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Resource allocation explains lactic acid production in mixed-culture anaerobic fermentations

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1 **Resource allocation explains lactic acid production in mixed-culture anaerobic**
2 **fermentations**

3 Running title: Resource allocation explains lactate production

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12

Abstract

Lactate production in anaerobic carbohydrate fermentations with mixed cultures of microorganisms is generally observed only in very specific conditions: the reactor should be run discontinuously and peptides and B vitamins must be present in the culture medium as lactic acid bacteria are typically auxotrophic for amino acids. State-of-the-art anaerobic fermentation models assume that microorganisms optimise the ATP yield on substrate and therefore they do not predict the less ATP efficient lactate production, which limits their application for designing lactate production in mixed-culture fermentations. In this work, a metabolic model taking into account cellular resource allocation and limitation is proposed to predict and analyse under which conditions lactate production from glucose can be beneficial for microorganisms. The model uses a flux balances analysis approach incorporating additional constraints from the resource allocation theory and simulates glucose fermentation in a continuous reactor. This approach predicts lactate production is predicted at high dilution rates, provided that amino acids are in the culture medium. In minimal medium and lower dilution rates, mostly butyrate and no lactate is predicted. Auxotrophy for amino acids of lactic acid bacteria is identified to provide a competitive advantage in rich media because less resources need to be allocated for anabolic machinery and higher specific growth rates can be achieved. The Matlab™ codes required for performing the simulations presented in this work are available at https://doi.org/10.5281/zenodo.4031144.

1. Introduction

Lactate is one of the most widely produced carboxylic acids with an annual market growth of over 15% that has applications as food additive, precursor of pharmaceutical compounds or building block of biodegradable plastics (polylactic acid). Currently, pure bacterial fermentations account for 90% of the world production using as substrate mainly sugars (Alves de Oliveira, Komesu, Vaz Rossell, & Maciel Filho, 2018). Lactate is therefore a good candidate to be produced from cheap organic wastes by open mixed-culture fermentations (MCF) in an economical and efficient way within the biorefinery paradigm. The use of undefined and mixed microbial cultures is an attractive option due to its intrinsic economic (e.g. substrate sterilisation is not needed and organic wastes can be used as substrate) and operational advantages (e.g. continuous processes are feasible).

Recently, it was reported that lactate can dominate the product spectrum in MCF dominated by species from the genus *Lactobacillus* and *Lactococcus*, but only under very specific operational conditions (Rombouts et al., 2020). A complex medium with peptides and vitamins is needed to grow lactic acid bacteria (LAB) because they are auxotrophic for amino acids and some vitamins meaning that they are not capable of producing these compounds from the primary carbon substrate (Cocaign-Bousquet, Garrigues, Novak, Lindley, & Loublere, 1995; Magnúsdóttir, Ravcheev, de Crécy-Lagard, & Thiele, 2015; Wang, Tashiro, & Sonomoto, 2015). Rombouts et al. (2020) performed glucose mixed-culture fermentations experiments in a sequential batch reactor and observed that when using a rich medium containing peptides and B vitamins the community was dominated by LAB (*Lactococcus* and *Lactobacillus* species) but when the employing a mineral medium glucose was fermented to mainly acetate and butyrate by an *Ethanoligenens* species from the *Clostridia* class.

Also environmental conditions that select for maximum specific growth rate, as in batch processes, are a prerequisite for LAB to outcompete other bacterial groups, as shown in

59 (Rombouts et al., 2020). Fermentations carried out under other conditions lead inevitably to
60 the production of a mixture of other carboxylic acids and are dominated by species of the
61 genus *Clostridium* or *Ethanololigenes* (Rombouts et al., 2020; Rombouts, Mos, Weissbrodt,
62 Kleerebezem, & van Loosdrecht, 2019; Temudo, Kleerebezem, & van Loosdrecht, 2007).

63 State-of-the-art models that aim for prediction of the product spectrum in MCF are based
64 on the assumption that selection of the dominant fermentation pathway is based on
65 maximisation of the ATP yield on substrate (González-Cabaleiro, Lema, & Rodríguez, 2015;
66 Regueira, Lema, Carballa, & Mauricio-Iglesias, 2020; Zhang, Zhang, Chen, van Loosdrecht,
67 & Zeng, 2013). Using this criterion, lactate would never be produced as a major end-product
68 since it yields 2 ATP molecules per glucose by substrate level phosphorylation (the major
69 contributor to ATP production in fermentations), significantly less than the obtained in the
70 production of other carboxylates such as acetate or butyrate, which yield 4 and 3 ATP
71 molecules per glucose, respectively (Regueira, González-Cabaleiro, Ofiţeru, Rodríguez, &
72 Lema, 2018). Accordingly, models predict that the product spectrum of glucose MCF can
73 only be dominated by acetate, butyrate and ethanol (González-Cabaleiro et al., 2015). Thus,
74 it is clear that only bioenergetic efficiency-based arguments are not enough to explain lactate
75 production and other factors, such as kinetic arguments, should be also considered. In this
76 way, the design of MCF processes producing lactate could also benefit from the use of these
77 models. Some authors consider that on top of maximising the ATP production cells
78 minimise the metabolic fluxes or that the ratio between biomass production rate and the sum
79 of the overall metabolic fluxes is maximised (Schuetz, Kuepfer, & Sauer, 2007). Another
80 criterion used by some authors is that pathway length is minimised by microorganisms
81 (Melendez-Hevia, 1990) and, in fact, this factor has been proposed to be one of the main
82 aspects explaining cross-feeding between microbial populations in some microbial
83 conversions, as in nitrification or methane production (Costa, Pérez, & Kreft, 2006; Kreft,
84 Griffin, & González-Cabaleiro, 2020).

85 Recently the resource allocation theory has been gaining interest as it can explain different
86 metabolic regulatory strategies that could not be explained from an ATP yield maximisation
87 perspective (Bachmann, Molenaar, Branco dos Santos, & Teusink, 2017; van Hoek & Merks,
88 2012). Resource allocation theory states that the different cellular processes (e.g. catabolism,
89 growth or transport) compete for a finite protein (i.e. enzymatic) pool. Well-known
90 phenomena, such as ethanol production by yeasts in aerobic conditions, also known as
91 Crabtree effect (Nilsson & Nielsen, 2016; Schumacher, 2018), acetate overflow (acetate
92 excretion in aerobic conditions) in bacteria (Zeng & Yang, 2019) or lactate production by
93 cancer or red blood cells in humans (Vazquez & Oltvai, 2011) were correctly reproduced by
94 integrating the resource allocation theory in the metabolic models (Goelzer & Fromion,
95 2011; Nilsson & Nielsen, 2016; Zeng & Yang, 2019). Beg *et al.* (2007) developed an extended
96 model for *E. coli* incorporating a constraint accounting for a maximum number of enzymes
97 fitting in a cell and named it Flux Balance Analysis with Molecular Crowding (FBAwMC).
98 Following the same approach, Van Hoek & Merks (2012) developed a FBAwMC that
99 predicts a change in the product spectrum in glucose fermentation by an auxotrophic LAB
100 (*L. lactis*) from a mixture of acetate and ethanol at low growth rates to lactate at high growth
101 rates. All the mentioned models are centred in explaining the changes in production of pure
102 species and the resource allocation theory has never been applied to explain the dynamics
103 observed in open systems. The conclusions drawn in the previous works with pure cultures
104 cannot be extrapolated to an open mixed-culture community where changing the catabolism
105 might not be enough as microbial competition only allow fittest species to survive.

106 The objective of this work is to test if a resource allocation model can provide a plausible
107 line of reasoning to explain the switch in product spectrum from lactate to acetate and
108 butyrate depending on the environmental conditions and the competitive advantage of
109 lactate production in anaerobic mixed culture fermentations. The developed model
110 incorporates constraints from the resource allocation theory to a flux balance analysis model

111 and simulates the metabolism of lactic acid bacteria and butyrate producing bacteria with
112 auxotrophic and prototrophic anabolisms. The model looks for proposing a mechanistic
113 explanation of the observed changes in both the microbial community structure and product
114 spectrum observed experimentally in glucose mixed-culture anaerobic fermentations under
115 different environmental conditions.

116 ## 2. Model development

117 The model simulates the conversion of glucose by two groups of microorganisms, LAB and
118 butyrate-producing bacteria (BPB), in a continuous stirred tank reactor (CSTR) assuming
119 that the system is only limited by the carbon source. The model is focused on central carbon
120 metabolism and includes catabolism, anabolism and transmembrane transport of substrates
121 and products. The model development follows the approach developed by Schumacher
122 (Schumacher, 2018) to simulate the metabolism of the yeast *S. cerevisiae*.

123 ### 2.1 Resource allocation

124 The resource allocation theory considers the cell as a self-replicating system in which the
125 different growth-related processes (e.g. protein production by the ribosomes, catabolism to
126 obtain energy for growth, etc.) compete for limited resources (Molenaar, Van Berlo, De
127 Ridder, & Teusink, 2009). The core hypothesis of resource allocation is that the most limiting
128 resource is protein (i.e. enzymes concentration), which is restricted by a maximum content
129 in the cells. The protein pool can be divided schematically in its main different functions:
130 anabolism, catabolism, metabolite transport across the membrane and a growth-independent
131 fraction that do not participate directly in growth-related processes (Fig. 1). As the specific
132 growth rate increases, the cell needs more energy to grow (and consequently more catabolic
133 enzymes to extract energy from the substrate) but, at the same time, a higher specific growth
134 rate requires more ribosomes to sustain the cellular protein production rate, and more
135 anabolic enzymes to achieve the higher biomass specific growth rate imposed. In this way,

cells face a challenging optimisation problem as they have to allocate the protein pool to the different functions in order to grow as fast as possible and dominate the community.

Each of the enzymes involved in the growth-related functions has a characteristic enzymatic activity (i.e. the catalysed flux per unit of enzyme mass) and thus the different pathways have different enzymatic requirements for catalysing a given flux. Consequently, pathways requiring a low enzymatic concentration are favoured when the total protein concentration is limiting (either because they have few enzymes involved or because the activities of these enzymes are high). On the other hand, when the protein concentration is not limiting (i.e. at low specific growth rates), pathways with higher enzymatic concentration requirements but also higher ATP yields may be more beneficial and generate a higher growth rate. Thus, there is a trade-off between being efficient in ATP yield and being efficient in terms of enzyme requirements and resource allocation models can predict which strategy prevails under a given conditions and therefore the product spectrum.

<Figure 1 should be placed approximately here>

For example, in the case of the Crabtree effect resource allocation models predict that, at certain environmental conditions, strategies providing a lower ATP yield but low enzymatic requirements (such as ethanol production in presence of oxygen) can sustain a higher specific growth rate than high ATP yield but high enzymatic requirements options (complete respiration of the substrate).

2.2 Model assumptions

- Biomass is divided in two fractions: i) raw biomass (RBM) representing the carbohydrates, lipids, DNA and RNA and ii) protein. The molecular composition of RMB is considered to be constant regardless of the specific growth rate. This has the advantage that the concentration of the different protein fractions (Fig. 1) can be

160 expressed over RBM in the form of a mass ratio ($g_{protein}/g_{RBM}$) as a growth-
161 independent measurement. The elemental composition of the two fractions and its
162 ATP requirements are assumed as reported in (Schumacher, 2018).

- 163 Protein is modelled to constitute up to 50% of the total cell weight (i.e. for each gram
164 of protein in the cell, there is one gram of raw biomass resulting in a concentration
165 of 1 $g_{protein}/g_{RBM}$). Values in the same order of magnitude were measured and used in
166 previous resource allocation models (Basan et al., 2015; Mori, Hwa, Martin, De
167 Martino, & Marinari, 2016; Nilsson & Nielsen, 2016).
- 168 Proteins can be located in the cytoplasm (ribosomes and catabolic enzymes) or in the
169 membrane (transport proteins). The maximum content of membrane proteins is 20%
170 of the total protein pool as experimentally measured in different bacterial species (Liu
171 & Rost, 2001).
- 172 of the proteins are not used in growth-related processes and their fraction is constant
173 regardless independent of the specific growth rate. In consequence, of the maximum
174 protein concentration of 1 g_{PROT}/g_{RBM}, 0.5 are not used for growth and the remaining
175 0.5 may be used for growth purposes, of which a maximum of 0.1 g_{PROT}/g_{RBM} can be
176 located in the membrane. This value is in line with the values used in similar
177 approaches (Basan et al., 2015; Mori et al., 2016) and a value of the order of 50% was
178 estimated for *E. coli* (Scott, Gunderson, Mateescu, Zhang, & Hwa, 2010).
- 179 The flux catalysed by enzymes follows a first-order kinetics with respect to the
180 enzyme concentration and activity (Eq. 1) (Goelzer & Fromion, 2011; Hui et al.,
181 2015; Scott, Klumpp, Mateescu, & Hwa, 2014). This assumption was used as well in
182 the previous modelling approaches using the resource allocation theory (Basan et al.,
183 2015; Mori et al., 2016; Nilsson & Nielsen, 2016; Zeng & Yang, 2019).

$$v_i = p_i \cdot \sigma_i \cdot a_i \qquad (1)$$

184 where v_i is the flux, p_i is the protein (i.e. enzyme) concentration, σ_i is the enzyme
185 saturation degree and a_i is the maximum activity of the enzyme catalysing flux i at
186 substrate saturation conditions.

187 • All enzymes are considered to be half-saturated (σ_i = 0.5), except for the glucose
188 transporters whose kinetics are governed by the extracellular glucose concentration
189 according to the Michaelis-Menten equation. This assumption is based on the
190 previously hypothesised trade-off between low metabolite concentrations and high
191 enzyme saturation (Tepper et al., 2013) and on the observation that most glycolytic
192 enzymes are half saturated in *E. coli* (Bennett et al., 2009). Previous resource
193 allocation models also followed this hypothesis (Nilsson & Nielsen, 2016;
194 Schumacher, 2018)

195 • LAB are considered auxotrophic in amino acids (i.e. they cannot synthesise them and
196 need to be transported). The main implication is that LAB do not need to allocate
197 anabolic enzymes to amino acid production from inorganic nitrogen and can dedicate
198 the freed protein fraction to other growth tasks. Experimental measurements
199 revealed that in *E. coli,* the share of amino acid biosynthesis enzymes drops from
200 20% to 6% when switching from rich to mineral growth medium while the share of
201 other synthetic enzymes increases (Li, Nimtz, & Rinas, 2014). This may be
202 interpreted as the enzymatic activity related to anabolism (i.e. the flux of new biomass
203 formed per unit of mass of anabolic protein) is higher as amino acids are not
204 synthesised. In the model, this observation is represented by a 15% higher enzymatic
205 activity of the anabolism in LAB with respect to BPB. Information on this subject
206 for LAB or other prokaryotic microorganisms was not available in literature.

207 • Energy requirements for transport of substrates (glucose, inorganic nitrogen or
208 amino acids) and end-products is not considered as it depends highly on the
209 intracellular concentrations of the substrates and products to be transported, which

are not simulated in the model. This assumption does not affect the competition between auxotrophic LAB and BPB since the energy required for transporting amino acids or inorganic nitrogen is similar (Rombouts et al., 2020; Stouthamer, 1973).

2.3 Model description

The model follows the formulation of a flux balance analysis (FBA), in which the different cellular fluxes are optimised by linear programming to maximise the specific growth rate (Eq. 2). The optimisation has common FBA constraints of steady state condition (as in a CSTR, Eq. 3) and positive fluxes (Eq. 4). Additionally, two inequality constraints related with resource allocation are included. As previously stated, the total concentration of growth-related protein (i.e. ribosomes and catabolic and transport enzymes) has an upper limit of 0.5 g_{PROT}/g_{RBM} (Eq. 5), of which 20% can be located in the membrane (i.e. transport enzymes), which is expressed in the model by an upper limit on membrane protein concentration of 0.1 g_{PROT}/g_{RBM} (Eq. 6). The total and membrane protein concentration are determined by addition of the concentration of all growth-related or membrane enzymes, respectively, for a given set of metabolic fluxes using Eq. 1, which relates the needed enzyme mass to catalyse a flux. Formally, the model is similar to the approach of van Hoek & Merks (2012), which is a FBA with constraints describing a maximum molecular crowding (equivalent to the maximum protein concentration in the model of this work) and was coined flux balance analysis with molecular crowding (FBAwMC).

$$\max_{v} \mu \qquad \text{h}^{-1} \qquad (2)$$

$$N \cdot v = 0 \qquad \text{mol/mol}_{BM}\cdot\text{h} \qquad (3)$$

$$v_i > 0 \qquad \text{mol/mol}_{BM}\cdot\text{h} \qquad (4)$$

$$\sum \frac{v_i}{\sigma_i \cdot a_i} \leq p_{max,total} = 0.5 \qquad g_{PROT}/g_{RBM} \qquad (5)$$

$$\sum \frac{v_j}{\sigma_j \cdot a_j} \leq p_{max,membrane} = 0.1 \qquad g_{PROT}/g_{RBM} \qquad (6)$$

229 where N is the stoichiometric matrix, v are the fluxes, σ is the saturation degree of the
230 corresponding enzyme, which is set to a value of 0.5 for all enzymes except the glucose
231 transporter, a is the enzymatic activity and p_{max} is the maximum protein concentration. There
232 are i fluxes of which j correspond to membrane-related (i.e. transport) processes.

233 Since steady state is assumed and glucose is the only substrate in limiting conditions, all fluxes
234 depend on the glucose import flux, which in turn depends on the activity of its transporter
235 (Eq. 1). As stated in the previous section, the enzymatic activity of this transporter depends
236 on glucose concentration following Michaelis-Menten kinetics (Eq. 7).

$$\sigma_{GT} = \frac{[Glucose]}{K_m + [Glucose]} \qquad (7)$$

237 where σ_{GT} is the glucose transporter saturation degree for a given glucose concentration and
238 K_m is the affinity constant.

239 Combining this equation with Eq. 1 results in the glucose import flux depending on the
240 external glucose concentration (Eq. 8).

$$v_{Glucose\ transport} = a_{GT} \cdot p_{GT} \cdot \frac{[Glucose]}{K_m + [Glucose]} \qquad (8)$$

241 where a_{GT} is the activity of the glucose transporter and p_{GT} is its concentration. The form of
242 this equation is equivalent to a Michaelis-Menten equation in which the maximum rate is
243 represent by $a_{GT} \cdot p_{GT}$.

244 Three model microorganisms with different catabolic and anabolic characteristics are
245 simulated varying the glucose concentration to discern whether catabolism or anabolism is
246 responsible for the different behaviours of LAB and BPB:

247 i) An amino acid auxotrophic LAB, i.e. with a higher anabolic enzyme activity.

248 ii) A hypothetical prototrophic (i.e. non auxotrophic) LAB.

249 iii) A prototrophic BPB.

250 **2.4 Metabolic network**

251 The experiments of Rombouts et al. (2020) show that there is duality in the ecological
252 domination of the microbial consortia in MCF operated in a glucose-fed sequential batch
253 reactor. LAB of the genus *Lactobacillus* and *Lactococcus* dominate the microbial community in
254 rich medium fermentations and bacteria belonging to the genus *Clostridium* dominate the
255 community when mineral medium is used. For the sake of simplicity, the metabolic network
256 used in this model to simulate the three hypothetical microorganisms presented in the
257 previous section only considers one possible catabolism for each of the microorganisms
258 dominating the culture: acetate and butyrate in the case of BPB and lactate in the case of
259 LAB (Table 1). The stoichiometric matrixes (N in Eq. 3) used in the model for both
260 microorganisms are available in the Supplementary Materials File 1 (Table S1 and Table S2).

261 The glycolytic part of the network (conversion of glucose to pyruvate) is considered equal
262 for both microorganisms and follow the EMP pathway. To produce acetate and butyrate at
263 an equimolar ratio, as observed experimentally with BPB, the electron bifurcation
264 mechanism must be included in the butyrate pathway to allow for a closed electron balance
265 in the conversion reaction from glucose (Buckel & Thauer, 2013; Regueira et al., 2018).
266 Biomass production is described in a lumped reaction with glucose as substrate and without
267 consumption of electron equivalents. As in some anabolic reactions carbon dioxide is
268 produced as a by-product, usually lumped biomass pathways used in metabolic models
269 consider a certain degree of decarboxylation of the substrate in the lumped anabolic reaction.
270 Therefore, the biomass formation pathway includes a 10% conversion of the substrate to
271 carbon dioxide in terms of carbon moles. The influx of amino acids or inorganic nitrogen is

272 omitted from the metabolic network and the model, as glucose is considered to be the only
273 limiting substrate in the system.

274 <Table 1 should be placed approximately here>

275 A more comprehensive model, with a metabolic network also including production of
276 ethanol and acetate as another possible catabolism of the three microorganisms, was also
277 developed to test model robustness (available in the Supplementary File 2). The model
278 predictions regarding competition between the two microbial groups, LAB and BPB, are
279 identical. Therefore, to isolate and evaluate individually the effect of having a different
280 anabolism (auxotrophic or prototrophic) or catabolism (lactate or butyrate and acetate
281 production), we preferred to describe in detail in the main text the base model.

282 **2.5 Enzymatic activities**

283 The activities of the enzymes involved in the steps described in the metabolic network were
284 collected in the BRENDA database (Schomburg et al., 2002) and in literature (Table S3).
285 Glycolysis activity values are referred to enzymes found in *E. coli* as we could not find values
286 for the genus of the microorganisms considered in the metabolic network. These glycolysis
287 enzymatic activity values are used in the catabolism of the three microorganisms considered
288 in the model and, in consequence, do not interfere in the competition between them. For
289 BPB, activity values related with different species belonging to the genus *Clostridium* were
290 chosen. The activity value of the lactate dehydrogenase (the only enzyme needed for
291 converting pyruvate into lactate) was selected from the species *L. plantarum*, a LAB found in
292 dairy, meat and vegetables fermentations involved in food spoilage (de Vries, Vaughan,
293 Kleerebezem, & de Vos, 2006).

294 Due to lack of available information in literature and databases, some approximations had
295 to be made. We could not find information on the enzymatic activity of any monocarboxylate

296 transporter in bacteria. For the acetate, butyrate and lactate exporter we decided to use for
297 the three of them the activity value used in (Schumacher, 2018) for the lactate exporter in *S.*
298 *cerevisiae*. Glucose importer activity could not be found either and its value was set to reflect
299 a maximum glucose uptake rate of 40 $mmol/g_{BM}\cdot h$ (Batstone et al., 2002), assuming that
300 glucose is converted to two lactate molecules that have to be transported out of the cell by a
301 system that also occupies space in the membrane. Anabolism activity was also set to reflect
302 a LAB maximum specific growth rate of 0.35 h^{-1} similar to that observed experimental in
303 lactate producers in Rombouts et al. (2020).

304 **2.6 Uncertainty and sensitivity analysis**

305 Given that the model relies on a number of assumptions on parameter values, we performed
306 a global uncertainty and sensitivity analysis of the parameter space. The parameters selected
307 for this analysis (Table S4) are assumed to have a uniform probability distribution bounded
308 25% or 50% around the default value, depending on the parameter type. The first four
309 parameters relate to assumptions about the cellular structure and the differences in
310 proteomics between auxotrophic and prototrophic microorganisms and are assumed to have
311 an intermediate uncertainty. Therefore, and following the approach of Vangsgaard, Mauricio-
312 Iglesias, Gernaey, Smets, & Sin (2012), their dispersion distribution is bounded 25% around
313 the default value. The remaining correspond to the enzyme requirements of different
314 sections of the metabolic network, growth and the affinity constant and are assumed to have
315 the highest uncertainty with 50% variability around the default value (Vangsgaard et al.,
316 2012). All the parameters are considered to be uncorrelated to avoid underestimating the
317 uncertainty of the model solution. The parameter space was sampled using the Latin
318 Hypercube Sampling methods to ensure a maximal coverage of the parameter space (Helton
319 & Davis, 2003). Following a Monte Carlo procedure, a total of 1000 random samples of the

320 parameter space were selected and used to solve the model. The model outputs (specific
321 growth rate values) are the basis for the subsequent sensitivity analysis.

322 A global sensitivity analysis was carried out to determine what parameters (and assumptions)
323 exert a higher influence in the model output and check the need of refining the model
324 hypotheses. The method of standardised regression coefficients (SRC) was chosen, whereby
325 a first order linear multivariable model relating the model outputs taken from the Monte
326 Carlo procedure (y_k) to the parameter values (θ_i) is fitted with the least squares method
327 (Saltelli et al., 2008):

$$y_k = b_{k,0} + \sum_i b_{k,i} \cdot \theta_i \tag{9}$$

328 where y_k are the model outputs, $b_{k,0}$ and $b_{k,i}$ are the linear regression coefficients and θ_i the
329 parameters. The subindex *k* denotes each of the model outputs and the subinxed *i* refers to
330 the parameters analysed.

331 In our case, only the ratio of the specific growth rates of LAB and BPB is used as model
332 output (denoted as Y) and the parameters included in Table 2 were considered. If Eq. 9 is
333 made dimensionless by mean-centred sigma-scaling, to make the coefficients directly
334 comparable regardless of their absolute value, the standardised linear regression coefficients,
335 $\beta_{k,I}$, are obtained:

$$\frac{y_k - \mu_{y_k}}{\sigma_{y_k}} = \sum_i \left(\beta_{k,i} \cdot \frac{\theta_i - \mu_{\theta_i}}{\sigma_{\theta_i}} \right) \tag{10}$$

336 where μ_{yk} and $\mu_{\theta i}$ are the mean values and σ_{yk} and $\sigma_{\theta i}$ are the standard deviation of the model
337 outputs and input parameters, respectively.

338 If the multivariable model of Eq. 10 is linearly additive, then $\sum_i \beta_i^2 = 1$ for each of the model
339 outputs should be fulfilled. The parameter β_i^2 represents the contribution to the variance of
340 parameter *i* and can be used as a measure of its importance on the output of the model. To

341 assume the model linear, the squared coefficient of correlation (R^2) between the Monte Carlo
342 simulation output (Y) and the values produced with the regression model with the estimated
343 SRC (Eq. 10) regressed linear output should be above 0.7 (ref. 34).

344 3. Results and discussion

345 The Matlab™ codes required for performing the simulations (including the comprehensive
346 version of the model) and the uncertainty and sensitivity analysis presented in this work are
347 available at https://doi.org/10.5281/zenodo.4031144.

348 3.1 Evaluation of the enzymatic activities for the different strategies

349 Table 2 shows the enzyme requirements (i.e. the mass of enzyme needed to catalyse one unit
350 of flux) for different parts of the metabolic network and of the different catabolic strategies.
351 Glycolysis requires a high enzyme mass as butyrate production and, to a lower extent, acetate.
352 In comparison, yielding lactate from pyruvate needs much less enzyme mass because lactate
353 is only one metabolic step away from pyruvate and the enzyme catalysing that conversion,
354 lactate dehydrogenase, has a remarkable high activity. As a consequence, lactate
355 fermentations only need 3.85 mg of enzyme to catalyse one flux unit while butyrate
356 fermentations need 6.13 mg, which is 40% more, reinforcing the hypothesis that shorter
357 pathways are faster due to lower enzymatic requirements (Kappler, Janssen, Kreft, & Schink,
358 1997; Thomas Pfeiffer & Bonhoeffer, 2004). However, lactate formation is energetically less
359 favourable, as the ATP yield is 40% lower (Table 1). As a result, the mass of enzymes needed
360 to produce a unit of ATP flux in catabolism, which is proportional to the specific growth
361 rate, is very similar for both strategies (Table 2).

362 <Table 2 should be placed approximately here>

363 Lactate fermentations cannot be explained through the Crabtree effect, where the differences
364 between fermentation and respiration in terms of enzymatic requirements are noticeable

365 higher than in this case. In this sense, the Crabtree effect can be already explained by only
366 the ATP flux enzymatic requirements. Moreover, if only producing lactate was the key to
367 attain a very high specific growth rate, fermentations dominated by *Clostridium* bacteria
368 potentially could be producing lactate as well, since they have the genes to synthetize lactate
369 dehydrogenase and lactate transporters (Biddle et al., 2014; Geer et al., 2010) and were
370 reported to produce lactate under specific environmental conditions (Payot, Guedon,
371 Gelhaye, & Petitdemange, 1999).

372 **3.2 LAB auxotrophic anabolism explains its fast growth**

373 The predicted specific growth rates of the three microorganisms presented in section 2.3 at
374 different glucose concentration are shown in Fig. 2. Glucose concentration values are divided
375 by the affinity constant (K_m, Eq. 7) to isolate the influence of the value of this parameter on
376 the analysis. Two areas can be clearly identified in the graph. At high glucose concentrations,
377 auxotrophic LAB are the fastest growers and, on the contrary, at low glucose concentrations,
378 BPB can achieve a higher specific growth rate and will likely dominate the microbial
379 community under these conditions. LAB grow slower and at a very similar rate regardless of
380 their anabolism (auxotrophic or prototrophic).

381 <Figure 2 should be placed approximately here>

382 LAB display a maximum specific growth rate of 0.35 h^{-1}, which is 9% higher than that of
383 BPB. This result suggests that auxotrophic LAB outcompete BPB in environments that
384 select for microorganisms with the highest maximum specific growth rate, as for example in
385 sequencing batch reactors. The estimated kinetic parameters of the two microbial
386 communities in the experiments of Rombouts et al. (2020) also show that LAB in rich
387 medium also developed a higher maximum growth rate (0.23 h^{-1}) than BPB in mineral
388 medium. (0.19 h^{-1}), which represent a 20% increase.

389 Prototrophic LAB behave similarly to auxotrophic LAB at low glucose concentrations but
390 their maximum specific growth rate is lower, which means that an auxotrophic anabolism
391 offers a competitive advantage in terms of maximum specific growth rate. The model
392 predictions also show that the value of the maximum specific growth rate of a hypothetical
393 prototrophic LAB is similar to that of BPB, as already predicted in section 3.1 based on the
394 enzyme requirements per ATP flux for the different catabolic strategies. In this way, in a
395 mineral medium, where auxotrophic LAB cannot grow, the two catabolic strategies
396 considered (lactate or butyrate and acetate production) allow cells to attain a similar
397 maximum specific growth rate.

398 At the low range of glucose concentrations, BPB grow faster or, equivalently, at the same
399 dilution rate (equivalent to the specific growth rate in a CSTR) BPB can achieve lower
400 effluent glucose concentrations, assuming that the affinity for glucose uptake is similar in
401 both types of microorganisms. For example, at a dilution rate of 0.25 h^{-1}, BPB will lower the
402 residual glucose concentration to 0.3 times the affinity constant, while LAB could only lower
403 it to 0.7 (Fig. 2). Hence, at low specific growth rates catabolism does play an important role
404 as a high ATP yield strategy gives a competitive advantage to BPB. This prediction fully
405 explains the observations of Thomas, Ellwood, & Longyear (1979) that a pure culture of an
406 homolactic fermenter (*Streptococcus lactic*) produced lactate in a CSTR at high dilution rates but
407 at low dilution rates switched production to acetate and ethanol. The model predictions show
408 some parallelism with the work of van Hoek & Merks (2012), in which a FBA model with
409 resource allocation constraints predicted that a pure culture of L. lactic changes its catabolism
410 depending on its growth rate. While at high growth rates lactate is produced, at low growth
411 rates a catabolism consisting of acetate and ethanol is predicted due to its increased ATP
412 yield on the substrate. Additionally, several experiments co-fermenting of glucose and
413 gelatine in a mixed-culture CSTR at relatively low dilution rates (between 0.1 and 0.2 h^{-1}) and
414 at pH 7 do not produce lactic acid according to their carbon mass balances (Breure, Beeftink,

415 Verkuijlen, & Andel, 1986; Breure, Mooijman, & van Andel, 1986). In these experiments,
416 peptides are available as a result of the protein hydrolysis, but the main product of glucose
417 fermentation is butyrate as indicated by the comparison with the product spectra obtained
418 in gelatine mono-fermentation. These set of experiments validate the prediction of the model
419 that at low dilution rates (i.e. at low growth rate conditions), BPB dominate the community
420 (and therefore butyrate is the main product of glucose fermentation) regardless of the
421 presence of peptides in the broth (i.e. LAB could grow under those conditions but are not
422 competitive enough). An auxotrophic anabolism does not appear to offer a competitive
423 advantage at low substrate concentration as both the auxotrophic and prototrophic LAB
424 growth curves overlap in the low glucose concentration area of the graph (Fig. 2).

425 Accordingly, if the competition between an auxotrophic LAB and a BPB is simulated with
426 the FBAwMC model, we observed that different products dominate the spectrum at
427 different substrate concentrations (Fig. 3). At low glucose concentration, the environmental
428 conditions usually found in a well-operated CSTR where substrate consumption is virtually
429 complete, butyrate and acetate dominate the product spectrum as under these conditions
430 BPB can grow faster and outcompete LAB. At increasing glucose concentration values
431 lactate starts to be produced simultaneous with butyrate and acetate and, eventually would
432 dominate the product spectrum at high glucose concentrations, the conditions expected in
433 the initial stages of a discontinuous operational or in a CSTR operated at a high dilution rate
434 and incomplete substrate consumption, as under these conditions LAB are capable of
435 growing faster and of attaining a higher maximum specific growth rate (Fig. 2). This
436 behaviour is in close agreement with the observations of Rafrafi et al. (2013) in CSTR
437 experiments, who reported significant lactate production under high residual glucose
438 concentration, which indicates that the biomass of the reactor was close to being washed
439 out.

440 <Figure 3 should be placed approximately here>

441 **3.3 The maximum protein concentration constraint triggers the change in**
442 **production strategy.**

443 The behaviour shown in Fig. 2 and 3 can be explained by the total and membrane protein
444 concentration in LAB and BPB attained at different specific growth rates (Fig. 4). By using
445 Eq. 1, the concentration of the main metabolic enzymatic groups involved in the two studied
446 microorganisms can be calculated using the optimum fluxes determined by the model at
447 different specific growth rates (equivalent to the dilution rate (D) of a CSTR in which there
448 is only one microbial species). Fig. 4A and 4C show the concentration of transport enzymes,
449 which are located in the membrane, and Fig. 4B and 4D focus on the concentration of all
450 the enzymes involved in the metabolism of BPB and LAB, respectively. With that
451 information it can be determined which constraint (membrane or total protein maximum
452 concentration, represented by Eq. 5-6) shapes the model outcome for each of the
453 microorganisms under different conditions.

454 <Figure 4 should be placed approximately here>

455 In Fig. 4A we observe that a low and medium dilution rates, BPB are restricted by the glucose
456 transport capacity, as their membrane protein concentration reaches the maximum allowed
457 value proposed of 0.10 g_{PROT}/g_{RBM} (Eq. 6). The increasing specific growth rate observed in
458 Fig. 2 under these conditions is well justified by the increasing values of glucose
459 concentration (Eq. 8). However, at a value of 0.28 h^{-1}, an abrupt change in the specific growth
460 rate slope happens. As observed in Fig. 4B, BPB reach at this point the maximum total
461 protein concentration imposed by the model (Eq. 5). From this point on, BPB must optimise
462 enzyme allocation and therefore the opportunities of increasing their specific growth rate at
463 higher glucose concentration are drastically limited. The factor limiting BPB growth at higher

464 dilution rates is the cytoplasm protein concentration, rather than membrane protein
465 concentration, as shown in Fig. 4A and 4B.

466 LAB display a quite similar behaviour and the factors limiting their growth are also the same
467 as in BPB. However, as observed in Fig. 4C and 4D, their maximum total protein
468 concentration is attained at a higher dilution rate, highlighting the competitive advantage
469 given by an auxotrophic anabolism, which has a higher anabolic enzymatic activity. Unlike
470 BPB, the unrestricted LAB do not face enzyme allocation issues in the cytoplasm and can
471 keep increasing the glucose consumption rate between dilution rates of 0.28 to 0.33 h^{-1}. At
472 their maximum specific growth rate, BPB have a concentration of glycolysis-related enzymes
473 of 0.11 g/g_{RBM} (Fig. 4B) while LAB attain a concentration of 0.20 g/g_{RBM}, indicating that
474 glucose consumption rate almost doubles in LAB (Fig. 4D). In fact, the observed glucose
475 consumption rate of lactate producers in the experiments of Rombouts et al. (2020) is about
476 twice as much as that of butyrate producers, which is in line with the predictions of the
477 model. The protein concentration related with lactate production from pyruvate is very small
478 and hardly noticeable in Fig. 4D (its concentration is represented between the light blue and
479 yellow areas) as the activity of the enzyme catalysing this conversion (LDH) is particularly
480 high (Table S3).

481 Up to dilution rates of around 0.28 h^{-1}, protein-unrestricted BPB would dominate the
482 microbial community due to their higher ATP yield, as glucose transport is limited in a similar
483 way in both BPB and LAB. Consequently, the predicted product spectrum consists mostly
484 of butyrate and acetate (Fig. 3). At higher dilution rates, however, LAB eventually overcome
485 their lower ATP yield on glucose and outcompete BPB due to a higher substrate
486 consumption capacity. From dilution rates values of around 0.28 h^{-1}, the product spectrum
487 starts to switch to lactate and eventually it becomes the dominant product (Fig. 3).

3.4 The model captures proteome regulation at different environmental conditions

The model predicts at low substrate concentrations, most of the enzymes are dedicated to substrate transport (Fig. 5) as the glucose transporter is far from saturation, which, following Eq. 7, results in a very low enzymatic activity. Conversely, at high substrate concentrations growth is limited by the cytoplasmatic protein space while the membrane protein concentration constraint is not reached (Fig. 4). Experimental measurements confirm that cells tune the concentration of the different enzymatic groups depending on the environmental conditions and show that under high substrate concentration conditions cells allocate most of their proteome to anabolic functions (Nielsen & Villadsen, 1994). Under these conditions, the model predicts that about 40% of the proteome consists of anabolic enzymes (Fig. 5), which is in agreement with experimental measurements for E. Coli (Bosdriesz, Molenaar, Teusink, & Bruggeman, 2015; Hui et al., 2015; Nielsen & Villadsen, 1994).

<Figure 5 should be placed approximately here>

Experimental evidence also show that cells modify their morphology as a way of coping and relieving these changing limitations at different environmental conditions (Dennis & Bremer, 2008; Molenaar et al., 2009). At low substrate concentrations, as in a well-operated CSTR, cells tend to shrink as a mean of optimising the surface to volume ratio and of increasing the membrane protein capacity per volume. At high substrate conditions, the opposite trend is observed: cells tend to be larger to maximise their protein capacity in the cytoplasm, in line with the results of section 3.3.

The proteome of L. lactis, a known LAB, was analysed in a chemostat and showed poor proteome regulation when the dilution rate of the reactor was varied and in general glycolytic enzymes remained overexpressed at low growth rates (Goel et al., 2015), contradicting the

513 results of the model simulations (Fig. 4-5). However, as previously stated, the conclusions
514 drawn from a study using pure microbial species cannot be extrapolated to a system using
515 open mixed microbial cultures. For example, in this case, the lack of proteome regulation at
516 low growth rates could indicate that L. lactis is not competitive under these conditions and
517 that it would be possibly outcompeted by microorganism that have that ability. In fact,
518 mixed-culture CSTR fermenting sugars operated at relatively low dilution rates are usually
519 dominated by bacteria of the genus Clostridium or Enterobacter (Rombouts et al., 2019;
520 Temudo, Muyzer, Kleerebezem, & Van Loosdrecht, 2008). In particular, L. lactis is the result
521 of human-directed evolutionary adaptation to high-substrate environments (e.g. milk) and to
522 growing as fast as possible for its use in industrial processes (Goel et al., 2015). Therefore, it
523 could be argued that L. lactis has never had or has lost its ability to tune its proteome to low
524 substrate environments (i.e. low growth rate conditions). The results of protein regulation at
525 different dilution rates in Fig. 4 should be interpreted as the optimal proteome distribution
526 dictated by the selective pressures acting on the microorganisms at different environmental
527 conditions (i.e. dilution rate in a chemostat and therefore metabolic fluxes through the
528 microorganisms). Our hypothesis is that It is likely that the dominating microbial guild arising
529 from microbial competition has a proteome that is closely regulated to the results predicted
530 by the model."

531 **3.5 Uncertainty and sensitivity analysis**

532 The goal of the uncertainty analysis is to evaluate the influence of different parameter values,
533 especially of those parameters with values set based on assumptions (anabolic enzyme
534 activity, glucose transporter activity, growth-related share of the proteome, etc.), on the
535 model outcome, which is represented by the output Y (the ratio between the specific growth
536 rate of auxotrophic LAB and BPB). For that Monte Carlo simulations with a variability of
537 the input parameters of 25% for the model assumptions and of 50% for the enzyme

538 requirement data were carried out (Fig. 6). As observed in Fig. 6, at high glucose
539 concentrations (around 0.2 mmol/L) in 85% of the Monte Carlo simulations the value of
540 the specific growth rate ratio was above 1. Besides, at very low glucose concentrations (0.01
541 mmol/L), 75% of the Monte Carlo simulations give as a result a specific growth rate ratio
542 below 1, indicating thus that at low glucose concentrations BPB grow faster for the same
543 glucose residual concentration than LAB. These two results would confirm the robustness
544 of the model outcome.

545 <Figure 6 should be placed approximately here>

546 The sensitivity of the model was analysed at two glucose concentrations (0.01 and 0.2
547 mmol/L) to identify in each case the mechanisms that affect the most the specific growth
548 rate of each microbial group. Table S5 features the squared standardised linear coefficients,
549 which represent the contribution of each parameter to the variance of the model outcome at
550 the two points analysed. At low glucose concentrations, where BPB dominate, membrane-
551 related parameters have the highest influence on the model outcome. The enzymatic
552 requirements for glucose transport represent 35% of the variance observed in the model
553 outcome and the affinity constant and the protein that is located in the membrane contribute
554 each 13% to the observed variance. The high sensitivity of these parameters on the model
555 output is in close agreement with section 3.3, as transport was already identified to limit
556 growth under these conditions. At high glucose concentrations, where LAB are predicted to
557 dominate the microbial community, the most sensitive parameters are related with
558 cytoplasmatic processes. The enzymatic requirements of butyrate and glycolysis yielding
559 explain 31% and 21% of the variance, respectively. This is in line with the conclusions of
560 section 3.3, where it was shown that cytoplasmatic protein capacity was the factor limiting
561 growth at high substrate concentrations.

562 **3.6 Resource allocation and process design**

563 Microorganisms strive for energy in their pursuit to grow and dominate their ecosystem. In
564 this sense, strong selective pressures are believed to have been exerted to the ATP-producing
565 pathways (T. Pfeiffer, Schuster, & Bonhoeffer, 2001). Apparently, based on the results of
566 this model, these selective pressures might have led LAB to lose the ability to synthesise
567 amino acids from inorganic compounds as it gives them a competitive advantage, which is
568 in agreement with a previous study regarding auxotrophies prediction. Several auxotrophies
569 for amino acids were predicted in gram-negative bacteria using genome-scale metabolic
570 reconstruction and some of them were postulated to confer a fitness in *in vivo* experiments
571 depending on the environmental conditions (Seif, Sonal, Hefner, Anand, & Yang, 2020).

572 According to the results of this work, LAB can dominate the microbial community when the
573 system selects on specific growth rate (as in discontinuous processes or very high dilution
574 rate CSTR) because they are capable of attaining a very high substrate consumption rate.
575 Their anabolism is more enzymatically efficient allowing LAB to allocate a higher share of
576 the proteome to catabolic processes. In consequence, LAB are common in habitats with
577 available peptides as for example wine, milk or grass (Carr, Chill, & Maida, 2002) as there
578 they can outcompete other fermenting bacteria, which is in complete agreement with the
579 conclusions of this work.

580 Resource allocation might also help explaining other metabolic behaviours such as polymer
581 accumulation in some bacteria. In environmental conditions in which growing is limited (e.g.
582 nitrogen is the limiting factor), these bacteria store the substrate in the form of a polymer
583 (e.g. polyhydroxyalkanoate) to use it afterwards when growth is not hampered. Under such
584 conditions, the accumulating bacteria outcompete other non-storing bacteria because they
585 can develop a much higher substrate uptake rate (Jiang, Marang, Kleerebezem, Muyzer, &
586 van Loosdrecht, 2011; Johnson, Jiang, Kleerebezem, Muyzer, & Loosdrecht, 2009). From a
587 resource allocation perspective, it could be hypothesised that the hampered anabolism leaves

588 room for additional catabolic enzymes, allowing in this way consuming substrate at a higher
589 rate.

590 Cross feeding between species (or labour division) is another candidate to be analysed using
591 resource allocation. The most accepted interpretation is that dividing the catabolism in
592 several microbial groups allows for attaining a higher consumption rate and therefore
593 outcompete microorganisms that perform the conversion completely but at a slower rate. A
594 process that is usually accomplished in a two-step conversion performed by two microbial
595 groups is nitrification of ammonia to nitrate (Kreft et al., 2020). Commonly, a first microbial
596 group partially nitrifies ammonia to nitrite and finally a second microbial group ends the
597 nitrification process converting nitrite to nitrate. Only at very low substrate concentration
598 conditions, the longer complete nitrification to nitrate by one microbial group (comammox)
599 was experimental reported (Hu & He, 2017). These observations are in agreement with the
600 resource allocation theory prediction that longer pathways (i.e. requiring a higher enzyme
601 concentration) but providing more energy are favoured when substrate is scarce and mainly
602 the transport capacity is limiting. In fact, lactate production could also be considered as a
603 case of cross feeding as lactate can be further metabolised to typical fermentation end
604 products. In the experiments of Rombouts et al. (2020) lactate is consumed by a non-glucose
605 consumer microbial group (bacteria belonging to the genus *Megasphaera* mainly) producing a
606 mixture of acetate, propionate, butyrate and valerate. In this sense, resource allocation is in
607 line with the most-recent cross feeding interpretations, as it also predicts that in high
608 substrate concentration conditions an association of two microbial groups consuming the
609 substrate faster and in two steps is fitter than one single microbial group converting the
610 substrate to the end products by its own. The faster consumption rate can be given by a
611 more enzymatically efficient catabolism (Crabtree effect or acetate overflow) or anabolism,
612 as shown in this work for lactate fermentation.

613 This mechanistic understanding of the mechanisms favouring lactate production under very
614 specific environmental conditions has immediate applications for bioprocess design. MCF
615 processes that use carbohydrate-rich wastes as substrate can now be directed to produce
616 lactate with a high selectivity. It was already shown in this work that lactate production is
617 promoted in batch reactor configurations and butyrate production is favoured in a CSTR
618 (except at very high dilution rates) (Fig. 3). To avoid lactate consumption the operational
619 conditions should be tuned to avoid the presence of lactate consumers (e.g. at a dilution rate
620 incompatible with the survival of these microbial populations). Apart from lactate-producing
621 systems, the mechanistic insight gained in this work can also be applied in the design of other
622 processes aiming at the intermediate compounds in two-step microbial conversions.

623 ## 4. Conclusions

624 A possible mechanism explaining lactate production in anaerobic mixed-culture
625 fermentations was identified by means of a resource allocation FBA model. Simulation
626 results indicate that the characteristic anabolic auxotrophy on amino acids of lactate acid
627 bacteria is advantageous and enables a higher maximum specific growth rate than butyrate-
628 producing bacteria, provided peptides are available. Maximum total and membrane protein
629 concentration constraints explain the different metabolic strategies and proteome regulation
630 behaviours observed experimentally. The model is in line with different empirical
631 observations and predicts lactate production only in rich cultivation medium and at high
632 dilution rates in a CSTR or a (repeated) batch process. On the contrary, prototrophic
633 butyrate-producing bacteria are predicted to dominate the community under any other
634 operational condition. To fully validate the predictions of the model, additional experiments
635 focusing on the composition of the microbial community and its meta-proteomics are
636 needed to corroborate the predicted link between proteome and the change in production
637 strategy. The model described in this work for the first time uses resource allocation theory

638 to identify and mechanistically explain the selective pressures acting in a mixed microbial
639 consortium at different environmental conditions that create different ecological niches for
640 specific groups of microorganisms.

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650 **Competing interests**

651 The authors declare that they have no conflict of interest.

652 **Authors contribution**

653 A. Regueira, J. L. Rombouts, S. A. Wahl and R. Kleerebezem conceived and discussed the
654 ideas leading to this manuscript. A. Regueira implemented the mathematical model. A.
655 Regueira wrote the manuscript draft, and all authors discussed the results and critically
656 revised and approved the final manuscript.

657

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856 Tables

857 **Table 1.** Summarised catabolic reactions considered in the metabolic network.

Microorganism	Conversion reaction	ATP yield
LAB	Glucose → 2 $lactate^-$ + 2 H_2O	2
BPB	Glucose → 0.67 $acetate^-$ + 0.67 $butyrate^-$ + 0.67 H_2O + 2.67 H_2 + 2 HCO_3^-	3.33

858

859 **Table 2.** Enzymatic requirements for metabolic fluxes of different pathways.

Pathway	Enzyme requirement
Glucose → 2 Pyruvate	2.52 mg Prot/(mmol glucose/h)
Pyruvate → Lactate	0.007 mg Prot/(mmol pyruvate/h)
Pyruvate → ½ Butyrate	1.46 mg Prot/(mmol pyruvate/h)
Pyruvate → Acetate	0.68 mg Prot/(mmol pyruvate/h)
Complete catabolism	
Glucose → 2 Lactate	3.85 mg Prot/(mmol glucose/h)
Glucose → 2/3 Butyrate + 2/3 Acetate	6.13 mg Prot/(mmol glucose/h)
Catabolism in terms of ATP flux	
Lactate (2 ATP)	1.93 mg Prot/(mmol ATP/h)
Butyrate fermentation (3.33 ATP)	1.86 mg Prot/(mmol ATP/h)

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864 Figure captions

865 **Figure 1.** Allocation of the cellular protein pool. The share of each fraction is linearly
866 dependent on the flux it catalyses. p is the share of the different protein functions, μ is the
867 specific growth rate, v represents the fluxes, a are the activities of the different groups of
868 enzymes and k represent the constant share of the proteome not related with growth tasks.

869 **Figure 2.** Predicted specific growth rate of three model microorganisms at different glucose
870 concentrations. The horizontal axis represents the dimensionless glucose concentration
871 determined by division by K_m (Eq. 7). ■ Auxotrophic lactic acid bacteria (LAB), ■
872 prototrophic lactic acid bacteria (LAB) and ■ butyrate-producing bacteria (BPB). The dashed
873 line represents specific growth rate value of 0.28 h^{-1}.

874 **Figure 3.** Molar product yields resulting from the competition of the three model
875 microorganisms at different glucose concentrations. The horizontal axis represents the
876 dimensionless glucose concentration determined by division by K_m (Eq. 7). ■ Lactic acid ■
877 butyrate and acetate (sum of the equimolar butyrate and acetate yields).

878 **Figure 4.** Contribution of each of the enzymatic groups to the membrane proteome (A) and
879 to the total proteome (B) of BPB and to the membrane proteome (C) and to the total
880 proteome (D) of LAB. ■ Glucose transport, ■ glycolysis, ■ lactic acid production, ■
881 butyrate production, ■ acetate production, ■ acid transport, ■ growth. The dashed lines
882 represent dilution rates of 0.28 and 0.33 h^{-1}.

883 **Figure 5.** Proportion of each enzymatic group in the proteome of butyrate-producing
884 bacteria (BPB, left graph) and lactic acid bacteria (LAB, right graph) at different dilution
885 rates. ■ Glucose transport ■ glycolysis ■ butyrate production ■ acetate production ■ lactic
886 acid production ■ acid transport ■ growth.

887 **Figure 6.** Uncertainty analysis results for the ratio between specific growth rate of LAB and
888 BPB at different non-dimensional glucose concentrations. The line represents the mean
889 value of the Monte Carlo simulations and the shaded area the region of ratio values within a
890 95% confidence interval (percentiles 2.5%-97.5%).

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