Table S1 Plackett-Burman design experiments

Run Order	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	Y
1	40	100	2	10	10	0.2	0.2	0.01	0.005	4.19±0.01
2	40	100	2	10	3	0.2	0.05	0.05	0.02	3.75±0.02
3	40	50	8	3	3	0.2	0.2	0.05	0.02	1.52±0.30
4	30	50	2	3	3	0.2	0.05	0.01	0.005	5.18±0.23
5	40	50	8	10	3	0.6	0.05	0.01	0.005	3.80±0.19
6	30	100	2	3	3	0.6	0.2	0.05	0.005	5.04±0.47
7	30	100	8	3	10	0.2	0.05	0.01	0.02	1.94±0.04
8	40	100	8	3	10	0.6	0.05	0.05	0.005	0.69±0.01
9	30	100	8	10	3	0.6	0.2	0.01	0.02	6.49±0.18
10	30	50	8	10	10	0.2	0.2	0.05	0.005	5.20±0.02
11	40	50	2	3	10	0.6	0.2	0.01	0.02	2.67±0.20
12	30	50	2	10	10	0.6	0.05	0.05	0.02	5.42±0.29

Table S2 Statistical analysis of Plackett-Burman design.

Factors	Coefficient estimate	Standard error	T-value	P-value	significant
Intercept	3.822	0.138	27.77	0.001	
(X_1) Temperature	-1.054	0.138	-7.66	0.017	Yes
(X_2) Volume	-0.140	0.138	-1.02	0.416	No
(X_3) Corn steep liquor	-0.550	0.138	-4.00	0.057	No
(X_4) $(NH_4)_2SO_4$	0.985	0.138	7.15	0.019	Yes
(X_5) Yeast extract	-0.473	0.138	-3.44	0.075	No
(X_6) KCl	0.196	0.138	1.42	0.291	No
(X_7) $MgSO_4$	0.360	0.138	2.62	0.120	No
(X_8) $FeSO_4$	-0.220	0.138	-1.60	0.251	No
(X_9) $MnSO_4$	-0.192	0.138	-1.40	0.297	No

Table S3. FCCD of the variables and experimental results for 2,3-dihydroxyisovalerate production (Y)

by *E. cloacae* ΔbudA-ΔilvD.

Run Order	X_1	X_3	X_4	Y (g/L)
1	28	1	15	6.27±0.14
2	33	3	10	8.64±0.01
3	28	3	10	8.02±0.31
4	33	3	10	8.27±0.05
5	33	3	15	8.56±0.02
6	33	3	10	8.31±0.06
7	33	3	10	8.72±0.05
8	33	3	10	9.16±0.18
9	33	3	10	8.94±0.08
10	38	1	15	7.45±0.02
11	28	5	15	7.09±0.13
12	38	3	10	8.08±0.18
13	33	5	10	6.71±0.18
14	33	1	10	7.08±0.04
15	28	1	5	6.67±0.03
16	28	5	5	4.08±0.15
17	38	5	5	4.70±0.29
18	38	1	5	7.28±0.08
19	38	5	15	7.34±0.02
20	33	3	5	8.48±0.13

Table S4. Analysis of variance of the fitted quadratic polynomial model for 2,3-dihydroxyisovalerate production by *E. cloacae* ΔbudA-ΔilvD.

Factor	Coefficient estimate	Standard error	F-value	P-value
Model	8.64	0.144	20.76	<0.001
X_1	0.28	0.132	4.56	0.058
X_3	-0.49	0.132	13.94	0.004
X_4	0.54	0.132	16.73	0.002
X_1^2	-0.53	0.252	4.45	0.061
X_3^2	-1.68	0.252	44.84	<0.001
X_4^2	-0.061	0.252	0.06	0.814
$X_1 X_3$	-0.13	0.148	0.75	0.408
$X_1 X_3$	0.013	0.148	0.01	0.934
$X_3 X_4$	0.75	0.148	25.64	<0.001
R^2=0.95	R^2 (adj)=0.90	R^2 (Pred)=0.67		