Electronic Supplementary Material (ESI) for Green Chemistry.

Supplementary File 3

Model Assumptions and Constraints

1. Cell metabolism reached isotopic pseudo-steady state under cell growth phase and 3HB production phase. This assumption is supported by our observations that ^{13}C-enrichment of proteinogenic amino acids are consistent in these two phases.
2. In our model, CO_2 exchange reaction with atmospheric $^{12}CO_2$ was not included since we use screwed flasks to prevent unlabeled atmospheric CO_2 diluting the tracer system. Supply of substrates ($NaH^{13}CO_3$ and glucose) is sufficient in both cell growth and 3HB production phases.
3. Our previous research[1] demonstrates that addition of ^{13}C-glutamate to the *Synechocystis* culture in the light leads to the labeling of succinate but not fumarate, suggesting a very low activity of succinate dehydrogenase under our experimental conditions. Therefore, we introduce the following constraint for succinate dehydrogenase reaction:

$$V_{(SUC \rightarrow FUM)} = 0$$

4. You et al.[2] reported that feeding glyoxylate into the *Synechocystis* culture resulted in altered labeling pattern in glycine, but had little effects on serine and other proteinogenic amino acids. Accordingly, we set:

$$V_{(GOX + GOX \rightarrow GA + CO2)} = 0$$

Reactions and Carbon Transitions for ^{13}C-MFA

1. Glucose (abcdef) -> G6P (abcdef)
2. G6P (abcdef) <-> F6P (abcdef)
3. F6P (abcdef) <-> FBP (abcdef)
4. FBP (abcdef) <-> DHAP (cba) + GAP (def)
5. DHAP (abc) <-> GAP (abc)
6. GAP (abc) <-> 3PGA (abc)
7. 3PGA (abc) <-> 2PGA (abc)
8. 2PGA (abc) <-> PEP (abc)
9. PEP (abc) <-> PYR (abc)
10. PYR (abc) -> ACA (bc) + CO2 (a)
11. 2.47*ACA (ab) + 1.47*ACA (cd) -> AC (ab) + 0.735*3HB (abcd) + 0.735*3HB (cdab)
12. OAA (abcd) + ACA (ef) -> CIT (dcbfea)
13. CIT (abcdef) <-> ICI (abcdef)
14. ICI (abcdef) <-> AKG (abcde) + CO2 (f)
15. AKG (abcde) -> SUC (bcde) + CO2 (a)
16. SUC (abcd) <-> FUM (abcd)
17. FUM (abcd) <-> MAL (abcd)
18. MAL (abcd) <-> OAA (abcd)

19. MAL (abcd) -> PYR (abc) + CO2 (d)
20. PEP (abc) + CO2 (d) -> OAA (abcd)
21. G6P (abcdef) -> RU5P (bcdef) + CO2 (a)
22. RU5P (abcde) -> RUBP (abcde)
23. RUBP (abcde) + CO2 (f) -> 3PGA (cde) + 3PGA (fba)
24. RU5P (abcde) <-> X5P (abcde)
25. RU5P (abcde) <-> R5P (abcde)
26. X5P (abcde) <-> GAP (cde) + EC2 (ab)
27. F6P (abcdef) <-> E4P (cdef) + EC2 (ab)
28. X5P (abcde) -> GAP (cde) + ACA (ab)
29. F6P (abcdef) -> E4P (cdef) + ACA (ab)
30. S7P (abcdefg) <-> R5P (cdefg) + EC2 (ab)
31. F6P (abcdef) <-> GAP (def) + EC3 (abc)
32. S7P (abcdefg) <-> E4P (defg) + EC3 (abc)
33. DHAP (cba) + E4P (defg) -> SBP (abcdefg)
34. SBP (abcdefg) -> S7P (abcdefg)
35. RUBP (abcde) -> 3PGA (cde) + 2PG (ba)
36. 2PG (ab) -> GLC (ab)
37. GLC (ab) -> GOX (ab)
38. GOX (ab) + GOX (cd) -> GA (abd) + CO2 (c)
39. GA (abc) <-> 2PGA (abc)
40. AKG (abdce) -> GLU (abcde)
41. GLU (abdce) -> GLN (abcde)
42. GLU (abcde) -> PRO (abcde)
43. GLU (abdce) + CO2 (f) + GLN (ghijk) + ASP (lmno) -> ARG (abcdef) + AKG (ghijk) + FUM (lmno)
44. OAA (abcd) + GLU (efghi) -> ASP (abcd) + AKG (efghi)
45. ASP (abcd) -> ASN (abcd)
46. PYR (abc) + GLU (defgh) -> ALA (abc) + AKG (defgh)
47. 3PGA (abc) + GLU (defgh) -> SER (abc) + AKG (defgh)
48. SER (abc) -> GLY (ab) + MTHF (c)
49. GLY (ab) -> MTHF (b) + CO2 (a)
50. GOX (ab) -> GLY (ab)
51. SER (abc) -> CYS (abc)
52. ASP (abcd) -> THR (abcd)
53. ASP (abcd) + PYR (efg) + GLU (hijkl) -> 0.5*LYS (bcdgfe) + 0.5*LYS (fgdcba) + 0.5*CO2 (a) + 0.5*CO2 (e) + AKG (hijkl)
54. ASP (abcd) + MTHF (e) + CYS (fgh) -> MET (abcde) + PYR (fgh)
55. PYR (abc) + PYR (def) + GLU (ghijk) -> VAL (abefc) + AKG (ghijk) + CO2 (d)
56. ACA (ab) + PYR (cde) + PYR (fgh) + GLU (ijklm) -> LEU (abdghe) + AKG (ijklm) + CO2 (c) + CO2 (f)
57. THR (abcd) + PYR (efg) + GLU (hijkl) -> ILE (abfcdg) + AKG (hijkl) + CO2 (e)
58. PEP (abc) + PEP (def) + E4P (ghij) + GLU (klmno) -> PHE (abcefghij) + AKG (klmno) + CO2 (d)
59. PEP (abc) + PEP (def) + E4P (ghij) + GLU (klmno) -> TYR (abcefghij) + AKG (klmno) + CO2 (d)

60. SER (abc) + R5P (defgh) + PEP (ijk) + PEP (lmn) + E4P (opqr) + GLN (stuvw) -> TRP (abcedkopqrj) + GAP (fgh) + PYR (lmn) + GLU (stuvw) + CO2 (i)
61. R5P (abcde) + MTHF (f) + GLN (ghijk) + ASP (lmno) -> HIS (edcbaf) + AKG (ghijk) + FUM (lmno)
62. 0.284*G6P + 0.495*R5P + 0.046*GAP + 0.173*DHAP + 0.046*PYR + 3.707*ACA + 0.366*MTHF + 0.53*GLY + 0.158*PRO + 0.387*ALA + 0.201*VAL + 0.31*LEU + 0.171*ILE + 0.048*MET + 0.03*CYS + 0.087*PHE + 0.058*TYR + 0.027*TRP + 0.043*HIS + 0.105*LYS + 0.104*ARG + 0.136*GLN + 0.11*ASN + 0.2*GLU + 0.657*ASP (abcd) + 0.199*SER + 0.165*THR -> Biomass + 0.210*FUM (abcd)

[*]**Notes**: **1**. Reaction 11 is used for 3HB production. TesB uses both Acetyl CoA and 3-hydroxybutyryl CoA as the substrates and produces acetate and 3HB, respectively. We lumped the two reactions for simplicity. The stoichiometry for acetate and 3HB production is based on experimental measurements, which is 1:1.47. **2**. Reaction 62 is biomass formation based on a previous report[3] and adopted for ^{13}C-MFA[2].

[**]**Abbreviations:** 2PGA, 2-phosphoglyceric acid; 2PG, 2-phosphoglycolate; 3HB, 3-hydroxybutyrate; 3PGA, 3-phosphoglyceric acid; AC, acetate; ACA, acetyl-CoA; AKG, α-ketoglutarate; ALA, alanine; ARG, arginine; ASN, asparagine; CIT, citrate; CYS, cysteine; DHAP, dihydroxyacetone phosphate; E4P,erythrose 4-phosphate; F6P, fructose 6-phosphate; FBP, fructose-1,6-bisphosphate; FUM, fumarate; G6P, glucose 6-phosphate; GA, glycerate; GAP, glyceraldehyde 3-phosphate; GLC, glycolate; GLN, glutamine; GLX, glyoxylate; GLY, glycine; GLU, glutamate; HIS, histidine; ICI, isocitrate; ILE, isoleucine; LEU, leucine; MAL, malate; MTHF, 5,10-Methylenetetrahydrofolate (5,10-CH2-THF); OAA, oxaloacetate; PEP, phosphoenolpyruvate; PHE, phenylalanine; PRO, proline; PYR, pyruvate; R5P, ribose 5-phosphate; Ru5P, ribulose-5-phosphate; RuBP, ribulose-1,5-diphosphate; S7P, sedoheptulose-7-phosphate; SBP, sedoheptulose-1,7-bisphosphate; SER, serine; SUC, succinate; SucCoA, succinyl-CoA; THR, threonine; TRP, tryptophan; VAL, valine; X5P, xylulose-5-phosphate.

References:

1. W. Xiong, D. Brune and W. F. J. Vermaas, *Molecular Microbiology*, 2014, **93**, 786-796.
2. L. You, B. Berla, L. He, H. B. Pakrasi and Y. J. Tang, *Biotechnology Journal*, 2014, **9**, 684-692.
3. R. Saha, A. T. Verseput, B. M. Berla, T. J. Mueller, H. B. Pakrasi and C. D. Maranas, *PLoS One*, 2012, **7**, e48285.