Reviving the Weizmann process for commercial n-butanol production

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Developing a commercial process for the biological production of n-butanol is challenging as it needs to combine high titer, yield, and productivities. Here we engineer Clostridium acetobutylicum to stably and continuously produce n-butanol on a mineral media with glucose as sole carbon source. We further design a continuous process for fermentation of high concentration glucose syrup using in situ extraction of alcohols by distillation under low pressure and high cell density cultures to increase the titer, yield, and productivity of n-butanol production to the level of 550 g/L, 0.35 g/g, and 14 g/L/hr, respectively. This process provides a mean to produce n-butanol at performance levels comparable to that of corn wet milling ethanol plants using yeast as a biocatalyst. It may hold the potential to be scaled-up at pilot and industrial levels for the commercial production of n-butanol.
The Weizmann process was developed at the beginning of the 20th century for the biological conversion of corn into acetone and n-butanol by *Clostridium acetobutylicum*\(^1\)–\(^3\). It was the second largest fermentation process (after ethanol) of enormous industrial, social and historical importance\(^1\)–\(^3\). Beyond its use during the First World War to produce acetone for smokeless gunpowder (cordite) manufacturing, it became a process used worldwide to produce these two industrial solvents from a variety of renewable substrates\(^3\)–\(^5\). Its demise in the early 1960s was the result of the superior petrochemical-process economics due to drastic increase of the substrate costs\(^1\), the low yield, titer, and productivity of the fermentation\(^6\) (resulting in high operating and capital expenses), and the inability to use continuous process technologies (as in the case of petrochemical processes) due to the loss of *C. acetobutylicum*'s capacity to produce solvents because of degeneration\(^7\),\(^8\).

Here we first use an advanced metabolic engineering approach to engineer *C. acetobutylicum* to stably and continuously produce n-butanol from glucose\(^9\). We further design a continuous process for the fermentation of high concentration glucose syrup using (i) in situ extraction of alcohols by distillation under low pressure, and (ii) high cell density cultures to increase both the titer, yield, and productivity of n-butanol production.

**Results**

**Engineering *C. acetobutylicum* for the production of n-butanol.**

This step requires to first eliminate the production of undesired by-products namely butyrate, acetone, and lactate (Fig. 1a). In the CAB1057 strain, we have then deleted *ptb* and *buk* that code for the phosphotransbutyrylase and the butyrate kinase involved in the last two steps of butyrate formation\(^10\)–\(^12\), *ctfAB* that code for the acetoacetyl-CoA-acetate CoA-transferase involved in the first specific step of acetone formation\(^13\) and *ldhA* that codes for the lactate dehydrogenase involved in the last step of L-lactate formation\(^13\). When CAB1057 was evaluated in chemostat culture and subjected to a metabolic flux analysis (Supplementary Fig. 1),

![Fig. 1 Metabolic engineering of *Clostridium acetobutylicum* for n-butanol production.](http://www.nature.com/naturecommunications)

**a** Elimination of by-products formation (strain CAB1057) by gene deletion. *ldhA* lactate dehydrogenase, *ctfAB* acetoacetyl-CoA-acetate CoA-transferase, *adc* acetoacetate decarboxylase, *ptb* phosphotransbutyrylase, *buk* butyrate kinase, *adhE2* bifunctional NAD\(^+\)-dependent aldehyde and alcohol dehydrogenase.

**b** Optimization of acetyl-CoA conversion to n-butanol (Strain CAB1060) using a chimeric pathway derived from three different microorganisms. Blue *C. acetobutylicum*; black *E. coli*, red *C. kluyveri*; Green boxes are Rex binding sites. *atoB* acetoacetyl-CoA thiolase/synthase, *hbd1* NAD\(^+\)-dependent 3-hydroxybutyryl-CoA dehydrogenase, *ctr* crotonase, *bcd-etfAB* butyryl-CoA dehydrogenase.
the expected phenotype was obtained for the deleted pathways but the n-butanol yield (Fig. 2a) and fluxes (Supplementary Fig. 1) were not improved as this mutant produces more acetate and ethanol than the wild-type strain. The redox-sensing transcriptional repressor (Rex) has been found to modulate its DNA-binding activity in response to the NADH/NAD+ ratio and to repress the expression of several operons including thlA (that codes for a thiolase), crtbcd-efAB-hbd, (that code, respectively, for a crotonase, a butyryl-CoA dehydrogenase, and a 3-hydroxybutyryl-CoA dehydrogenase) and adhE2 (that codes for a bifunctional aldehyde-alcohol14,15 (Fig. 1b)). As thlA and crtbcd-efAB-hbd code for the enzymes converting acetyl-CoA to butyryl-CoA and acetyl-CoA to n-butanol and ethanol, respectively, the rexA gene, encoding Rex, was deleted in CAB1057 (to yield CAB1058) with the objective of improving the C4/C2 ratio and the flux of alcohol formation. The yield and flux of alcohol formation by CAB1058 were significantly improved, associated with a four-fold decrease of the hydrogen yield and fluxes as the electrons from reduced ferredoxin were mainly used for n-butanol yield remained almost unchanged and, consequently, the C4/C2 ratio was not improved (Fig. 2b). When the thiolase (catalyzed by ThlA) and 3-hydroxybutyryl-CoA dehydrogenase (catalyzed by Hbd) activities were measured in CAB1057 and CAB1058, ThlA remained unchanged while Hbd was 1.5 fold higher, indicating that the rexA deletion has the expected effect on the expression of the crtbcd-efAB-hbd operon coding for the enzymes that convert acetoacetyl-CoA to butyryl-CoA but not on thlA. ThlA is one of the most abundant intracellular protein16 representing more than 5% of the cytosolic proteins. Furthermore, the condensation reaction by ThlA was shown to be very sensitive to CoA-SH, with micro molar levels totally inhibiting the enzyme17 and it was also found to be regulated by a redox-switch through a reversible disulfide bond that is formed between two catalytic cysteine residues (Cys88 and Cys378)18. As it might be difficult to further overexpress ThlA and as its biochemical properties might also contribute to the low C4/C2 ratio in CAB1058 (Fig. 2b), ThlA was replaced by AtoB, a thiolase from E. coli that has a higher catalytic efficiency, is less sensitive to CoA-SH17,19,20 and not subject to a redox-switch. For this purpose, a synthetic atoB gene was designed, codon harmonized21,22 for C. acetobutylicum, and used to replace in frame thlA in CAB1058 (to yield CAB1059). In CAB1059, the butyral to ethanol ratio (mol/mol) and the butanol flux (in % of the glucose flux) only increased from 0.59 to 0.84 (Fig. 2b) and from 45 to 55.1, respectively, while the thiolase activity increased 1.7 fold (Supplementary Fig. 2) suggesting that the C4/C2 ratio is not controlled by the thiolase level or by the levels of the other enzymes of the pathway and might be thermodynamically controlled. The condensation of two acetyl-CoA to acetoacetyl-CoA is catalyzed by the thiolase is thermodynamically unfavorable23. Therefore, it is important that acetoacetyl-CoA is efficiently reduced by Hbd (Fig. 1b) to pull the reaction23. Hbd is an NADH-dependent enzyme24 and, as it likely works near the thermodynamic...
Table 1 Representative studies of high level butanol production in *C. acetobutylicum* and *C. beijerinckii*

| Strain               | Type         | Substrate/Medium                     | Fermentation Type | Butanol Yield (g/g) | Butanol Titer (g/L) | Butanol Productivity (g/L/hr) | Reference |
|----------------------|--------------|--------------------------------------|-------------------|--------------------|---------------------|-----------------------------|-----------|
| *C. acetobutylicum* ATCC824 | Wild type    | Glucose/synthetic medium             | Batch             | 0.18               | 10                  | 0.25                        | Ref.38    |
| *C. acetobutylicum* ATCC824 | Wild type    | Glucose/synthetic medium + 1 mM MV   | Batch             | 0.27               | 13.5                | 0.22                        | Ref.38    |
| *C. acetobutylicum* ATCC824 | Wild type    | Glucose/synthetic medium             | Continuous chemostat | 0.185          | 8.8                | 1.1                         | Ref.33    |
| *C. acetobutylicum* X Y16 | Wild type    | Glucose/complex medium               | Continuous immobi lized distillation | 0.31           | 5.7                | 11.3                        | Ref.29    |
| *C. acetobutylicum* ATCC824 | Wild type    | Glucose/complex medium               | Continuous extractive pervaporation | 0.23           | 132                | 0.74                        | Ref.40    |
| *C. acetobutylicum* ATCC824 (pPGROEL) | Engineered | Glucose/complex medium               | Batch             | 0.2               | 18.2                | 0.38                        | Ref.42    |
| *C. acetobutylicum* BEKW (pPlhAAD) | Engineered | Glucose/complex medium               | Batch             | 0.29               | 18.9                | 0.33                        | Ref.43    |
| *C. acetobutylicum* ATCC824 (pPykA + pykA) | Engineered | Glucose/complex medium               | Fed-batch         | 0.21               | 19.1                | 0.19                        | Ref.44    |
| *C. acetobutylicum* BKM19 | Engineered   | Glucose/synthetic medium             | Continuous cell recycle distillation | 0.17           | 11.9                | 10.7                        | Ref.45    |
| *C. acetobutylicum* CAB1060 | Engineered   | Glucose/complex medium               | Continuous extractive distillation | 0.35           | 550                 | 14                          | This study |
| *C. beijerinckii* P260 | Wild type    | Glucose/complex medium               | Batch             | 0.26               | 11.8                | 0.25                        | Ref.46    |
| *C. beijerinckii* P260 | Wild type    | Glucose/complex medium               | Batch vacuum fermentation | 0.20           | 35.9                | 0.27                        | Ref.46    |
| *C. beijerinckii* DSM 2152 | Wild type    | Glucose/complex medium               | Continuous extractive gas stripping | 0.19           | NA                  | 0.63                        | Ref.47    |

In *C. acetobutylicum*, the acetoacetyl-CoA/3-hydroxybutyryl-CoA ratio will be dependent on the NADH/NAD\(^+\) ratio. As it has been demonstrated in solventogenic *C. acetobutylicum* cells that the NADPH/NAD\(^+\) ratio is at least 70 times higher than the NADH/NAD\(^+\) ratio\(^25\), we anticipate that it would be advantageous to replace Hbd by a strictly NADPH-dependent enzyme to decrease the acetoacetyl-CoA/3-hydroxybutyryl-CoA ratio and potentially improve the butanol/ethanol ratio. Such an enzyme has been identified and characterized in *Clostridium klyveri*\(^26\). In strain CAB1060, we have replaced *hbd* by *hbd1* from *C. klyveri*. A dramatic improvement in the butanol/ethanol ratio (Fig. 2b) and the n-butanol fluxes (Supplementary Fig. 1) were observed, respectively, 6- and 1.6-fold increase. Enzyme assays on crude extract confirm that in the genetic modification of CAB1060, the NADH-dependent 3-hydroxybutyryl-CoA dehydrogenase activity was very low while the NADPH-dependent activity was high; the reverse was observed for CAB1059 (Supplementary Fig. 1). The stability of CAB1060 was evaluated in chemostat culture. We could maintain stable biomass concentrations (Fig. 3a) for more than 45 days and after 13 days of cultures only slight changes in the ethanol/acetate ratio between 1 and 0.8 (mol/mol) were observed (Fig. 3a and Supplementary Fig. 1).

Development of continuous process for n-butanol production. Having a stable strain that produces n-butanol at a high yield in a continuous culture is a prerequisite, but it is clearly not sufficient for a commercial process as both the titer and the productivities are too low. To improve those two parameters, several methods of in situ butanol recovery including gas stripping, vacuum fermentation, pervaporation, liquid–liquid extraction, perstraction, and adsorption, have been investigated\(^27\). However, high volumetric productivities or scaled-up industrial production are still hard to achieve. We then envisioned a continuous high cell density bioreactor with in situ extraction of the alcohols (n-butanol + ethanol) by distillation under low pressure (0.18–0.46 \(\times\) 10\(^4\) Pa, for a boiler temperature regulated between 35 and 37 °C) without using any microfiltration membrane (Fig. 4). The advantages of this method are (1) that the fermentor is at atmospheric pressure which allows the fermentation gas to be eliminated without pumping and limit the cost for low pressure distillation compared to vacuum fermentation and (2) high cell density can be obtained without the use of microfiltration membranes. Three runs of continuous extractive fermentation were performed (Supplementary Table 1) but only the run III will be presented in detail below. After an initial batch phase without distillation, this setup was run in a fed-batch mode by (i) starting vacuum distillation and (ii) exponentially feeding the reactor with a medium at a high concentration of glucose (~530 g/L) while maintaining full cell retention until reaching an OD 600 nm of 90–100 (Fig. 3b), which corresponds to ~28–30 g/L cell dry weight. As shown in Fig. 3b, the targeted cell density was reached in ~70 hours after vacuum distillation and feeding began, at which point a cell bleeding was implemented at a dilution rate of 0.05 h\(^{-1}\) to maintain a constant cell density and perform the fermentation in a continuous mode. The concentration of n-butanol in the fermentor was between 9 and 10.5 g/L (Supplementary Fig. 3) while the average concentration of n-butanol in the butanol-rich phase that was continuously collected was 550 g/L with concurrent average
ethanol and acetate titers of 60 and 3.3 g/L (Supplementary Fig. 3). Starting at hour 70 and for 170 hours afterwards (i.e., up to 240 hours of fermentation time), the volumetric productivities of butanol and ethanol remained essentially constant, along with the glucose volumetric consumption. For the three runs (Supplementary Table 1), the average volumetric productivities of n-butanol and ethanol were 14 and 1.5 g/L/hr, respectively, and the average glucose volumetric consumption rate was 39.4 g/L/hr. This resulted in an average n-butanol yield of 0.35 g/g (~84% of the theoretical maximum) and n-butanol + ethanol yield of 0.39 g/g. Such high values of n-butanol productivities, titer, and yield compare very well with the performances of the best corn wet milling continuous industrial ethanol processes that display ethanol titers of ~100 g/L, yield of 0.46 g/g (~90% of the theoretical maximum) and productivity of 10 g/L/hr. In the US the selling price of n-butanol is 2.3 times higher than ethanol ($1.2 per kg versus $0.5 per kg, ISIS data of September 2017) while the yield of alcohol formation of our process is only 1.25 lower than the ethanol process. If scaled-up at the industrial scale, the technology presented here should drastically decrease both the operational and capital expenditure costs of the historical Weizmann process and make the commercial production of n-butanol economical and more attractive than the production of ethanol.

Methods
Bacterial strains, plasmids, and primers. The bacterial strain and plasmids used in this study are listed in Supplementary Table 2 and Supplementary Table 3. The specific oligonucleotides used for PCR amplification were synthesized by Eurogentec (Supplementary Table 4).

General protocol for gene deletion. Gene disruption in specified chromosomal loci was carried out by homologous recombination with the modifications of patent application WO2008/04038730. Deletion cassettes consisting of the upstream homologous region-FRT-catP-FRT-downstream homologous region were cloned in the pSOS95-MLSr-upp plasmid. The pSOS95-MLSr-upp plasmids with the different deletion cassettes were used to transform the successive recipient C. acetobutylicum strains which were constructed from the MGC Δcac1502 Δupp platform strain. As a general procedure, thiamphenicol-resistant transformants were selected on CGM 50 g/L Glucose 0.1 M MES pH 6.1 (CGMMG) plates containing thiamphenicol (10 μg/mL) (Tm). One colony was cultured for 24 hours in liquid CGMMG with thiamphenicol (10 μg/mL) and 100 μL of undiluted culture was plated on CGMMG with clarithromycin (40 μg/mL) to select clones where 5-FU resistance is also associated with clarithromycin sensitivity. The genotype of the clones resistant to thiamphenicol and sensitive to clarithromycin was checked by PCR. The catP resistance cassette was removed by transforming the strain with plasmid pCLF1 expressing the flp1 gene encoding the Flp recombinase from S. cerevisiae. One colony of a clarithromycin resistant clone was cultured on

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Fig. 3 Continuous cultures of C. acetobutylicum CAB1060. a In phosphate-limited chemostat culture at pH 5, b in phosphate-limited high cell density culture at pH 5 with in situ extraction of the alcohols (n-butanol + ethanol) by distillation under low pressure. The arrow indicate the start of the cell bleeding. For both continuous cultures, the initial batch phase is not presented.
Figure 4 Schematic representation of the extractive continuous fermentation process. The fermenter, boiler, and decantor have working volumes of 0.5 L, 0.3 L, and 0.1 L, respectively. For more details see Methods

Construction of pEryUppAtoB plasmid and CAB1059 strain. A synthetic atob operon coding for the AtoB thiolase of E. coli was synthesized as follow. A synthetic atob gene was designed with both an optimized synthetic RBS (translation initiation rate of \(\text{thlA} \)) and a harmonized codon usage for \(\text{C. acetobutylicum}\). The synthetic gene was PCR amplified with the Phusion DNA polymerase with pSOSatob8s as a template and the AtoB-1 and AtoB-2 oligonucleotides as primers. This DNA fragment was directly cloned into the StuI linearized pEryUppAthIA for the construction of pEryUppAthIA by homologous recombination using the GeneArt® Seamless Cloning & Assembly kit to yield the pEryUppAtoB plasmid.

The pEryUppAtoB plasmid was used to transform by electroporation to CAB1058 strain. After selection on Petri plates for clones having inserted the pEryUppAtoB plasmid. CAB1058 was constructed from CAB1057 by applying the same protocol to the deletion of \(\text{catP}\) cassette to generate the marker-less strain CAB1060.

Continuous extractive high density cell recycle fermentation. A 10% inoculum of mid-exponential phase (\(\text{OD}_{600}\) 0.8–1.5) CAB1060 culture was used to inoculate a 1 L glass bioreactor with a 0.5 L working volume of anaerobic synthetic medium with 60 g/L glucose. Fermentation pH was controlled from dropping below 5.0 using 4 M NH4OH. Once the cultures reached an OD600 of 0.8, fed batch mode was initiated. Low pressure distillation was accomplished by sending the whole broth to a 3 L Pyrex boiler (0.3 L working volume) equipped to maintain a constant temperature (35–37 °C) and a constant low pressure (0.18-0.46 x 10^5 Pa) above the boiler. The distillation column has an internal diameter of 7.8 cm, a height of 60 cm and was filled with mesh column packing from Multikinet Ltd. The decanter was maintained at 2 °C, has a working volume of 0.1 L, and consists of a Pyrex tube with an internal diameter of 2.4 cm and a height of 25 cm. The initial residence time of the broth in the boiler was 0.5 hour. The fermenter was fed exponentially with a feed medium (see the composition below) containing high glucose concentration (530 g/L) by

prime 1-hbd1-sacII and primer 4 to obtain the fourth fragment of 1778 bp. After that, this fourth fragment was fused with the second fragment by primer 1-hbd1-sacII and primer 6-hbd1-sacII to produce the fifth fragment of 2692 bp. The colE1 origin on pSOS95-MLsr-upp was replaced by the repA origin of pSc101. The repA origin was amplified from pSc101 by two primers orirepA-XbaI-F and orirepA-5′R to obtain a PCR product of 2296 bp. The bacterial ampicillin resistant gene from pSOS95-upp was amplified with Amp3′-F and Amp-Scal-R to obtain a PCR product of 573 bp. The two resultant PCR fragment was then fused by PCR using orirepA-Xbal-F and Amp-Scal-R. This 2849 bp fragment was introduced to pSOS95-upp to replace repl fragment by digestion with XbaI and Scal restriction enzymes and ligation to obtain pSOS95-upp-orirepA. After that, the homologous fragment containing hbd1 CDS was introduced to this pSOS95-upp-orirepA by the SacI site to obtain pSOS95-hbd1-orirepA plasmid. The catP cassette (flanking by FRT sequences) was inserted into the StuI site carried by primer 4 and primer 5 in the homologous region to generate the final plasmid pSOS95-hbd1-catP-orirepA.

Chemostat culture of recombinant strains. The strains were evaluated in phosphate-limited continuous culture fed at 30 g/L glucose at a pH of 5.0. Metabolic flux analysis was performed using the genome scale model previously developed.
just keeping the volume of the fermentor constant. The distillate was condensed (2°C and de-mixed in a butanol-rich- and a water-rich phase; the water-rich phase was recycled to the distillation column while the butanol-rich phase was collected. Once cell concentration reached an OD_{600} of 90–100 (20–30 g/L cell dry weight), a cell bleeding was started at a dilution rate of 0.05 h^{-1} while still keeping volume of the fermentor constant by the addition of feed medium. The residence time of the broth in the boiler was decreased to 0.25 h.

The feeding medium has the following composition (per liter of tap water): glucose, 530 g; KH_{2}PO_{4}, 1.8 g; KCl, 5.7 g; MgSO_{4} ·7H_{2}O, 3.6 g; FeSO_{4} ·7H_{2}O, 0.5 g; NH_{4}Cl, 18 g; Biotin, 0.18 mg; and PAB, 0.14 g.

**Measurement of fermentation parameters.** Biomass concentration was determined by a DCW method that includes three steps: a centrifugation (16,000 g, 5 min, room temperature) of 1.5 mol broth in an Ependorf tube, two washes with Milli-Q water, and drying under vacuum at 80 °C. The concentrations of glucose, and of all the fermentation products were determined based on high-performance liquid chromatography (HPLC) using a HPX87H (Biorad) column, H2SO4 at 0.5 mM, as mobile phase and a refractometer to quantify the different compounds.

**Enzyme activity measurements.** Cell crude extract of *C. acetobutylicum* mutants was prepared in 100 mM Tri-HCl (pH 7.6), 5 mM dithiothreitol, and 2.5% glycerol. The cell suspension was sonicated in an ultrasonic disintegrator (VibraCell 71434; Bioflow, Ilkirk, France), at 0 °C in four cycles of 30 s with 2-min intervals between each cycle. Cell debris was removed by three centrifugations at 13,000×g for 5 min. The supernatant was loaded to PD10 column.

Thiolase activity was measured in the reverse thiololytic cleavage direction. The reaction containing 0.1 M Tris-HCl pH 8.8, 0.2 mM CoASH; 60 μM Acetoacet-CoA; 10 mM MgCl_{2} and 1 mM DTT and diluted crude extracts was incubated at 30 °C and followed the decrease of acetoacet-CoA at 303 nm. The enzyme activity was calculated by the difference in the slope values of the sample and control and using the molar extinction coefficient of 14,000 M^{-1} cm^{-1}.

HBD activity was measured in the physiological direction. The reaction containing 0.1 mM MOPS (pH 7.0) buffer, 1 mM DTT, 0.1 mM acetoacet-CoA, and 0.15 mM NADH, and crude extract at different dilution was incubated at 35 °C. The decreasing of NADH was followed spectrophotometrically at 345 nm. The enzyme activity was calculated by the difference in the slope values of the sample and control and using the molar extinction coefficient of 6220 M^{-1} cm^{-1}.

HD1 activity was measured in the physiological direction. Reaction components were 0.1 M potassium phosphate, 25 mM potassium citrate, 0.15 mM NADPH, 0.6 mM acetoacet-CoA, and crude extract. The assay was done at 35 °C and the decrease of NADPH was followed at 340 nm. The molar extinction coefficient of 6220 M^{-1} cm^{-1} was used to calculate the enzyme activity.

**Data availability**

All relevant data are included with the manuscript (as figure or supplementary information files). All data are available upon reasonable request from the corresponding author.

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**References**

1. Jones, D. T. & Woods, D. R. Acetone-butanol fermentation revisited. *Microbiol. Rev.* 50, 484–524 (1986).
2. Weizmann, C. UK Patent 4845 (1915).
3. Weizmann, C. US Patent 1,315,585 (1919).
4. Noda, B., Chiyanzu, I. & Marx, S. N. Butanol derived from biochemical and chemical routes: a review. *Biotechnol. Rep.* 8, 1–9 (2015).
5. Tracy, B. P., Jones, S. W., Fast, A. G., Indurthi, D. C. & Papoutsakis, E. T. Clostridia: the importance of their exceptional substrate and metabolite diversity for biofuel and biorefinery applications. *Curr. Opin. Biotechnol.* 23, 364–381 (2012).
6. Dodd, D. R. & Gross, R. A. Chemicals from Biomass. *Science* 318, 1250–1251 (2007).
7. Cormilô, E. & Soucaille, P. Solvent-forming genes in clostridia. *Nature* 380, 489–489 (1996).
8. Cormilô, E., Nair, R. V., Papoutsakis, E. T. & Soucaille, P. The genes for butanol and acetone formation in Clostridium acetobutylicum ATCC 824 reside on a large plasmid whose loss leads to degeneration of the strain. *J. Bacteriol.* 179, 5442–5447 (1997).
9. Anbarasan, P. et al. Integration of chemical catalysis with extractive fermentation to produce fuels. *Nature* 491, 235–239 (2012).
10. Walter, K. A., Nair, R. V., Cary, J. W., Bennett, G. N. & Papoutsakis, E. T. Sequence and arrangement of two genes of the butyrate-synthesis pathway of Clostridium acetobutylicum ATCC 824. *Gene* 134, 107–111 (1993).
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Author contributions
N.P.T.M and C.R. designed and performed experiments. I.M.S. and P.S. conceived this study, analyzed the data, discussed results, and wrote the manuscript.

Additional information
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Competing interests: Three patent applications have been filed related to this work. C.R. and P.S. hold shares in Metabolic Explorer, a company pursuing applications of part of these technologies. The remaining authors declare no competing interests.

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