Electronic Supplementary Information

Supplementary Figures

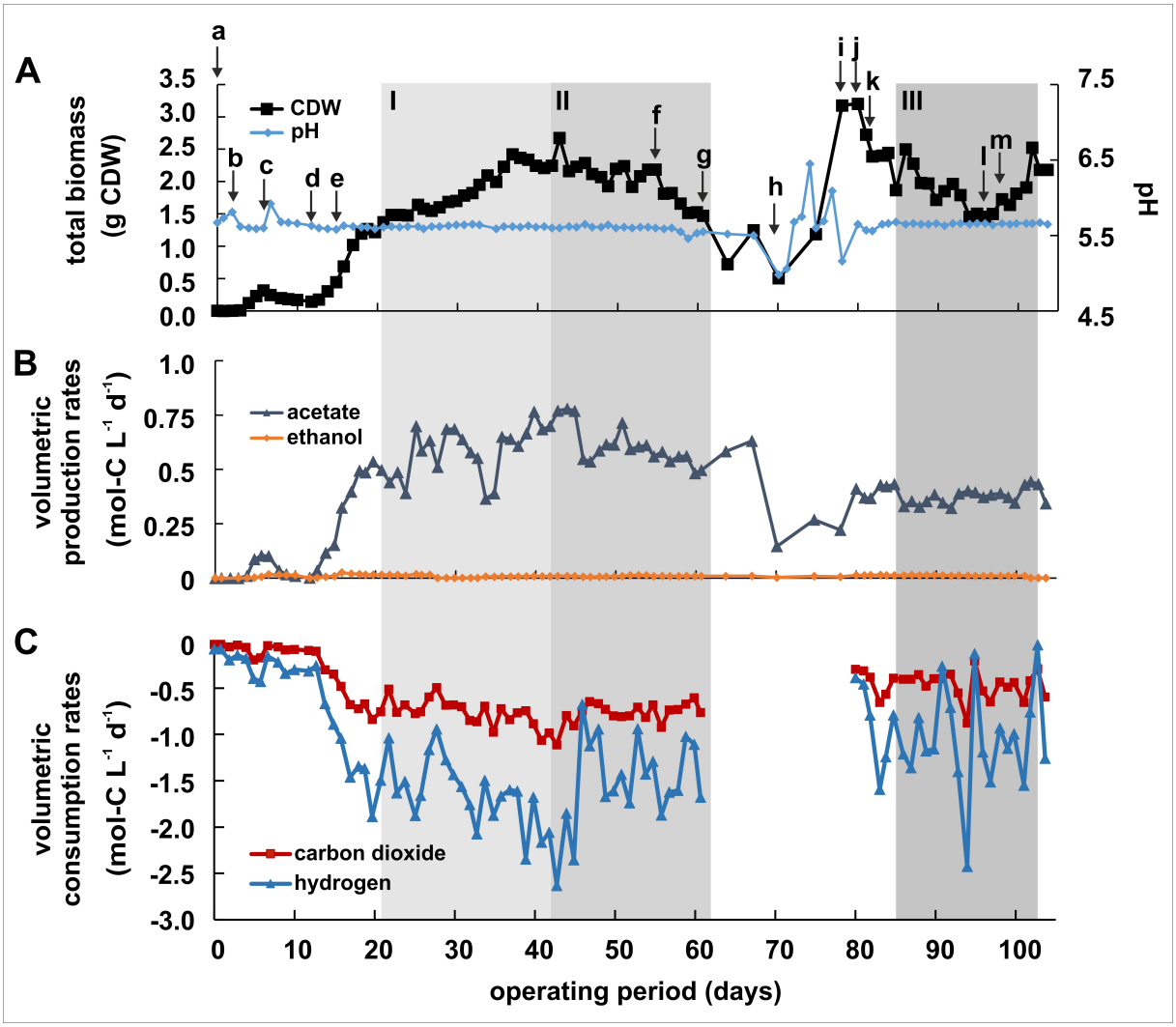

Figure S1. Details on the performance of Stage A with *C. ljungdahlii*. A, biomass concentration as grams cellular dry weight (CDW) and pH. **B,** production rates for acetate and ethanol in mol-C L^{-1} d^{-1}. **C,** consumption rates for carbon dioxide and hydrogen in mol-C L^{-1} d^{-1}. The grey areas labeled with I, II, and III indicate operating periods with batch or continuous feeding of Stage B (*S. cerevisiae*) with sterile-filtered Stage A effluent. Labels on the top of panel A indicate process changes and process perturbation: **a** (day 0), inoculate, batch, 200 rpm; **b** (day 2), re-inoculate, add 0.5 g L^{-1} yeast extract; **c** (day 6), start cell recycling, start continuous, start Na_2S feed; **d** (day 12), batch, keep cell recycling, no Na_2S feed, add 0.5 g L^{-1} yeast extract, 300 rpm; **e** (day 15), start continuous, start Na_2S feed; **f** (day 55), switch feed to medium without Vitamin B_{12} and folate; **g** (day 61), switch feed to medium with Vitamin B_{12} and folate; **h** (day 70), bad pH probe; **i** (day 78), black precipitate accumulating; **j** (day 80), exchange pH probe, OD measurements after addition of HCl to dissolve black precipitate; **k** (day 82), re-inoculate; **l** (day 96) OD measurements without addition of HCl (measurements with and without HCl are similar again); **m** (day 98), switch feed to medium without Vitamin B_{12} and folate.

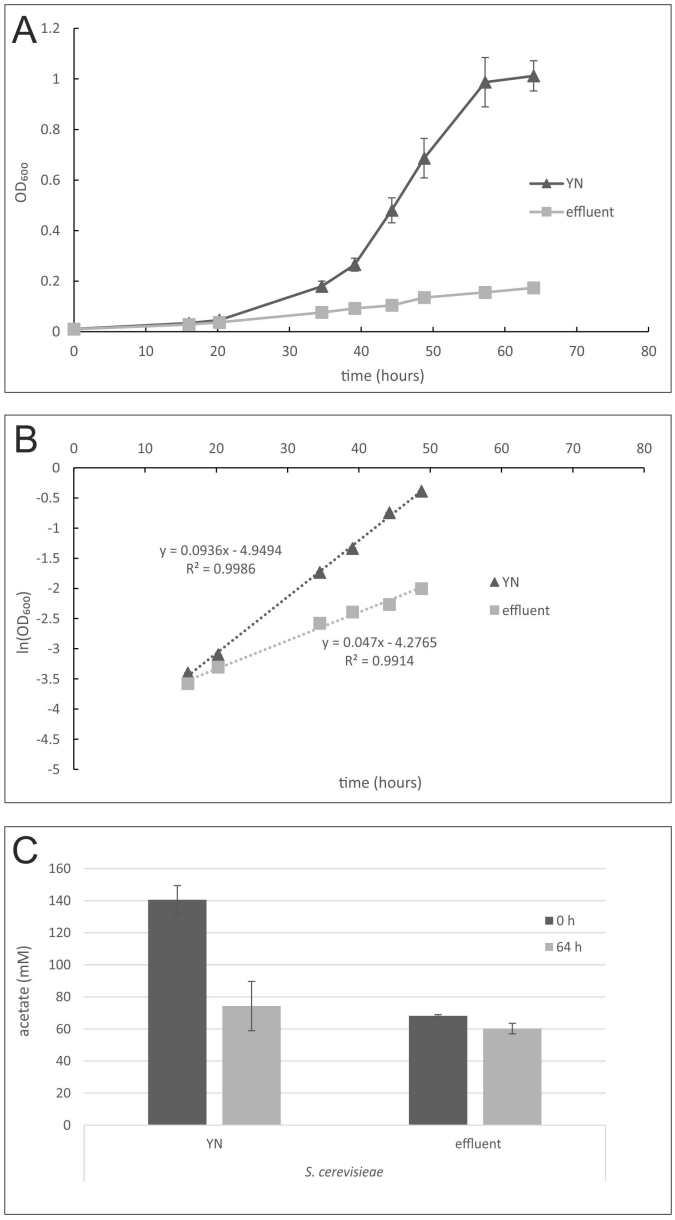

Figure S2. Growth and acetate consumption data for flask experiments with *S. cerevisiae*. A, cell concentrations given as OD_{600} during the experimental period in YNA medium with a 150-mM acetate concentration (YN) and in gas-fermentation effluent with a 70-mM acetate concentration (effluent). **B,** determination of the growth rate by plotting the natural logarithm of the OD_{600} during the exponential growth phase only**. C,** acetate concentration in the beginning (0 hours) and at the end (64 hours) of the experiment. Data is shown for biological triplicates. Error bars indicate standard errors.

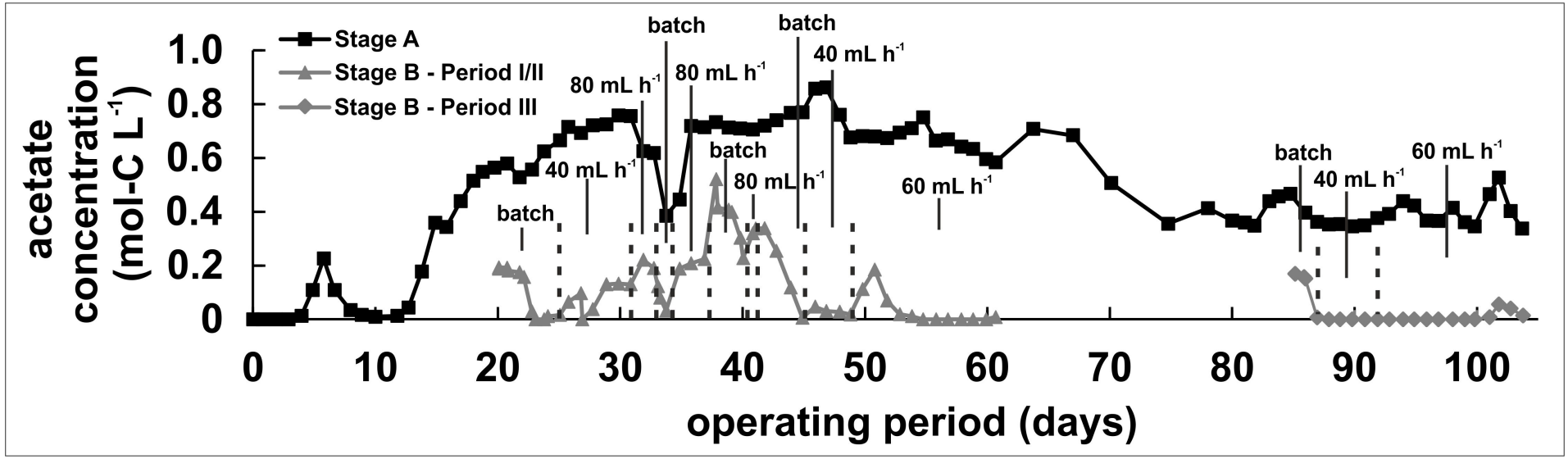

Figure S3. Acetate concentrations for Stage A (*C. ljungdahlii*) and Stage B (*S. cerevisiae*) during the continuous operating period of 104 days. Data is given in mol-C L^{-1}. For Stage B the flow rates of different operating periods are given.

Supplementary Tables

Table S1. Protein measurements for stage B (*S. cerevisiae*). Data for three technical replicates is given.

Operating period (days)	Dilution factor[a]	Concentration[b] (µg mL^{-1})			Biomass concentration (g CDW L^{-1})	Specific concentration (µg mg CDW^{-1})					Dairy One[c] (µg mg CDW^{-1})
		1	2	3		1	2	3	average	SD[d]	
Period I											
26	1	1164	1086	1154	2.3	255	238	253	248	9.32	
27	1	1186	1220	1211	2.2	274	282	279	278	4.10	
28	1	1080	1017	939	2.2	247	232	215	231	16.1	
29	1	1070	1055	1055	1.8	290	286	286	288	2.45	
30	1	730	652	816	1.8	200	179	224	201	22.5	
31	1	930	948	725	1.8	265	270	206	247	35.3	
32	1	707	681	525	0.9	374	360	278	337	52.0	
33	1	834	798	765	1.0	413	395	378	395	17.2	
34	1	995	953	805	2.0	247	237	200	228	24.7	
35	1	740	821	797	1.0	387	429	417	411	21.6	
36	1	904	865	813	1.1	420	402	378	400	21.2	
37	1	745	429	756	1.0	361	208	366	312	90.1	
38	1	507	403	556	0.6	438	348	481	422	67.6	
39	1	250	276	268	0.2	536	592	575	568	28.6	
41	1	445	377	391	0.4	579	491	509	526	46.4	
Period II											
42	1	259	212	202	0.2	557	456	434	482	65.7	
43	1	387	317	128	0.3	730	597	241	523	253	
44	2	1516	1462	1658	1.9	398	383	435	405	26.5	

45	2	1476	1853	1610	2.5	301	378	328	336	39.0	
46	2	1840	1995	2022	2.4	386	419	424	410	20.6	
47	2	1920	1698	2183	2.6	364	321	413	366	46.0	
48	2	2156	2197	1873	2.6	420	428	365	404	34.4	421
49	2	1725	2244	2210	2.7	324	421	415	387	54.5	
50	1	1428	1337	1434	2.3	315	295	317	309	11.9	
51	1	1356	1350	1350	1.7	393	392	392	392	1.01	
52	1	1901	2097	2007	2.4	388	429	410	409	20.0	544
53	2	2675	2789	2572	3.0	443	462	426	444	18.0	534
54	2	2699	2765	2771	3.3	406	416	417	413	6.04	547
55	2	2795	2916	2922	3.4	409	426	427	421	10.4	541
56	2	2576	2908	1730	3.2	399	451	268	373	94.2	
57	2	2410	2747	2041	3.1	392	447	332	390	57.5	
58	2	2456	2623	2472	3.2	378	404	381	388	14.1	547
59	2	2353	2612	2825	3.0	392	436	471	433	39.5	544
60	2	2711	2675	2654	2.9	474	468	464	469	5.06	554
61	2	2420	2223	2420	2.7	442	406	442	430	20.8	568
Period III											
90	1	1072	1182	1192	1.2	462	509	514	495	28.5	
91	1	1109	1096	1119	1.1	487	481	491	486	5.14	571
92	1	1091	1052	1166	1.1	479	462	512	484	25.5	
93	1	1259	1351	1341	1.8	359	385	382	375	14.5	
94	1	1194	1447	1387	1.4	430	521	500	484	47.6	
95	1	1408	1284	1346	2.0	350	319	334	334	15.4	534
96	1	1369	1423	1305	1.7	393	408	374	392	17.0	543
97	1	1274	1478	1354	1.7	384	446	408	413	30.9	557
98	1	1313	1380	1390	1.6	415	436	439	430	13.3	559
99	1	1315	1364	1367	1.7	377	391	392	387	8.33	
100	1	1256	1264	1279	1.7	367	369	374	370	3.45	

101	1	1508	1470	1449	1.8	415	404	398	406	8.27	597
102	1	1351	1354	1323	1.4	480	480	470	477	6.09	
103	1	1635	1735	1604	1.5	535	568	525	543	22.4	596
104	1	1741	1732	1782	1.8	473	471	484	476	7.21	

[a] the necessary dilution was determined in a pre-measurement. The triplicate measurement was conducted with no dilution (1) or with a 1:2 dilution (2) to stay within measurement range of our standard curve; [b] 2 mL samples were collected from the bioreactor, pelleted, and resuspended/stored in 1 mL 1 M NaOH. The concentration given here is referred to 1 mL NaOH; [c] biomass from stage B was collected over a period of one day, pelleted, dried, and analyzed by Dairy One Co-op for crude protein content. When the amount of dried biomass was not enough to conduct a separate crude protein analysis, samples were combined, and the measurement given is an average of the combined samples; [d] SD, standard deviation.

Table S2. Data used for economic calculations, including calculations, explanation of assumptions, and references for an existing Quorn™ process facility of 310,000 L operated by Marlow Foods Ltd.

Variable used in equations	Parameter	Value	Calculation (Variables in bold)	Assumptions	Reference
General considerations for 2014 Quorn™ process					
a	Quorn™ II Volume	155 m^3			[1,2]
b	Quorn™ III Volume	155 m^3			[1,2]
c	Quorn™ total Volume 2014	310 m^3	**a** + **b**		[1,2]
d	Vol. Prod. Rate DW protein	1.25 $g \cdot L^{-1} \cdot h^{-1}$		We assume an average production rate given from the cited reference	[1]
e	Vol. Prod. Rate DW protein	1.25 $kg \cdot m^{-3} \cdot h^{-1}$	**d**·(1kg/1000g) ·(1000L/1m^3)		
f	Loss in protein to reduce RNA content	0.3			[1,2]
g	Plant run-time efficiency	0.9		The process is operated for 6 weeks in continuous mode and then restarted with some down-time (~8 runs per year with each ~4 days down-time)	[1]
h	Kilograms protein per kilogram biomass	0.44 $kg \cdot kg^{-1}$			[1]
i	Total DW protein produced per year	3055050 $kg \cdot yr^{-1}$	**c**·**e**·**g**·(8760h/1yr)		
j	Total DW protein produced per year after RNA reduction	2138535 $kg \cdot yr^{-1}$	**i**·(1-**f**)		
k	Quorn™ Supermarket price per kg protein	150 $\$ \cdot kg^{-1}$		Sourced in German supermarket (www.rewe.de) in May 2019, converted with daily euro-to-dollar conversion rate in 2019	
l	Total revenue calc. from supermarket price in 2019	320,780,250 $	**j**·**k**	**l** > **n**	
m	Total revenue reported in 2014 (£)	150,300,000 £			[3]
n	Total revenue reported in 2014 ($)	247,637,694 $	**m**·(1.648$/£)	Average conversion rate £ to $ in 2014	[4]
o	Value ratio increase supermarket	30%	(**l**-**n**)/**n**		
p	Dietary requirement of protein per	0.056 $kg \cdot d^{-1}$		Average person with 70 kg body	[5]

	person			weight	
q	How many persons can be fed per day?	105,000 d^{-1}	**j**·(1yr/365d)/**p**		
r	One person spends how much daily?	8.40 $\$·d^{-1}$	**k**·**p**		
s	How many persons can be fed per day after 10x scale-up?	1,050,000 d^{-1}	**q**·10		
t	How many 10x scaled-up plants are required to feed 10 billion people?	9558	$10·10^9$/**s**	Assuming 10 billion people by 2050	
General considerations for 2010 Quorn™ process					
u	Total revenue reported in 2010 (£)	128,800,000 £			[6]
v	Profit reported in 2010 (£)	16,100,000 £			[6]
w	Employees reported in 2010	600			[6]
TOT1	Profit ratio	12.50%	**v**/**u**		
General considerations for 1997 Quorn™ process					
x	Total revenue reported in 1997 (£)	74,000,000 £			[6]
y	Reported investment for fermenters in 1997	75,000,000 £			[6]
	Ratio of revenue to investment	99%	(**x**/**y**)·100		
Calculations on Quorn™ process in 2014 – comparing implemented Quorn™ process and hypothetical two-stage bioprocess					
z	Value of £ in 2014 (162.56 £) *vs.* 1997 (100 £)	1.6256	162.56£/100£		[7]
A	Investment cost of 1997 fermenter in 2014 (£)	121,920,000 £	**y**·**z**		
B	Investment cost of 1997 fermenter in 2014 ($)	200,878,162 $	**A**·(1.648$/£)	Average conversion rate £ to $ in 2014	[4]
C	Investment cost for the two-stage bioprocess	401,756,324 $	**B**·2	Double investment cost for similar-sized second fermenter	
D	Weighted average cost of capital for UK food/beverage industry per year	8%			[8]
E	Capital cost per year for Quorn™ process	16,070,253 $	**B**·**D**		
F	Capital cost per year for two-stage bioprocess	32,140,506 $	**C**·**D**		
TOT2	Capital cost ratio *vs.* revenue (Quorn™ process)	6.49%	(**E**/**n**)·100		

	Capital cost ratio *vs.* revenue (two-stage bioprocess)	12.98%	(**F**/**n**)·100		
G	Average cost per employee per year	90,949 $\$\cdot yr^{-1}$		Based on 27,600 £ average salary in UK in 2015, converted with average conversion rate (**B**); doubled to include additional cost for employer (insurance, health, pension)	[9]
H	Labor cost per year (Quorn™ process)	54,569,264 $\$\cdot yr^{-1}$	**w**·**G**	Number of employees similar as in 2010	
I	Labor cost per year (two-stage bioprocess)	57,297,728 $\$\cdot yr^{-1}$	**H**+(**H**·5%)	5% more employees (30) for second fermenter	
TOT3	Labor cost ratio *vs.* revenue (Quorn™ process)	22.04%	(**H**/**n**)·100		
	Labor cost ratio *vs.* revenue (two-stage bioprocess)	23.14%	(**I**/**n**)·100		
J	DW protein yield per kg of glucose	0.136 $kg\cdot kg^{-1}$		This value includes that for DW protein production carbon also is "lost" as other biomass components and CO_2	[6]
K	Glucose price	0.37 $\$\cdot kg^{-1}$			[10]
L	Glucose cost per year in Quorn™ process	8,311,533 $	**i**/**J**·**K**		
TOT4	Glucose cost ratio *vs.* revenue (Quorn™ process)	3.36%	(**L**/**n**)·100		
M	Kilogram Ammonia required per DW protein	0.33 $kg\cdot kg^{-1}$	0.27/(14g-N/mol) ·(17g-NH_3/mol)	Based on average nitrogen content of dry biomass (0.12 kg/kg) and a protein content of 44% (kg/kg). This gives 0.27 kg-N/kg-DW protein	[11]
N	Ammonia price	0.53 $\$\cdot kg^{-1}$			[12]
O	Ammonia cost per year in Quorn™ process	530,859 $	**i**/**M**·**N**		
TOT5	Ammonia cost ratio *vs.* revenue (Quorn™ process and two-stage bioprocess)	0.21%	(**O**/**n**)·100		
P	Biotin required per DW protein	$1.36\cdot10^{-6}$ $kg\cdot kg^{-1}$			[1]

Q	Biotin price	2000 $\$\cdot kg^{-1}$			[13]
R	Biotin cost per year in Quorn™ process	8,310 $	**i**/**P**·**Q**		
TOT6	Biotin cost ratio *vs.* revenue (Quorn™ process and two-stage bioprocess)	0.00%	(**R**/**n**)·100		
S	Magnesium required per DW protein	$4.20\cdot10^{-3}$ $kg\cdot kg^{-1}$			[1]
T	Magnesium price	8.55 $\$\cdot kg^{-1}$			[14]
U	Magnesium cost per year in Quorn™ process	109,707 $	**i**/**S**·**T**		
TOT7	Magnesium cost ratio *vs.* revenue (Quorn process and two-stage bioprocess)	0.04%	(**U**/**n**)·100		
V	Dilution rate in Quorn™ process per hour	0.19 h^{-1}			[2,6]
W	Exchanges of reactor volume per day (dilution rate per day)	4.56 d^{-1}	**V**·(24h/1d)		
X	Water usage per year without recycling	464,368 $m^3\cdot yr^{-1}$	**W**·**c**·**g**·(365d/1yr)		
Y	Recycling rate	50%		Similar to corn-to-ethanol industry	[15]
Z	Water usage per year with recycling	232,184 $m^3\cdot yr^{-1}$	**X**·**Y**		
a1	Water cost	2.01 $\$\cdot m^{-3}$		Location of Marlow foods (https://www.google.com/maps/search/marlow+foods/@54.522888,-1.497877,10z)	[16]
b1	Water cost per year	466,628 $\$\cdot yr^{-1}$	**Z**·**a1**		
TOT8	Water cost ratio *vs.* revenue (Quorn™ process)	0.19%	(**b1**/**n**)·100		
c1	Water to wastewater	50%			
d1	Wastewater cost	1.74 $\$\cdot m^{-3}$		Location of Marlow foods (https://www.google.com/maps/search/marlow+foods/@54.522888,-1.497877,10z)	[16]
e1	Wastewater cost per year	201,670 $\$\cdot yr^{-1}$	**Z**·**c1**·**d1**		
TOT9	Wastewater cost ratio *vs.* revenue (Quorn™ process)	0.08%	(**e1**/**n**)·100		
f1	Glucose used per year	22,463,603 $kg\cdot yr^{-1}$	**i**/**J**		

g1	Millimoles glucose per hour	14,233,987 $mmol \cdot h^{-1}$	**f1**·(1yr/8760h)/(180.156g·mol^{-1})·(1000g/kg)·(1000mmol/mol)		
h1	Millimoles oxygen per Liter per hour needed for aeration without electrolyzer	275.49 $mmol \cdot (L \cdot h)^{-1}$	**g1**·6/**c**·(1m³/1000L)	Stoichiometric need of 6 moles oxygen per 1 mole glucose	
i1	Kilograms oxygen per year	23,940,428 $kg \cdot yr^{-1}$	**f1**·(1000mmol/mol) / (180.156g·mol^{-1}) ·6·(32g·mol^{-1}) ·(1kg/1000g)		
j1	Power to deliver oxygen to reactor per kg	2.75 $kWh \cdot kg^{-1}$		Extrapolated from data given Figure 3 in reference	[17]
k1	Power for aeration of reactor per year	$6.58 \cdot 10^{7}$ $kWh \cdot yr^{-1}$	**i1**·**j1**		
	Amount of overall electric power in process used for aeration	50%		Similar to wastewater industry	[18]
l1	Power for other pumping, etc.	$6.58 \cdot 10^{7}$ $kWh \cdot yr^{-1}$		Same as for aeration, due to assumption that this is 50% of overall electric power needs	
m1	Whole sale kWh cost in UK in 2014	0.07 $·kWh			[19]
n1	Electricity cost per year for Quorn™ process	9,330,830 $\$ \cdot yr^{-1}$	**(k1+l1)·m1**		
o1	Millimoles oxygen per Liter per hour with oxygen from electrolyzer present	142.39 $mmol \cdot (L \cdot h)^{-1}$	**h1**-(**C1**/2)·(1000mmol/mol)/**c**·(1000L/m³)	Offset by pressurized oxygen from electrolyzer; per two moles of H_2 one mole of O_2 is produced; the stoichiometric need of O_2 for the combustion is the same for glucose and acetate because carbon has the same oxidation number	
p1	Power to deliver oxygen to reactor per kg with electrolyzer	2.3 $kWh \cdot kg^{-1}$		From data given Figure 3 in reference	[17]
q1	Power for aeration of reactor per year with electrolyzer	$5.51 \cdot 10^{7}$ $kWh \cdot yr^{-1}$	**i1**·**p1**		
r1	Electricity cost per year with electrolyzer	8,567,398 $\$ \cdot yr^{-1}$	(**l1**+**q1**)·**m1**		
TOT10	Electricity cost ratio *vs.* revenue (Quorn™ process)	3.77%	(**n1**/**n**)·100		
	Electricity cost ratio *vs.* revenue (two-	3.46%	(**r1**/**n**)·100	Offset by oxygen from electrolyzer	

	stage bioprocess				
s1	Kilograms carbon per kilogram protein	1.16 $kg \cdot kg^{-1}$		Based on average carbon content of dry biomass (0.51 kg/kg) and a protein content of 44% (kg/kg)	[11]
t1	Carbon required for protein product produced per year	3,543,858 $kg \cdot yr^{-1}$	**i**·**s1**		
u1	Moles of carbon per year	295,321,500 $mol \cdot yr^{-1}$	**t1**/(12g/mol) ·(1000g/1kg)		
v1	Moles of acetate required for protein production	147,660,750 $mol \cdot yr^{-1}$	**u1**/2	2 moles carbon per acetate	
w1	Carbon yield in gas fermentation	81.7%		Observation from this manuscript	
x1	Moles of CO_2 required for gas fermentation per year	361,470,624 $mol \cdot yr^{-1}$	**v1**·2/**w1**	2 moles CO_2 per acetate	
y1	Kilogram of CO_2 required for gas fermentation per year	15,904,707 $kg \cdot yr^{-1}$	**x1**·(44g/mol) ·(1000g/1kg)		
z1	CO_2 cost	0.16 $\$ \cdot kg^{-1}$		Independent on how carbon taxes may influence the cost or cost benefit of utilizing CO_2, there will be a cost for CO_2 transportation infrastructure or shipping, therefore, we used a very conservative estimate here	[20,21]
A1	CO_2 cost per year	2,544,753 $\$ \cdot yr^{-1}$	**y1**·**z1**		
	CO_2 cost ratio *vs.* revenue (two-stage bioprocess)	1.03%	(**A1**/**n**)·100		
B1	Moles H_2 required for gas fermentation per year	722,941,249 $mol \cdot yr^{-1}$	**x1**·2	Stoichiometric ratio of 2 moles of H_2 per 1 mole of CO_2 in acetogenesis	[22]
C1	Moles H_2 required for gas fermentation per hour	82,528 $mol \cdot h^{-1}$	**B1**·(1yr/8760h)		
D1	Normal cubic meter H_2 required per hour	1,849 $Nm^3 \cdot h^{-1}$	**C1**·(22.4L/mol) ·(1Nm^3/1000L)	22.4 $L \cdot mol^{-1}$ of a gas under normal conditions	
E1	Normal cubic meter H_2 produced from 1 MWh electrolyzer per hour	200 $Nm^3 \cdot h^{-1}$			[23], Electrochaea LLC (personal communication
F1	Required electrolyzer capacity	9.24 MWh	**D1**/**E1**		
G1	Electrolyzer cost 1-10 MW per MW	1,000,000 $\$ \cdot MW^{-1}$			Electrochaea LLC (personal

					communication
H1	Capital cost for electrolyzer	9,243,084 $	**F1**·**G1**		
I1	Capital cost for electrolyzer per year (two-stage bioprocess)	739,447 $	**H1**·**D**		
	Capital cost electrolyzer ratio *vs.* revenue (two-stage bioprocess)	0.30%	(**I1**/**n**)·100		
J1	Electricity required to operate electrolyzer	9243 kWh	**F1** ·(1000kWh/1MWh)		
K1	Cost to operate electrolyzer per hour	655.00 $\$\cdot h^{-1}$	**J1**·**m1**		
L1	Cost to operate electrolyzer per year	5,737,817 $\$\cdot yr^{-1}$	**K1**·(8760h/1yr)		
	Operation cost electrolyzer ratio *vs.* revenue (two-stage bioprocess)	2.32%	(**L1**/**n**)·100		
Summary of comparing implemented Quorn™ process and hypothetical two-stage bioprocess					
M1	Percentage of accountable revenue for 2014 Quorn™ process	49%	SUM(**TOT1** to **TOT10**)		
	Percentage of revenue for fermentation for 2014 Quorn™ process	14%	SUM(**TOT2**, **TOT4** to **TOT10**)		
	Percentage of revenue for profit and labor for 2014 Quorn™ process	35%	SUM(**TOT1**, **TOT3**)		
N1	Residual revenue percentage for PR, research, packaging, overhead, eggs, etc. for 2014 Quorn™ process	51%	100%-**M1**		
	Total accountable revenue per year for 2014 Quorn™ process	120,553,765 $	**n**·**N1**		
	Total residual revenue per year for 2014 Quorn™ process	127,083,929 $	**n**·**M1**		
	Glucose cost credit per year in two-stage bioprocess	-8,311,533 $	-**L**		
	Aeration cost credit per year by oxygen from electrolyzer in two-stage bioprocess	-763,432 $	-(**n1**-**r1**)		
	Additional labor cost per year in two-stage bioprocess	2,728,463 $	**I**-**H**		
	Additional capital cost for fermenter per year in two-stage bioprocess	16,070,253 $	**F**-**E**		
	Additional CO_2 cost per year in two-	2,544,753 $	**A1**		

	stage bioprocess				
	Additional capital cost for electrolyzer per year in two-stage bioprocess	739,447 $	**I1**		
	Additional operating cost for electrolyzer per year in two-stage bioprocess	5,737,817 $	**L1**		
O1	Total cost (required revenue) for two-stage bioprocess	266,892,414 $	SUM(all above excluding percentage values)		
	Overall increase in cost to produce protein in two-stage bioprocess compared to Quorn™ process	7.57%	(**O1**-**n**)/**n**·100		

Supplementary Experimental Procedures

Media compositions:
The 2xP7 medium was composed of mineral salts (per Liter: 2.4 g NaCl, 3 g NH_4Cl, 0.3 g KCl, 0.3 g KH_2PO_4, 1.01 g $MgCl_2 \cdot 6H_20$, 0.16 g $CaCl_2 \cdot 2H_20$), trace elements (per Liter: 20 mg NTA, 1.32 mg $MnCl_2 \cdot 4H_20$, 8 mg $(NH_4)_2Fe(SO_4)_2$, 2 mg $CoCl_2 \cdot 6H_2O$, 3.56 mg $ZnSO_4 \cdot 7H_2O$, 0.2 mg $CuCl_2 \cdot 2H_2O$, 0.2 mg $NiCl_2 \cdot 6H_2O$, 0.2 mg $Na_2MoO_4 \cdot 2H_20$, 0.27 mg $Na_2SeO_3 \cdot 5H_2O$, 0.22 mg $Na_2WO_4 \cdot 2H_2O$), and vitamins (per Liter: 0.1 mg pyridoxine, 0.05 mg thiamine, 0.05 mg riboflavin, 0.05 mg D-pantothenic acid hemicalcium salt, 0.05 mg thioctic acid, 0.02 mg aminobenzoic acid, 0.02 mg nicotinic acid, 0.1 mg vitamin B_{12}, 0.05 mg biotin, 0.05 mg folic acid, 0.05 mg mesna). The vitamin B_{12} and folate was omitted from the vitamin solution during the last 6 days of Period II and III without noticeable changes in performance. Vitamins were added after autoclaving. Oxygen was completely removed from the medium by sparging with sterile nitrogen gas, and then adding cysteine (1 mM final concentration). Antifoam (Sigma 204) was added to the feed medium for Stage A according to the following scheme: below OD_{600} of 5, no antifoam; above OD_{600} of 5, addition of 10 µL L^{-1} antifoam; and above OD of 7, addition of 20 µL L^{-1} antifoam.

The YN medium for flask experiments was composed of mineral salts (per Liter: 1 g $(NH_4)_2SO_4$, 1 g KH_2PO_4, 1 g $MgSO_4 \cdot 7H_2O$, NaCl 0.1 g, 0.033 $CaCl_2 \cdot 2H_2O$), trace elements (per Liter: 5 mg $Fe(II)SO_4 \cdot 7H_2O$, 1.6 mg $ZnSO_4 \cdot 7H_2O$, 1.12 mg $Mn(II)SO_4 \cdot 1H_2O$, 0.28 mg Na_2MoO_4, 0.18 mg $Co(II)Cl_2$, 0.18 mg $Cu(II)Cl_2 \cdot 2H_2O$), and vitamins (per Liter: 20 mg myo-inositol, 4.4 mg thiamine, 1.2 mg pyridoxine, 1 mg D-pantothenic acid hemicalcium salt, 0.03 mg biotin). For the YN medium for the bioprocessing experiment, the $(NH_4)_2SO_4$ was replaced by 0.4 g NH_4Cl, and the $MgSO_4 \cdot 7H_2O$ was replaced by 0.82 g $MgCl_2 \cdot 6H_2O$. Instead, we added 0.6 g L-1 (2xYN) or 1.2 g L-1 (4xYN) cysteine as the sulfur source. The by-pass feed was supplemented with antifoam (Sigma 204) according to the following scheme: 10 µL L^{-1} in the 2xYN; 50 µL L^{-1} in the 4xYN.

Stage A set-up:
The pH was controlled with the internal pH controller of the Stage A unit and a pH probe (Mettler Toledo, Columbus, OH). The temperature was controlled at 35 °C with a recirculating water bath. Stirring was at 300 rpm with two vertical flat-blade impellers each with four blades. The gas was provided through a microsparger (0.5 microns, More Beer, Pittsburg, CA). The headplate was equipped with ports for pH control (5 M KOH), gas in and out, medium feed (40 mL h^{-1}), Na_2S feed, and cell recycling. The additional Na_2S feed was used during continuous operation to provide an additional source of sulfur and to keep Stage A anaerobic. The Na_2S feed pump was triggered with a timer (1 s on, 59 s off) resulting in a feed rate of 0.84 mL h^{-1} with an anaerobic 1 M Na_2S stock, which resulted in an apparent concentration of 2 mM Na_2S per day at the 40 mL h^{-1} medium feed rate. Cell recycling with full cell retention was performed through a Cellflo polyethersulfone hollow fiber module (cell guard) with 500-cm^2 membrane surface area and 0.2 µm pore size (C22E-011-01N, Spectrum Laboratories, Inc., Rancho Dominguez, CA) at 180 mL min^{-1}.

Stage B set-up:
The pH was controlled with an Alpha pH 800 controller (Eutech Instruments, Vernon Hills, IL) and a pH probe (Cole-Parmer, Vernon Hills, IL). The temperature was controlled at 30°C with the electric heating jacket from Stage B. Stirring was at 200 rpm with a magnetically coupled stirrer with two vertical flat-blade impellers each with six blades. Compressed air was provided through a microsparger (0.5 microns, More Beer, Pittsburg, CA) at an average flow-rate of 88.5 ± 19.6 mL min^{-1} (n=60). The headplate was equipped with ports for pH control (2 M HCl, 2 M NaOH), gas in and out, medium feed from Stage A, and a by-pass feed for additional YN feeding.

Analytical Procedures:
The OD_{600} for the flask experiments was measured in a plate reader (BioTek Synergy 2, Winooski, VT). The OD_{600} for the bioprocessing experiments was measured using a photometer (Milton Roy Spectronic 1201, Houston, TX) in a quartz glass cuvette (SCC 282, 1.000, Hellma, Müllheim, Germany). Cell dry weight (CDW) was determined by using correlation factors of 242 mg CDW OD^{-1} L^{-1} for *C. ljungdahlii*,[24] and 270 mg CDW OD^{-1} L^{-1} for *S. cerevisiae* as determined in this study. For calculation of carbon in biomass a factor of 0.53 (bacterial biomass composition: $C_5H_7O_2N$) was used

for *C. ljungdahlii*, and a factor of 0.51 (yeast biomass composition: $C_{100}H_{174}O_{45}N_{20}$) was used for *S. cerevisiae*,[11] considering the molecular weight for carbon of 12 g mol^{-1}. Ethanol and acetate concentrations were quantified by HPLC as described before.[25] Inlet and outlet gas flow rates were measured with water displacement flow-meters and bubble flow meters, respectively. Gas pressure was measured with a digital pressure gauge (Cole Parmer, Vernon Hills, IL). Gas samples were collected at the inlet and the outlet of the gas lines to analyze the relative partial pressures of the gases (H_2/CO_2 for Stage A; $N_2/O_2/CO_2$ for Stage B) by GC as described before.[24] The oxygen and nitrogen peaks in our GC system overlapped. Therefore, the oxygen partial pressure was determined as the difference of the signal for air (78.09% nitrogen plus 20.95% oxygen) and the gas outlet, assuming that nitrogen is inert and at a constant concentration. Oxygen partial pressure in the gas outlet of Stage B was additionally determined with an electrochemical oxygen sensor that correlated the oxygen partial pressure to a potential (S101 Diffusion Sensor, Qubit Systems, Kingston, ON, Canada). The electrochemical oxygen sensor was calibrated with 100% nitrogen gas, and with compressed air. The partial pressures, the volumetric flow rates, and the gas pressure were used to determine the gas consumption and production rates.

Lowry determination of proteins:
Frozen samples were thawed at room temperature and samples were boiled for 10 min in a heating block at 105 °C. A 2 mg mL^{-1} BSA standard was mixed with 10 M NaOH to give a final concentration of 1 M NaOH with 1.8 mg mL^{-1} BSA. The standard was also boiled at 105°C in a heating block. Samples were either used directly or diluted further with 1 M NaOH. Standard dilutions were prepared with 1 M NaOH. 10 µL of standards and samples were loaded into a 96-well plate (265301, Nunc MicroWell 96-Well Plates, Thermo Scientific, Waltham, MA). 100 parts of Na_2CO_3 in 0.1 M NaOH were mixed with 1 part of 1% $CuSO_4$ and 1 part of 2% NaH-tartrate (*e.g.*, 10 mL + 100 µL + 100 µL). 200 µL of this mixture was added to every well. The plate was read in a plate reader (BioTek Synergy 2, Winooski, VT) at 750 nm. Then 20 µL of a mixture of 1 part water and 1 part Folin-Ciocalteu's phenol reagent was added to each well, and the plate was incubated at room temperature in the dark for 15 min. The plate was read again at 750 nm, and the background (first reading) was subtracted from the measurement (second reading).

Supplementary Results and Discussion

S1. Operating Stage B with *S. cerevisiae* during Period I to test for nutrient limitations and a feasible dilution rate

Only a few studies have been published with acetate as a substrate for SCP production. Goldberg *et al.*[26] and Verduyn *et al.*[27] reported growth of *S. cerevisiae* (CBS 8066) and *Candida utilis* (CBS 621; fodder yeast), respectively, in a continuously fed bioreactor without a cell guard (chemostat), while feeding a 125-mM acetate solution as the sole carbon source. Nevertheless, we performed a flask experiment to observe growth for *S. cerevisiae* under our conditions with: **1)** a 150-mM acetate concentration in YNA medium (**Figure S2**); and **2)** an acetate-rich effluent that we had obtained from a gas fermenter with *C. ljungdahlii* (**Figure S2**), which had been producing acetate concentrations of 70 mM from hydrogen and carbon dioxide (H_2:CO_2, 80:20 vol-%) during an experiment similar to Richter *et al.*[28]

We found that for the YNA medium, 53% of the acetate remained, while this was 88% for the gas-fermentation effluent (**Figure S2C**). Since for both growth media the exponential phase of the growth curve had ended (**Figure S2A**), the presence of ample substrate indicated to us that one or more unknown nutrients had been limiting the growth of *S. cerevisiae*. Obviously, future studies would need to identify the growth-limiting nutrients to minimize unnecessary medium and nutrient usage.

Next, we used Period I to learn about bioprocessing of *S. cerevisiae* in Stage B with a filtered, acetate-rich Stage-A solution and a bypass of fresh medium without yeast extract. Unlike for the flask experiment (**Figure S2C**), the initial acetate concentration of 95 mM was completely diminished during the batch-mode operating condition for the first 5 days of the operating period for Stage B (**Figure S3**). Similarly, for all continuous-mode conditions the acetate was consumed efficiently with little residual acetate concentrations left in Stage B (**Figure S3**). Thus, with a bypass of fresh medium, growth nutrients were no longer limiting yeast growth.

An important bioprocessing parameter to determine is the dilution rate for maintaining high enough concentrations of cells. We had switched to continuous mode with an initial dilution rate of 0.04 h^{-1} on Day 25 of the operating period (**Figure 3**), which was similar to the dilution rate of Stage A. Without a cell guard, the performance at this dilution rate was stable, indicating that the growth rate could keep up with the dilution rate. To increase the yeast-protein production rate by providing more acetate and nutrients, the dilution rate was doubled to 0.08 h^{-1}. However, the growth rate was not sufficiently high enough, which ultimately led to a complete wash-out of the yeast cells during Period I (**Figure 3A**), although we had observed a growth rate of 0.09 h^{-1} in the flask experiment (**Figure S2B**). Verduyn *et al.*[27], on the other hand, was able to achieve a dilution rate of 0.1 h^{-1} for an aerobic *S. cerevisiae* (CBS 8066) culture with acetate as the sole carbon substrate and without yeast extract. In our case and with a different *S. cerevisiae* strain, we decided to use a lower dilution rate of 0.06 h^{-1} during the last 12 days of both Periods II and III for Stage B (**Figure 3**).

S2. Determining the protein mass-fraction of the yeast biomass

The average protein mass-fraction for *S. cerevisiae* was 40.2% ± 3.65% (n=16) and 53.3% ± 4.32% (n=9) during Period II and 43.7% ± 5.95% (n=15) and 56.5% ± 2.44% (n=7) during Period III for the Lowry method and Dumas method, respectively (g g^{-1} in **Table S1**). In another study, Verduyn *et al.*[27] measured a 47% protein mass-fraction (g g^{-1}) for *S. cerevisiae* CBS 8066 when grown aerobically with acetate and without yeast extract (by using a modified Biuret method). The protein mass-fraction during Period III was slightly higher than during Period II for *S. cerevisiae* by both methods in our study. This relatively high protein mass-fraction agrees with the relatively low crude-fat (lipid) mass-fraction during Period II (0.59% ± 0.22% [n=9]), and during Period III (1.07% ± 0.35% [n=7]), with ash values of 13.6% ± 1.14% (n=9) and 14.2% ± 0.76% (n=7) for Period II and Period III, respectively (all g g^{-1}). The remaining ~30% likely consisted of mainly fiber and nucleic acids, but this would need to be confirmed during future research.

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