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Culture conditions affect *Lactobacillus reuteri* DSM 17938 ability to perform glycerol bioconversion into 3-hydroxypropionic acid

Thi-Lan-Phuong Nguyen, Claire Saulou-Bérion^{*}, Jérôme Delettre, Catherine Béal

Université Paris-Saclay, AgroParisTech, INRAE, UMR 0782 SayFood, F-78850, Thiverval-Grignon, France

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Lactobacillus reuteri, Plackett and Burman experimental design, growth medium composition, environmental culture conditions, glycerol bioconversion, 3-hydroxypropionic acid production yield

Abstract

The platform molecule 3-hydroxypropionic acid (3-HP) can be produced using *Lactobacillus reuteri* through a two-step bioprocess that involves a growth phase followed by a bioconversion phase. The bioproduction is performed by resting cells that convert glycerol into 3-HP and 1,3-propanediol in fed-batch mode. This work aimed at studying the effect of the growth conditions of *L. reuteri* DSM 17938 during the first step, on the glycerol bioconversion into 3-HP during the second step. A Plackett and Burman design was carried out to test, in controlled bioreactors, the effect of 11 growth conditions simultaneously, at fixed bioconversion conditions. The supplementation of the growth medium with vitamin B12 and cysteine displayed a negative effect on the 3-HP bioproduction. The addition of glucose, phytone peptone, Tween 80, 1,2-propanediol and betaine in the growth medium, together with a low temperature and an optimal pH of 6.0 during the growth phase increased the bioconversion duration from 56 h to 89 h at a glycerol feeding rate of 0.5 g·h⁻¹. A validating experiment displayed that the 3-HP titer, 3-HP

^{*} Corresponding author: Claire Saulou-Bérion. E-mail: claire.saulou@agroparistech.fr; phone number: +33 1 30 81 54 17; fax number: +33 1 30 81 55 97

23 production yield and 3-HP specific production rate were significantly improved by 25 %, 150
24 % and 61 %, respectively.

25 **Introduction**

26 Using microorganisms to produce valuable biomolecules from renewable resources has gained
27 great interest in the two last decades due to limitation of fossil fuel resources and overwhelming
28 environmental issues. In such context, the development of a biotechnological process to
29 produce the platform chemical 3-hydroxypropionic acid (3-HP) is a key issue as this weak
30 organic acid displays many applications. Firstly, it can be used as preservative in the food or
31 feed industry (1, 2). But its major attraction comes from its bi-functionality, i.e. the presence
32 of a carboxyl group and a β -hydroxyl group, which makes 3-HP the precursor of a large variety
33 of chemicals for multipurpose applications. It thus serves as a versatile agent for chemical
34 reactions such as dehydration, oxidation, reduction, cyclization or polymerization that lead to
35 the formation of acrylic acid, malonic acid, 1,3-PDO, propiolactone, valuable homopolymers
36 (poly 3-HP) or copolymers (containing 3-HP), respectively (3). This explains why it was
37 ranked as one of the top value-added platform molecules to be produced from biomass by the
38 US Department of Energy (4). Some chemical synthesis routes have been proposed to obtain
39 3-HP from acrylic acid, 3-propiolactone, 3-hydroxypropionitrile, allyl alcohol, vinyl acetate
40 and 1,3-PDO (5). However, these processes display several drawbacks such as the use of
41 expensive raw materials, the high costs for operating processes and associated environmental
42 issues (3).

43 Nowadays, a better alternative to produce 3-HP is based on biotechnological processes, by
44 employing either natural microbial producers or genetically modified organisms (GMO)
45 working as cell factories, including GMOs that do not produce 3-HP naturally and
46 microorganisms producing 3-HP but genetically modified to improve their performances.
47 Detailed information about genetically modified microorganisms is available in recent reviews

48 (6, 7). *L. reuteri* is an attractive candidate due to its safety characteristics, its probiotic
49 properties and its anaerobic but aerotolerant attributes (8). In considering 3-HP bioproduction,
50 *L. reuteri* is naturally capable of converting glycerol into 3-HP and 1,3-PDO in equimolar
51 proportions, as it possesses all the enzymes of the metabolic pathway for 3-HP biosynthesis
52 encoded by the *pdu* operon (9). It has the ability to synthesize vitamin B12 that is an essential
53 co-factor for the first enzyme of the glycerol metabolism (10), but cannot use glycerol as a
54 carbon source (11), which allows glycerol to be completely valorized into 3-HP and 1,3-PDO
55 through the bioconversion pathway.

56 The 3-HP bioproduction has to be achieved through a two-step process, which includes a
57 growth phase to produce a high cellular concentration and a second step to perform the glycerol
58 bioconversion into 3-HP by resting cells (12). Glycerol is firstly converted to 3-
59 hydroxypropionaldehyde (3-HPA) by a vitamin-B12 dependent glycerol dehydratase. 3-HPA
60 is then either converted to 1,3-PDO or 3-HP through parallel reductive and oxidative branches
61 that maintain the redox balance between NADH and NAD⁺. 1,3-PDO is obtained from 3-HPA
62 using the enzyme 1,3-propanediol oxidoreductase (13), while 3-HP is the product of three
63 successive reactions using propionaldehyde dehydrogenase, phosphotransacylase and
64 propionate kinase (12, 14). The conditions to be employed during the bioconversion step have
65 been identified with the aim of increasing the 3-HP concentration and productivity. The use of
66 fed-batch cultures with glycerol supply is required to prevent accumulation of the toxic
67 intermediate 3-HPA, since the rate of 3-HPA formation is higher than that of its subsequent
68 conversion (12). The maximum rate of glycerol supply to *L. reuteri* DSM 20016 has been
69 determined at 0.75 g·h⁻¹ to avoid 3-HPA accumulation (16). The use of micro-aerobiosis was
70 recommended to improve the 3-HP bioproduction (17). Finally, the absence of glucose was
71 shown to reduce 1,3-PDO production due to a redox cofactor imbalance (12) and to prevent
72 the synthesis of undesirable fermentation products (i.e. lactic acid, ethanol, acetic acid, CO₂).

73 The influence of some conditions implemented during the growth step, prior to bioconversion,
74 has been studied, in the vast majority of cases with the aim of improving the growth
75 performances. By considering the nutritional requirements of *L. reuteri*, the influence of carbon
76 and nitrogen sources has been determined. Different carbon sources have been used to trigger
77 *L. reuteri* growth, such as glucose alone (18, 19), glucose plus fructose, saccharose (19),
78 galactose, lactose, melibiose, raffinose, saccharose (20) and industrial wheat or sugar beet
79 syrup by-products that contained glucose and fructose (21). From these studies, glucose was
80 demonstrated as the better carbon source to maximize *L. reuteri* growth (21). The suggested
81 concentrations of glucose were comprised between 20 g·L⁻¹ (22) and 30 g·L⁻¹ (21). As *L.*
82 *reuteri* displays a heterofermentative metabolism, glucose is catabolized through two pathways
83 (phosphoketolase (PK) and Embden-Meyerhof-Parnas (EMP) pathways) that operate
84 simultaneously and lead to the production of lactic acid, acetic acid, ethanol, CO₂ and energy
85 (19).

86 The effects of various nitrogen sources have been established on *L. reuteri* cell growth. Yeast
87 extracts and peptones are commonly used for growth of lactic acid bacteria due to their
88 composition in amino acids and vitamins which meets cell needs. Yeast extract is the most
89 common source employed in the published studies concerning *L. reuteri* (21, 23). In addition,
90 phytone peptone, which is obtained from papain digestion of soybean meal, displays an
91 interesting composition with 18 of the 20 natural amino acids (asparagine and glutamine are
92 absent) and is a cheaper nutrient source than yeast extract. Phytone peptone was demonstrated
93 to lead to the highest cell concentration by comparison with other nitrogen sources such as
94 peptones of animal origin, tryptone, proteose peptone, tryptic soy broth, yeast extract, and beef
95 extract, in *L. reuteri* DSM 20016 (24) and other *L. reuteri* strains (PTA-4965, 23272, and
96 55730) (25).

97 The addition of the amino acid cysteine was also considered as this component is an essential
98 growth factor of some lactobacilli (26). For *L. reuteri* DSM 20016 and SD 2112 strains, it was

99 recommended at concentrations between $0.1 \text{ g}\cdot\text{L}^{-1}$ (24) to $0.5 \text{ g}\cdot\text{L}^{-1}$ (20). However, the addition
100 of $2 \text{ g}\cdot\text{L}^{-1}$ cysteine in the culture medium was mentioned to decrease the production of vitamin
101 B12 by *L. reuteri* JCM1112 by a factor 3 to 5 (10). This amino acid is also known as a potent
102 reducing agent, thus displaying antioxidative properties that help the cells to cope with the
103 oxidative stress encountered during the recovery and concentration steps, as demonstrated with
104 *L. fermentum* (27).

105 Tween 80 is a key growth factor in culture media for lactobacilli, as it brings unsaturated fatty
106 acids that allow reducing intracellular energy consumption, thanks to the down-regulation of
107 de novo fatty acid synthesis in membrane phospholipids (28). It is for instance present at a
108 concentration of $1 \text{ g}\cdot\text{L}^{-1}$ in MRS medium (12, 29) or added to reach $5 \text{ g}\cdot\text{L}^{-1}$ (21).

109 Besides, the influence of some other components in the culture medium of lactobacilli has been
110 investigated. Supplementation with vitamin B12 ($0.1 \text{ mg}\cdot\text{L}^{-1}$), as a mandatory cofactor for the
111 first enzyme in the *pdu* pathway, although not influencing *L. reuteri* growth, positively impacts
112 the 3-HP bioproduction (21). The addition of 1,2-propanediol (1,2-PDO) has been studied as it
113 is involved in the activation of genes of the *pdu* pathway (16, 30). It was added at a
114 concentration of $5 \text{ g}\cdot\text{L}^{-1}$ into *L. reuteri* DSM 20016 growth medium prior to glycerol
115 conversion (14). Betaine is a methylated derivative of the amino acid glycine that acts as an
116 osmolyte to help lactic acid bacteria counteract the osmotic shocks they face during the
117 harvesting and concentration stages (31). By considering *L. buchneri* R1102, it was
118 demonstrated that the combined supplementation with $2 \text{ mmol}\cdot\text{L}^{-1}$ betaine and $0.1 \text{ mol}\cdot\text{L}^{-1}$ KCl
119 in the growth medium, together with a further addition of $0.6 \text{ mol}\cdot\text{L}^{-1}$ KCl at the end of growth,
120 led to intracellular accumulation of betaine and improved cell survival during freeze-drying.

121 Formulating the environmental parameters (i.e. growth temperature, pH or the base used for
122 pH control) to improve cell growth and further bioconversion is also challenging. The
123 temperature for *L. reuteri* growth is generally established at $37 \text{ }^\circ\text{C}$ (18, 24, 32). However, the
124 optimum temperature for growth may differ from that maximizing a given metabolic activity.

125 For example, cultures of *L. fermentum* CRL 251 and *Bifidobacterium longum* CRL 849
126 implemented at 30 °C showed a slower growth but a stimulated alpha-galactosidase activity,
127 in comparison to that observed at 37 °C (33). In order to reduce inhibitory effects of acidic
128 conditions, the pH must be controlled during growth with an appropriate base solution. The
129 optimal pH for *L. reuteri* growth is generally between 6.0 and 6.8 (34). Recently, different pH
130 values between 3.7 and 6.7 were tested during batch cultures of *L. reuteri* DSM 12246 and the
131 final biomass concentration was enhanced at pH 5.5 (35). Finally, the use of two kinds of bases
132 have been reported for pH control during *L. reuteri* growth phase: NaOH (36, 37) and NH₄OH
133 (12, 38).

134 However, they have never been compared for bacterial growth or 3-HP bioproduction.

135 From this information, it can be seen that most previous works studied the effects of culture
136 conditions on *L. reuteri* growth but not on 3-HP bioproduction. They show that many culture
137 conditions during the growth phase affect the growth performances of *L. reuteri*. However the
138 effects of these culture conditions were scarcely investigated on cell ability to subsequently
139 convert glycerol into 3-HP. In addition, the reported studies focus on a limited number of
140 factors, thus making it difficult to define the optimal conditions by taking all of them into
141 account. In such context, the present work aimed at quantifying the effects of relevant culture
142 conditions on *L. reuteri* capacity to produce 3-HP during the subsequent glycerol bioconversion
143 stage. As 11 factors were selected, a Plackett and Burman experimental design was retained as
144 it allowed identifying the key factors that act on 3-HP bioproduction and quantifying their main
145 effects, together with reducing the number of experiments. All experiments were performed in
146 bioreactors in order to evaluate the effects of the factors in well-controlled conditions.

147 **Material and methods**

148 **Bacterial strain**

149 The strain *L. reuteri* DSM 17938 was obtained from BioGaia AB, Stockholm, Sweden. It was
150 stored at -80 °C in cryotubes with glycerol 20 % as cryoprotectant. The inoculum was prepared
151 by cultivating the cells in MRS broth (Biokar Diagnostic, Beauvais, France) for 16 hours at 37
152 °C and 100 rpm and then in MRS broth supplemented with glucose 20 g·L⁻¹ (VWR BDH
153 Prolabo, Leuven, Belgium) for 8 hours in the same conditions. Inoculation of the culture
154 medium was performed at a concentration of 5.1.10⁻⁶ g of cell dry weight (CDW) per liter
155 (g_{CDW}·L⁻¹), corresponding to 5.8.10⁴10³ cells·mL⁻¹. The specific growth rate was calculated by
156 plotting the natural logarithm of CDW against time.

157 **Culture and bioconversion media**

158 The reference culture medium used for bacterial growth was composed of MRS broth added
159 with 20 g·L⁻¹ glucose. In accordance with the experimental design, it was supplemented or not
160 with the following components: glucose 50 g·L⁻¹ (instead of 20 g·L⁻¹), yeast extract 25 g·L⁻¹
161 (Organotechnie S.A.S, La Courneuve, France), phytone peptone 25 g·L⁻¹ (Merck KGaA,
162 Darmstadt, Germany), Tween 80 4 g·L⁻¹ (VWR), vitamin B12 0.1 mg·L⁻¹ (Sigma-Aldrich,
163 Saint Louis, MO, USA), 1,2-PDO 3 g·L⁻¹ (Sigma-Aldrich), cysteine 1 g·L⁻¹ (Sigma-Aldrich),
164 betaine 0.234 g·L⁻¹ (Sigma-Aldrich) together with KCl 7.455 g·L⁻¹ (Prolabo, Fontenay-sous-
165 Bois, France). All media were autoclaved at 110 °C for 20 minutes. Additional glucose was
166 autoclaved separately to prevent its degradation due to the Maillard reaction (18).

167 The bioconversion medium was composed of sterile osmosis water into which a glycerol
168 (Sigma-Aldrich) solution at 100 g·L⁻¹ was added at a constant flow rate (29).

169 **Two-stage process for 3-HP production**

170 The two steps of 3-HP bioproduction were carried out in controlled bioreactors. Cell growth
171 was conducted in batch mode in a 5-L Sartorius B bioreactor (Sartorius, Dourdan, France). The
172 agitation rate was set at 100 rpm thanks to two Rushton propellers. The temperature and pH
173 during growth were defined according to the experimental design (33 or 37 °C, pH 5.5 or 6.0).
174 The pH was controlled either with NH₄OH 14.8 mol·L⁻¹ (Sigma-Aldrich) or with NaOH 8.75

175 mol·L⁻¹ (VWR). The growth step was stopped when the cells reached stationary phase. Bacteria
176 were harvested by centrifugation (Avanti[®] J-E centrifuge; Beckman Coulter, Fullerton, CA) at
177 6,200 g for 10 min at 4 °C. Cell pellets were then suspended in the same volume of sterile
178 osmosis water to reach about 26.7 ± 9.5 g_{CDW}·L⁻¹, corresponding to about 3.2·10¹⁰ ± 1.3·10¹⁰
179 cells·mL⁻¹.
180 Bioconversion was achieved in a 2-L bioreactor (Sétric Génie Industriel, Toulouse, France)
181 with an initial working volume of 0.8 L. It was performed in fed-batch mode, defined by a
182 constant glycerol feeding rate of 0.5 g·h⁻¹ to avoid 3-HPA accumulation. The pH was controlled
183 at 6.0 by addition of NH₄OH 1.48 mol·L⁻¹, the temperature was set at 37 °C and the agitation
184 rate was maintained at 100 rpm with two Rushton propellers. Bioconversion was stopped when
185 no more 3-HP was produced, which was evaluated by the stop of NH₄OH consumption.

186 **Evaluation of cell concentration and analysis of cell physiological state by flow** 187 **cytometry**

188 The bacterial cell concentration was determined indirectly by measuring optical density at 600
189 nm (OD₆₀₀) (spectrophotometer UV-Vis Evolution™ 201, Fisher Scientific SAS, Illkirch,
190 France). Then, bacterial concentrations expressed in CDW were obtained from OD₆₀₀ values
191 using the correlation: CDW = 0.2639 × OD₆₀₀ (R² = 0.99).

192 The physiological state of bacterial cells was assessed by flow cytometry after double
193 fluorescent staining with carboxyfluorescein diacetate (cFDA) and propidium iodide (PI) (39).
194 The cFDA allowed assessing cellular enzymatic activity after internalization and cleavage by
195 intracellular esterases, thus forming green fluorescent carboxyfluorescein (cF). Nucleic acid
196 dye PI was used to characterize cells with damaged membranes, in which it formed a
197 fluorescent DNA-complex. Samples were first diluted with McIlvaine buffer pH 7.3 (0.2
198 mol·L⁻¹ disodium dihydrogen phosphate (J. T. Baker, Deventer, NL) and 0.1 mol·L⁻¹ citric acid
199 (Fisher Chemical, Elancourt, France)) to reach a cell concentration of 10⁶ cells·mL⁻¹. One
200 milliliter of diluted sample was added simultaneously with 10 µL cFDA (Chemchrom V8,

201 Biomérieux, Marcy-l'Etoile, France) diluted at $100 \text{ mL}\cdot\text{L}^{-1}$ in acetone (Fisher Scientific,
202 Leicestershire, UK) and $10 \text{ }\mu\text{L}$ PI ($1 \text{ g}\cdot\text{L}^{-1}$ in distilled water, Sigma-Aldrich) before incubation
203 at $40 \text{ }^\circ\text{C}$ for 10 min. Flow cytometry analyses were performed with a BactiFlow cytometer
204 (Sysmex Partec, Roissy, France) equipped with the Flowmax software (Partec). Cell
205 fluorescence was measured at 530 nm for cF and 670 nm for PI after excitation by a 488-nm
206 emitting laser. Cells emitting in green only (530 nm) were considered as viable, while cells
207 emitting in red only (670 nm) were considered as dead. The data collected included cell
208 concentrations (in $\text{cells}\cdot\text{mL}^{-1}$) and percentages of viable and dead cells (29, 39).

209 **Quantification of substrates and metabolites**

210 Substrates used for growth (glucose) and bioconversion (glycerol) as well as growth products
211 (lactic acid, ethanol and acetic acid) and bioconversion metabolites (3-HP, 1,3-PDO and 3-
212 HPA) were quantified by high-performance liquid chromatography (Waters Associates,
213 Molsheim, France). Growth samples were diluted twice with trichloroacetic acid (Sigma-
214 Aldrich) at $60 \text{ g}\cdot\text{L}^{-1}$, then centrifuged at $13,000 \text{ g}$ for 10 min at $4 \text{ }^\circ\text{C}$ before filtration through a
215 $0.22 \text{ }\mu\text{m}$ pore-size filter (Sartorius Stedim Biotech, Gottingen, Germany). Bioconversion
216 samples were added with citric acid $5 \text{ g}\cdot\text{L}^{-1}$, centrifuged and filtered in the same conditions.
217 For each sample, a volume of $20 \text{ }\mu\text{L}$ was injected (Waters 717 plus) in mobile phase (H_2SO_4 ,
218 Sigma-Aldrich) on a cation-exchange column Aminex HPX-87H ($300 \text{ mm} \times 7.8 \text{ mm}$, Biorad,
219 Richmond, USA). Two sets of conditions including column temperature, H_2SO_4 concentration
220 and flow rate of the mobile phase, were applied to separate the components properly (Table 1).
221 Detection was done by a refractive index detector (Waters 2414) and an UV detector (Waters
222 2489). Quantification was achieved using the Empower software (Waters Associates) with the
223 help of external standards. All analyses were duplicated and concentrations are given in $\text{g}\cdot\text{L}^{-1}$.

224

Table 1. Conditions of HPLC analyses

	Condition A	Condition B
Temperature (°C)	35	60
H ₂ SO ₄ concentration in mobile phase (mmol·L ⁻¹)	5	0.5
Flow rate of mobile phase (mL·min ⁻¹)	0.6	0.4
Compounds detected in growth samples (and corresponding retention times, in min)	Glucose (9.3), lactic acid (13.2), acetic acid (15.6), ethanol (21.2)	
Compounds detected in bioconversion samples (and corresponding retention times, in min)	3-HPA (14.9), 1,3-PDO (17.5), acetic acid (15.6), ethanol (21.2) (the two latter remaining from growth)	Lactic acid (18.7, remaining from growth), 3-HP (19.7), glycerol (20.7)

225

226 **Assessment of carbon mass balance**

227 The carbon mass balance has been calculated during growth (CMB_G, in % mol·mol⁻¹)
 228 according to the central metabolism of *L. reuteri* via PK and EMP pathways and during
 229 bioconversion (CMB_B, in % mol·mol⁻¹), from the equations below:

$$230 \text{ CMB}_G = (3 \cdot n_{\text{Lactic acid}} + 2 \cdot n_{\text{Ethanol}} + 2 \cdot n_{\text{Acetic acid}} + n_{\text{CO}_2} + n_{\text{C in Biomass}}) / 6 \cdot n_{\text{Glucose}} \quad (\text{eq. 1})$$

$$231 \text{ CMB}_B = (n_{3\text{-HP}} + n_{1,3\text{-PDO}} + n_{3\text{-HPA}}) / n_{\text{Glycerol}} \quad (\text{eq. 2})$$

232 With *n* being the number of moles of consumed glucose (*n*_{Glucose}) and glycerol (*n*_{Glycerol}),
 233 produced lactic acid (*n*_{Lactic acid}), ethanol (*n*_{Ethanol}), acetic acid (*n*_{Acetic acid}), CO₂ (*n*_{CO₂})
 234 (hypothesized to be equal to the number of moles of produced [ethanol + acetate]), carbon in
 235 cells produced during growth (*n*_{C in Biomass}), 3-HP (*n*_{3-HP}), 1,3-PDO (*n*_{1,3-PDO}) and 3-HPA (*n*₃₋
 236 HPA).

237 **Experimental design and statistical analyses**

238 A Plackett and Burman matrix (40) allowed creating an experimental design to test the effects
 239 of 11 medium components and culture conditions on growth and subsequent 3-HP
 240 bioproduction by *L. reuteri* DSM 17938. The general form of the model is given as follows:

241 $Y_i = k_i + \sum a_i \cdot Y_{(+i)}$ (eq. 3)

242 Where Y_i is the response variable; k_i , the constant value of Y_i when all 11 variables are at their
243 Minus levels; a_i , the linear coefficient of each variable to express the effect of the Plus level;
244 and $Y_{(+i)}$, the value of the Plus level of each variable i .

245 The multiple regression analysis was performed using the XLSTAT software (Paris, France).
246 The 11 experimental factors tested in this study are listed in Table 2 that specifies the
247 concentrations or values characterizing the Minus and Plus levels in the experimental design.
248 The conditions used in each experiment are listed in Table S1. They were randomized and the
249 reference condition was performed in triplicate. The final cell concentration ($g_{CDW} \cdot L^{-1}$) was
250 selected as the response variable to describe the effect of the factors on the growth step. The
251 percentage of viable cells at the beginning of the bioconversion stage was included in the
252 analysis. Three response variables were chosen to characterize the effect of the experimental
253 factors on the bioconversion performance: total quantity of 3-HP produced at the end of the
254 fed-batch bioconversion (g_{3-HP}), 3-HP production yield ($g_{3-HP} \cdot g_{CDW}^{-1}$) and duration of the
255 bioconversion (h).

256 **Table 2. Factors included in the Plackett-Burman experimental design to study the**
 257 **effects of culture conditions of *L. reuteri* DSM 17938 on its bioconversion performances**

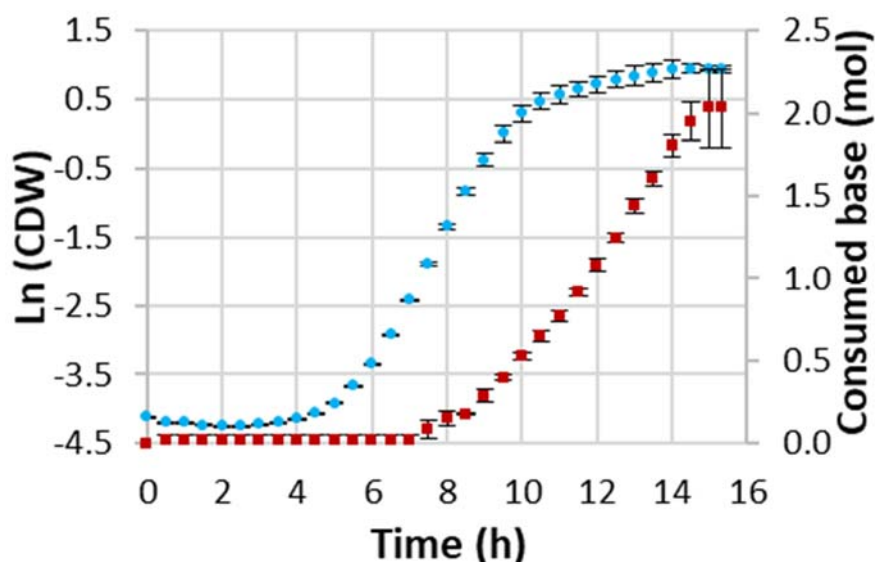
Medium components	Minus level	Plus level
Additional glucose (g·L ⁻¹)	20	50
Additional yeast extract (g·L ⁻¹)	0	25
Phytone peptone (g·L ⁻¹)	0	25
Tween 80 (g·L ⁻¹)	1	5
Vitamin B12 (mg·L ⁻¹)	0	0.1
1,2-propanediol (3 g·L ⁻¹)	0	3
Cysteine (g·L ⁻¹)	0	1
Betaine (g·L ⁻¹) and KCl (g·L ⁻¹)	0	0.234 0.745
Culture conditions		
Temperature (°C)	33	37
pH	5.5	6.0
Base type (and concentration, in mol·L ⁻¹)	NH ₄ OH (14.8)	NaOH (8.75)

258

259 **Results and Discussion**

260 **Kinetics of cell growth and glycerol bioconversion in the reference condition of the** 261 **experimental design**

262 In order to assess the kinetics of *L. reuteri* DSM 17938 in the reference condition, three
 263 replicates were performed. The experimental conditions are summarized with the code T1 in
 264 the Table S1. Figure 1 shows the cell growth kinetics during the first phase, whereas Figure 2
 265 displays the bioconversion kinetics of during the second step.



266

267 **Figure 1. Kinetics of *L. reuteri* DSM 17938 growth and acidification in the reference**
 268 **condition**

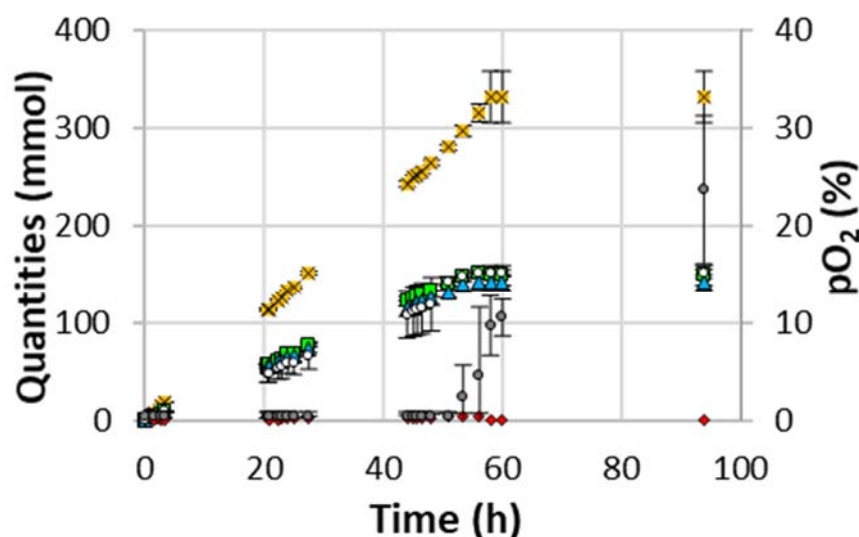
269 Blue circle: cell dry weight (CDW) expressed in $\text{g}_{\text{CDW}} \cdot \text{L}^{-1}$; red square: consumed base; error
 270 bars correspond to standard errors from 3 replicates.

271

272 From Figure 1, two successive exponential growth phases were observed, with different
 273 specific growth rates that were calculated at $0.99 (\pm 0.002) \text{ h}^{-1}$ from 6 h to 9.5 h, and then 0.13
 274 $(\pm 0.01) \text{ h}^{-1}$, between 10.5 h and 14 h, indicating that a substrate limitation occurred. This
 275 limitation cannot be due to a lack of carbon source as the glucose concentration was equal to
 276 $8.5 (\pm 1.7) \text{ g} \cdot \text{L}^{-1}$ at 10 h. It may be caused by a lack of another nutrient source that was not
 277 searched in this study or by an inhibition of bacterial growth by the accumulation of lactic acid
 278 (41) and ethanol (42) that reached $4.6 (\pm 0.9)$ and $2.0 (\pm 0.4) \text{ g} \cdot \text{L}^{-1}$, respectively after 10 h.
 279 Meanwhile, no limitation was observed on organic acid production since the base was
 280 consumed regularly during the logarithm phase. However, when no more glucose was available
 281 in the medium, the base consumption stopped suddenly, together with growth, indicating that
 282 no more organic acid was synthesized. These results were confirmed by HPLC analysis. The
 283 base profile was then used to precisely detect the growth stop. Quantitative results obtained
 284 within the three reference experiments are summarized with the codes T1a, T1b and T1c in
 285 Table S2. The carbon mass balance during growth was verified at $104.33 (\pm 3.82) \%$ for all the

286 experiments performed. This value was slightly higher than 100%, which could be explained
287 by the fact that some carbon used for the growth may come from other sources than glucose
288 (for instance yeast extract) that could not be quantified. A final CDW value of $3.3 (\pm 0.3)$
289 $\text{g}_{\text{CDW}}\cdot\text{L}^{-1}$ was achieved, which was similar to previous results obtained with the same *L. reuteri*
290 strain at pH 5.5 ($3.1 \text{ g}_{\text{CDW}}\cdot\text{L}^{-1}$) (12). From HPLC analyses, the presence of lactic acid ($16.4 (\pm$
291 $1.2) \text{ g}\cdot\text{L}^{-1}$), acetic acid ($3.7 (\pm 0.4) \text{ g}\cdot\text{L}^{-1}$) and ethanol ($7.6 (\pm 0.8) \text{ g}\cdot\text{L}^{-1}$) was detected. The
292 molar ratio of the sum of (ethanol + acetic acid) to lactic acid was close to 1 ($0.97 (\pm 0.05)$
293 $\text{mol}\cdot\text{mol}^{-1}$). This value indicated that the PK pathway was prevalent in this heterofermentative
294 *L. reuteri* strain, as previously observed by Burgé et al. (18). This value indicated that the PK
295 pathway was prevalent in this heterofermentative *L. reuteri* strain, as previously observed by
296 Burgé et al. (2015) (18). This result, obtained at controlled pH, is consistent with previous
297 studies on *L. reuteri* ATCC 55730 (19) and *L. reuteri* DSM 17938 grown in free-pH conditions
298 (18). Regarding the efficiency of ATP synthesis, the domination of PK pathway is unfavorable
299 compared to EMP pathway (production of 1 instead of 2 ATP). Nevertheless, by producing
300 less organic acid, the PK pathway reduced the inhibition of the bacteria by lactic acid, thus
301 improving their survival in an acidic environment (18).

302 Kinetics of glycerol bioconversion into 3-HP and 1,3-PDO are illustrated in Figure 2. The
303 carbon mass balance during bioconversion was verified at $100.12 (\pm 4.39) \%$. This step was
304 performed using resting cells of *L. reuteri*, to allow the NAD^+ regeneration through 3-HP
305 production in place of lactate production. The glycerol was fed into the bioreactor at a constant
306 feeding rate $0.5 \text{ g}_{\text{glycerol}}\cdot\text{h}^{-1}$ until the bioconversion stopped. The slope of glycerol consumption
307 was constant (Figure 2), which indicated that all substrate was consumed as and when it was
308 supplied. The glycerol consumption ceased at the same time as the base consumption, which
309 was confirmed from HPLC analyses. This indicated that the base profile was a relevant
310 indicator of the cessation of the bioconversion.



311

312 **Figure 2. Kinetics of glycerol bioconversion into 3-HP and 1,3-PDO by *L. reuteri* DSM**
 313 **17938 in the reference condition**

314 Yellow square with cross: consumed glycerol; green square: 3-HP; blue triangle: 1,3-PDO;
 315 red diamond: 3-HPA; white circle: consumed NH₄OH; grey circle: dissolved O₂; error bars
 316 correspond to standard errors from 3 replicates.

317

318 After 55.8 (± 2.2) h of bioconversion, two final products 3-HP and 1,3-PDO were obtained at
 319 final concentrations of 11.8 (± 0.5) g·L⁻¹ and 9.5 (± 0.5) g·L⁻¹, respectively. In comparison to
 320 the previous study of Dishisha et al. (2015) (16) using *L. reuteri* DSM 20016, the duration of
 321 bioconversion was similar (56 h compared to 58 h), but the final 3-HP titer was 16 % lower
 322 which was explained by the lower substrate feeding rate used in our study (0.5 g_{glycerol}·h⁻¹
 323 compared to 0.75 g_{glycerol}·h⁻¹). The maximal 3-HPA content detected into the bioconversion
 324 broth was equal to 0.4 \pm 0.1 g·L⁻¹. This value was about 10 times lower than the minimum
 325 inhibition concentration reported for *L. reuteri* DSM 20016 (2.2 – 3.7 g·L⁻¹) (43), thus
 326 protecting it from detrimental effects (44). 3-HP was produced equimolarly to 1,3-PDO, as the
 327 molar ratio was equal to 1.04 \pm 0.06 mol·mol⁻¹, which indicated that the redox balance between
 328 NAD⁺ and NADH was well maintained during the glycerol bioconversion. These results were
 329 close to those obtained by (12) who obtained a molar ratio of 1 mol·mol⁻¹. The molar ratio
 330 between 3-HP and NH₄OH was equal to 1.08 \pm 0.01 mol·mol⁻¹, which is a little bit higher than

331 1. As no other organic acid was detected from HPLC analyses, this small divergence can be
332 explained by a slight volatilization of the ammonia solution during the bioconversion. The total
333 amount of 3-HP and 1,3-PDO produced at the end of the fed-batches were respectively equal
334 to 13.4 ± 0.9 g and 10.8 ± 0.7 g. These values were lower than those obtained by (16) due to
335 the lower supply rate of glycerol as aforementioned. The 3-HP production yield was equal to
336 0.78 ± 0.11 g_{3-HP}.g_{CDW}⁻¹, which was also lower than the higher value reported by (16).

337 Micro-aerobic conditions were applied in this study to facilitate glycerol metabolism toward 3-
338 HP production (17). The partial pressure of dissolved oxygen (pO₂) in the medium remained
339 at a value lower than 0.5 % until 51 h (Figure 2). At that time, the base consumption started to
340 decrease, 3-HP and 1,3-PDO production ceased and pO₂ increased. This link between the pO₂
341 increase and the cessation of bioconversion is consistent with previous works (17, 45). This
342 pO₂ increase may be due to a reduction of the activity of the enzyme NAD(P)H oxidase that
343 uses molecular oxygen as a substrate (45). We may hypothesize that the stopping of the
344 bioconversion corresponded to a global dysfunction of the whole enzyme pool of bacterial
345 cells. In that case, the catalytic work of the NAD(P)H oxidase stopped concomitantly with that
346 of the enzymes of the *pdu* operon that drives the 3-HP bioproduction. The decrease in oxygen
347 consumption is thus observed simultaneously to the reduction in 3-HP bioproduction.
348 Moreover, our results indicate that the pO₂ measurement may constitute an early on-line
349 indicator of the cessation of bioconversion, which is an original approach for controlling the
350 fed-batch bioconversion of glycerol into 3-HP.

351 **Effect of culture conditions on *Lactobacillus reuteri* DSM 17938 growth performances**

352 According to the Plackett and Burman experimental design (Table S1), various growth medium
353 recipes and culture conditions were designed to screen, among 11 factors, the best combination
354 to improve bacterial growth. The final biomass concentration (g_{CDW}·L⁻¹) was retained as the
355 variable to characterize the bacterial growth performance. Indeed, as the growth occurred
356 during night without being monitored by a biomass probe, the effect of the conditions on the

Table 3. Effect of growth conditions on cell concentration and on glycerol bioconversion performances of *L. reuteri* DSM 17938

	Cell concentration at the end of growth (g_{CDW}·L⁻¹)	Total quantity of 3- HP produced (g)	Bioconversion duration (h)	3-HP production yield (g_{3-HP}·g_{CDW}⁻¹)
Constant	3.90	14.12	64.1	0.75
Additional glucose	0.58**	ns	4.0**	-0.23**
Additional yeast extract	ns	ns	ns	ns
Phytone peptone	ns	0.94*	5.7**	0.11*
Tween 80	ns	0.84*	2.1*	0.10*
Vitamin B12	ns	-2.16**	-10.6***	-0.15**
1,2-propanediol	ns	1.03*	4.5**	0.093*
Cysteine	ns	ns	ns	-0.10*
Betaine and KCl	ns	ns	4.6**	0.10*
Temperature	0.53**	ns	2.1*	-0.15**
pH	ns	ns	ns	0.096*
Base	ns	ns	ns	ns
R²	0.873	0.893	0.978	0.919

Confidence level: * 90 %; ** 95 %; *** 99 %; ns: not significant; R² adjusted for degree of freedom.

357 specific growth rate could not be established. The results obtained from the 14 experiments are
358 summarized in Table S2 and were used for statistical analyses. From Table 3, only two factors
359 displayed a significant effect on *L. reuteri* DSM 17938 final concentration: the addition of
360 glucose and the higher temperature, which positively influenced the final biomass
361 concentration, by 15 % and 20 %, respectively. This result was confirmed by considering the
362 number of viable cells at the end of the culture, enumerated by flow cytometry. It was improved
363 by 34 % in the presence of 50 g·L⁻¹ glucose, which matches with the aforementioned result.
364 This positive effect of glucose on *L. reuteri* growth is consistent with early studies (18, 19, 21).
365 Conversely, the addition of yeast extract or phytone peptone to the MRS medium as nitrogen
366 sources did not improved neither the biomass concentration nor the cell viability. This result is
367 contrary to those obtained in previous studies (21, 24) and suggested that the growth limitation
368 previously observed in the growth phase cannot be justified by a lack of these nutrients. The
369 existence of interactions between factors, which cannot be considered by the type of
370 experimental design used in our study, can partly explain this observation.

371 Tween 80 and cysteine are both growth factors for lactobacilli. However, the current
372 supplementation of these compounds at 5 g·L⁻¹ and 1 g·L⁻¹ respectively, seemed not enough to
373 increase the cell concentration. The addition of vitamin B12, 1,2-PDO and betaine together
374 with KCl did not affect the growth, which was expected as they were added to act during the
375 bioconversion stage.

376 The temperature positively affected the biomass concentration that was higher at 37 °C than at
377 33 °C, which was confirmed by flow cytometry measurements (5.6.10⁹ cell·mL⁻¹ instead of
378 4.5.10⁹ cell·mL⁻¹). This result is consistent with the conditions found in the digestive tract, the
379 ecological niche of *L. reuteri* (46).

380 Finally, the pH value (pH 5.5 or pH 6.0) and the base used for pH control (NH₄OH or NaOH)
381 did not significantly modify the final cell concentration of *L. reuteri* DSM 17938. Therefore,
382 they can be used equally to maintain the pH during the growth phase.

383 **Effect of culture conditions on *L. reuteri* DSM 17938 ability to convert glycerol into 3-**
384 **HP**

385 The Plackett and Burman experimental design allowed identifying the growth medium
386 composition and culture conditions that affected the ability of *L. reuteri* DSM 17938 to further
387 perform glycerol bioconversion into 3-HP. During all experiments, no residual glycerol was
388 detected in the bioconversion broth. Table 3 summarizes the results obtained from the 14
389 different experiments (including three replicates of the reference condition), to explain the
390 effects of the 11 factors on three variables that characterized the bioconversion step: quantity
391 of 3-HP produced, bioconversion duration and 3-HP production yield. From Table 3, nine
392 factors displayed a statistically significant effect on these variables. Only two factors did not
393 show any significant effect on the characteristic variables of the 3-HP production step: the
394 addition of yeast extract and the type of base used for pH control. The three variables were
395 positively influenced by the addition of phytone peptone, Tween 80 and 1,2-PDO but
396 negatively by a vitamin B12 supplementation. A high glucose concentration and a high
397 temperature positively affected the bioconversion duration but negatively the 3-HP production
398 yield. The addition of betaine enhanced the bioconversion duration and the 3-HP production
399 yield. Finally, the addition of cysteine and a low pH value negatively influenced the 3-HP
400 production yield. In addition to these three variables, the quantity of 1,3-PDO produced and
401 the molar ratio between 3-HP and 1,3-PDO were also determined and will be analyzed when
402 required.

403 As explained above, the growth medium supplementation with a higher glucose concentration
404 led to a higher quantity of harvested biomass and a higher percentage of viable cells at the
405 beginning of bioconversion. However, in the experimental conditions chosen during the
406 bioconversion step, no increase of 3-HP production was observed, which is explained by the
407 moderate glycerol feeding rate ($0.5 \text{ g}_{\text{glycerol}} \cdot \text{h}^{-1}$) used in this study. In other words, the quantity
408 of glycerol provided was insufficient to feed the higher cell quantity obtained in this condition.

409 As a consequence, the specific feeding rate of glycerol was lower in the condition of the higher
410 level of glucose addition ($18.1 \pm 3.5 \text{ mg}_{\text{glycerol}} \cdot \text{g}_{\text{CDW}}^{-1} \cdot \text{h}^{-1}$) as compared to that observed with
411 the lower level of glucose addition ($32.9 \pm 8.6 \text{ mg}_{\text{glycerol}} \cdot \text{g}_{\text{CDW}}^{-1} \cdot \text{h}^{-1}$). This observation was
412 supported by the decrease of the 3-HP production yield (- 30.7 %). The longer duration of
413 bioconversion (+ 6.2 %) noticed when the cells were previously cultivated in the presence of a
414 higher glucose concentration might be explained by a better enzymatic activity of the total
415 bacterial population at the beginning of bioconversion stage, as observed by cF fluorescence
416 measurements. This better activity allowed the cells to convert glycerol for a longer time.
417 Results showed that phytone peptone slightly promoted the 3-HP bioproduction whereas yeast
418 extract did not (Table 3). As these two compounds act as amino acid sources, the positive effect
419 of phytone peptone can be related to a specific amino acid composition that differs from that
420 of yeast extract. From the information available from the suppliers, phytone peptone contained
421 more arginine, histidine, tyrosine (+ 200 %) and glycine (+ 90 %) than yeast extract.
422 Particularly, the amino acids arginine and glycine have been shown to be used for energy
423 production and were linked to the survival of *Lactococcus lactis* under starvation (47) and
424 *Lactobacillus sakei* under acidic conditions (48).
425 A positive effect of Tween 80 supplementation was observed on cell ability to produce 3-HP
426 (Table 3) that is consistent with (21). This component is known to support cell division of
427 lactobacilli by modifying the cell membrane fatty acids composition (49). In addition, it
428 induces a down-regulation of the *de novo* fatty acid synthesis that helps the cells to save
429 intracellular energy (28).
430 Addition of 1,2-PDO significantly improved the three variables characterizing the
431 bioconversion step (Table 3), in agreement with (16). Here we consider that due to the
432 structural similarity between glycerol and 1,2-PDO, the addition of the latter in the growth
433 medium might help the cells to prepare their enzymatic machinery.

434 A negative effect of the addition of $0.1 \text{ mg}\cdot\text{L}^{-1}$ vitamin B12 in the growth medium was observed
435 on the quantity of 3-HP produced, the bioconversion duration and the 3-HP production yield
436 (Table 3). This result was an unintended outcome because this compound is a required cofactor
437 of the first enzyme of the *pdu* metabolic pathway (12). It was reported that a supplementation
438 of vitamin B12 at $0.1 \text{ mg}\cdot\text{L}^{-1}$ in the bioconversion medium showed a positive impact on 3-HP
439 production by *L. reuteri* DSM 17938 (21). Thus, it could be hypothesized that the early addition
440 of exogenous vitamin B12 in the growth medium may reduce the ability of the cells to produced
441 endogenous vitamin B12 during the further step of bioconversion.

442 The addition of betaine together with KCl led to a longer bioconversion, together with a higher
443 production yield (Table 3) that can be explained by the protective effect of betaine to osmotic
444 stress (50). This result can be linked to that of (31) who related a higher intracellular betaine
445 content to a more rigid membrane that led to the reduction of water exchange between the
446 intracellular and extracellular compartments in *L. buchneri* R1102 (31).

447 The addition of cysteine was related to a reduction of the 3-HP production yield (Table 3). This
448 observation can be explained by its negative effect on vitamin B12 biosynthesis by *L. reuteri*,
449 as previously demonstrated (10). As another hypothesis, cysteine may counteract the oxygen
450 utilization by the cells, as suggested by (27).

451 Regarding the influence of the growth temperature on the further bioconversion step, a higher
452 3-HP production yield but shorter bioconversion duration was observed when the growth was
453 conducted at $33 \text{ }^{\circ}\text{C}$ instead of $37 \text{ }^{\circ}\text{C}$ (Table 3). As the glycerol feeding rate was fixed at a
454 moderate value of $0.5 \text{ g}_{\text{glycerol}}\cdot\text{h}^{-1}$ during bioconversion, this higher 3-HP production yield was
455 the consequence of the lower cell concentration achieved at the end of the growth phase.

456 The pH value during growth had a very little effect on the bioconversion step. Only the 3-HP
457 production yield decreased slightly when the pH was reduced from 6.0 to 5.5 (Table 3). This
458 difference could be ascribed to a small, even not significant, increase in the final biomass
459 concentration when the growth was conducted at pH 5.5. Finally, it was demonstrated that the

460 type of base used had no effect, thus allowing us to use them indiscriminately. However, as the
461 base NH_4OH prevents the formation of salt in the medium, this base might be preferred to
462 facilitate further downstream processes.

463 **Selected growth conditions that improve *L. reuteri* DSM 17938 ability to convert** 464 **glycerol into 3-HP**

465 In order to enhance the capacity of *L. reuteri* DSM 17938 to perform 3-HP bioproduction at a
466 glycerol feeding rate of $0.5 \text{ g}_{\text{glycerol}} \cdot \text{h}^{-1}$, the growth conditions leading to the best 3-HP titer,
467 bioconversion duration and 3-HP production yield have been identified. They consist in MRS
468 medium added with glucose ($20 \text{ g} \cdot \text{L}^{-1}$), phytone peptone ($25 \text{ g} \cdot \text{L}^{-1}$), Tween 80 ($4 \text{ g} \cdot \text{L}^{-1}$), 1,2-
469 PDO ($3 \text{ g} \cdot \text{L}^{-1}$), betaine ($0.234 \text{ g} \cdot \text{L}^{-1}$) plus KCl ($0.745 \text{ g} \cdot \text{L}^{-1}$), temperature ($33 \text{ }^\circ\text{C}$) and pH 6.0.
470 No additional yeast extract, no supplementation with vitamin B12 nor cysteine were required,
471 and the base to control the pH was likely unimportant. This set of conditions has been assayed
472 to demonstrate its positive effects on the bioconversion performances. In comparison to the
473 reference condition, the 3-HP titer was increased from $11.8 \pm 0.5 \text{ g} \cdot \text{L}^{-1}$ to $14.7 \text{ g} \cdot \text{L}^{-1}$, the
474 bioconversion duration from $55.8 \pm 2.2 \text{ h}$ to 88.6 h , and the 3-HP production yield from $0.8 \pm$
475 $0.1 \text{ g}_{3\text{-HP}} \cdot \text{g}_{\text{CDW}}^{-1}$ to $2.0 \text{ g}_{3\text{-HP}} \cdot \text{g}_{\text{CDW}}^{-1}$. In addition, the specific 3-HP production rate was
476 improved from $14.0 \pm 2.5 \text{ mg}_{3\text{-HP}} \cdot \text{g}_{\text{CDW}}^{-1} \cdot \text{h}^{-1}$ to $22.5 \text{ mg}_{3\text{-HP}} \cdot \text{g}_{\text{CDW}}^{-1} \cdot \text{h}^{-1}$. As the cell concentration
477 at the end of the growth phase ($3.0 \text{ g}_{\text{CDW}} \cdot \text{L}^{-1}$) was similar to that of the reference condition (3.3
478 $\pm 0.3 \text{ g}_{\text{CDW}} \cdot \text{L}^{-1}$), this improvement could be explained by a better ability of each cell to produce
479 3-HP from glycerol. This was supported by the higher ratio between 3-HP production to
480 enzymatically-active cells determined by flow cytometry, which increased from $0.98 \pm 0.21 \text{ g}_{3\text{-HP}}$
481 $\cdot \text{g}_{\text{viable cells}}^{-1}$ in the reference condition to $2.05 \text{ g}_{3\text{-HP}} \cdot \text{g}_{\text{viable cells}}^{-1}$ in the optimized recipe.

482

483 In conclusion, the influence of various growth conditions was assessed on the growth efficiency
484 and on the ability of resting cells of *L. reuteri* DSM 17938 to produce 3-HP at a given glycerol
485 feeding rate of $0.5 \text{ g} \cdot \text{h}^{-1}$. The implementation of a Plackett and Burman experimental design

486 enabled 11 factors to be tested. The supplementation of MRS medium with glucose and the use
487 of a higher temperature (i.e. 37 °C) induced a greater cell quantity at the end of the growth
488 phase. Meanwhile, the addition of phytone peptone, Tween 80, 1,2-PDO, betaine with KCl and
489 the use of a suboptimal temperature together with an optimal pH, were recognized as options
490 to improve the bioconversion duration and the 3-HP production yield. The best set of conditions
491 has been validated as it enhanced the 3-HP titer (+ 25 %), the 3-HP production yield (+ 150 %)
492 and the 3-HP specific production rate (+ 61 %).

493 With the aim of further improving the 3-HP bioproduction by *L. reuteri* DSM 17938, the
494 environmental conditions during the bioconversion step will have to be optimized, together
495 with avoiding 3-HPA accumulation. Finally, in order to better understand the cellular
496 modifications that are linked to the improvement of 3-HP bioproduction, a more in-depth
497 analysis of the physiological state of the cells may be done, mainly by using omic methods.

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Table S1. Experimental factors and their levels in the Plackett and Burman experimental design

Experiment code	Experimental factors										
	Additional glucose (g·L ⁻¹)	Additional yeast extract (g·L ⁻¹)	Phytone peptone (g·L ⁻¹)	1,2-propanediol (g·L ⁻¹)	Cysteine (g·L ⁻¹)	Betaine and KCl (g·L ⁻¹)	Tween 80 (g·L ⁻¹)	Vitamin B12 (mg·L ⁻¹)	Temperature (°C)	pH	Base type (and concentration, in mol·L ⁻¹)
T1	20	0	0	0	0	0 and 0	1	0	37	6.0	NH ₄ OH (14.8)
T2	50	0	25	0	0	0 and 0	5	0.1	33	6.0	NaOH (8.75)
T3	50	25	0	3	0	0 and 0	1	0.1	37	5.5	NH ₄ OH (14.8)
T4	20	25	25	0	1	0 and 0	1	0	37	5.5	NaOH (8.75)
T5	50	0	25	3	0	0.234 and 0.745	1	0	33	5.5	NaOH (8.75)
T6	50	25	0	3	1	0 and 0	5	0	33	6.0	NaOH (8.75)
T7	50	25	25	0	1	0.234 and 0.745	1	0.1	33	6.0	NH ₄ OH (14.8)
T8	20	25	25	3	0	0.234 and 0.745	5	0	37	6.0	NH ₄ OH (14.8)
T9	20	0	25	3	1	0 and 0	5	0.1	33	5.5	NH ₄ OH (14.8)
T10	20	0	0	3	1	0.234 and 0.745	1	0.1	37	6.0	NaOH (8.75)
T11	50	0	0	0	1	0.234 and 0.745	5	0	37	5.5	NH ₄ OH (14.8)
T12	20	25	0	0	0	0.234 and 0.745	5	0.1	33	5.5	NaOH (8.75)

Table S2. Data characterizing *L. reuteri* DSM 17938 growth and glycerol bioconversion performances

	Cell concentration (g_{CDW}·L⁻¹)	Top 3-HP titer (g·L⁻¹)	Top 1,3-PDO titer (g·L⁻¹)	Total 3-HP produced (g)	Total 1,3-PDO produced (g)	3-HP production yield (g_{3-HP}·g_{CDW}⁻¹)	Bioconversion duration (h)
T1a	3.4	11.2	9.4	12.4	10.4	0.76	57.7
T1b	3.0	11.9	9.0	13.7	10.4	0.90	53.4
T1c	3.6	12.3	10.0	14.1	11.5	0.68	56.2
T2	3.0	10.9	10.0	12.4	11.3	0.60	51.4
T3	4.8	9.9	10.4	11.1	11.6	0.42	52.2
T4	3.2	11.0	10.0	13.3	12.1	0.88	63.2
T5	5.1	13.9	14.0	18.9	19.0	0.61	92.3
T6	5.7	12.7	12.8	16.1	16.1	0.50	75.6
T7	4.5	9.9	11.3	11.9	13.5	0.35	64.3
T8	3.0	14.7	10.9	19.6	15.7	1.99	88.6
T9	4.5	11.7	10.8	14.3	13.2	0.68	58.8
T10	2.4	10.1	8.0	11.0	8.7	0.83	44.1
T11	3.8	12.9	12.7	16.3	16.0	0.63	72.7
T12	3.5	9.8	9.2	11.1	10.4	0.68	50.1