The Enzymes that beyond Non-Oxidative Glycolysis

Zixiang Xu[12] Qiaqing Wu[2]* Dunming Zhu[2]*

1 Key laboratory of systems microbial biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, 32 Xi Qi Dao, Tianjin Airport Economic Area, Tianjin 300308, China.

2 National Engineering Laboratory for Industrial Enzymes and Tianjin Engineering Center for Biocatalytic Technology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, 32 Xi Qi Dao, Tianjin Airport Economic Area, Tianjin 300308, China.

* Correspondence and requests for materials should be addressed to email: ZX (email: xzx21c@163.com) or QW (email: wu_qq@tib.cas.cn) or DZ (email: zhu_dm@tib.cas.cn).

Abstract

High yield is an important objective of cell factory. One or several genes cloned into the bacterial may make the synthetic pathway much more optimal, so can increase the yield. But the global benefit enzymes are rare, which can increase the yields of many chemical products for a cell factory such as *E.coli*. Two of these kinds of global benefit enzymes are the famous enzymes, D-fructose-6-phosphate D-erythrose-4-phosphate-lyase and D-Xylulose 5-phosphate D-glyceraldehyde-3-phosphate-lyase, of non-oxidative glycolysis (NOG) published in *Nature*, which can improve the utilization ratio of carbon. We expect to find other global benefit enzymes. We use an integrated model, which integrated *in silico* model of *E.coli* and KEGG. By computation, we analyze the effect of adding each reaction from KEGG on the theoretical yields of several products with *E.coli* and find about 80 enzymes that may be potentially global benefit enzymes. By comparison, we find many of the 80 enzymes are better in improving the theoretical yields than the two enzymes of NOG.

In order to compare the global benefit enzymes with NOG, as an example, we select "Glycerol:NADP+ oxidoreductase" (GNO) which can increase the supply of NADPH in *E.coli.* But To increase the supply of reducing power, such as NADPH will probably increase the yield of chemicals in a cell factory. We use flux balance analysis method to testify our assumption. By comparing the maximum yields of 80 products produced by *E.coli* with respectively using GNO and NOG, we find GNO has better performance in the product production of *E.coli*. So GNO is a global benefit enzyme which can increase the yields of many chemical products in *E.coli*.

keywords

Synthetic biology, Flux balance analysis, Maximum yield, Cell factory, Non-oxidative glycolysis

Introduction

Synthetic biology has developed rapidly in recent years and the construction of cell factory is one of the main tasks of synthetic biology. For microorganisms to produce a variety of chemicals, cell factories have greatly improved industrial bio-economy. The construction of synthetic pathway is the key aspect for the development of cell factories. If a bacterial can't produce a chemical natively, some genes should be cloned into the bacterial and make it can produce the chemical. High yield is an important objective of cell factories. Gene knockout and overexpression are two

ways to increase the yields of cell factories. Sometimes, we may clone one or several genes into the bacterial and this will make the synthetic pathway much more optimal, so can increase the yield as well. But the global benefit enzymes are rare, which can increase the yields of many chemical products for a cell factory such as *E.coli*. The famous global benefit enzyme is the non-oxidative glycolysis (termed NOG) that was found by James Liao group in the year 2013 and published in *Nature* [1], which can improve the utilization ratio of carbon. One enzyme name of NOG is D-fructose-6-phosphate D-erythrose-4-phosphate-lyase, its reaction formula is "D-Fructose 6-phosphate + Orthophosphate <=> Acetyl phosphate + D-Erythrose 4-phosphate + H2O" and its KEGG id is R00761; another enzyme name of NOG is "D-Xylulose 5-phosphate D-glyceraldehyde-3-phosphate-lyase", its reaction formula is "D-Xylulose 5-phosphate + Orthophosphate <=> Acetyl phosphate + D-Glyceraldehyde 3-phosphate + H2O" and its KEGG id is R01621. KEGG (Kyoto Encyclopedia of Genes and Genomes) is a database resource for understanding high-level functions and utilities of the biological system [2]. NOG can increase the yields of many chemical products produced by *E.coli*.

We expect to find other global benefit enzymes and we utilize the computational method. Computation biology has been used as testification method widely. We use an integrated model, which integrated *in silico* model of *E.coli* and KEGG [3]. By computation, we analyze the effect of adding each reaction from KEGG on the theoretical yields of several products and find about 80 enzymes that are potentially global benefit enzymes. By comparison, we find many of the 80 enzymes are better in improving the theoretical yields than NOG.

As we know, to increase the supply of, such as NADPH, will probably increase the yields of chemicals. In order to compare the global benefit enzymes with NOG, as an example, we select the enzyme, "Glycerol:NADP+ oxidoreductase" (termed GNO) from the 80 enzymes, its KEGG id is R01041, its reaction formula is "Glycerol + $NADP^+$ <=> D-Glyceraldehyde + NADPH + H^+", and we make our test in this study. We have compared the maximum yields of 80 products produced by *E.coli* respectively using GNO and NOG.

Methods

About 80 enzymes that are potentially global benefit enzymes

An integrated model [3] was used, which integrated *in silico* model of *E.coli* and KEGG. The reactions from KEGG that added to *E.coli* model were balance checked and reversibility checked. There were 4733 reactions that were added to the *E.coli* model. When we used one reaction from KEGG, the up/low flux bounds were not constrained, while the flux bounds of other reactions were set to zero. So it is equivalent to add one reaction to *E.coli* every time.

E.coli can produce many chemicals, and here we did not calculate all the yields of them. We choose Acetate (Ace), L-Glutamate (Glu), L-Lysine (Lys), L-Threonine (Thr), Succinate (Suc), Formate (For) as the representatives.

Firstly, we calculated the growth rate (Grw) and the theoretical yields of above 6 chemicals with wild-type *E.coli*. The following constraints were applied, when we did the calculation: glucose consumption rate was -10 mmol $g^{-1}(Dw)h^{-1}$; there was no constraint for oxygen consumption rate. The FBA (flux balance analysis) calculation was carried out by COBRA Toolbox [4] with a loopless function which eliminates all steady-state flux solutions that are incompatible with the loop-law [5]. The optimization solver is Gurobi. The growth rate and the theoretical yields were calculated by

setting the corresponding flux as objectives of FBA model.

Max v_j (j respectively equals to the reaction number of Grw, Ace, Glu, Lys, Thr, Suc, For)

$S \cdot v = 0$ (S is the stoichiometric matrix of $E.coli$ model iOJ1366)

$\alpha_i \leq v_i \leq \beta_i$ (α_i and β_i are the bounds of flux v_i of i-th reaction)

Secondly, we calculated the growth rate and the theoretical yields of above 6 chemicals with *E.coli* but added one reaction every time. That is to say, when we used one reaction from KEGG, the up/low flux bounds were not constrained, while the flux bounds of other reactions were set to zero. The computational condition was the same as above.

Max v'_j (j respectively equals to the reaction number of Grw, Ace, Glu, Lys, Thr, Suc, For)

$S' \cdot v' = 0$ (S′is the stoichiometric matrix of an integrated model of $E.coli$ and KEGG)

$\alpha'_i \leq v'_i \leq \beta'_i$ (α'_i and β'_i are the bounds of flux v'_i of i-th reaction)

$0 \leq v'_k \leq 0$ (if $k \geq 2584$, and the k-th reaction is not the added reaction)

The example of GNO (Glycerol:NADP+ oxidoreductase)

In order to compare the impact of GNO and NOG for the products of *E.coli*, we add both reactions to the model iAF_1260 of *E.coli* [6]. In iAF_1260, there are about 300 exchange reactions, we calculate the maximum yield of each product with FBA (flux balance analysis) method, which has been described in Ref [7]. Here we use the exchange reaction as the objective but not the biomass (growth). The following constraints were applied, when we do the calculation: glucose consumption rate is 10 mmol $g^{-1}(Dw)h^{-1}$; there is no constraint for oxygen consumption rate. The FBA calculation was carried out with COBRA Toolbox [4]. Many of the exchange reactions take no flux, while about 80 exchange reactions have fluxes, i.e. *E.coli* may produce about 80 products actually according to the model of iAF_1260.

We first add NOG (R00761) into the iAF_1260 model and calculate, one by one, the maximum yields of all the 80 products which *E.coli* may produce actually. Then, we add GNO into the iAF_1260 model and calculate the maximum yields of all the 80 products with the same method. We compare GNO and NOG, and hope to find out which will get a larger number with higher yields. We classify the result into three groups, i.e. “NOG is better” (product yields are higher when adding NOG), “GNO is better” (product yields are higher when adding GNO) and “No significant difference between NOG and GNO” (product yields are nearer when adding NOG or GNO).

Results and Discussion

About 80 enzymes that are potentially global benefit enzymes

The growth rate and the theoretical yields of 6 chemicals, i.e. Acetate (Ace), L-Glutamate (Glu), L-Lysine (Lys), L-Threonine (Thr), Succinate (Suc), Formate (For), were obtained with wild-type *E.coli* and with *E.coli* but added one reaction every time (4733 cases in total). In order to find which reactions added would make the theoretical yields of 6 chemicals improved, if any one of the 6 theoretical yields of *E.coli* (added one reaction every time) is larger than (1.01 times) the corresponding value of wild-type *E.coli*, we select out the enzyme (reaction), and we get 83 enzymes (reactions) in total. We list them in **Table 1** with descending order in Ace and Glu yield values. The whole names and equations of these reactions are illustrated in **Supplementary Table S1**. These 83 enzymes are enzymes that have potential ability to improve the theoretical yields of some chemical products in *E.coli*.

Among these 83 enzymes, many can get Acetate yield to 3.0 (wild-type *E.coli* is 2.9),

L-Glutamate yield to 1.33 (wild-type *E.coli* is 1.19), L-Lysine yield to 0.86 (wild-type *E.coli* is 0.78), L-Threonine yield to 1.5 (wild-type *E.coli* is 1.28), and Formate yield to 12 (wild-type *E.coli* is 10.3), but the yield of Succinate was not improved significantly. Many can improve the growth of the cell as well and the growth rate can get to 1.38, while the growth rate of wild-type *E.coli* is 0.98.

Two reactions of NOG, R00761 and R01621, are on the list of **Table 1**. But they are not the best in improving the yields of products and they are just at the average level of improving the yields of L-Glutamate, L-Lysine, L-Threonine, and Formate. Among these about 80 enzymes, many have better performance in improving the product yields with *E.coli* than the two reactions of NOG.

But the first two reactions, R07409 and R00790, have seemingly abnormal values in yields improved. We checked the reason and it may lie in the reversibility of the two reactions. We list all the fluxes through the added reactions when calculating the yields of the six chemicals, in **Supplementary Table S2**, following on the column of the yield of the corresponding chemicals. By comparing the direction of every added reaction, the flux through the reaction and the property of the reaction, we marked all the possible error of reversibility of added reactions in the column **Rev** with red color in **Supplementary Table S2**. The property of the reactions we mention here refer to CO_2 and H_2O as metabolites in the reactions, and it is not easy for CO_2 and H_2O to be decomposed *in vivo*. Although the reaction directions of these added reactions were checked in Ref [3], the reversibility quality of marked reaction may result in wrong calculations for improving yields.

Global benefit enzymes which can increase the theoretical yields of many chemical products for cell factories are what we want. NOG is one of them. In this study, by computation method, we find many other global benefit enzymes, which can increase the theoretical yields of some chemical products in *E.coli*. Especially, some of these global benefit enzymes are better than NOG in many products from the comparison result above. For the number of these global benefit enzymes we find is large, it is not possible to give testification with experiments. Computation method has been used as testification method widely. We think these global benefit enzymes we find will be potentially effective.

The example of GNO (Glycerol:NADP+ oxidoreductase)

We add NOG (R00761) into the iAF_1260 model and calculate, one by one, the maximum yields of all the 80 products which *E.coli* may produce actually. Then we add GNO into the iAF_1260 model and calculate the maximum yields of all the 80 products with the same method. The maximum yields of *E.coli* for wild-type were listed as a comparison. From the yields of 80 products, we find that all of them are higher than wild-type when adding with GNO, so GNO is also a global benefit enzyme. We classify the result into three groups, i.e. “NOG is better”, “GNO is better” and “No significant difference between NOG and GNO”. “NOG is better” of 30 products was shown in **Table 2**, “GNO is better” of 43 products was shown in **Table 3**, and “No significant difference between NOG and GNO” of 7 products was shown in **Table 4**. GNO gets 43 products better than NOG, while NOG gets 30 better than GNO, so GNO has better performance in the product production of *E.coli*.

Global benefit enzymes which can increase the theoretical yields of many chemical products for cell factories are our hope. NOG is one of them. In this study, we find another global benefit enzyme, GNO, which can increase the theoretical yields of 80 chemical products in *E.coli*. Especially, GNO is better than NOG in many products from the comparison result above. GNO is an enzyme related to NADPH production. NADPH is an important cofactor in the construction of cell factory. The cofactor optimization will increase the supply of reducing power, so increase the theoretical

yields.

References

1. Igor W Bogorad, Tzu-Shyang Lin, James C Liao. Synthetic non-oxidative glycolysis enables complete carbon conservation. *Nature*. 2013, 502: 693–697.
2. Kanehisa, M., Goto, S., Sato, Y., Kawashima, M., Furumichi, M., and Tanabe, M.; Data, information, knowledge and principle: back to metabolism in KEGG. *Nucleic Acids Res*. 2014, 42, D199–D205.
3. Xiaolin Zhang, Christopher J. Tervo, Jennifer L. Reed. Metabolic assessment of E. coli as a Biofactory for commercial products. *Metabolic Engineering*. 2016, 35:64-74.
4. Scott A Becker, et al.. Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox. *Nature Protocols*. 2007, 2: 727 – 738.
5. Jan Schellenberger, Nathan E Lewis, Bernhard Ø Palsson. Elimination of Thermodynamically Infeasible Loops in Steady-State Metabolic Models. *Biophysical Journal*. 2011, 100(3): 544–553.
6. Feist AM, Henry CS, Reed JL, Krummenacker M, Joyce AR, et al. A genome-scale metabolic reconstruction for Escherichia coli K-12 MG1655 that accounts for 1260 ORFs and thermodynamic information. *Molecular Systems Biology*. 2007, 3: Art. No. 121.
7. Zixiang Xu, Xiao Sun, Shihai Yu. Genome-Scale Analysis to the Impact of Gene deletion on the Metabolism of E. coli: Constraint-Based Simulation Approach. *BMC Bioinformatics*. 2009, 10 (Suppl 1): S62.

Acknowledgments

Support for this work was provided by “National Natural Science Foundation of China (31370829)’’, “Tianjin Research Program of Application Foundation and Advanced Technology (15JCYBJC23600)”. The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

Additional Information

The authors declare no competing financial interests.

Author contributions

Conceived and designed the experiments: ZX. Performed the experiments: ZX. Analyzed the data: ZX QW DZ. Contributed reagents/materials/analysis tools: QW DZ. Wrote the paper: ZX.

Table 1. Reactions that have potential ability to improve the theoretical yields of some chemical products in *E.coli*.

No.	KEGG id	Rev	Growth	Ace	Glu	Lys	Thr	Suc	For
	Wild_Type		0.982371813	29.09347	11.93731959	7.832592593	12.85294	17.09643	103.74
1	R07409	rev	6.664134773	207.9191176	80.3323863	60.94181034	98.18402777	132.9534588	1000
2	R00790	rev	6.677312281	195.8289141	76.02769607	56.43977435	93.8123188	118.033866	1000
3	R00659	rev	1.384066012	29.99999999	13.33333334	8.571427526	14.99999817	17.1428571	119.9999853
4	R00206	rev	1.384066012	30	13.33333333	8.571428572	14.99999999	17.14285714	120
5	R02537	rev	1.384066012	29.99999999	13.33333333	8.571428572	14.99999996	17.14285714	120
6	R01050	rev	1.384066012	30	13.33333333	8.571428568	14.99999999	17.14285714	120
7	R00709	rev	1.384066012	29.99999999	13.33333333	8.571427526	14.99999999	17.14285714	119.9999997
8	R00149	rev	1.384066012	29.99999993	13.33333333	8.571428571	14.99999817	17.14285714	119.9999938
9	R00224	rev	1.384066012	29.99999634	13.33333333	8.571427526	14.99999817	17.1428571	120
10	R08515	rev	1.384066012	29.99999999	13.33333333	8.571428573	14.99999998	17.14285714	120
11	R01224	rev	1.384066012	29.99999993	13.33333333	8.571427526	15	17.14285714	119.9999999
12	R00572	rev	1.384066012	29.99999993	13.33333333	8.571428568	15	17.14285714	120
13	R03004	rev	1.38406594	29.99999999	13.33333333	8.571428568	14.99999996	17.14285714	120
14	R02301	rev	1.384066012	29.99999993	13.33333333	8.571428551	14.99999997	17.14285506	119.9999854
15	R02145	rev	1.384066012	29.99999993	13.33333333	8.571428568	14.99999998	17.14285714	120
16	R07159	rev	1.384066012	30	13.3333333	8.571428558	14.99999817	17.1428571	119.9999938
17	R01138	rev	1.384066012	29.99999999	13.3333333	8.571428572	15	17.14285506	120
18	R00397	rev	1.384066012	29.99999999	13.3333333	8.571427525	14.99999996	17.14285714	120
19	R07758	rev	1.384066012	29.99999844	13.33333264	8.571427526	14.99999922	17.14285714	120
20	R00724	rev	1.384066012	29.99999634	13.33333264	8.571428572	14.99999999	17.14285506	119.999998
21	R06846	rev	1.384066014	29.99999999	13.33333264	8.571427526	14.99999922	17.14285714	120
22	R00430	rev	1.384066012	30	13.33333264	8.571428572	14.99999996	17.14285714	120

23	R00885	rev	1.384066012	29.99999999	13.33333264	8.571428569	14.99999996	17.14285714	120
24	R00522	rev	1.384065939	29.99999866	13.33333171	8.571428558	15	17.14285714	120
25	R01858	rev	1.384066012	29.99999993	13.33333171	8.571428572	15	17.1428571	120
26	R00126	rev	1.384066012	29.99999999	13.33333171	8.571428127	14.99999996	17.14285714	119.9999938
27	R00502	rev	1.384066012	30	13.33333171	8.571427526	14.99999998	17.14285714	119.9999854
28	R01217	rev	1.384066012	29.99999993	13.33333171	8.571428551	14.99999999	17.14285714	119.9999938
29	R01197	rev	0.990497673	29.99999999	13.18947364	7.832592566	12.89512102	17.1428571	104.7399999
30	R00265	rev	0.982371813	30	13.07297072	7.832592429	12.855813	17.09642857	104.7399999
31	R01621	irr	0.982371813	29.99999999	12.43333327	7.832592593	12.85294118	17.09642857	103.7399925
32	R00761	irr	0.982371813	29.99999999	12.4333314	7.832592593	12.85294118	17.09642563	103.7399997
33	R01817	rev	0.989631556	29.99999999	12.20776699	8.135087691	13.44057741	17.09642856	109.7999999
34	R00834	rev	0.989631483	29.99999844	12.20776699	8.135086327	13.4405797	17.09642851	109.8
35	R07164	rev	0.989631556	29.99999634	12.20776699	8.13508769	13.44057741	17.09642567	109.8
36	R01063	rev	0.989631556	29.99999634	12.20776699	8.135086328	-7.48113E-10	17.09642567	109.7999999
37	R09281	rev	0.989631557	29.99999993	12.20776699	8.135086328	13.4405797	17.09642857	109.8
38	R01434	rev	0.989631556	29.99999844	12.20776699	8.135087715	13.44057966	17.09642857	109.7999997
39	R07140	rev	0.989631557	29.99999634	12.20776699	8.135087719	13.44057971	17.09642732	109.7999999
40	R01976	rev	0.989631556	29.99999999	12.20776699	8.135087719	13.4405797	17.09642851	109.7999997
41	R00978	rev	0.989631556	30	12.20776699	8.135087719	13.44057966	17.09642856	109.8
42	R01218	rev	0.989631556	29.99999634	12.20776699	8.135087719	13.44057971	17.09642567	109.8
43	R00396	rev	0.989631556	29.99999999	12.20776699	8.135087118	13.44057971	17.09642856	109.8
44	R00094	rev	0.989631556	30	12.20776698	8.135087719	13.44057966	17.09642857	109.7999997
45	R00688	rev	0.989631556	29.99999993	12.20776698	8.135087691	13.44057952	17.09642567	109.7999831
46	R01738	rev	0.989631556	29.99999999	12.20776698	8.135087691	13.44057971	17.09642567	109.7999997
47	R00343	rev	0.989631556	29.99999999	12.20776698	8.135087719	13.44057741	17.09642567	106.2428406

48	R01747	rev	0.989631556	29.99999999	12.20776698	8.135087715	13.44057741	17.09642856	109.8
49	R00746	rev	0.989631556	29.99999634	12.20776698	8.135087718	13.44057741	17.09642851	109.8
50	R01773	rev	0.989631556	29.99999999	12.20776696	8.135087719	13.44057966	17.09642856	109.7999999
51	R02196	rev	0.989631558	29.99999993	12.20776695	8.135086327	13.4405797	17.09642857	109.7999997
52	R00936	rev	0.989631556	29.99999634	12.20776695	8.135087691	13.4405797	17.09642851	109.8
53	R00243	rev	0.989631483	29.99999999	12.20776695	8.135086328	13.44057966	17.09642732	109.8
54	R02259	rev	0.989631556	29.99999999	12.20776695	8.135087719	13.44057741	17.09642732	109.7999999
55	R00842	rev	0.989631556	30	12.20776616	8.135087715	13.2254545	17.09642857	109.8
56	R06847	rev	0.989631557	29.99999999	12.20776506	8.135087715	13.44057971	17.09642857	109.8
57	R02577	rev	0.989631556	29.99999993	12.20776506	8.135087716	13.44057971	17.09642857	109.7999999
58	R07759	rev	0.989631556	29.99999993	12.20776506	8.13508761	13.44057971	17.09642732	109.7999999
59	R01041	rev	0.989631556	29.99999999	12.20776506	8.135087719	13.4405797	17.09642851	109.7999999
60	R00848	rev	0.989631556	30	12.20776505	8.135087722	13.4405797	17.09642857	109.7999997
61	R02165	rev	1.039574999	29.99999993	12.11777379	7.959509177	13.22742642	17.14285714	118.4249999
62	R02965	rev	1.039574999	29.99999993	12.11777378	7.959509202	13.22742857	17.1428571	100.4357064
63	R01058	rev	1.384066012	29.99999999	9.999999982	8.571428127	14.99999999	17.14285714	119.9999938
64	R02639	rev	0.989631556	29.99999999	0	8.135087719	13.44057971	17.09642567	109.7999996
65	R00726	rev	0.996797059	29.94347826	12.52181818	8.133846106	13.55641026	17.14285714	104.7399999
66	R00346	rev	0.996797059	29.94347824	12.52181734	8.133844799	13.55641026	17.14285506	104.7399827
67	R00431	rev	0.996796986	29.94347359	12.52181818	8.133846105	13.55641025	17.14285714	104.7399926
68	R01967	rev	0.982371813	29.75294109	12.10151515	7.832591264	12.02916666	17.09642857	103.7399999
69	R02089	rev	0.982371813	29.75294109	12.10151515	7.832592587	12.85294117	17.09642857	103.7399999
70	R01010	rev	1.013832283	29.73749991	12.24705882	8.011023618	13.04358974	17.14285506	104.3090909
71	R01059	rev	0.982371813	29.71363635	12.04864865	7.895	12.93783784	17.09642856	106.7399924
72	R07417	rev	0.982371813	29.71363635	12.04864861	7.894999999	12.93783779	17.09642563	106.74

73	R00585	rev	0.982371814	29.4064516	12.01238937	7.832592593	12.85294117	17.09642856	103.7399999
74	R00941	rev	0.982541207	29.39148471	11.99131944	7.832592588	12.85294117	17.09642857	103.7399999
75	R00519	rev	0.982373062	29.27916667	11.97103918	7.832592021	12.85294118	17.09642857	117.6714286
76	R01199	rev	0.985637935	29.2247403	11.96118143	7.83259127	12.85294117	17.09642856	114.3090909
77	R00210	rev	0.982371813	29.13061223	11.94590164	7.832592565	12.85581395	17.09642856	113.7399997
78	R09280	rev	0.987171241	29.09346732	11.93731952	7.832592588	12.86967742	17.09642731	105.7399996
79	R00352	rev	1.001066004	29.0934628	12.3445629	7.832592588	12.89512195	17.14285506	107.945447
80	R04198	rev	0.98274982	29.09346279	11.93731959	7.945453178	12.85294118	17.09642851	103.7399999
81	R07613	rev	0.983506707	29.09346279	11.93731959	8.010606056	12.85294118	17.09642857	103.7399997
82	R06975	rev	5.84298E-12	29.09346279	11.93731772	7.832592593	12.53076707	17.09642563	109.7399817
83	R00859	rev	0.982624369	29.09346278	11.93731772	7.639250936	12.85294118	17.0964273	110.3812499

*: All the rate unit is mmol $g^{-1}(Dw)h^{-1}$; glucose consumption rate is -10 mmol $g^{-1}(Dw)h^{-1}$; there is no constraint for oxygen consumption rate;

*: Rev: Reversibility; Acetate(Ace), L-Glutamate(Glu), L-Lysine(Lys), L-Threonine(Thr), Succinate(Suc), Formate(For).

*: Descending order in Ace and Glu values.

Table 2. NOG is better (30 products)

Product_Reaction_ab	Product_Reaction_Name	Wild_Type	NOG	GNO
EX_12ppd_R(e)	R Propane 1 2 diol exchange	13.88545	14.53389	13.88545
EX_4abut(e)	4 Aminobutanoate exchange	11.83424	12.2481	12.12196
EX_ac(e)	Acetate exchange	28.4675	30	30
EX_acald(e)	Acetaldehyde exchange	23.16	24	23.65569
EX_ade(e)	Adenine exchange	10.73562	11.01516	10.91
EX_adn(e)	Adenosine exchange	5.417281	5.53672	5.455
EX_akg(e)	2 Oxoglutarate exchange	12.69491	12.88026	12.87958
EX_cytd(e)	Cytidine exchange	6.252926	6.374651	6.298924
EX_dha(e)	Dihydroxyacetone exchange	18.39308	18.78899	18.39308
EX_enter(e)	Enterochelin exchange	1.764448	1.803355	1.791633
EX_etha(e)	Ethanolamine exchange	13.95312	14.33479	14.09908
EX_feenter(e)	Fe enterobactin exchange	1.764448	1.803355	1.791633
EX_for(e)	Formate exchange	60.22207	83.60167	65.49739
EX_fum(e)	Fumarate exchange	18.00846	18.274	18.00846
EX_g3pe(e)	sn Glycero 3 phosphoethanolamine exchange	7.301553	7.474571	7.390645
EX_g3pg(e)	Glycerophosphoglycerol exchange	6.406267	6.623038	6.453803
EX_glu_L(e)	L Glutamate exchange	11.73479	12.17341	12.00427
EX_glyc3p(e)	Glycerol 3 phosphate exchange	11.41311	11.89136	11.4555
EX_glyc(e)	Glycerol exchange	15.31661	15.61851	15.53288
EX_gua(e)	Guanine exchange	11.14265	11.49934	11.1761
EX_hxan(e)	Hypoxanthine exchange	11.41311	11.75775	11.4555
EX_indole(e)	Indole exchange	5.496417	5.651753	5.51661
EX_ins(e)	Inosine exchange	5.617921	5.74967	5.622331
EX_pyr(e)	Pyruvate exchange	21.50355	22.04879	21.94208
EX_succ(e)	Succinate exchange	16.72214	17.13187	16.72214
EX_trp_L(e)	L Tryptophan exchange	4.521346	4.5685	4.536832
EX_uri(e)	Uridine exchange	6.650919	6.726626	6.704821
EX_xan(e)	Xanthine exchange	12.47268	12.76146	12.55397
EX_xtsn(e)	Xanthosine exchange	5.863092	5.979657	5.874615
Ec_biomass_iAF1260 _core_59p81M	E coli biomass objective function iAF1260 core with 5981 GAM estimate	0.929292	0.949731	0.937114

*: All the rate unit is mmol $g^{-1}(Dw)h^{-1}$.

Table 3. GNO is better (43 products)

Product_Reaction_ab	Product_Reaction_Name	Wild_Type	NOG	GNO
EX_15dap(e)	1 5 Diaminopentane exchange	7.677333	7.694412	7.95122807
EX_LalaDgluMdapDala(e)	L alanine D glutamate meso 2 6 diaminoheptanedioate D alanine exchange	2.785664	2.790507	2.841504702
EX_LalaDgluMdap(e)	L alanine D glutamate meso 2 6 diaminoheptanedioate exchange	3.240662	3.259938	3.308175182

EX_acolipa(e)	4 Amino 4 deoxy L arabinose modified core oligosaccharide lipid A exchange	0.257744	0.257744	0.261548524
EX_acser(e)	O Acetyl L serine exchange	11.74243	12.03389	12.21978495
EX_agm(e)	Agmatine exchange	8.56562	8.577377	8.886666667
EX_ala_D(e)	D Alanine exchange	14.24909	14.43366	14.50304
EX_alaala(e)	D Alanyl D alanine exchange	7.124545	7.216828	7.25152
EX_alltn(e)	Allantoin exchange	12.33857	12.60771	12.72833333
EX_arg_L(e)	L Arginine exchange	8.49541	8.507642	8.80038835
EX_asn_L(e)	L Asparagine exchange	16.97545	17.13188	17.34148148
EX_asp_L(e)	L Aspartate exchange	16.97545	17.13188	17.34148148
EX_cgly(e)	Cys Gly exchange	6.746341	6.839477	6.995725191
EX_colipa(e)	core oligosaccharide lipid A exchange	0.264453	0.264453	0.268283346
EX_cys_L(e)	L Cysteine exchange	9.878571	10.06192	10.41409091
EX_eca4colipa(e)	enterobacterial common antigen x4 core oligosaccharide lipid A exchange	0.181196	0.181265	0.183655311
EX_enlipa(e)	phosphoethanolamine KDO 2 lipid A exchange	0.379557	0.379557	0.389726675
EX_glyald(e)	D Glyceraldehyde exchange	17.274	17.40381	17.62384615
EX_glyc_R(e)	R Glycerate exchange	15.34549	16.34705	16.48586667
EX_glyclt(e)	Glycolate exchange	28.4675	30	30
EX_gthrd(e)	Reduced glutathione exchange	4.391695	4.415359	4.545825243
EX_h2s(e)	Hydrogen sulfide exchange	19.9288	20.9288	21.58190476
EX_his_L(e)	L Histidine exchange	8.56562	8.720333	8.728
EX_hom_L(e)	L Homoserine exchange	12.91813	13.0805	13.52895522
EX_hxa(e)	Hexanoate n C60 exchange	4.486392	4.559651	4.566448363
EX_ile_L(e)	L Isoleucine exchange	7.298873	7.317762	7.617142857
EX_kdo2lipid4(e)	KDO 2 lipid IV A exchange	0.521135	0.521135	0.533618524
EX_leu_L(e)	L Leucine exchange	7.549392	7.68751	7.885228758
EX_lipa_cold(e)	cold adapted KDO 2 lipid A exchange	0.373595	0.373595	0.383524425
EX_lipa(e)	KDO 2 lipid A exchange	0.388145	0.388145	0.398340304
EX_lys_L(e)	L Lysine exchange	7.677333	7.694412	7.95122807
EX_orn(e)	Ornithine exchange	9.422182	9.427387	9.746666667
EX_phe_L(e)	L Phenylalanine exchange	5.392431	5.427921	5.455
EX_pheme(e)	Protoheme exchange	1.332481	1.35698	1.367743363
EX_pro_L(e)	L Proline exchange	9.965769	9.966095	10.30366667
EX_ptrc(e)	Putrescine exchange	9.508624	9.513091	9.852608696
EX_ser_L(e)	L Serine exchange	20.44794	20.74263	20.9644
EX_thr_L(e)	L Threonine exchange	12.57273	12.76146	13.13681159
EX_thym(e)	Thymine exchange	10.68495	11.13234	11.2597996
EX_thymd(e)	Thymidine exchange	5.342474	5.421969	5.470925024
EX_tyr_L(e)	L Tyrosine exchange	5.602378	5.622769	5.692173913
EX_ura(e)	Uracil exchange	16.97545	17.13187	17.34148148
EX_urea(e)	Urea exchange	30.35739	32.38566	32.53789474

*: All the rate unit is mmol g^{-1}(Dw)h^{-1}.

Table 4. No significant difference between NOG and GNO (7 products)

Product_Reaction_ab	Product_Reaction_Name	Wild_Type	NOG	GNO
EX_5dglcn(e)	5 Dehydro D gluconate exchange	10	10	10
EX_anhgm(e)	N Acetyl D glucosamine anhydrous N Acetylmuramic acid exchange	2.713329	2.727463	2.719406528
EX_etoh(e)	Ethanol exchange	20	20	20
EX_glcn(e)	D Gluconate exchange	10	10	10
EX_idon_L(e)	L Idonate exchange	10	10	10
EX_lac_D(e)	D lactate exchange	20	20	20
EX_val_L(e)	L Valine exchange	10	10	10

*: All the rate unit is mmol $g^{-1}(Dw)h^{-1}$.