathway

18. G6P + 2NADP => RU5P + 2 NADPH + CO_2

19. RU5P <=> R5P
20. RU5P <=> XU5P
21. R5P + XU5P <=> S7P + G3P
22. S7P + G3P <=> F6P + E4P
23. XU5P + E4P <=> F6P + G3P

TCA cycle

24. $ACCoA_{mit}$ + Oaa => CIT
25. CIT => ICIT
26. ICIT => AKG + CO_2 + $NADH_{mit}$
27. AKG => SUCCoA + CO_2 + $NADH_{mit}$
28. SUCCoA + Pi + ADP => SUC + ATP
29. SUC + ATP => SUCCoA + ADP + Pi
30. SUC => FUM + $FADH_2$
31. FUM <=> MAL
32. MAL => Oaa + $NADH_{mit}$

Biosynthesis of amino acids

Serine Family

33. 3PG + Glu => Ser + AKG + NADH +Pi
34. Ser + THF => Gly + MetTHF
35. Ser + ACCoA + H_2S => Cys

Alanine Family

36. Pyr + NADPH => Ala + NADP

37. Pyr + Glu => AKG

38. 2 Pyr + $NADPH_{mit}$ => Kval + CO_2

39. Kval + Glu => Val + AKG

40. Kval + $ACCoA_{mit}$ + Glu => Leu + AKG + NADH + CO_2

Histidine Family

41. R5P + ATP => PRPP + AMP

42. PRPP + ATP + Gln => His + AKG + Pi + 2 NADH

Aspartic Family

43. OAA + Glu => Asp + AKG

44. Asp + Gln + ATP => Asn + Glu + AMP

45. Asp + ATP + 2 NADPH => Ser + ADP + Pi

46. Thr + $NADPH_{mit}$ + Glu + Pyr => Ile + AKG + NH_4 + CO_2

47. ACCoA + Ser + H_2S + MTHF => Met + THF

Aromatic Family

48. 2 Pep + E4P + ATP + NADPH => CHOR + ADP + 4 Pi

49. CHOR + Glu => Phe + AKG + CO_2

50. CHOR + Glu => Tyr + AKG + NADH + CO_2

51. CHOR + Gln + PRPP + Ser => Trp + Glu + Pyr + G3P + CO2

Glutamic Family

52. AKG +NH_4 + NADPH => Glu

53. Glu + ATP + NH_4 => Gln + ADP + Pi

54. Glu + ATP + 2 NADPH => Pro + ADP + Pi
55. Gln + CO_2 + 2 ATP => CaP + Glu + 2 ADP + Pi
56. Glu + ACCoA + 4 ATP + $NADPH_{mit}$ + CaP + Asp => Arg + AKG + 4 ADP + FUM + 5 Pi
57. 2 Glu + ACCoA + 3 ATP + 2 $NADPH_{mit}$ => Lys + AKG + CO_2 + 2 NADH

Biosynthesis and Interconversion of One-carbon Units

58. DHF + NADPH => THF
59. Gly + THF => MetTHF + NH_4 + NADH + CO_2
60. MetTHF + NADH => THF

Transport Reactions

61. CO_2 => exp
62. imp => CO_2
63. imp => NH_4
64. NH_4 => exp
65. imp => Pi
66. Pi => exp
67. NAD => $NADH_{mit}$

Biomass Synthesis

Protein synthesis

X-33 control strain

68. 0.1488 Pyr + 0.00725 R5P + 0.0147 E4P + 0.0633 AKG_{mit} + 0.044 Oaa_{mit} + 0.0137 $ACCoA_{cyt}$ => 1 C-mol Protein + 0.004 G3P + 0.0448 CO_2

X-33 ROL 1-copy strain

69. 0.1419 Pyr + 0.0068 R5P + 0.014 E4P + 0.0419 Oaa_{mit} + 0.0681 AKG_{cyt} + 0.0136 $ACCoA_{cyt}$ => 1 C-mol Protein + 0.0029 G3P + 0.0445 CO_2

X-33 ROL 2-copy strain

70. 0.1491 Pyr + 0.00699 R5P + 0.0153 E4P + 0.044 Oaa_{mit} + 0.062 AKG_{cyt} + 0.01454 $ACCoA_{cyt}$ => 1 C-mol Protein + 0.0031 G3P + 0.0502 CO_2

Carbohydrate Synthesis

71. 0.113 G6P + 0.053 F6P => 1 C-mol Carbohydrate

Lipids Synthesis

72. 0.002 G6P + 0.0055 Pyr + 0.011 G3P + 0.006 CO_2 + 0.039 $ACCoA_{mit}$ + 0.441 $ACCoA_{cyt}$ + 0.065 O_2 => 1 C-mol Lipid

RNA Synthesis

73. 0.056 Pyr + 0.1136 CO_2 + 0.105 R5P + 0.0479 Oaa_{mit} => 1 C-mol RNA

DNA Synthesis

74. 0.051 Pyr + 0.132 CO_2 + 0.102 R5P + 0.051 Oaa_{mit} => 1 C-mol DNA

ROL Synthesis

75. 0.171 Pyr + 0.0067 R5P + 0.022 E4P + 0.0472 Oaa_{mit} + 0.029 AKG_{cyt} + 0.0164 AKG_{mit} + 0.0112 $ACCoA_{cyt}$ => 1 C-mol Protein + 0.0022 G3P + 0.0689 CO_2