Electronic Supplementary Material (ESI) for Green Chemistry

A novel nutritional induction strategy flexibly switching biosynthesis of food-like products from methane by a methanotrophic bacterium

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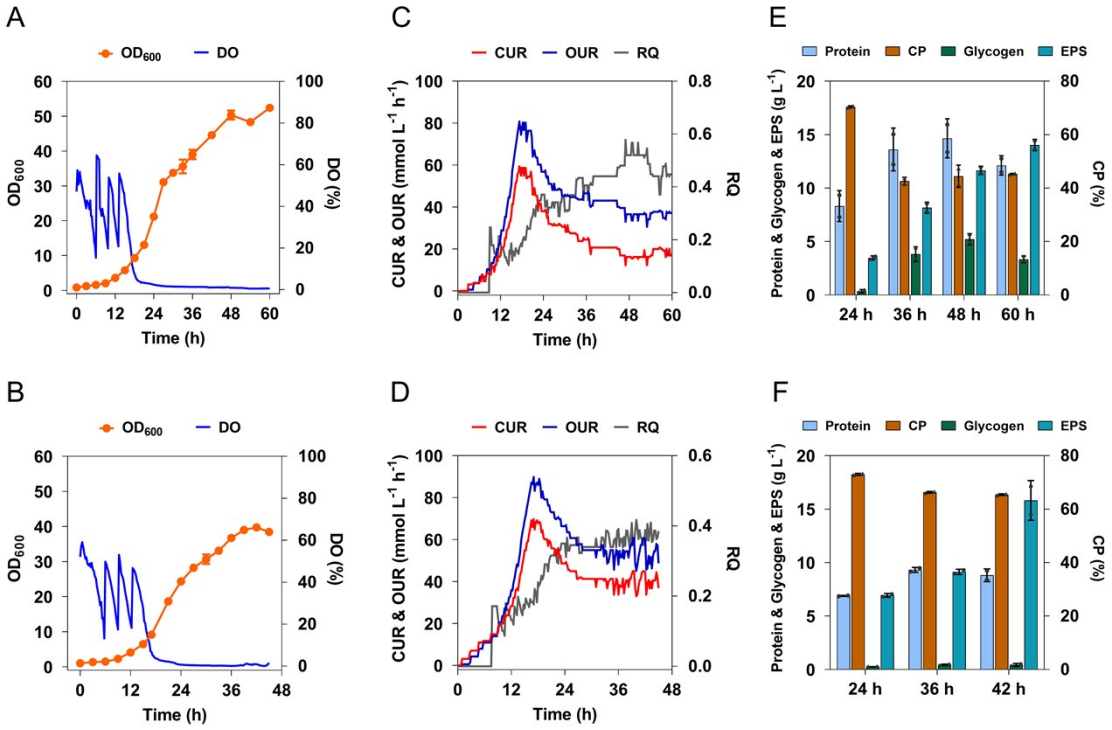

Fig. S1 Comparison of process parameters and synthesis of cell protein and carbohydrates by *M. buryatense* 5GB1 in scenario Ⅰ and scenario Ⅱ. (A and B) Time course of cell growth (OD_{600}) and dissolved oxygen (DO) concentration in scenario Ⅰ (A) and scenario Ⅱ (B) of *M. buryatense* 5GB1, respectively. (C and D) Time course of methane (CH_4) and oxygen (O_2) uptake rate, and respiratory quotient (RQ) in scenario Ⅰ (C) and scenario Ⅱ (D) of *M. buryatense* 5GB1, respectively. (E and F) Time course of cell protein, glycogen, and extracellular polysaccharides (EPS) titer and crude protein (CP) content in scenario Ⅰ (E) and scenario Ⅱ (F) of *M. buryatense* 5GB1, respectively. Scenario Ⅰ and scenario Ⅱ were terminated at 60 and 45 h, respectively, due to cessation of growth and uncontrollable foaming.

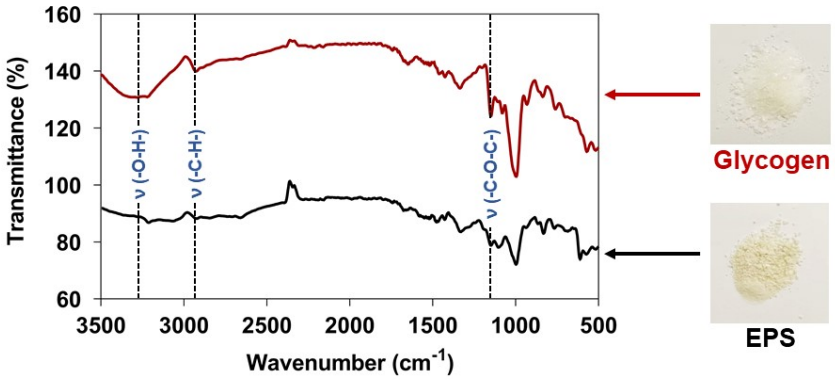

Fig. S2 Characterized methane-derived carbohydrates (glycogen and extracellular polysaccharides (EPS)) produced by *M. buryatense* 5GB1. Samples of glycogen and EPS were analyzed by a Fourier-transform infrared spectroscopy. Typical spectrum assignments from 3300-1000 cm^{-1}, where *v* = stretching vibrations. The intense band at about 3270 cm^{-1} and the weak absorption band at approximately 2930 cm^{-1} were the correlative characteristic peaks of O-H and C-H stretching vibrations in the sugar ring, respectively. The absorption of 1150 cm^{-1} was caused by C-O stretching vibration, indicating the presence of a cyclic C-O-C structure.

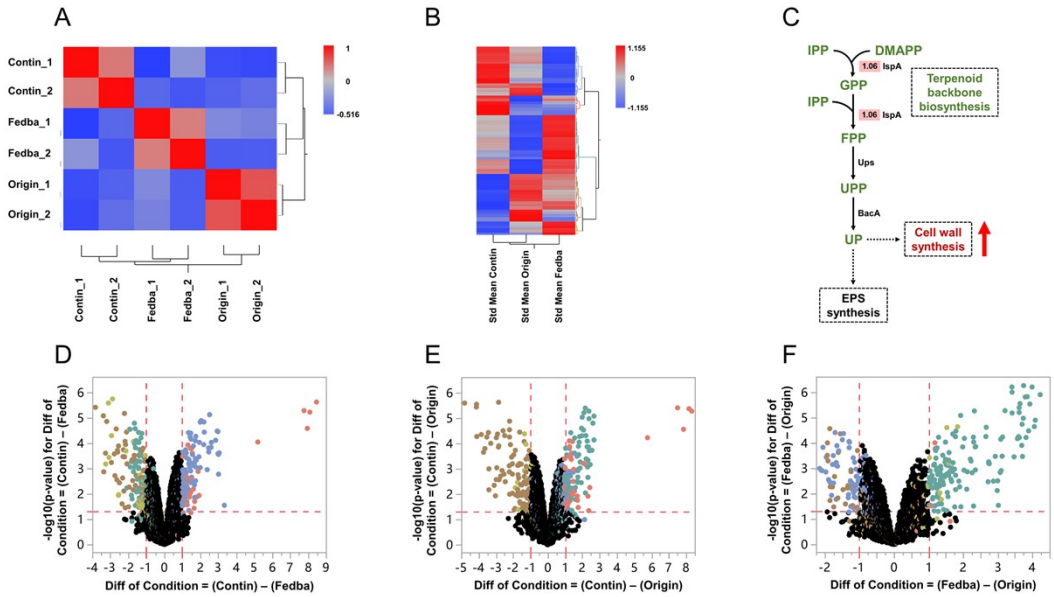

Fig. S3 Identification of differentially expressed genes (DEGs) of *M. buryatense* 5GB1 in scenario III versus scenario II. (A) Repeatability analysis for biological replicates between RNA-sequence samples. (B) Hierarchical clustering performed using the least-squares means of DEGs. (C) DEG involved in the terpenoid backbone synthesis. Full-names of the enzymes are as below. IspA, farnesyl diphosphate synthase. Ups, undecaprenyl diphosphate synthase. BacA, undecaprenyl-diphosphatase. Full-names of the compounds are as below. IPP, isopentenyl diphosphate. DMAPP, dimethylallyl diphosphate. GPP, geranyl diphosphate. FPP, farnesyl diphosphate. UPP, undecaprenyl diphosphate. UP, undecaprenyl phosphate. EPS, exopolysaccharides. (D-F) Volcano maps of DEGs (log_2(fold change) ≥1 and ≤-1 regarded as significantly up- and down-regulated respectively, *P*-value ≤0.05 for both). In (D), pairwise comparison of scenario III (Contin, sampled at the steady state under dilution rate 0.07 h^{-1}) relative to scenario II (Fedba, sampled at 32 h) was performed (n=2). In (E), pairwise comparison of scenario III relative to the late exponential phase (Origin, sampled at 21 h) was performed (n=2). In (F), pairwise comparison of scenario II relative to the late exponential phase was performed (n=2).

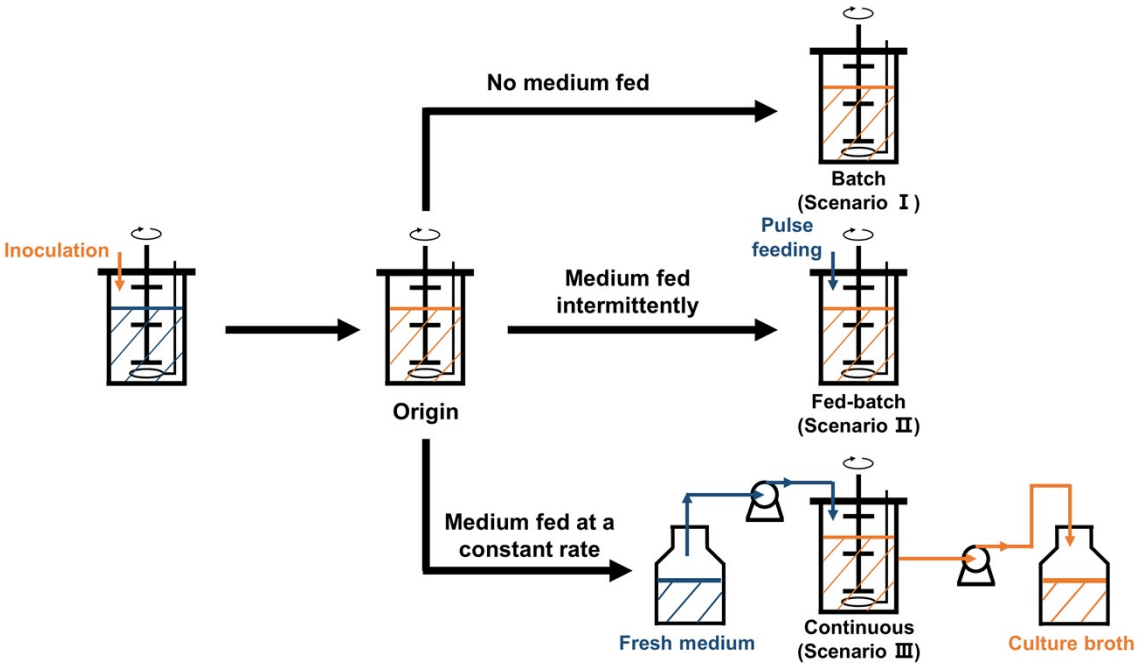

Fig. S4 Schematic of *M. buryatense* 5GB1 cultures in the three different scenarios. A gas mixture containing methane and air was sparged into the fermenter at a fixed flow rate throughout cultures. After inoculation, cell growth went through an exponential phase after adapting to the new environment, named "Origin" in this study. Nutrients were all sufficient in the "Origin" period. No additional medium was fed during scenario Ⅰ, while a concentrated medium was fed at several pulses in the scenario Ⅱ to maintain residual nitrate concentration. In scenario Ⅲ, fresh medium modified based on the initial medium as well as culture broth was pumped in and out of the fermenter by peristaltic pumps at a constant rate equal to the growth rate multiplied by working volume.

Table S1 Cell protein production by methanotrophic bacteria using CH_4 as the sole substrate.

Strain	Culture type	Titer ($g\ L^{-1}$)	Productivity ($mg\ L^{-1}\ h^{-1}$)	Content (%)	Reference
Methylosinus trichosporium OB3b	Batch	14.00	58.33	n.a.	Han et al. 2009 (1)
Methylotuvimicrobium alcaliphilum 20Z	Batch	3.00	31.25	n.a.	Cho et al. 2020 (2)
Methylomonas rapida MP1T	Continuous (μ=0.218 h^{-1})	3.26	710.68	69.55	Tikhonova et al., 2023 (3)
Methylotuvimicrobium buryatense 5GB1	Batch	14.80	308.33	n.a.	Hu et al. 2020 (4)
M. buryatense 5GB1	Scenario Ⅰ	14.65	305.21	44.42	This study
M. buryatense 5GB1	Scenario Ⅱ	9.32	258.89	66.28	This study
M. buryatense 5GB1	Scenario Ⅲ (μ=0.102 h^{-1})	6.28	640.05	71.60	This study

Note: μ, growth rate; **n.a.**: not applicable.

Table S2 Influence of growth rate on cell protein production performance in scenario III.

μ (h^{-1})	**0.050**	**0.063**	**0.076**	**0.089**	**0.102**
CP content (%, w/w)	66.77	68.09	68.11	69.06	71.60
$Y_{X/S}$ (g g^{-1} CH_4)	0.82	0.78	0.77	0.78	0.68
q_{O2} (mmol g^{-1} DCW h^{-1})	6.22	7.71	9.46	10.83	13.29
O_2/CH_4 uptake rate	1.64	1.53	1.53	1.51	1.42
Residual NO_3^--N (mg L^{-1})	7.85	102.43	143.02	186.25	196.40
Supernatant TN (mg L^{-1})	189.21	249.14	247.04	288.16	295.31
EPS yield (g g^{-1} protein)	0.59	0.60	0.82	1.26	1.33

Note: μ, growth rate. CP, crude protein. $Y_{X/S}$, cell protein yield on methane (CH_4). q_{O2}, specific oxygen (O_2) uptake rate. TN, total nitrogen. EPS, extracellular polysaccharides.

Table S3 Elemental composition of cell protein produced in scenario III.

μ (h^{-1})	**0.050**	**0.063**	**0.076**	**0.089**	**0.102**
C (%)	45.21±0.56	45.51±0.22	45.60±0.39	45.37±0.12	45.42±0.12
H (%)	7.21±0.12	7.26±0.01	7.35±0.07	7.38±0.02	7.30±0.05
O (%)	30.74±2.01	30.26±0.11	29.40±0.89	29.15±0.38	28.96±0.42
N (%)	11.02±0.08	11.25±0.06	11.34±0.02	11.35±0.05	11.63±0.06
S (%)	0.65±0.10	0.59±0.07	0.59±0.02	0.56±0.00	0.56±0.02
In total (%)	94.82	94.86	94.28	93.81	93.86
Others (%)	5.18	5.14	5.72	6.19	6.14

Note: μ, growth rate. C, carbon. H, hydrogen. O, oxygen. N, nitrogen. S, sulfur. Phosphorus could not be detected due to the limits of the elemental analyzer. Four replicates were assayed for each μ, and the results (%, w/w) were expressed as (average value) ± (standard deviation).

Table S4 Transcriptomic response to induction of oxygen deficiency.

Pathway	Name	ID (KEGG)	log_2(fold-change)	*P*-value	Regulation	Function
Methane assimilation	*pmoB*	EQU24_19300	1.32	1.20E-04	Up	Particulate methane monooxygenase alpha subunit
	pmoA	EQU24_19305	1.53	1.59E-04	Up	Particulate methane monooxygenase beta subunit
	eno	EQU24_13315	1.02	1.16E-03	Up	Phosphopyruvate hydratase
Ribosome small subunit synthesis	*rpsL*	EQU24_03850	1.37	4.42E-04	Up	30S ribosomal protein S12
	rpsG	EQU24_03855	1.11	7.55E-03	Up	30S ribosomal protein S7
	rpsK	EQU24_03990	1.04	2.30E-04	Up	30S ribosomal protein S11
	rpsI	EQU24_18070	1.30	6.12E-03	Up	30S ribosomal protein S9
Ribosome large subunit synthesis	*rplJ*	EQU24_03830	1.56	2.51E-04	Up	50S ribosomal protein L10
	rplL	EQU24_03835	1.12	1.71E-03	Up	50S ribosomal protein L7/L12
	rplM	EQU24_18065	1.12	2.67E-02	Up	50S ribosomal protein L13
Regulation of nitrogen transportation	*nifA*	EQU24_17280	1.13	5.50E-03	Up	*nif*-specific transcriptional activator
	nifL	EQU24_17275	1.38	4.52E-02	Up	Nitrogen fixation negative regulator
	glnK	EQU24_07180	2.57	1.51E-04	Up	P-II family nitrogen regulator
	glnL	EQU24_07190	1.26	5.14E-03	Up	Nitrogen regulation protein NR(II)
	glnG	EQU24_07195	1.76	5.50E-05	Up	Nitrogen regulation protein NR(I)
Nitrogen assimilation	*nrt*	EQU24_08975	1.68	4.50E-03	Up	MFS transporter
	n.a.	EQU24_12620	3.02	2.82E-04	Up	ABC transporter substrate-binding protein
	n.a.	EQU24_12625	2.95	3.40E-05	Up	ABC transporter permease
	n.a.	EQU24_12630	2.99	2.64E-04	Up	Nitrate ABC transporter ATP-binding protein
	amtA	EQU24_07165	2.06	1.33E-05	Up	Ammonium transporter
	amtB	EQU24_07175	2.48	2.27E-04	Up	Ammonium transporter
	nasA	EQU24_12645	2.99	3.59E-05	Up	Nitrate reductase
	nirB	EQU24_08985	2.86	7.86E-05	Up	Nitrite reductase large subunit

	nirD	EQU24_08990	2.63	9.53E-05	Up	Nitrite reductase small subunit
	n.a.	EQU24_12640	3.06	2.46E-04	Up	FAD-dependent oxidoreductase
	glnA	EQU24_07185	1.48	4.13E-05	Up	Glutamine synthetase
Terpenoid backbone synthesis	*ispA*	EQU24_15335	1.06	9.71E-03	Up	(2E,6E)-farnesyl diphosphate synthase

Note: pairwise comparison of scenario III (sampled at the steady state under dilution rate 0.07 h^{-1}) relative to scenario II (sampled at 32 h) was performed (n=2). Differentially expressed genes (log_2(fold-change) ≥1, *P*-value ≤0.05) corresponding to Fig. 4 B and C and Fig. S3C were listed above, including 27 up-regulated genes. **n.a.**: not applicable.