The Enzymes that beyond Non-Oxidative Glycolysis

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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 83 enzymes that may be potentially global benefit enzymes. By comparison, we find about 30 of the 83 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 83 enzymes that are potentially global benefit enzymes. By comparison, we find about 30 from the 83 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 83 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), L-Alanine (Ala), L-Tryptophan (Try), L-Phenylalanine (Phe), L-Malate (Mal) as the representatives.

Firstly, we calculated the growth rate (Grw) and the theoretical yields of the above 10 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, 10 products respectively)

$S \cdot v = 0$ (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 10 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, 10 products respectively)

$S' \cdot v' = 0$ (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)

Thirdly, we calculated the growth rate and the theoretical yields of above 10 chemicals with *E.coli* by adding one reaction every time. But for this time, considering the enzyme activity, when we used one reaction from KEGG, up/low flux bounds were constrained to not more than 100 in abstract value. At the same time, we calculated the smallest balanced pathway for every chemical by the tool FIND_tfSBP [8].

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

83 enzymes that are potentially global benefit enzymes

The growth rate and the theoretical yields of 10 chemicals, i.e. Acetate (Ace), L-Glutamate (Glu), L-Lysine (Lys), L-Threonine (Thr), Succinate (Suc), Formate (For) , L-Alanine (Ala), L-Tryptophan (Try), L-Phenylalanine (Phe), L-Malate (Mal), 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 10 chemicals improved, if any one of the 10 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 83 enzymes, about 30 enzymes 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 10 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.

At the same time, considering the enzyme activity, when we calculated the growth rate and the theoretical yields of above 10 chemicals with *E.coli* by adding one reaction every time, we constrained the up/low flux bounds to not more than 100 in abstract value. We list all the yields of the 10 chemicals in this condition in **Supplementary Table S3**. Of course, they were lower than the corresponding yield value if without constraint. We also calculated the smallest balanced pathway for every chemical by the tool FIND_tfSBP [8] and provided the pathways of Excel table in **Supplementary Material**. At the same time, we drew the pathways for every chemical by an improved Paint4Net [9] in **Supplementary Pictures**, where the highly correlated nodes, such as the co-factors of ATP, ADP, NADP, NADPH, H, H2O and Pi, are splited with differrent labels, in order to reduce the network complexity. Metabolic flux is proportional to line thickness.

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.

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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 10 chemical products in *E.coli*.

No.	KEGG id	Rev	Growth	Ace	Glu	Lys	Thr	Suc	For	Ala	Try	Phe	Mal
	Wild_Type		0.982	29.093	11.937	7.833	12.853	17.096	103.740	20.000	4.622	5.507	18.412
1	R07409	rev	6.664	207.919	80.332	60.942	98.184	132.953	1000.000	164.401	30.603	3.222	131.302
2	R00790	rev	6.677	195.829	76.028	56.440	93.812	118.034	1000.000	144.410	29.143	35.589	122.509
3	R00659	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
4	R00206	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
5	R02537	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
6	R01050	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
7	R00709	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
8	R00149	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
9	R00224	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
10	R08515	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
11	R01224	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
12	R00572	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
13	R03004	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
14	R02301	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
15	R02145	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
16	R07159	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
17	R01138	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
18	R00397	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
19	R07758	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
20	R00724	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
21	R06846	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
22	R00430	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000

23	R00885	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
24	R00522	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
25	R01858	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
26	R00126	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
27	R00502	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
28	R01217	rev	1.384	30.000	13.333	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
29	R01197	rev	0.990	30.000	13.189	7.833	12.895	17.143	104.740	20.000	4.626	5.507	18.403
30	R00265	rev	0.982	30.000	13.073	7.833	12.856	17.096	104.740	20.000	4.399	5.507	18.412
31	R01621	irr	0.982	30.000	12.433	7.833	12.853	17.096	103.740	20.000	4.622	5.507	18.412
32	R00761	irr	0.982	30.000	12.433	7.833	12.853	17.096	103.740	20.000	4.622	5.507	18.412
33	R01817	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
34	R00834	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
35	R07164	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
36	R01063	rev	0.990	30.000	12.208	8.135	0.000	17.096	109.800	20.000	4.641	5.580	18.412
37	R09281	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
38	R01434	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
39	R07140	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
40	R01976	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
41	R00978	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
42	R01218	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
43	R00396	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
44	R00094	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
45	R00688	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
46	R01738	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
47	R00343	rev	0.990	30.000	12.208	8.135	13.441	17.096	106.243	20.000	4.641	5.580	18.412

48	R01747	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
49	R00746	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
50	R01773	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
51	R02196	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
52	R00936	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
53	R00243	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
54	R02259	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.346	18.412
55	R00842	rev	0.990	30.000	12.208	8.135	13.225	17.096	109.800	20.000	4.641	5.580	18.412
56	R06847	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
57	R02577	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
58	R07759	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
59	R01041	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
60	R00848	rev	0.990	30.000	12.208	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
61	R02165	rev	1.040	30.000	12.118	7.960	13.227	17.143	118.425	20.000	4.727	5.592	18.668
62	R02965	rev	1.040	30.000	12.118	7.960	13.227	17.143	100.436	20.000	4.727	5.592	18.668
63	R01058	rev	1.384	30.000	10.000	8.571	15.000	17.143	120.000	20.000	5.217	6.000	20.000
64	R02639	rev	0.990	30.000	0.000	8.135	13.441	17.096	109.800	20.000	4.641	5.580	18.412
65	R00726	rev	0.997	29.943	12.522	8.134	13.556	17.143	104.740	20.000	4.623	5.507	19.945
66	R00346	rev	0.997	29.943	12.522	8.134	13.556	17.143	104.740	20.000	4.623	5.507	19.945
67	R00431	rev	0.997	29.943	12.522	8.134	13.556	17.143	104.740	20.000	4.623	5.507	19.148
68	R01967	rev	0.982	29.753	12.102	7.833	12.029	17.096	103.740	20.000	4.473	5.507	18.412
69	R02089	rev	0.982	29.753	12.102	7.833	12.853	17.096	103.740	20.000	4.622	5.507	18.412
70	R01010	rev	1.014	29.737	12.247	8.011	13.044	17.143	104.309	20.000	4.710	5.652	19.181
71	R01059	rev	0.982	29.714	12.049	7.895	12.938	17.096	106.740	20.000	4.625	5.523	18.412
72	R07417	rev	0.982	29.714	12.049	7.895	12.938	17.096	106.740	20.000	4.625	5.523	18.412

73	R00585	rev	0.982	29.406	12.012	7.833	12.853	17.096	103.740	20.000	4.622	5.507	18.412
74	R00941	rev	0.983	29.391	11.991	7.833	12.853	17.096	103.740	20.000	4.622	5.507	18.412
75	R00519	rev	0.982	29.279	11.971	7.833	12.853	17.096	117.671	20.000	4.622	5.507	18.412
76	R01199	rev	0.986	29.225	11.961	7.833	12.853	17.096	114.309	20.000	4.622	5.507	18.412
77	R00210	rev	0.982	29.131	11.946	7.833	12.856	17.096	113.740	20.000	4.623	5.507	18.412
78	R09280	rev	0.987	29.093	11.937	7.833	12.870	17.096	105.740	20.000	4.628	5.507	18.412
79	R00352	rev	1.001	29.093	12.345	7.833	12.895	17.143	107.945	20.000	4.638	5.507	18.525
80	R04198	rev	0.983	29.093	11.937	7.945	12.853	17.096	103.740	20.000	4.622	5.507	18.412
81	R07613	rev	0.984	29.093	11.937	8.011	12.853	17.096	103.740	20.000	4.622	5.507	18.412
82	R06975	rev	0.000	29.093	11.937	7.833	12.531	17.096	109.740	20.000	4.622	5.507	18.412
83	R00859	rev	0.983	29.093	11.937	7.639	12.853	17.096	110.381	20.000	4.622	5.507	18.412

*: 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) , L-Alanine (Ala), L-Tryptophan (Try), L-Phenylalanine (Phe), L-Malate (Mal).

*: 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}.