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Model-driven approach for the production of butyrate from CO$_2$/H$_2$ by a novel co-culture of C. autoethanogenum and C. beijerinckii

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One-carbon (C1) compounds are promising feedstocks for the sustainable production of commodity chemicals. CO$_2$ is a particularly advantageous C1-feedstock since it is an unwanted industrial off-gas that can be converted into valuable products while reducing its atmospheric levels. Acetogens are microorganisms that can grow on CO$_2$/H$_2$ gas mixtures and syngas converting these substrates into ethanol and acetate. Co-cultivation of acetogens with other microbial species that can further process such products, can expand the variety of products to, for example, medium chain fatty acids (MCFA) and longer chain alcohols. Solventogens are microorganisms known to produce the variety of products to, for example, medium chain fatty acids (MCFA) and alcohols via longer chain alcohols. Solventogen in a consortium provides a potential platform to produce valuable chemicals from CO$_2$. In this study, metabolic modeling was implemented to design a new co-culture of an acetogen and a solventogen to produce butyrate from CO$_2$/H$_2$ mixtures. The model-driven approach suggested the ability of the studied solventogenic species to grow on lactate/glycerol with acetate as co-substrate. This ability was confirmed experimentally by cultivation of Clostridium beijerinckii on these substrates in batch serum bottles and subsequently in pH-controlled bioreactors. Community modeling also suggested that a novel microbial consortium consisting of the acetogen Clostridium autoethanogenum, and the solventogen C. beijerinckii would be feasible and stable. On the basis of this prediction, a co-culture was experimentally established. C. autoethanogenum grew on CO$_2$/H$_2$ producing acetate and traces of ethanol. Acetate was in turn, consumed by C. beijerinckii together with lactate, producing butyrate. These results show that community modeling of metabolism is a valuable tool to guide the design of microbial consortia for the tailored production of chemicals from renewable resources.

**Keywords**
microbial communities, Clostridium autoethanogenum, Clostridium beijerinckii, constraint-based metabolic modeling, CO$_2$, H$_2$, lactate, butyrate
1. Introduction

The energy crisis and the effects of climate change have emphasized the need to accelerate the transition toward a circular bio-based economy (Göttinger et al., 2020). Current circular approaches focus on the application of microbial conversion to convert low-value carbon feedstocks, such as biomass waste streams, into commodity chemicals (Casau et al., 2022). Recalcitrant lignocellulosic biomass can be pretreated and hydrolyzed into sugars (Loow et al., 2016) or gasified to produce synthesis gas (syngas), a one-carbon (C1) feedstock consisting of a mixture of CO, CO2 and H2 (Richardson et al., 2015). In addition, C1-rich industrial waste gases from steel and thermal power plants can be directly used as microbial feedstocks (Hwang et al., 2020). In this regard, CO2 is an advantageous one-carbon feedstock (C1) since it can be obtained from natural and industrial sources, and can be converted into valuable products reducing the release of contaminant gases to the environment.

Acetogens are strict anaerobes that can grow on CO2/H2 and syngas as their sole carbon source using the Wood-Ljungdahl metabolic pathway (Ragsdale and Pierce, 2008; Bertsch and Müller, 2015). Acetogenic fermentation of C1 gases leads mainly to the production of acetate and ethanol, as well as, 2,3-butanediol or lactate (Köpke et al., 2014; Valgepea et al., 2018), and the production of ethanol has been commercialized recently (Marcellin et al., 2016; Bae et al., 2022). Acetogens that grow autotrophically on these slightly soluble gases are energy limited and produce a constrained product spectrum and low product titers. This can be overcome by exploring alternative strategies, such as mixotrophic growth or co-cultivation (Lee et al., 2022).

Solventogenic Clostridia have been widely applied to ferment sugars into mixtures of the solvents acetone, butanol, and ethanol (ABE) (Tracy et al., 2012) or in some cases isopropanol, butanol, ethanol (IBE) (Yang et al., 2016). These fermentations consist of an acidogenic phase followed by a solventogenic phase. During acidogenesis, solventogens produce carboxylic acids (mainly acetate and butyrate), and CO2 and H2. Accumulation of carboxylic acids and the concomitant lowering of the pH trigger solventogenesis during which solventogens reduce the carboxylic acids into solvents (Liao et al., 2015).

Cross-feeding strategies have been used to establish synthetic microbial communities that produce a wider product range (Diender et al., 2016, 2019; Du et al., 2020; Moreira et al., 2021). Therefore, co-cultivation of an acetogen and a solventogen has the potential to overcome the drawbacks associated to acetogens by increasing the product spectrum. Charubin and Papoutsakis (2019) recently established a co-culture of the solventogen Clostridium acetobutylicum and the acetogen Clostridium ljungdahlii. In this setup, glucose was metabolized by C. acetobutylicum to butanol, ethanol, acetone, acetoin, CO2, and H2. Subsequently, CO2 and H2 were fixed, and acetone and acetoin were reduced to isopropanol and 2,3-butanediol, respectively, by C. ljungdahlii.

Acetate is one of the most abundant products in acetogens (Bengelsdorf et al., 2018), and while solventogenic strains cannot grow on acetate as sole carbon source, they can reassimilate acetate and convert it into carboxylic acids such as butyrate, or solvents when glucose is used as co-substrate (Monot et al., 1982; Kuit et al., 2012; Diao et al., 2021). Therefore, in a co-culture of acetogens/solventogens on CO2/H2, butyrate could be produced as main product. Butyrate is a valuable product as it is used in many commercial applications, as a solvent, cosmetic, food, animal feed, or as a precursor of pharmaceuticals (Dwidar et al., 2012; Brändle et al., 2016).

In this study, we followed a model-driven approach to find an alternative route for production of butyrate. We produced butyrate from CO2 using a co-culture of two strains, one acetogen producing acetate from CO2/H2, and one solventogenic strain that co-metabolized acetate with an alternative carbon source into butyrate. To select the solventogenic strain, we systematically assessed growth on several carbon sources using the genome-scale metabolic models (GEMs) of C. acetobutylicum and Clostridium beijerinckii. The most promising carbon sources were experimentally tested and validated. On the basis thereof, we constructed a community model of Clostridium autoethanogenum and C. beijerinckii, and qualitatively assessed the fermentation of CO2/H2 and the new carbon source through scenario simulations. Model predictions guided the experimental work and led to the successful establishment of this new co-culture.

2. Materials and methods

2.1. GEM availability and curation

The GEM of C. autoethanogenum DSM 10061, iCLAU786 (Valgepea et al., 2017) was downloaded in sbml and table format and used without modification.

The GEM of C. acetobutylicum ATCC 824, iCac802 (Dash et al., 2014) was downloaded in sbml and table format, and modified as follows: Two reactions were defined as reversible: ATP:3-phospho-D-glycerate 1-phosphotransferase (with model identifier R0239), and Hydrogenase (R1563). Formate dehydrogenase (R1562) was removed since it was not found in the genome of C. acetobutylicum. Three new reactions were added in the model: Pyruvate transport (pyrT),
2.2. In-silico carbon source screening in *C. acetobutylicum* and *C. beijerinckii*

We systematically assessed growth capabilities on the updated versions of the GEMs of *C. acetobutylicum* and *C. beijerinckii* on a wide range of carbon sources. Model simulations were done using COBRApy, version 0.15.4 (Ebrahim et al., 2013), and Python 3.9. Growth capabilities were assessed using Flux Balance Analysis (FBA). The biomass synthesis reaction (termed “Biomass” or “biomass” in the respective *C. acetobutylicum* and *C. beijerinckii* models), was defined as the objective function for maximization. Growth was considered when the growth rate was higher than 0.0001 h⁻¹. For each assessed carbon source, the maximum uptake (corresponding to minus the lower bound of the associated exchange reaction denoted "EX_xx") was constrained to 20 mmol gDW⁻¹ h⁻¹, and the minimum uptake (corresponding to minus the upper bound of the associated exchange reaction) was constrained to 0.1 mmol gDW⁻¹ h⁻¹. In addition, uptake of small metabolites and ions was allowed by setting the lower bound of the corresponding exchange reaction to −1,000 as described in the corresponding script in the git repository.

2.3. Co-culture GEM reconstruction

A compartmentalized co-culture model of *C. autoethanogenum* and *C. beijerinckii* was obtained by combining single species models iCLAU786 (Valgepea et al., 2017) and iCM943 (Milne et al., 2011), following a previous approach (Benito-Vaquerizo et al., 2020). In this approach, each species is considered a single compartment. The compartment associated with *C. autoethanogenum* was defined as “cytosol_ca” and the compartment associated with *C. beijerinckii* was defined as “cytosol.” Intracellular metabolites were assigned to their respective compartment and the flag “_ca” was added to the identifier of metabolites belonging to “cytosol_ca” to distinguish them from the *C. beijerinckii* metabolites. In addition, the combined model included an extracellular compartment, defined as “extracellular,” common to both species. Metabolites in this compartment are either secreted, metabolized, or exchanged by both species, and separated from metabolites present in the cellular compartments by adding the “_e” flag to the identifier. All extracellular metabolites follow the same naming system (namespace) for both species. Therefore, the same namespace was applied to metabolites secreted by both species. All metabolites present in both intracellular compartments and the extracellular compartment can be exchanged between species if favored by the directionality. Interchanged metabolites are assumed to be first transported into the extracellular compartment, before taken up by the other species using the corresponding exchange reaction. The co-culture GEM contains one biomass synthesis reaction per species, termed “biomass_auto” and “biomass_beije” for *C. autoethanogenum* and *C. beijerinckii*, respectively. Additionally, the model contains a community biomass synthesis reaction (“Community_biomass”), which incorporates the biomass of *C. autoethanogenum* and *C. beijerinckii* in the form of metabolite “biomass_ca” and metabolite “biomass,” respectively. The combined model incorporates a transport reaction of butyrate (“BUTex_au”) from the extracellular compartment to the intracellular compartment of *C. autoethanogenum*, and the reaction to produce butyraldehyde from butyrate ("butobut") in *C. autoethanogenum*. In addition, we incorporated the transport reaction of acetone (“ACETONE_ca”) from the extracellular compartment to the intracellular compartment of *C. autoethanogenum*; an alcohol dehydrogenase to convert acetone into isopropanol (“ISOBIO”); a transport reaction of isopropanol from the intracellular compartment of *C. autoethanogenum* to the extracellular compartment (“ISOPRO_ca”), and an exchange reaction of isopropanol (“EX_IPRO_e”).

The final three-compartment co-culture model was translated into SBML level 3 version 1 (see git repository).

2.4. Co-culture modeling framework

Co-culture model simulations were carried out using a previously described modeling framework (Benito-Vaquerizo et al., 2020), similar to SteadyCom (Chan et al., 2017), and based
on Community FBA (cFBA) (Khandelwal et al., 2013). Specific fluxes (mmol gDW⁻¹ h⁻¹) were substituted by environmental fluxes (mmol l⁻¹ h⁻¹), and thus, the biomass synthesis reaction of each species was changed accordingly accounting for the growth rate and biomass of each species (gDW l⁻¹ h⁻¹). The relative contribution of each species to the community biomass was calculated from the total biomass of the community and the species ratio.

2.5. Co-culture model simulations

In this study, we simulated hypothetical scenarios varying biomass species ratios, growth rates, and substrates environmental fluxes to explore the feasible solution space of the co-culture. We selected a community biomass of 0.22 gDW l⁻¹ based on the average value measured for a similar co-culture of C. autoethanogenum and Clostridium kluyveri on syngas (Diender et al., 2019). The biomass of each species was calculated based on the indicated species ratio and the community biomass. The biomass of each species was multiplied by the indicated growth rate, and the value was used to constrain the flux through the biomass synthesis reaction of each species. We assessed conditions with C. autoethanogenum–C. beijerinckii ratios ranging from 0.1–0.9 to 0.9–0.1, and growth rates from 0.005 to 1.0 h⁻¹. For this exploratory analysis, we assumed equal growth rates for each species and steady-state. For each condition, we fixed the uptake rate of CO₂ and H₂ to 5 mmol l⁻¹ h⁻¹ or to 2.5 mmol l⁻¹ h⁻¹, covering values found in literature for a similar co-culture (Diender et al., 2019). The maximum lactate uptake rate was constrained to 2.5 or 5 mmol l⁻¹ h⁻¹ and a minimum uptake rate of 0.1 mmol l⁻¹ h⁻¹ was imposed. We defined the community biomass reaction (“Community_biomass”) as the objective function and we performed FBA to assess the feasibility of each condition. For a selected number of feasible conditions, the solution space and the set of fluxes compatible with the measured constraints were sampled using the sample function in the flux_analysis submodule of COBRApy. Presented results show the average and standard deviation based on 5,000 iterations generated at each condition (git repository).

2.6. Bacterial strains

The laboratory strains C. beijerinckii NCIMB 8052 and C. acetobutylicum ATCC 824 were stored as spore suspensions in 20% glycerol at −20°C. Spores of C. beijerinckii and C. acetobutylicum were heat-activated for 1 min at 95°C and 10 min at 78°C, respectively, before inoculation. C. autoethanogenum DSM 10061 was kindly provided by Professor Diana Z. Sousa from the Laboratory of Microbiology, Wageningen University and Research, Wageningen, the Netherlands, and was stored as vegetative cells suspended in 25% glycerol buffered with phosphate and reduced with Ti(III)citrate under anoxic conditions at −80°C.

2.7. Experimental carbon source screening of C. acetobutylicum and C. beijerinckii

Cultures were prepared in serum bottles containing CM2 medium consisting of the following components: 2.5 g l⁻¹ yeast extract (Duchefa Biochemie), 1.0 g l⁻¹ KH₂PO₄ (Fischer Scientific), 0.61 g l⁻¹ K₂HPO₄ (Sigma-Aldrich), 1 g l⁻¹ MgSO₄ · 7H₂O (Roth), 2.9 g l⁻¹ ammonium acetate (USB), 0.10 g l⁻¹ 4-aminobenzoic acid (Duchefa Biochemie), 6.6 mg l⁻¹ Fe(II)SO₄ · 7H₂O (Sigma-Aldrich), and 0.5 mg l⁻¹ Na-resazurin (Sigma-Aldrich). Acetic acid (Sigma-Aldrich), l-lactic acid (~90%, Merck), ethanol (Merck) and glycerol (Sigma-Aldrich) were added to final concentrations of 40 mM. pH was set to pH 6.1–6.2 with KOH and/or HCl. Media were made anoxic with N₂(g) and autoclaved. D-Glucose was made anoxic and autoclaved separately and added to a final concentration of 40 mM. Media were inoculated with 4% (v/v) culture made from heat-activated spore suspension grown overnight at 37°C in CM2 medium supplemented with 40 g l⁻¹ D-glucose (Duchefa Biochemie). Cultures were incubated at 37°C and sampled at t₀ and after 4 d. Cell density was measured as optical density at 600 nm (OD₆₀₀), extracellular metabolites were analyzed with high-performance liquid chromatography (HPLC) as described in Section 2.9, and pH was measured. Acetate co-accumulation was determined by calculating the fraction of the total converted carbon coming from consumed acetate.

2.8. Cultivation experiments in pH-controlled bioreactors

pH-controlled bioreactor experiments were performed in a working volume of 2 l in Infors HT Labfors 5 bioreactors (Infors HT, Switzerland). The stirrer, set at 150 rpm, consisted of at equidistance from top to bottom a pitch-blade and two Rushton impellers. Temperature was controlled at 37°C and pH at pH 5.5 ± 0.1 using 3 M KOH and 2 M H₃PO₄. Foaming was controlled with Antifoam 204 (Sigma-Aldrich). 2.9 g l⁻¹ ammonium acetate in the CM2 medium was replaced by 2.5 g l⁻¹ ammonium sulfate (Merck). 0.75 g l⁻¹ anoxic and sterilized L-cysteine HCl · H₂O (Merck) was added after autoclaving.

In pH-controlled batch fermentations of C. beijerinckii growing on different concentrations of acetate and lactate, the adapted CM2 medium was supplemented with acetic acid and L-lactic acid prior to autoclaving. 10 ml min⁻¹ N₂(g) was flushed across the head space to keep anoxic
conditions. Reactors were inoculated with 1% (v/v) C. beijerinckii culture growing overnight at 37°C in CM2 medium supplemented with 20 g 1 L-1 D-glucose and 0.75 g 1 L-1 L-cysteine HCl. Cultures were sampled at regular time intervals for analysis of cell density, extracellular metabolites, and morphology with phase-contrast microscopy. The overall stoichiometry was calculated by scaling the difference of the concentrations of the main extracellular metabolites between $t_0$ and $t_{end}$ to the difference in the measured lactate concentration.

In pH-controlled fed-batch co-cultivation experiments of C. autoethanogenum and C. beijerinckii, reactors were equipped with sinter spargers to flush 40 ml min$^{-1}$ H$_2$(g) and 10 ml min$^{-1}$ CO$_2$ through the medium. At $t_0$, reactors were inoculated with <1% (v/v) C. autoethanogenum culture growing at 37°C in CM2 medium supplemented with 10 g 1 L-1 D-fructose (VWR Chemicals) and 0.75 g 1 L-1 L-cysteine HCl. Furthermore, the continuous L-lactic acid feed was started. Cultures were sampled at regular time intervals for analysis of cell density, extracellular metabolites, and morphology with phase-contrast microscopy. Theoretical acetate production from CO$_2$ was calculated for each time point after the start of the L-lactic acid feed as follows: the amount of lactate converted was calculated from the difference in the amount of lactate fed and calculated amount of lactate remaining in the reactor. The conversion of lactate via pyruvate yields the intermediate metabolite acetyl-CoA and CO$_2$ in a 1:1 ratio. The fraction of the amount of carbon from lactate available for the formation of products was subtracted from the amount of carbon present in the produced (iso)butyrate to obtain a theoretical amount of carbon coming from a difference source than lactate, i.e., from converted acetate. This theoretical amount of converted acetate was added to the calculated amount of acetate in the reactor to get a theoretical amount of acetate produced from CO$_2$. Subsequently, the stoichiometry for the production of (iso)butyrate from lactate and acetate was calculated by scaling the difference of (iso)butyrate produced, theoretical acetate converted, and lactate converted between $t_0$ and the selected time points to lactate converted.

2.9. Analysis of extracellular metabolites

Concentrations of acetate, acetone, butanol, (iso) butyrate, ethanol, fructose, glycerol, glucose, and lactate were analyzed with HPLC. Supernatant was mixed with an equal volume of 1 M H$_2$SO$_4$ with 30 mM 4-methylpentanoic or 100 mM pentanoic acid as internal standard. This was filtered through a 0.2 μm regenerated cellulose filter followed by analysis on a Waters HPLC system with a Shodex KC-811 column at 65°C, 1 ml/min 3 mM H$_2$SO$_4$ mobile phase, and a refractive index and UV detector.

3. Results

Figure 1 shows the steps for the model-driven approach followed to establish a novel co-culture of C. autoethanogenum and C. beijerinckii for the production of butyrate from CO$_2$/H$_2$. GEMs of solventogens were used to evaluate candidate species and possible carbon sources. After the experimental validation of the model predictions, the co-culture was successfully established.

3.1. Updated GEMs of C. autoethanogenum, C. acetobutylicum, and C. beijerinckii

The GEM of C. autoethanogenum, iCLAU786 was used as the original version (Valgepea et al., 2017) with no modification. The updated version of the GEM of C. acetobutylicum, iCac803 (Dash et al., 2014), had 1,254 reactions, 1,465 reactions, and 803 genes, and the GEM of C. beijerinckii, iCM943 (Milne et al., 2011), had 881 metabolites, 941 reactions, and 943 genes.

Regarding the GEM of C. beijerinckii, we included the Na$^+$-translocating ferredoxin:NAD$^+$ oxidoreductase (Rnf) complex (EC 7.2.1.2) in the model of C. beijerinckii. Rnf is formed by the following gene cluster: rnfC, rnfD, rnfG, rnfE, rnfA, rnfB, (locus_tag: Cbei_2449, Cbei_2450, Cbei_2451, Cbei_2452, Cbei_2453, and Cbei_2454, respectively). Additionally, we have identified an electron-bifurcating, ferredoxin-dependent transhydrogenase (Nfn) complex that catalyzes NADH-dependent reduced Ferredoxin:NAD$^+$ oxidoreductase activity in C. beijerinckii. The Nfn complex has two subunits: NAD(P)-binding subunit, and a glutamate synthase subunit. These two subunits showed 56–79% identity with the Nfn subunits of C. kluyveri, C. autoethanogenum, C. difficile (Kremp et al., 2020), and of the recently annotated Anaerotignum neopropionicum (Benito-Vaquerizo et al., 2020), forming two possible complexes: (Cbei_2182 and Cbei_2183) or (Cbei_0661 and Cbei_0662). To our knowledge, this is the first time the Nfn complex is reported in C. beijerinckii NCIMB 8052. Ferredoxin NAD$^+$ reductase (EC 1.18.1.3) is not found in the genome of C. beijerinckii, and the ferredoxin NAD$^+$ reductase (EC 1.18.1.2) showed lower percentage identity to the FNR of C. acetobutylicum, and thus, we hypothesized that the former FNR corresponds to the NADP-binding subunit of the Nfn complex.
3.2. In-silico carbon source screening of *C. acetobutylicum* and *C. beijerinckii*

We explored growth on 25 carbon sources individually and pairs of these carbon sources with one another in the model of *C. acetobutylicum* (Figure 2). Growth was predicted on sugars, glycerol, lactate, serine, and pyruvate as single carbon sources, reaching the highest growth rates on cellobiose, sucrose and maltose. As expected, acetate did not sustain growth as the sole carbon source and neither was sustained on acetone, succinate, acetoacetin (not shown here). Pairwise combinations of most carbon sources that led to growth as single carbon source, also led to growth in combination with an alternative carbon source. However, L-methionine, L-cysteine and CO2 did not show growth in combination with carbon sources that sustained growth alone since the model was forced to uptake a minimum amount of each carbon source, leading in some cases, to infeasible solutions. The highest growth rates were obtained with cellobiose, sucrose or maltose in combination with serine, glycine or ethanolamine; the combination of glucose or fructose with glycerol, and xylene, or arabinose with ribose. Interestingly, acetate, in combination with lactate or glycerol, could sustain growth in *C. acetobutylicum*, as previously described for other solventogens (Diez-Gonzalez et al., 1995; Kumar et al., 2022).

Additionally, we assessed growth on glucose, glycerol, ethanol, formate, butyrate, lactate, and acetate in the model of *C. beijerinckii* (Figure 3). As observed for *C. acetobutylicum*, *C. beijerinckii* only sustained growth on glucose, glycerol, and lactate as single carbon sources. Pairwise combinations of the latter carbon sources sustained growth in combination with the rest of carbon sources. The highest growth rates were obtained with glucose in combination with glycerol or lactate. Here, acetate with lactate or glycerol also sustained growth,
FIGURE 2
Growth capabilities predicted for *C. acetobutylicum*. The color scale shows growth rates in h⁻¹. Squares on the diagonal correspond to single carbon sources. Squares below the diagonal correspond to the combination of the carbon source presented on the x-axis with the carbon source presented on the y-axis. Non-colored squares show that no growth was predicted for the specified carbon source(s). Meth, methionine; Etha, ethanolamine. The maximum uptake rate for each carbon source was set to 20 mmol g⁻¹ h⁻¹ and the minimum to 0.1 mmol g⁻¹ h⁻¹.

being the growth rate higher with addition of acetate in both scenarios.

3.3. Growth of *C. acetobutylicum* and *C. beijerinckii* on lactate and acetate

The substrate space provided by the model was used in an initial screening to assess growth and co-assimilation of acetate on various carbon sources by the solventogens *C. acetobutylicum* and *C. beijerinckii* (Figure 4). On all assessed carbon sources *C. beijerinckii* grew to higher cell densities after 4 days than *C. acetobutylicum*, which is known to produce autolysins toward the end of the exponential growth phase (Croux et al., 1992). As predicted by the models (Figures 2, 3), neither strain grew on acetate alone (Figure 4; Condition 4), as both strains only converted the residual metabolites from the inoculum, i.e., glucose. This showed the need for an additional carbon source to co-assimilate acetate. Contrary to the model predictions (Figure 2), *C. acetobutylicum* did not grow on acetate with lactate, glycerol and ethanol as co-substrates under the tested conditions. However, the model predictions for *C. beijerinckii* (Figure 3) were confirmed, and acetate was co-assimilated using all lactate and part of the glycerol into butyrate (Figure 4; Condition 1). Both solventogens further reduced butyrate to butanol with the addition of glucose (Figure 4; Condition 2). The fraction of carbon coming from acetate in the products produced by *C. beijerinckii* was not improved by the addition of glucose to the medium, and was highest in the medium containing only acetate, ethanol, glycerol, and lactate (Figure 4; Condition 1–3, Supplementary Table 1).

Growth of *C. beijerinckii* on lactate and acetate was further explored in a bioreactor at a controlled pH of 5.5 (Supplementary Figure 1). This pH is close to the optimal pH of *C. autoethanogenum* (Abrini et al., 1994), and acid re-assimilation and ABE production in *C. beijerinckii* (Diallo et al., 2020, 2021). Butyrate was the most abundant product and
the stoichiometry was as follows: consumption of one mol lactate and 0.4–0.5 mol acetate produced 0.6–0.7 mol butyrate. This was similar to the stoichiometry reported for Clostridium saccharobutylicum NCP 262, previously known as Clostridium acetobutylicum P262 (Keis et al., 2001), growing on lactate and acetate (Diez-Gonzalez et al., 1995).

The screening of the solventogens C. acetobutylicum and C. beijerinckii on various carbon sources showed that the combination of C. beijerinckii and lactate was most promising for the re-assimilation of acetate produced from CO₂/H₂ by C. autoethanogenum in a future co-culture.

### 3.4. Fermentation of lactate and acetate by C. beijerinckii

In C. beijerinckii, lactate is oxidized to pyruvate via NAD-independent l-lactate dehydrogenase (EC 1.1.1.27) encoded by the following isoenzymes: Cbei_4072, Cbei_4903, or Cbei_2789 (Figure 5). Pyruvate is decarboxylated to acetyl-CoA via pyruvate:ferredoxin oxidoreductase (PFOR; EC 1.2.7.10, encoded by Cbei_1853, Cbei_4318, or Cbei_1458), generating reduced ferredoxin and CO₂. Model predictions showed that reduced ferredoxin is partly spent to produce H₂ and oxidized ferredoxin via ferredoxin hydrogenase (EC 1.12.7.2, Cbei_0327, Cbei_4000, or Cbei_3796), and partly spent to regenerate oxidized ferredoxin and NADH via the Rnf complex (EC 7.2.1.2, Cbei_2449-55), translocating Na⁺/H⁺ (Patakova et al., 2019). The Rnf complex is coupled to an ATPase (EC 7.1.2.2) encoded by the cluster Cbei_0412 to Cbei_0419, that pumps in Na⁺/H⁺ for energy generation.

Model predictions suggested that acetate is converted into acetyl phosphate (acetyl-P) investng ATP by acetate kinase (EC 2.7.2.1, Cbei_1165), and acetyl-P is converted into acetyl-CoA via phosphate acetyltransferase (EC 2.3.1.8, Cbei_3402 or Cbei_1164). As previously mentioned, C. beijerinckii produces butyrate via acetyl-CoA (Chen and Blaschek, 1999). First, two acetyl-CoA molecules are converted into one acetoacetyl-CoA by acetoacetyl-CoA thiolase (EC 2.3.1.9, Cbei_0411 or Cbei_3630). Acetoacetyl-CoA is reduced to 3-hydroxybutyryl-CoA via (5)-3-Hydroxybutanoyl-CoA:NAD⁺ oxidoreductase (EC 1.1.1.157, Cbei_0325) or via NAD(P)-dependent acetoacetyl-CoA reductase (EC 1.1.1.36, Cbei_5834).

Then, 3-hydroxybutyryl-CoA is converted to crotonyl-CoA by 3-hydroxybutyryl-CoA dehydratase (EC 4.2.1.55, Cbei_2034 or Cbei_4544). Crotonyl-CoA is reduced via the butyryl-CoA dehydrogenase/electron-transferring flavoprotein complex (Bcd-EtfAB) producing reduced ferredoxin. Two complete clusters were identified in the genome: Cbei_0322 (Bcd), Cbei_0323 (EtfB), and Cbei_0324 (EtfA) or Cbei_2035 (Bcd), Cbei_2036 (EtfB) and Cbei_2037 (EtfA). An acyl-CoA dehydrogenase (Acd) showed 79.4% similarity with the Bcd subunit of C. acetobutylicum ATCC 824. Butyrate can be produced from butyryl-CoA via two routes in C. beijerinckii. The first route is a linear pathway in which butyryl-CoA is first converted into butyryl phosphate via butanoyl-CoA-phosphate butanoyltransferase (Ptb; EC 2.3.1.19, Cbei_0204). Butyryl phosphate is then converted into butyrate producing ATP via butyrate kinase (Buk; EC 2.7.2.7, Cbei_0204). The second route is catalyzed by a butyryl-CoA-acetoacetate CoA-transferase (EC 2.8.3.9, Cbei_2654 or Cbei_2653 or Cbei_3834 or Cbei_3833 or Cbei_4614 or Cbei_4612), where the CoA moiety of butyryl-CoA is transferred to acetate producing acetyl-CoA and butyrate. However, model predictions showed that butyrate is mostly produced generating ATP via Ptb and Buk.

### 3.5. Community model simulations of C. autoethanogenum and C. beijerinckii for the fermentation of CO₂/H₂ and lactate

The GEM of the co-culture consisted of 2,005 metabolites, 2,107 reactions, and 1,659 genes. Community model simulations supported the co-existence of the co-culture of C. autoethanogenum and C. beijerinckii for the fermentation of CO₂/H₂ and lactate in a wide range of growth rate and species ratio combinations.
Initial screening of *C. beijerinckii* and *C. acetobutylicum* for growth on various combinations of carbon sources. The bars indicate substrate and product concentrations, and cell density at the time of inoculation and after 4 days. CM2 medium, containing 38 mM acetate, was supplemented with 40 mM of each of the various carbon sources as follows: Condition 1: acetic acid, ethanol, glycerol, and L-lactic acid; Condition 2: acetic acid, ethanol, glycerol, L-lactic acid, and glucose; Condition 3: glucose; Condition 4: none. Error bars show standard deviations between two cultures inoculated with the same inoculum.

Figure 6 shows the feasible solution space for multiple combinations of species ratios, growth rates, CO$_2$, H$_2$, and lactate feeds. When the maximum uptake rate of lactate is 2.5 mmol l$^{-1}$ h$^{-1}$ (Figure 6; green figures), the feasibility of the co-culture becomes more limited. In these conditions, the co-culture is only feasible at low growth rates (<0.02 h$^{-1}$) for all species ratios, and feasible at higher growth rates (up to 0.07 h$^{-1}$) when *C. autoethanogenum* and *C. beijerinckii* are similarly present in the community for CO$_2$/H$_2$ feed ratio of 0.5. The co-culture is infeasible in all conditions when the CO$_2$/H$_2$ feed ratio is 2, and only feasible when the presence of *C. autoethanogenum* is low and the CO$_2$/H$_2$ feed ratio is 1. The range of feasible solutions becomes wider when the lactate feed rate is 5 mmol l$^{-1}$ h$^{-1}$. When *C. autoethanogenum* and *C. beijerinckii* are equally present in the community, the co-culture can be established with all explored growth rates, except when the CO$_2$/H$_2$ feed ratio is 2, that is only feasible up to 0.04 h$^{-1}$. Again, only at lower
growth rates ($<0.02 \text{ h}^{-1}$), the co-culture is feasible for all tested species ratios. Figure 7 shows the steady-state consumption and production rates observed in co-culture compared to the consumption and production rates associated to C. autoethanogenum or C. beijerinckii. Part of the acetate produced by C. autoethanogenum is taken up by C. beijerinckii since the steady-state production rates in the co-culture are lower than the production rates of C. autoethanogenum. The fermentation of acetate and lactate leads to the production of butyrate in C. beijerinckii. A small amount of butyrate is reassimilated by C. autoethanogenum and by C. beijerinckii and converted into butanol (not shown). Furthermore, ethanol is being produced in smaller amounts by C. autoethanogenum and C. beijerinckii. Model predictions also showed an exchange of CO$_2$ and H$_2$ from C. beijerinckii to C. autoethanogenum. C. beijerinckii produces CO$_2$ and H$_2$ that are taken up by C. autoethanogenum, since the flux through C. autoethanogenum is higher than the flux through the exchange reaction in the co-culture. Model predictions suggested that acetate consumption by C. beijerinckii varied depending on the lactate feed rate, being lower when the lactate feed rate was higher than 2.5 mmol l$^{-1}$ h$^{-1}$ ($\approx 5$ mmol l$^{-1}$ h$^{-1}$). A higher lactate feed rate also led to more CO$_2$ and H$_2$ produced by C. beijerinckii, and thus, to more
Feasible solution space of the co-culture of *C. autoethanogenum* and *C. beijerinckii* for several species ratio and growth rate combinations under different CO\(_2\), H\(_2\), and lactate feed rates. The y-axis shows the biomass species ratio of *C. autoethanogenum*/*C. beijerinckii* and the x-axis shows the growth rate in h\(^{-1}\). Colored areas indicate feasible solutions predicted by the model. Figures in purple and green show results when the lactate feed rate is set to a maximum of 5 mmol l\(^{-1}\) h\(^{-1}\), and 2.5 mmol l\(^{-1}\) h\(^{-1}\), respectively. Predictions shown on the first row were obtained with a CO\(_2\) and H\(_2\) feed rate of 5, and 2.5 mmol l\(^{-1}\) h\(^{-1}\), respectively. Predictions shown on the second row were obtained with a CO\(_2\) and H\(_2\) feed rate of 5 mmol l\(^{-1}\) h\(^{-1}\), and on the third row, with a CO\(_2\) and H\(_2\) feed rate of 2.5, and 5 mmol l\(^{-1}\) h\(^{-1}\), respectively.

Furthermore, we observed traces of formate, 2,3-butanediol, acetone, isopropanol, and butanol (see git repository).
3.6. Fed-batch fermentation of CO$_2$/H$_2$ and lactate by the novel co-culture of *C. autoethanogenum* and *C. beijerinckii*

Production of butyrate from CO$_2$/H$_2$ and the co-substrate lactate by the modeled co-culture of *C. autoethanogenum* and *C. beijerinckii* was experimentally verified with two biologically independent pH-controlled fed-batch fermentations (Figure 8 and Supplementary Figure 2). Both fermentations showed similar trends in biomass production and metabolites profiles. Below, the results are described for the fermentation shown in Figure 8. Initially, *C. autoethanogenum* was grown...
solely on a continuous CO₂/H₂ feed, and after 3 d, the OD₆₀₀ had reached a value of 0.29 and the acetate concentration a value of 34 mM. This acetate concentration was considered sufficient to support C. beijerinckii. C. beijerinckii was added and the L-lactic acid feed was started. The rate of the L-lactic acid feed was set lower than the rate of acetate production from CO₂/H₂ by C. autoethanogenum to prevent complete depletion of acetate. Upon inoculation with C. beijerinckii and the start of the L-lactic acid feed, a continued growth phase was observed till 10 d in which butyrate was produced up to 28 mM.

A theoretical acetate production from CO₂ was calculated from which the corresponding stoichiometry for butyrate production at each time point was calculated (Supplementary Figure 2). Between 4 and 7 d, during butyrate production in the first growth phase, for each mol of consumed lactate, 0.2–1 mol acetate was reassimilated, and 0.5–0.6 mol butyrate was produced.

The drop in cell density observed between 10 and 13 d could be explained by the accumulation of biomass observed at the reactor wall above the fermentation medium from 6 d onward (data not shown). No production of butyrate was observed in this period and microscope observations showed that the consortium consisted almost entirely of vegetative cells (data not shown). These cells could not be assigned to either species as the morphologies of C. autoethanogenum and C. beijerinckii could not be clearly distinguished. As a result, the species ratio in the co-culture was not determined experimentally. In future studies, the species ratio could be obtained from transcriptomic (Benito-Vaquerizo et al., 2020), amplicon (Ibarbalz et al., 2014), or qPCR data (Charubin and Papoutsakis, 2019).

After this adaptation period, a second growth phase was observed between 13 and 15 d coinciding with a larger fraction of sporulating cells in the culture and the co-production of butyrate and isobutyrate to final concentrations of 53 and 31 mM, respectively. While measured in both replicates (Figure 8 and Supplementary Figure 2), the production of isobutyrate by the consortium was not predicted by the models, and will be further investigated in a follow-up research. The calculated stoichiometry indicated a shift toward the conversion of lactate during this second growth and production phase (Supplementary Figure 2).

3.7. Analysis of substrate consumption and product formation by the co-culture model

C. autoethanogenum takes-up CO₂ and H₂ through the Wood-Ljungdahl pathway, where H₂ is used as an electron donor for CO₂ reduction to acetyl-CoA (Figure 9). Acetyl-CoA is mainly converted into acetate producing ATP, and ethanol. In addition, traces of 2,3-butanediol, formate and lactate were predicted by the model (not shown here). Part of the acetate was in turn taken-up by C. beijerinckii together with the external lactate feed, following the metabolism described in Section 3.3. Ethanol was produced by C. autoethanogenum and by C. beijerinckii, as observed in the experiments. Model simulations suggested the production of butyrate, CO₂ and H₂ by C. beijerinckii, and traces of acetone and butanol. We observed that most of the CO₂ and H₂ produced by C. beijerinckii was metabolized by C. autoethanogenum (Figure 8). Furthermore, we observed traces of isopropanol formed from the conversion of the assimilated acetone by C. autoethanogenum through an alcohol dehydrogenase. The community model suggested that butanol was produced by C. beijerinckii and by C. autoethanogenum (Figure 9). As Diender et al. (2016) already observed, butyrate could be exchanged between C. beijerinckii and C. autoethanogenum, and converted into butanol by an alcohol dehydrogenase and the aldehyde ferredoxin oxidoreductase.

In addition, the model predicts traces of formate and lactate produced by C. autoethanogenum being assimilated by C. beijerinckii (not shown here).

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**FIGURE 8** pH-controlled fed-batch fermentation of the C. autoethanogenum–C. beijerinckii co-culture on 1:4 CO₂/H₂ with an L-lactic acid feed. Concentrations of the main substrates, products, and cell density are shown. At 0, cultures were inoculated with C. autoethanogenum. The dotted black line marks inoculation with C. beijerinckii and the start of the L-lactic acid feed. L-lactic acid was fed at a rate of 3 ml d⁻¹ till 19 d. Traces of ethanol (3–8 d, max. 4.6 mM at 7 d and 13–20 d, max. 5.6 mM at 20 d), butanol (14–20 d, max. 3.1 mM at 20 d) and glucose from the C. beijerinckii inoculum (1.4 mM at 3 d) were detected. pH was controlled at pH 5.5 ± 0.1. Results of a single biological replicate are shown here and the results of a second independent biological replicate are shown in Supplementary Figure 2.
Supplementary Figure 3 represents the metabolic profile of the novel co-culture with glucose, instead of lactate, as additional carbon source. As observed in Figure 4, the addition of glucose would lead to an increase of ABE production by C. beijerinckii, since there are more reducing equivalents when glucose is converted to pyruvate. Acetate would still be the main product in C. autoethanogenum and ethanol would be produced in minor amounts. Part of the acetate could be metabolized by C. beijerinckii, but also produced together with butyrate during the acidogenic phase. Once the pH drops enough, the acids could be partly reassimilated during solventogenesis to produce solvents. Acetone and butyrate could be partly taken up by C. autoethanogenum producing isopropanol and more butanol. Possibly, part of the CO₂ and H₂ produced by C. beijerinckii would be consumed by C. autoethanogenum as described for the co-culture growing on lactate.

4. Discussion

GEMs are mathematical representations of the metabolism and have been successfully employed to gain insights into metabolic capabilities of single species (Milne et al., 2011; Dash et al., 2014; Valgepea et al., 2017; Gu et al., 2019; Benito-Vaquerizo et al., 2022), and to elucidate possible strategies to optimize the performance of microorganism(s) in mono- and co-cultivation (Kim et al., 2019; Benito-Vaquerizo et al., 2020; Foster et al., 2021; García-Jiménez et al., 2021). In this study, the use of constrained-based modeling has been key to design an alternative way to produce butyrate from CO₂/H₂ and lactate. We have proven the capacity of C. beijerinckii NCIMB 8052 to grow on lactate and acetate as the sole carbon and energy source. Moved by the need to upcycle sustainable feedstocks, we have used this new found capacity of C. beijerinckii to established a novel synthetic co-culture of C. autoethanogenum and C. beijerinckii for the fermentation of CO₂/H₂ and lactate into butyrate.

The use of lactate as alternative carbon source by C. beijerinckii as co-substrate with acetate creates new possibilities for the production of butyrate. Acetate is the most abundant product of gas fermentation, and therefore, has an essential role in the establishment of the co-culture. Lactate is a minor fermentation product of acetogens grown on syngas or CO₂/H₂ (Valgepea et al., 2018; Im et al., 2022), but a major fermentation product of acetogens grown on sugars (Drake and Daniel, 2004). Acetogens could be engineered toward autotrophic lactate production from CO₂/H₂ (Mook et al., 2022) or syngas, thereby facilitating butyrate production in co-cultivation with C. beijerinckii without the need of adding an additional carbon source. Alternatively, lactate can be obtained from other sources, such as side-streams from the dairy industry (Sar et al., 2022), spoiled agri-food products (Xu et al., 2020), ensiled
agricultural biomass (Chen et al., 2007), and fermented grass (Sakarika et al., 2022).

C. beijerinckii has wide physiological versatility, which makes this microbe an ideal candidate to produce butyrate in a co-culture. However, butyrate production could also be achieved by the co-cultivation of an acetogen with a butyrate producing species, such as Clostridium butyricum, whose ability to grow on lactate and acetate was also proved recently (Detman et al., 2019).

Additionally, the use of the newly established co-culture could increase carbon recycling and electron transport, since model predictions indicated that CO$_2$ and H$_2$ produced by C. beijerinckii were almost fully reassimilated by C. autoethanogenum (Figure 7), which reduces the carbon footprint. Incorporation of an organism able to produce H$_2$ needed for CO$_2$ assimilation is interesting to consider for future approaches. Besides solventogenic Clostridia, other anaerobic bacterial species have been described that produce H$_2$ from the fermentation of sugars at high yields (Show et al., 2012). This opens up new alternatives for more efficient co-cultures without the need of external H$_2$.

Model predictions showed slow growth on lactate and acetate by C. acetobutylicum. However, this was not confirmed by experiments in which lactate was not consumed, and acetate was produced rather than consumed (Figure 4; condition 1). Diez-Gonzalez et al. (1995) showed growth on lactate and acetate in the solventogen C. saccharobutylicum NCP 262. They analyzed extracts of cells grown on lactate and acetate and observed NAD-dependent lactate dehydrogenase (d-LDH) as well as NAD-independent lactate dehydrogenase (i-LDH) activity. d-LDH regulated the conversion of pyruvate to lactate and required fructose-1,6-biphosphate to be active (Freier and Gottschalk, 1987; Diez-Gonzalez et al., 1995). i-LDH regulated the conversion of lactate to pyruvate (Figure 5) and had double the activity over d-LDH. In addition, i-LDH activity decreased fourfold when glucose was added to cultures growing on lactate and acetate. However, lactate was only converted by C. acetobutylicum ATCC 824 when glucose was added (Figure 4; Condition 2) suggesting that i-LDH from C. acetobutylicum ATCC 824 is activated by glucose. Interestingly, the LDH of C. beijerinckii NCIMB 8052 and C. acetobutylicum ATCC 824 showed 87.7 and 57% similarity with the LDH of C. saccharobutylicum NCP 262, respectively. Keis et al. (2001) showed that C. saccharobutylicum NCP 262 is more similar to C. beijerinckii NCIMB 8052 than to C. acetobutylicum ATCC 824. Therefore, we hypothesize that C. beijerinckii has an i-LDH activity comparable to C. saccharobutylicum, whereas i-LDH activity in C. acetobutylicum ATCC 824 is regulated differently.

Model predictions showed a high production of butyrate, acetate, and traces of ethanol, acetone, butanol, isopropanol, 2,3-butanediol, and formate. Fed-batch experiments also showed butyrate and acetate as major fermentations products, and ethanol and butanol as minor fermentation products. Charubin and Papoutsakis (2019) observed production of 2,3-butanediol from the assimilation by C. ljungdahlii of the acetoin produced by C. acetobutylicum. However, acetolactate decarboxylase was only annotated in the genome of C. autoethanogenum and not in the genome of C. beijerinckii (Siemerink et al., 2014), and thus, acetoin could not be produced by the solventogen. Lactate degradation results in less NAD(P)H available, and therefore, the production of solvents is lower compared to the standard ABE fermentation on sugars (Sreekumar et al., 2015; Charubin and Papoutsakis, 2019). In contrast, the co-culture in our study has a relatively high butyrate production (up to 53 mM). Furthermore, mono-culture experiments on lactate and acetate in our study produced higher concentrations of butyrate (up to 42 mM) than the reported co-assimilation of glycerol and acetate by C. beijerinckii (∼20 mM) (Kumar et al., 2022), and than the co-assimilation of lactate and acetate by C. saccharobutylicum (Diez-Gonzalez et al., 1995) (∼20 mM).

Model predictions indicated that C. beijerinckii could grow on lactate as the sole carbon and energy source, as was recently observed (Schwalm et al., 2019). However, the growth rate was improved by the addition of acetate (Figure 3), as was previously shown (Diez-Gonzalez et al., 1995). The addition of acetate favors lactate uptake, since the acetyl-CoA pool increases with addition of acetate as co-substrate, and thus, more acetyl-CoA would be converted into butyrate producing more ATP. Co-culture fed-batch experiments showed, however, accumulation of acetate in the fermentation broth. This showed that not all acetate produced by C. autoethanogenum was consumed by C. beijerinckii, as indicated by the model, and possibly that some acetate could also be produced by C. beijerinckii.

We should note that the deployed modeling approach predicts steady-state production or consumption rates, and thus, we cannot compare the results quantitatively with bioreactor data, which consist of concentrations over time. Instead, our study should be seen as an exploratory study assessing the feasibility of the co-culture. Future optimization of this co-culture could integrate current experimental data and relative abundance of species into dynamic modeling approaches to gain better insights into the concentration profiles over time. These results show that community modeling of metabolism is a valuable tool to guide the design of microbial consortia for the tailored production of important chemicals from renewable resources. It thereby expands the space of options to possibly accelerate the transition to a biobased economy.

5. Conclusion

Genome-scale metabolic modeling helped identifying the ability of C. beijerinckii to co-metabolize acetate and lactate for the production of butyrate. This ability was experimentally verified in batch serum bottles and pH-controlled batch bioreactor fermentations. A community
model of C. autoethanogenum and C. beijerinckii was then constructed to assess the feasibility of the co-culture to produce butyrate from CO$_2$/H$_2$ and lactate. Community modeling predicted the feasibility of the co-culture in several conditions and the interactions between species, especially, the exchange of acetate. Following model predictions, the co-culture of C. autoethanogenum and C. beijerinckii was established in pH-controlled fed-batch fermentations. The main products were acetate, butyrate and the newly identified metabolite, isobutyrate. Our study shows the strength of a model-driven approach to explore the high metabolic flexibility of clostridial species for the production of chemicals from renewable sources.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

SB-V and NN conceived and designed the study and drafted the manuscript. SB-V constructed the models and performed model simulations and data analysis. NN performed the experiments and data analysis. MS-D, AML-C, JH, SB, PS, and VM conceived, designed, and supervised the research. MS-D, VM, JH, and AML-C acquired project funding. All authors reviewed and edited the study. All authors read and approved the content of the submitted version.

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Conflict of interest

VM has interests in LifeGlimmer. JH has interests in NoPalm Ingredients BV.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1064013/full#supplementary-material

References


Ragdale, S. W., and Pierce, E. (2008). Acetogenesis and the wood-


