Supporting information (SI)

S1 Strains construction and preservation

Construction of recombinant *E. coli* to synthesize isoprene follows previous research. To be briefly, replacing the DXS, DXR, and IDI gene endogenic promoter on *E. coli* chromosome with strong mobilizer PGI or P_L**. Used the IDI from *Sacchromycess Cerevisiae* to replace the endogenic IDI to strengthen the MEP pathway in *E. coli* itself. In addition, two genes MVAE and MVAS from *Enterococcus Faecalis*, as well as PMK, PMD and IDI from *S. Cerevisiae* were integrated into *E. coli* chromosomes. And then introduce two grains, PES and PSK, to expresses ISPS and MK on the upstream routes of MVA, respectively. Eventually, engineered *E. coli* that can efficiently synthesize isoprene through both MEP and MVA pathways was obtained. Strains and plasmids used in this study was shown in Table S1.

Table S1 Strains and plasmids used in this study. (Martinez et al., 1988; Lerner and Inouye, 1990; Yang et al., 2016).

Strain	Genotype	Plasmids	Genotype
BL21	F^-dcm ompT hsdS(rB^-mB^-) gal λ^S	pCL1920	pSC101 origin, $Spec^r$
CIBTS1756	BL21, glmS-pstS: P_L^* MK_{MM} PMK_{SC} PMD_{SC} idi_{SC}	pTrcHis2B	pBR322 origin, Amp^r
CIBTS1757	BL21, Δidi: PGI^* idi_{SC}, P_L^{**} dxs, PGI^* dxr	pSU2718	P15A origin, Cm^r
CIBTS1758	BL21, glmS-pstS: P_L^* MK_{MM} PMK_{SC} PMD_{SC} idi_{SC}, Δidi: PGI^* idi_{SC}, P_L^{**} dxs, PGI^* dxr	pS1	Ptrc $ispS_{PA}$, pTrcHis2B ori, Amp^r
CIBTS1357	CIBTS1757/pAG+pHGFH+pTrcS1	pSK	Ptrc $ispS_{PA}$, MK_{MM}, pTrcHis2B ori, Amp^r
CIBTS1384	CIBTS1756/pSK	pAG	PGI^* fldA ispG, pCL1920 ori, $Spec^r$
CIBTS1385	CIBTS1756/pES+pSK	pHGFH	Ptac $ispH_{AS}$ $ispG_{TE}$ $petF_{TE}$ $petH_{TE}$, pSU2718 ori, Cm^r
CIBTS1439	CIBTS1758/pAG+ pHGFH+pSK	pES	Ptrc $mvaE_{EF}$ $mvaS_{EF}$, pCL1920 ori, $Spec^r$
CIBTS1440	CIBTS1758/pAGES+ pHGFH+pSK	pAGES	PGI^* fldA ispG, Ptrc $mvaE_{EF}$ $mvaS_{EF}$, pCL1920 ori, $Spec^r$
CIBTS1557	CIBTS1757/pAG+pHGFH+pEBI	pEBI	Ptrc $crtE_{PAN}$ $crtB_{PAN}$ $crtI_{PAN}$, pTrcHis2B ori, Amp^r
CIBTS1558	CIBTS1756/pES+pEBIK	pEBIK	Ptrc $crtE_{PAN}$ $crtB_{PAN}$ $crtI_{PAN}$ MK_{MM}, pTrcHis2B ori, Amp^r
CIBTS1559	CIBTS1758/pAGES+pHGFH+pEBIK		

S2 Engineered *E. coli* and wastewater

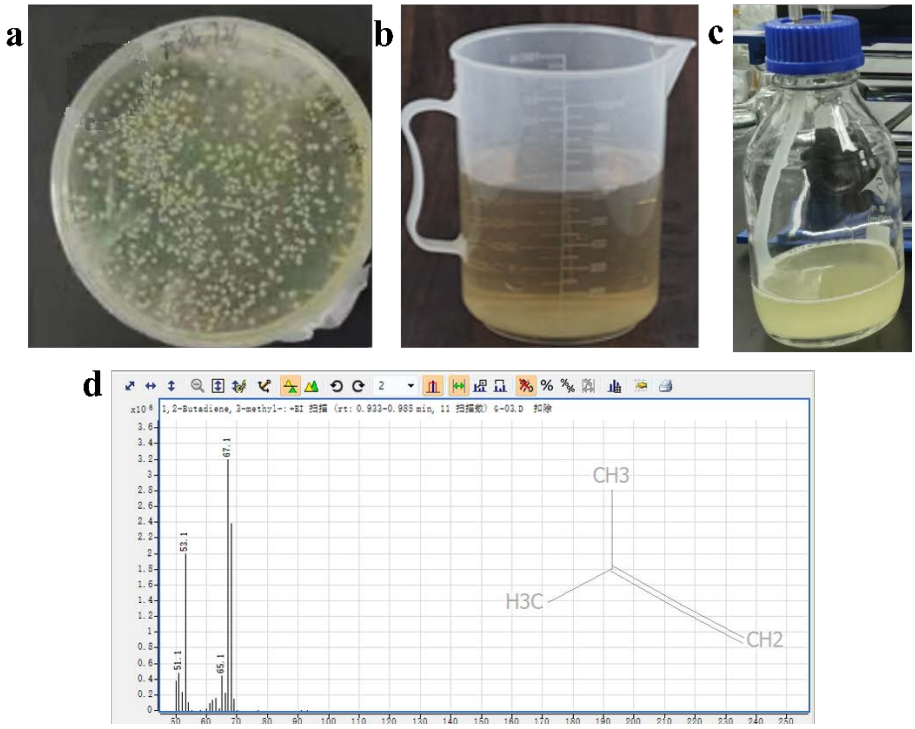


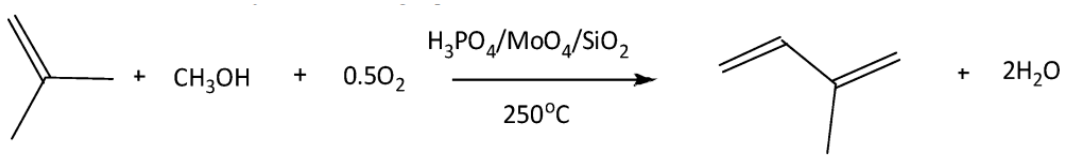
Fig. S1 Visual representation of (a) the engineered *E. coli* on plate, (b) the wastewater used in this study, and (c) the sealed shake-flask with wastewater and *E. coli*; and (d) the mass spectrometry of gas products.

S3 The economic and ecological effect evaluation

We compare the isoprene production by laboratory-scale wastewater based engineering bacteria fermentation with current chemical production from fossil-fuels and bacterial fermentation of sugars. The greenhouse gases (GHG) emissions and economic cost from conventional routes of fossil-fuels refining and bio-fermentation of clean sugars are calculated in Ecoinvent database and model from Argonne National Laboratory (Dunn et al., 2015; Wernet et al., 2016). A modified industrial modeling of biochemical fermentation applying the laboratory-data of engineering bacteria fermentation is adopted here to comprehensively indicate the environmental and economic impacts of the laboratory-scale operation with less uncertainties.

Route 1 Production of isoprene by fossil-fuels

The Sumitomo process (Morais et al., 2015) has been selected as the industrial production of isoprene by fossil-fuels method because it is beneficial by using less expensive components as well as the fact that it has lower investment costs for a single-step process. The Sumitomo process involves the use of isobutylene, methanol and oxygen, and it can be ascribed by the following equation (Eq. (S1)):



(S1)

Isobutene comes from the C4 component of liquefied gas from petroleum catalytic cracking (Morais et al., 2015). Methanol required is produced from the gasification and fractionation of coal. The stoichiometry of these reactions is $C_4H_8 + CH_4 + 0.5O_2 \rightarrow C_5H_8 + H_2O + H_2$ and the overall conversion is equal to 12% whereas selectivity is 60% (based on isobutene) and 40% (based on methanol). Fig. S2 exhibits the schematic of isoprene production. Table S2 summarizes the energy and chemicals consumed in the production of isoprene.

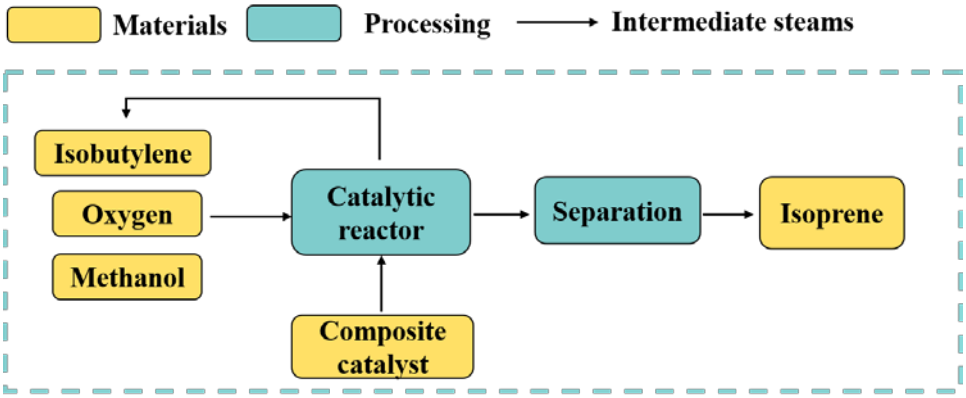


Fig. S2 Schematic and system boundary of isoprene production by fossil-fuels refining.

Table S2 Energy and material inventory of the production of isoprene by fossil-fuels refining.

Items	Inputs	Units	Price（$/kg）	Description
Isobutylene	1.37	kg	1.05, 1.25, 1.35	—
Oxygen	0.39	kg	0.057, 0.058, 0.059	—
Catalyzer	0.001	kg	97.82, 99.26, 100.70	(MoO_4 SiO_2 H_3PO_4)
Methanol	1.17	kg	0.48, 0.50, 0.56	—
Electricity	0.25	kWh	0.067, 0.070, 0.074	Material pumping and mixing
Natural gas	0.20	Nm^3	0.198, 0.253, 0.304	Heat and separation

Route 2 Production of isoprene by sugars fermentation

The production of isoprene by sugars fermentation is conducted in the bio-fermentation reactor. Slurry of sugars is converted to isoprene via feedstock sterilization, fermentation, and products separation. Slurry of sugars is mixed with accessory fermentation ingredient (corn steep liquor) and heated to 121℃ for ~15 min with natural cooling. Bio-fermentation is conducted at 37℃ with residence of 48 h, wherein corn steep liquor and added diammonium phosphate serve as the nitrogen sources and buffer. The fermentation broth is then flashed to purge the gases and transferred to the separation unit. A hybrid extraction-distillation was proposed here with solvents of 1-butanol and the mass-flow rate of solvent transferred to the multi-stage mixers is assumed as 1.2 times than the water in product streams of fermentation broth. Fig. S3 exhibits the schematic of isoprene production. Table S3 summarizes the energy and chemicals consumed in the production of isoprene.

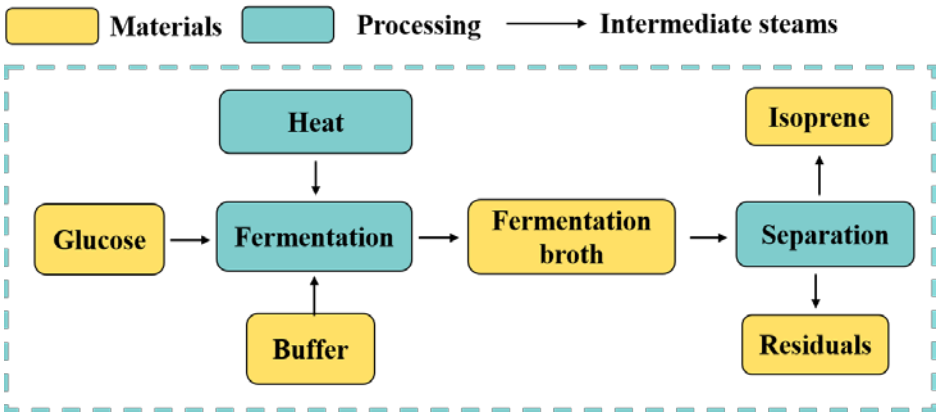


Fig. S3 Schematic and system boundary of isoprene production by sugars fermentation.

Table S3 Energy and material inventory of the production of isoprene by sugars fermentation.

Items	Inputs	Units	Price（$/kg）	Description
Glucose	37.5	kg	0.16, 0.17, 0.19	
Buffer	3125	L	0.005,0.021,0.037	Inorganic salt and buffer
Natural gas	0.30	Nm^3	0.198, 0.253, 0.304	Heat and separation
Electricity	0.00627	kWh	0.067, 0.070, 0.074	Material pumping and mixing
N,N-dimethylformamide	1.5	kg	1.02, 1.45, 1.89	Separation,1.5 times of isoperene
N,N-dimethylformamide	0.045	kg	1.02, 1.45, 1.89	3% wastage

Route 3 Production of isoprene by engineering bacteria fermentation from wastewater

To make the comparison of environmental and economic impacts of different routes of isoprene production credible, similar industrial fermentation of biochemicals is adopted here as the industrial modeling to investigate the potential advantages of wastewater-based engineering bacteria fermentation system. Thus, laboratory-scale operation of isoprene production by the genetically engineered *E. coli* via engineering bacteria fermentation from wastewater is assumed to be modified with an industrial modeling similar to the described routes of bio-fermentation, which includes the coculture of *E. coli* from mature seed with Corn fermentation wastewater (5553 mg/L COD content) in an fermenter, conditioning of pH by caustic soda, and separation of products and by-products. Fig. S4 exhibits the schematic of isoprene production. Table S4 summarizes the energy and chemicals consumed in the production of isoprene.

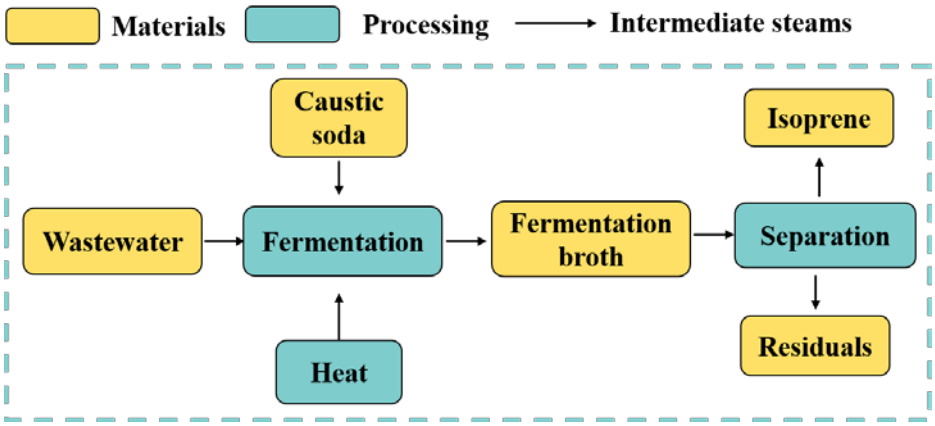


Fig. S4 Schematic and system boundary of isoprene production by wastewater-based engineering bacteria fermentation system.

Table S4 Energy and material inventory of the production of isoprene by wastewater-based engineering bacteria fermentation system.

Items	Inputs	Units	Price（$/kg）	Description
Wastewater	2877.78	L L	3.96, 4.40, 4.84×10^{-4}	Wastewater collection
Caustic soda	2.22	kg	0.20, 0.25, 0.30	Adjusting pH
Natural gas	0.53	Nm^3	0.198, 0.253, 0.304	Heat and separation
Electricity	0.01115	kWh	0.067, 0.070, 0.074	Material pumping and mixing
N,N-dimethylformamide	1.5	kg	1.02, 1.45, 1.89	Separation,1.5 times of isoperene
N,N-dimethylformamide	0.045	kg	1.02, 1.45, 1.89	3% wastage

Potential offset of GHG emissions and economic cost

Strategy of wastewater-based engineering bacteria fermentation system avoids the wastewater treatment and produces valuable products, and potential GHG emissions from wastewater treatment can be avoided. Estimated unit offset of GHG emission and economic cost are calculated in Eqs. (S2)–(S4), results are exhibited in Figs. 7(c) and 7(d).

$$\Delta COD = V_{WW} \times COD_{influent} - (V_{WW} \times COD_{effluent} - m_{isoprene} \times C_{isoprene-COD}) \quad , \quad \textbf{(S2)}$$

$$Offset_{GHG} = (V_{WW} \times GHG_{WWT} + C_{CH_4-CO_2} \times \Delta COD \times EF_{s-CH_4} + C_{N_2O-CO_2} \times \Delta TN \times EF_{s-N_2O})/V_{WW} \quad , \quad \textbf{(S3)}$$

$$Offset_{Cost} = (V_{WW} \times Cost_{WWT} + m_{Cds} \times Cost_{Cds})/V_{WW} \quad , \quad \textbf{(S4)}$$

where $\boldsymbol{COD_{influent}}$ and $\boldsymbol{COD_{effluent}}$ are the concentrations of COD (kg/m^3) in the influent and effluent of industrial wastewater, $\boldsymbol{m_{isoprene}}$ is the mass (kg) of produced isoprene in one cycle of operation, $\boldsymbol{C_{isoprene-COD}}$ (~1.95 kg/kg) is the conversion constant of COD to per kilogram of isoprene, $\boldsymbol{Offset_{GHG}}$ (kg CO_2-eq/m^3) is the offset of GHG (CO_2, CH_4, and N_2O) emission from wastewater treatment, $\boldsymbol{V_{WW}}$ (m^3) is the treated volume of wastewater, $\boldsymbol{GHG_{WWT}}$ (kg CO_2-eq/m^3) is the GHG emission of wastewater treatment, $\boldsymbol{\Delta COD}$ (kg) is the removal amount of COD before and after wastewater treatment, $\boldsymbol{\Delta TN}$ (kg) is the removal amount of total-nitrogen before and after wastewater treatment, $\boldsymbol{EF_{s-CH_4}}$ (10^{-3} kg CH_4/kg COD removal) and $\boldsymbol{EF_{s-N_2O}}$ (10^{-3} kg N_2O/kg TN removal) are the emission factors of CH_4 (generated from anaerobic zone) and N_2O (generated from aerobic zone) during A^2O treatment, $\boldsymbol{C_{CH_4-CO_2}}$ (~25, dimensionless) and $\boldsymbol{C_{N_2O-CO_2}}$ (~298, dimensionless) are the constant of GHG effects of CH_4 and N_2O compared against CO_2 according to the reports of Intergovernmental Panel on Climate Change, $\boldsymbol{Offset_{Cost}}$ ($/$m^3$) is the offset of economic burdens from wastewater treatment, $\boldsymbol{Cost_{WWT}}$ ($/$m^3$) is the economic expense of wastewater treatment.

Table S5 The significance test for comparisons of engineered bacteria fermentation of wastewater and fossil-fuels refining or sugars fermentation for isoprene production in terms of economic cost and carbon emissions.

Items	Economic cost		Carbon emissions	
	T Statistic	*p*-value	T Statistic	*p*-value
Wastewater vs. Fossil fuels	−39.20	0.00065	−40.24	0.00062
Wastewater vs. Sugars	−27.06	0.0014	−27.92	0.0013

S4 Isoprene production from wastewater

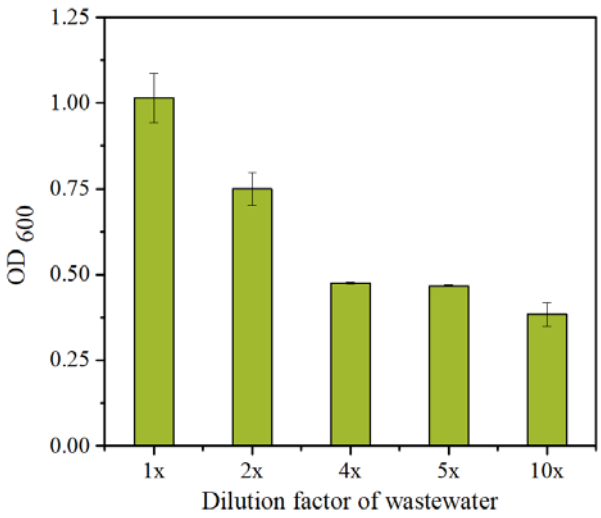


Fig. S5 The OD_{600} after 24 h culture of wastewater with different dilution factor.

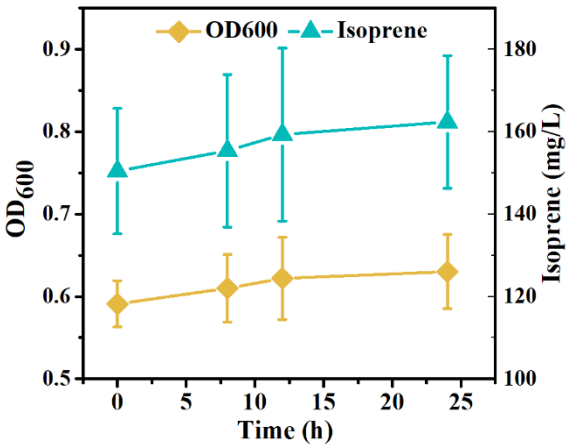


Fig. S6 The OD_{600} and accumulated concentration of isoprene after the mixed liquid of wastewater and bacterial fluid (after first stage of 24 h cultivation) was transferred to a new sealed shake-flask.

S5 The different role of each composition in *E. coli* proliferation and isoprene production

As shown in Fig. S7(a), compared to the control group (no substrate), the isoprene production was almost zero when protein was the substrate. Furthermore, the concentration of residual protein in the culture medium was measured and shown in Fig. S7(b). Obviously, the efficiency of protein utilization by the E. coli was quite low, and when the reaction proceeded to 4 h, 16 h, and 24 h, the protein concentration decreased from the initial 688 mg/L to 668, 676, and 655 mg/L, respectively.

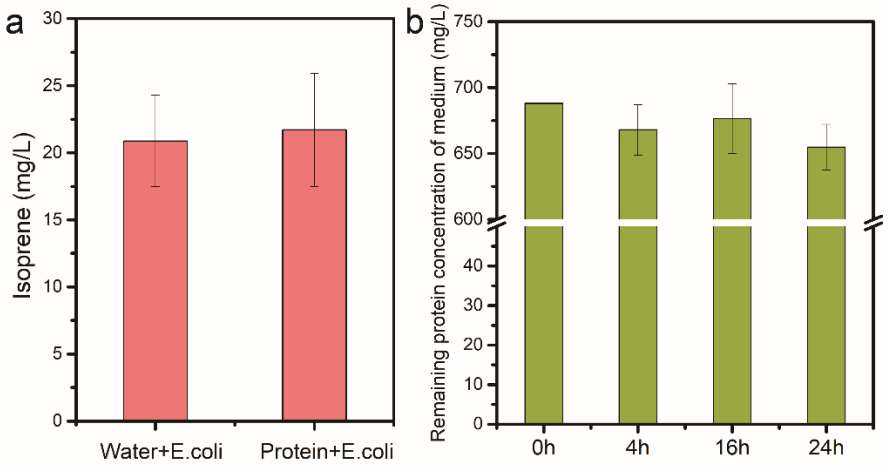


Fig. S7 (a) Isoprene production with protein as substrate and (b) the concentration of residual protein in the culture medium.

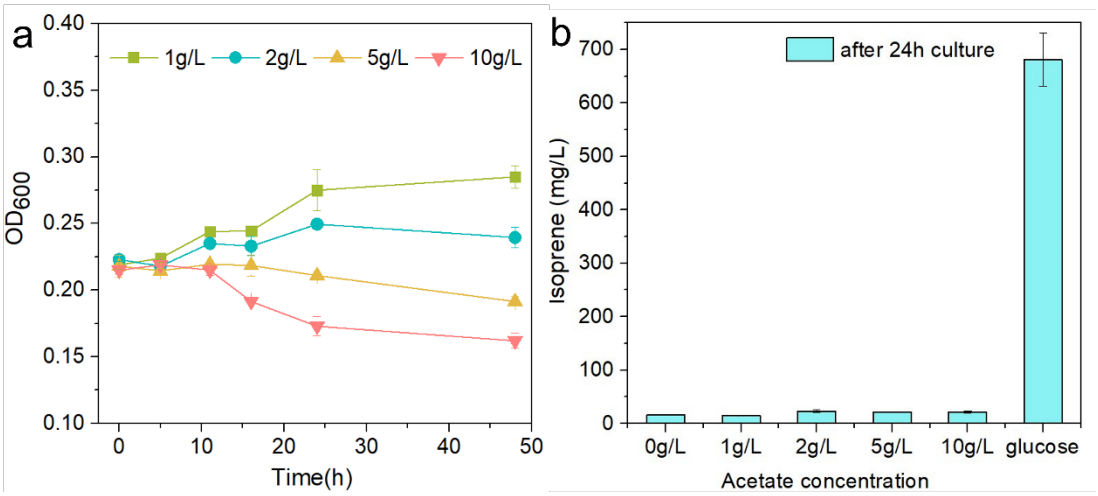


Fig. S8 The (a) OD_{600} and (b) isoprene production with acetic acid as a substrate.

References

Dunn J B, Adom F, Sather N, Han J, Snyder S, He C, Gong J, Yue D, You F (2015). Life-cycle analysis of bioproducts and their conventional counterparts in GREET (No. ANL/ESD-14/9 Rev.). Argonne, IL, USA: Argonne National Lab. (ANL)

Lerner C G, Inouye M (1990). Low copy number plasmids for regulated low-level expression of cloned genes in Escherichia coli with blue/white insert screening capability. Nucleic Acids Research, 18(15): 4631

Martinez E, Bartolomé B, De La Cruz F (1988). pACYC184-derived cloning vectors containing the multiple cloning site and lacZα reporter gene of pUC8/9 and pUC18/19 plasmids. Gene, 68(1): 159–162

Morais A R, Dworakowska S, Reis A, Gouveia L, Matos C T, Bogdał D, Bogel-Łukasik R (2015). Chemical and biological-based isoprene production: Green metrics. Catalysis Today, 239: 38–43

Wernet G, Bauer C, Steubing B, Reinhard J, Moreno-Ruiz E, Weidema B (2016). The ecoinvent database version 3 (part I): overview and methodology. International Journal of Life Cycle Assessment, 21(9): 1218–1230

Yang C, Gao X, Jiang Y, Sun B, Gao F, Yang S (2016). Synergy between methylerythritol phosphate pathway and mevalonate pathway for isoprene production in Escherichia coli. Metabolic Engineering, 37: 79–91