Supplementary information

Metabolic profiling of *Geobacter sulfurreducens* during scale-up

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NBAF medium contained: 9.3 g L^{-1}fumaric acid (electron acceptor), 4.1 g L^{-1} sodium acetate (electron donor), 0.04 g L^{-1} $CaCl_2.2H_2O$, 0.1 g L^{-1} $MgSO_4.7H_2O$, 1.8 g L^{-1} $NaHCO_3$, 0.5 g L^{-1} Na_2CO_3, 1 mL $L^{-1}$$Na_2SeO_4$ (1mM), 10 mL L^{-1} nitrogen and buffer mix, 10 mL L^{-1} mineral elixir mix, 15 mL L^{-1} vitamin mix (full details of these solutions are available in the supplementary information).The pH of the media was adjusted to 7.1 using NaOH and degassed by purging with N_2:CO_2 (80:20) gas under sterile conditions for 45-60 min.

Table S1. Additional components required for preparation of NBAF.

Component	Compound	Concentration
100× NB Mix	KH_2PO_4(monobasic)	42.0 g L^{-1}
	K_2HPO_4(dibasic)	22.0 g L^{-1}
	NH_4Cl	20.0 g L^{-1}
	KCl	38.0 g L^{-1}
	NaCl	36.0 g L^{-1}
NB Mineral Elixir	Nitrilotriacetic acid	2.14 g L^{-1}
	$MnCl_2.4H_2O$	0.1 g L^{-1}
	$FeSO_4.7H_2O$	0.3 g L^{-1}
	$CoCl_2.6H_2O$	0.17 g L^{-1}
	$ZnSO_4.7H_2O$	0.20 g L^{-1}
	$CuCl_2.2H_2O$	0.03 g L^{-1}
	$AlK(SO_4)_2.12H_2O$	0.005 g L^{-1}
	H_3BO_3	0.005 g L^{-1}
	Na_2MoO_4	0.09 g L^{-1}
	$NiSO_4.6H_2O$	0.11 g L^{-1}
	$Na_2WO_4.2H_2O$	0.02 g L^{-1}
Vitamin mix	Biotin	2.0 mg L^{-1}
	Folic acid	2.0 mg L^{-1}
	Pyridoxine HCl	10.0 mg L^{-1}
	Riboflavin	5.0 mg L^{-1}
	Thiamine	5.0 mg L^{-1}
	Nicotinic acid	5.0 mg L^{-1}
	Pantothenic acid	5.0 mg L^{-1}
	B-12	0.1 mg L^{-1}
	p-aminobenzoic acid	5.0 mg L^{-1}
	Thioctic acid	5.0 mg L^{-1}

Table S2. List of the top 17 significant metabolites identified by CCA. All identifications are based on minimum metabolite reporting standards (1).

Variable ID	RT	RI	QM	Metabolite	MSI ID level	chEBI code
9	403.843	1168.9	174	pyruvic acid	1	32816
16	444.843	1253.1	205	glycerol	2	17754
18	449.143	1261.9	174	unknown	4	-
22	467.643	1300	233	malonic acid	1	30794
43	523.893	1415.5	245	fumaric acid	1	18012
54	573.443	1524.3	233	malic acid	1	6650
59	598.743	1597.1	245	unknown	4	-
62	625.643	1674.5	158	oxaloacetic acid	2	30744
64	635.293	1702.3	230	5-oxoproline	1	16010
66	651.593	1749.2	179	nicotinamide	1	17154
70	669.293	1800.1	299	glycerol-3-phosphate	2	15978
71	674.093	1813.9	211	unknown	4	-
74	683.393	1840.7	211	citric acid	1	30769
77	708.593	1916	290	unknown	4	-
82	768.293	2124.3	264	adenine	1	16708
93	847.593	2400.9	121	unknown	4	-
97	873.043	2489.7	361	unknown	4	-

Code: ID, identifier on plots; RT, retention time; RI, retention index; QM, quant mass; MSI, Metabolomics Standards Initiative identification level; for chEBI codes see: https://www.ebi.ac.uk/chebi/

Note that these are the identities for the acids and not the charged species and that due to conventions we refer to fumaric acid as fumarate in the text.

(1) **Sumner LW, Amberg A, Barrett D, Beale MH, Beger R, Daykin CA, Fan TWM, Fiehn O, Goodacre R, Griffin JL and others**. 2007. Proposed minimum reporting standards for chemical analysis. Metabolomics; **3**:211-221.

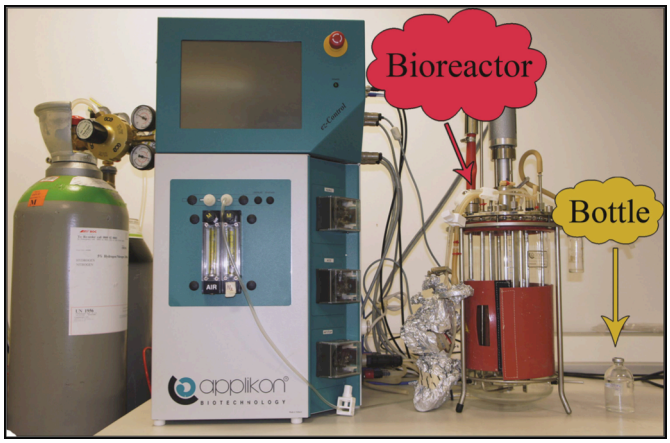

Figure S1. The 7 L bioreactor (which we filled with 5 L medium) and the 120 mL serum bottles (filled with 100 mL of medium) used for cultivation of *G. sulfurreducens* during the experiment.

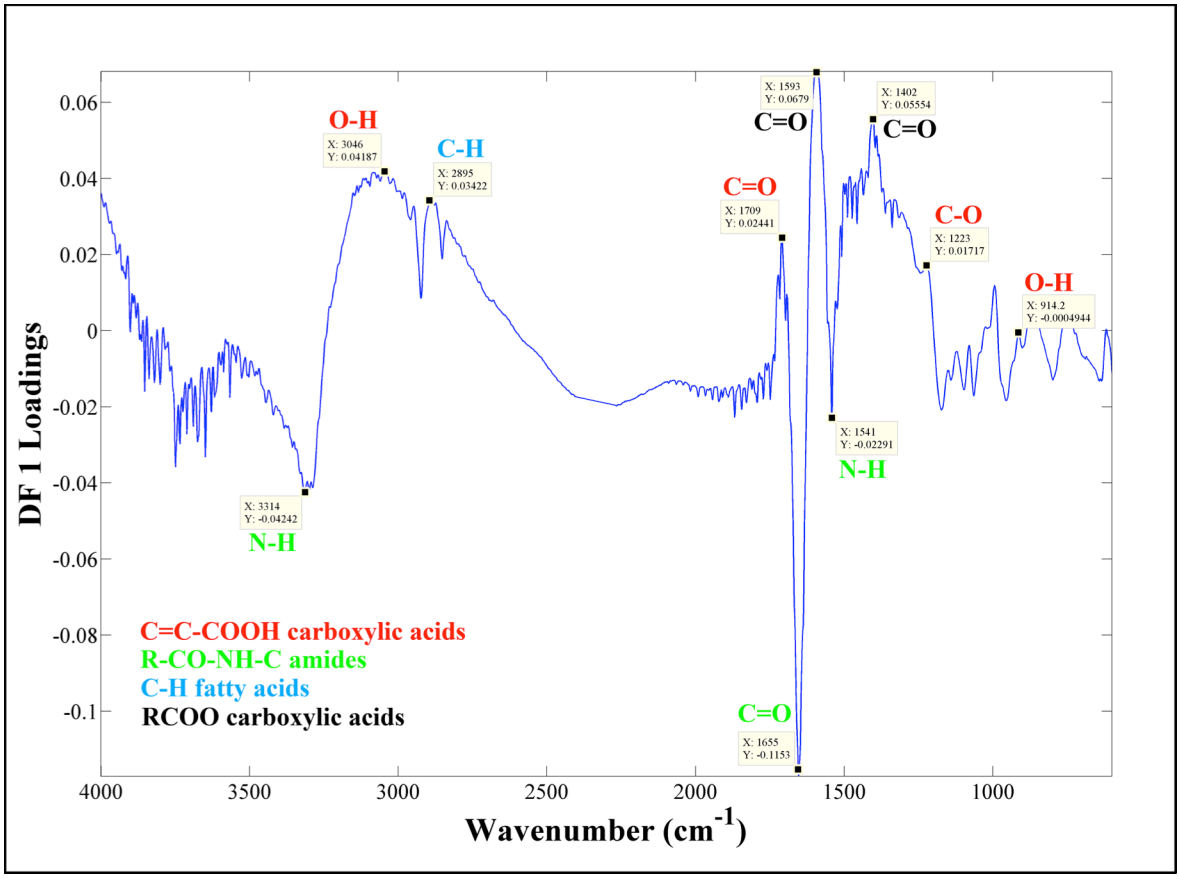

Figure S2. PC-DF1 loadings plot of the FT-IR spectra from the first 48 h samples collected from both cultivation conditions.

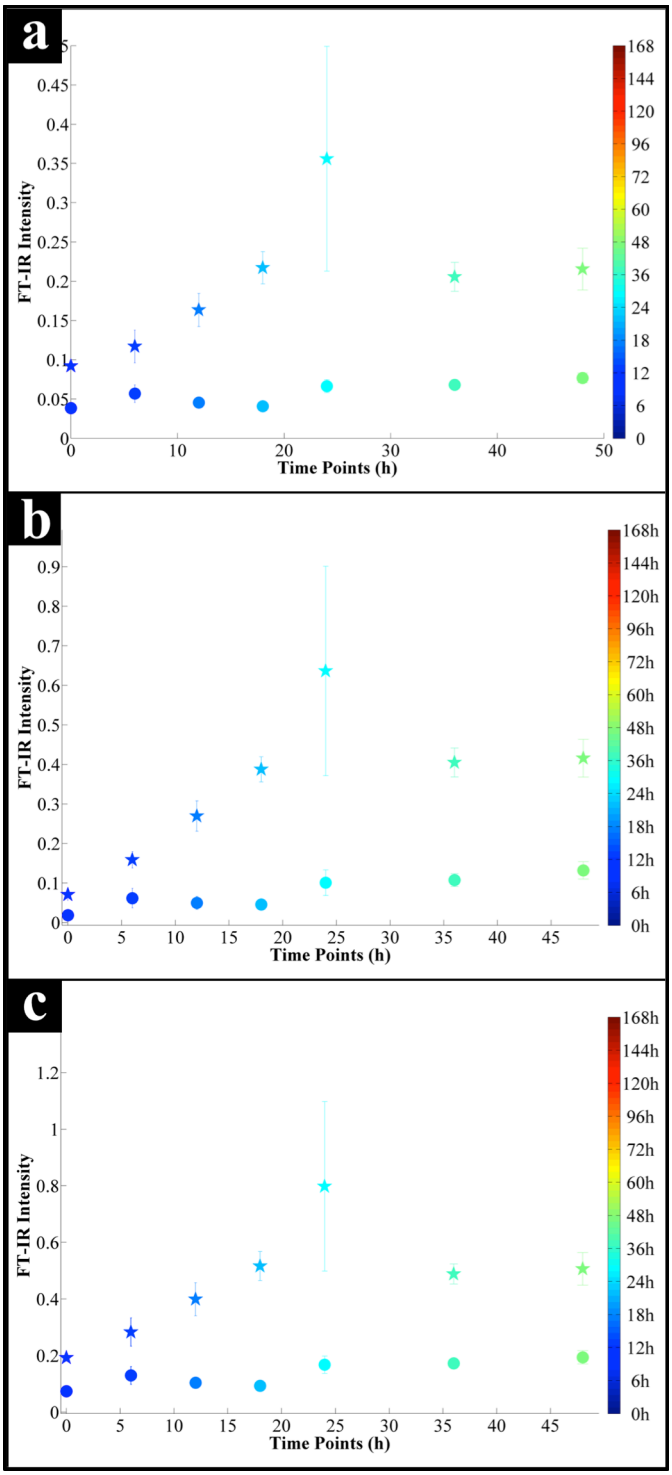

Figure S3. Comparison of the intensity of significant FT-IR vibrations identified by the PC-DFA loadings plot (Figure S2) of serum bottles (star) and bioreactor (circle) samples during the first 48 h of incubation. **(a)** 3046 cm^{-1} region of stretching of O-H bonds in carboxylic acids, **(b)** 1593 cm^{-1} region of asymmetric stretching of C=O bonds in carboxylic acids, **(c)** 3314 cm^{-1} region for stretching of N-H of amides where C=O and N-H are in *trans* configuration. Data points represent the mean centre of three replicates with bars indicating the relative standard deviation.

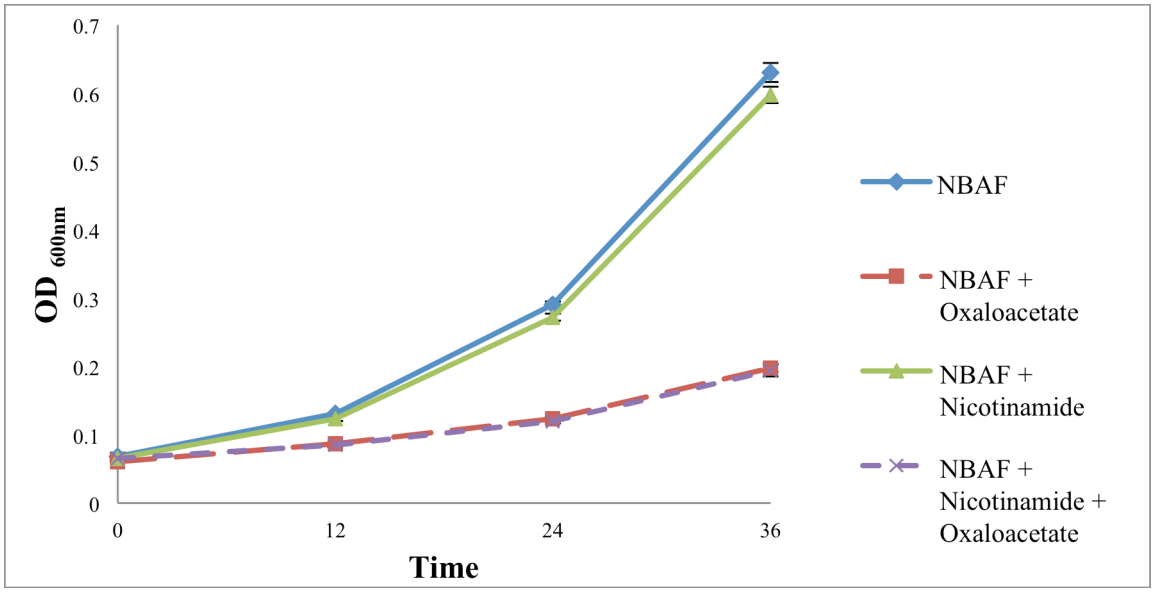

Figure S4. Growth curves of *G. sulfurreducens* incubated in 100 mL serum bottles at 30 °C for 36h using NBAF (blue line), and supplemented with oxaloacetate (red line), nicotinamide (green line) or both (purple dotted line). The data points are average of three biological replicates with bars representing the standard deviation.

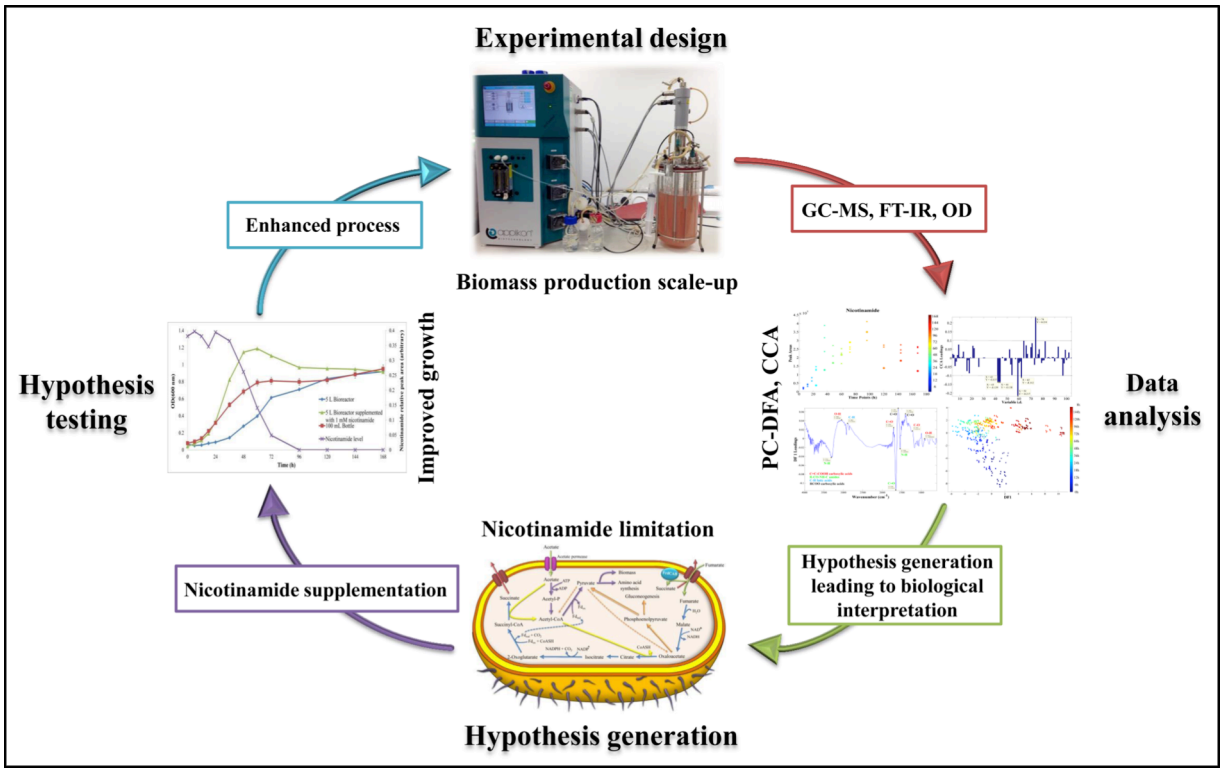

Figure S5. The cycle of inductive approach to knowledge discovery employed as the main strategy in this study for identification of growth-limiting metabolites during the scale-up process, and using these findings towards optimisation and enhancement of the bioprocess.