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1 **Isopropanol production from carbon dioxide in *Cupriavidus***  
2 ***necator* in a pressurized bioreactor.**

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12 **ABSTRACT**

13 A bioreactor was designed to provide high gas mass transfer to reach cell and product titres in  
14 the  $\text{g L}^{-1}$  level from  $\text{CO}_2$  for realistic, laboratory scale, engineered autotrophic strain  
15 evaluation. The design was based on independent  $\text{CO}_2$ ,  $\text{H}_2$  and air inputs and the ability to  
16 operate at high pressures. The bioreactor configuration and cultivation strategy enabled  
17 growth of *Cupriavidus necator* strains for long periods, to reach over  $3 \text{ g L}^{-1}$  dry cell weight.  
18 No negative impact of the high pressure was observed on viability of the strains up to more  
19 than 4 bar overpressure. The cultivation was then carried out using an engineered isopropanol  
20 producing strain; in this case,  $3.5 \text{ g L}^{-1}$  isopropanol was obtained from  $\text{CO}_2$  as the sole carbon  
21 source. This is the first reported demonstration of a successful production from engineered  
22 bacteria of product in the  $\text{g L}^{-1}$  range on  $\text{CO}_2$ , raising the prospect of future development of  
23  $\text{CO}_2$ -based bioprocesses.

24

25 **KEYWORDS**

26 *Ralstonia eutropha*; *Cupriavidus necator*; isopropanol; carbon dioxide; hydrogen; bioreactor  
27 design; gas fermentation; autotrophy; pressure

28 **ABBREVIATIONS**

29 P(3HB): poly(3-hydroxybutyrate); TSB: tryptic soy broth; DCW: dry cell weight.

30

## 31 INTRODUCTION

32 Developing bioconversion processes based on CO<sub>2</sub> has gained increasing interest in the  
33 microbial production of biofuel and chemical synthons. To develop future bioprocesses for  
34 bio-based chemical synthesis using CO<sub>2</sub>, the bacterium *Cupriavidus necator* is particularly  
35 attractive due to its efficient utilization of this sustainable carbon source. This facultative  
36 chemolithoautotroph, formerly also classified as *Ralstonia eutropha*, is the best-reported  
37 microorganism based on CO<sub>2</sub> as the sole carbon source. It has the ability to grow at high rates  
38 (in the range of 0.3-0.4 h<sup>-1</sup>) to high cell densities using gas mixtures of CO<sub>2</sub>/H<sub>2</sub>/O<sub>2</sub> [1]. It has  
39 also demonstrated higher energy efficiencies in producing biomass and bioproduct, poly(3-  
40 hydroxybutyrate) (P(3HB)) compared to plants or microalgae [2]. *C. necator* has also been  
41 extensively studied for P(3HB) production in heterotrophy [3-6] and autotrophy [1]. This  
42 natural ability to store excess carbon in the form of P(3HB) is of great interest as numerous  
43 interesting chemicals share the same production pathway precursor acetyl-coA, which implies  
44 that fewer genetic modifications would be required to divert P(3HB) precursors into the final  
45 product [7, 8].

46 Recently, *C. necator* was successfully engineered for the production of various compounds  
47 under autotrophic conditions from a CO<sub>2</sub>/H<sub>2</sub>/O<sub>2</sub> mixture. Up to 250 mg L<sup>-1</sup> isopropanol [8],  
48 180 mg L<sup>-1</sup> methyl ketones [9], 4.4 mg L<sup>-1</sup> alka(e)nes [7] and 6 mg L<sup>-1</sup> α-humulene [10] were  
49 obtained under autotrophic conditions. The limited amount of product reported here is due to  
50 a combination of strain efficiency and unsuitability of the laboratory-scale bioreactor for gas  
51 fermentation. The previous cultivation systems using classical bioreactor flushed with  
52 commercial gas mixtures have demonstrated production levels suitable only for proof of  
53 concept studies. In order to further develop the engineered strains for future CO<sub>2</sub>-based

54 bioprocesses, laboratory-scale bioreactor systems have to be designed specifically for gas  
55 fermentation, allowing higher titres of biomass to be achieved and to characterize the strains  
56 under more realistic conditions in terms of product titres, productivity and yields.

57 Bioreactor designs for gas fermentation have previously been reported for cultivation of H<sub>2</sub>  
58 oxidising bacteria such as *C. necator*. They generally consist of gas mixtures containing CO<sub>2</sub>,  
59 H<sub>2</sub> and O<sub>2</sub> added through a single sparger. The optimal gas composition employed in the  
60 literature for autotrophic growth of *C. necator* is typically H<sub>2</sub>/O<sub>2</sub>/CO<sub>2</sub>=7:1:1 [11-13],  
61 H<sub>2</sub>/O<sub>2</sub>/CO<sub>2</sub>=7:2:1 [12], or H<sub>2</sub>/O<sub>2</sub>/CO<sub>2</sub>=6:2:1 [14, 15]. However, these gas mixtures lie within  
62 the explosive composition range (which is normally regarded as being above 6% O<sub>2</sub> in the  
63 presence of H<sub>2</sub>) and limits the cell densities reached. In order to avoid this O<sub>2</sub> limitation  
64 during autotrophic growth of *C. necator*, pure oxygen has been fed to the bioreactor [1]. This  
65 was combined with a basket-type agitator which generated huge increases in the k<sub>L</sub>a (up to  
66 2970 h<sup>-1</sup>), allowing a high cell density of 91g L<sup>-1</sup> and a residual biomass containing 68% of  
67 PHB (29 g L<sup>-1</sup> of catalytic biomass concentration) to be reached.

68 The design of a small laboratory-scale bioreactor dedicated to CO<sub>2</sub> fermentation of H<sub>2</sub>-  
69 oxidizing bacteria is described here. The challenges tackled were (i) the danger of gas  
70 explosion above the limit of 6% O<sub>2</sub> in the presence of excess H<sub>2</sub> and (ii) the gas transfer  
71 efficiency at small scale. Reducing the size of a bioreactor is always more challenging for  
72 parameters such as gas transfer and necessitates the application of process engineering and  
73 biochemical engineering principles. The engineering solution to poor gas transfer is usually  
74 limited to the volumetric gas transfer coefficient, k<sub>L</sub>a, increase through changes in sparger and  
75 stirring arrangement as mentioned above. However, this offers relatively limited scope for  
76 improvement. An effective alternative is to operate the bioreactor at elevated pressure as this

77 can, in principle, increase the gas transfer rate several fold without changes to sparging or  
78 agitation. This concept has already been successfully applied in bioreactors with various  
79 microorganisms, such as *Arxula adenivorans*, *Corynebacterium glutamicum*, *C. necator*,  
80 *Escherichia coli*, and *Saccharomyces cerevisiae* [16-20]. There is, however, a lack of  
81 information on its impact on cell viability and productivity with engineered microorganisms  
82 during long fermentations runs.

83 Moreover, in order to have more flexibility in terms of gas composition and therefore higher  
84 gas solubility, the bioreactor gas-supply system was designed with three independent gas lines  
85 supplying three spargers (CO<sub>2</sub>, H<sub>2</sub> and air). A safety system was designed to control O<sub>2</sub> level  
86 in the headspace in order to avoid an explosive mixture within the bioreactor. Experimental  
87 validation was performed with autotrophic growth and production of isopropanol with an  
88 engineered *C. necator* strain.

## 89 **MATERIALS AND METHODS**

### 90 **Strains**

91 In this study, the reference strain used was *C. necator* Re2133, deficient for P(3-HB)  
92 biosynthesis (H16 Δ*phaCAB*) obtained from [21]. The isopropanol strain tested in this study  
93 was *C. necator* Re2133 bearing the plasmid pEG7b carrying a synthetic isopropanol operon  
94 under the constitutive pTac promoter [22]. The strains were stored at -80°C in liquid TBS  
95 medium (dextrose-free tryptic soy broth, Becton Dickinson, France) with 20 % glycerol (v/v).

### 96 **Media**

97 The rich medium consisted of 2.75% (w/v) dextrose-free tryptic soy broth (TSB, Becton  
98 Dickinson, France). The minimal medium A used for preculture in flask was previously

99 described by [21] and [7]. Fructose ( $20 \text{ g L}^{-1}$ ) was used as the only carbon source.  $\text{NH}_4\text{Cl}$  ( $0.5$   
100  $\text{g L}^{-1}$ ) was used as the nitrogen source to reach a biomass concentration of about  $1 \text{ g L}^{-1}$ . The  
101 minimal medium B used for the cultures in the bioreactor consisted of  $0.19 \text{ g L}^{-1}$   
102 nitrilotriacetic acid,  $0.06 \text{ g L}^{-1}$  ferrous ammonium citrate,  $0.5 \text{ g L}^{-1}$  of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $0.01 \text{ g L}^{-1}$   
103 of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , and 1 ml of trace element solution. The composition of the latter was  $0.3 \text{ g}$   
104  $\text{L}^{-1}$   $\text{H}_3\text{BO}_3$ ,  $0.2 \text{ g L}^{-1}$   $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $0.1 \text{ g L}^{-1}$   $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $0.03 \text{ g L}^{-1}$   $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ,  $0.03 \text{ g L}^{-1}$   
105  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ,  $0.02 \text{ g L}^{-1}$   $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ , and  $0.01 \text{ g L}^{-1}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ .  $(\text{NH}_4)_2\text{SO}_4$  ( $2 \text{ g L}^{-1}$ )  
106 was used as nitrogen source to reach a biomass concentration of about  $4 \text{ g L}^{-1}$ . After  
107 autoclaving the above medium, 40 ml of a sterile phosphate solution containing  $224 \text{ g L}^{-1}$   
108  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  and  $37.5 \text{ g L}^{-1}$   $\text{KH}_2\text{PO}_4$  was aseptically added to the fermenter.

### 109 **Seed cultivations**

110 After thawing at room temperature, one glycerol stock was streaked on a TSB agar Petri dish.  
111 and incubated for 36 h at  $30^\circ\text{C}$ . One isolated colony was used to inoculate the first seed  
112 culture grown for 24 h with 5 mL of TSB supplemented with  $10 \text{ mg L}^{-1}$  gentamycin and  $200$   
113  $\text{mg L}^{-1}$  kanamycin, in a 50 mL baffled Erlenmeyer flask. This culture was used to inoculate a  
114 250 mL Erlenmeyer baffled flask containing 50 mL of mineral medium A. The seed was  
115 cultivated at  $30^\circ\text{C}$ , 100 rpm for 18h. The culture was then centrifuged (5 min,  $10000g$ ), the  
116 supernatant was discarded and the cell pellet was resuspended in 30 mL sterile fresh medium  
117 B to eliminate the residual fructose. The suspension was then used to inoculate the bioreactor  
118 containing 300 mL of sterile mineral medium B.

### 119 **Culture conditions and bioreactor system**

120 The cultures were performed in a 500 ml total volume Xplorer® (HEL Ltd, UK) bioreactor  
121 that was modified specifically for this study. The working liquid volume was 330 mL. The

122 bioreactor was first used with a commercial gas mixture containing H<sub>2</sub>/O<sub>2</sub>/CO<sub>2</sub>/N<sub>2</sub> (molar %:  
123 60:2:10:28) (Air Liquide, France) provided into the medium through a sparger. For the  
124 experiments carried out with individual gases, the bioreactor was then set up with three  
125 spargers in order to deliver air, pure CO<sub>2</sub> and pure H<sub>2</sub> independently. A fourth inlet gas was  
126 added in the headspace for pure N<sub>2</sub> supply as a safety system. Each gas inlet was controlled  
127 by a gas mass flow meter. The bioreactor was equipped with pH, dissolved oxygen (DO),  
128 temperature and pressure controllers. The Xplorer® software handled the on-line monitoring  
129 and control systems of the reactor. Both CO<sub>2</sub> and O<sub>2</sub> in the outlet gases were analysed using a  
130 Tandem Pro gas analyzer (Magellan BioTech HEL Ltd , UK). The bioreactor was safely  
131 controlled by the O<sub>2</sub> concentration in the headspace in order to avoid any explosive risk. The  
132 headspace was flushed with pure N<sub>2</sub> if the O<sub>2</sub> surpassed 5% in the headspace. The DO level  
133 in the reactor was controlled below 5% of air saturation at atmospheric pressure by varying  
134 stirring speed and/or inlet air flow rate in order to restrict the known inhibition of *C. necator*  
135 hydrogenases by the dissolved O<sub>2</sub> concentration [23]. Temperature was maintained at 30°C.  
136 The pH was maintained at 7.0 by addition of a 14% (v/v) NH<sub>4</sub>OH solution.

### 137 **Characterization of the biomass**

138 Cell growth was followed spectrophotometrically at 600 nm with a DR3900  
139 spectrophotometer (Hach, Loveland, Colorado, USA) after calibration against cell dry weight  
140 measurements. For cell dry weight determination, culture medium was harvested and filtered  
141 through 0.2 µm pore-size polyamide membranes (Sartorius AG, Göttingen, Germany), which  
142 were then dried to a constant weight at 60°C under partial vacuum (200 mmHg, i.e.  
143 approximately 26.7 kPa).



144 Cell viability was measured with a BD Accuri C6® flow cytometer (BD Biosciences, US)  
145 after cell staining with propidium iodide (PI) dye (Molecular Probes, Invitrogen, USA)  
146 commonly used to monitor membrane integrity as indicator of viability [8]

#### 147 **Metabolites and statistical analyses**

148 Metabolites (isopropanol, acetone, pyruvic and acetic acids) were quantified by  
149 chromatography as described in [22].

#### 150 **Statistical analyses**

151 Concentrations of metabolites and biomass are given as the mean value of 2 to 3 independent  
152 analyses. The error on the specific growth rate (determined as being the slope of  $\ln[\text{Optical}$   
153  $\text{Density}] = f(t)$ ) was calculated as the standard deviation (SD) of the slope. The instantaneous  
154 specific growth rates were calculated upon fitting process of the experimental data and the  
155 derivative calculation. For the yield determination, the state variables were plotted pairwise in  
156 a scatter plot within the considered period of the culture. A linear regression was applied to  
157 determine the yield (as the slope) and the error (as the SD of the slope). For the calculation of  
158 the CO<sub>2</sub> to isopropanol conversion yield, the isopropanol amount was plotted versus the  
159 amount of consumed CO<sub>2</sub>, calculated from a classical mass balance from the analyses of inlet  
160 and outlet gas composition.

## 161 **RESULTS & DISCUSSION**

### 162 **Benefits of elevated pressure on autotrophic growth of *C. necator* Re2133 on a** 163 **commercial gas mixture**

164 Autotrophic production of chemicals by *C. necator* was previously reported in a classical  
165 CSTR bioreactor where gases were supplied by flushing the reactor with a commercial gas

166 mixture containing H<sub>2</sub>/O<sub>2</sub>/CO<sub>2</sub>/N<sub>2</sub> mix (molar %: 60:2:10:28) [7, 8]. Here, this mixture was  
167 used first to characterize the growth of the reference strain *C. necator* Re2133 in the newly  
168 designed bioreactor (**Figure 1**). After 6 h cultivation, the maximum values of agitation and  
169 ventilation rates were reached. The biomass concentration was low at 0.2 g L<sup>-1</sup>. An alternative  
170 way to provide higher gas transfer is to operate the bioreactor at elevated pressure. Then the  
171 dissolved O<sub>2</sub> concentration was kept at 2% saturation, at various pressures in the reactor up to  
172 3 bar overpressure. Growth was then extended to 15 h leading to a final biomass  
173 concentration of 0.5 g L<sup>-1</sup>. Growth followed an exponential profile throughout the cultivation  
174 with a maximum specific rate of 0.13 ± 0.01h<sup>-1</sup>. Cell viability was analysed by flow cytometry  
175 after staining with propidium iodide. The non-PI stained cells were considered viable, and the  
176 percentage was >95% over the duration of the culture, even when overpressure reached 3 bar,  
177 indicating their robustness towards high pressure.

178

### 179 **Benefits of independent gas sparging on autotrophic growth of *C. necator* Re2133**

180 The relatively low final biomass concentration obtained even under high pressure was likely  
181 due to the low gas transfer capacity when using the mixture containing H<sub>2</sub>/O<sub>2</sub>/CO<sub>2</sub>/N<sub>2</sub> (molar  
182 %: 60:2:10:28). This composition was far from optimal for autotrophic growth of *C. necator*.  
183 However, they are all within the explosive range for H<sub>2</sub> and O<sub>2</sub>. The cultivation of the Re2133  
184 strain was initiated by sparging the 3 independent gases in order to reach 88% H<sub>2</sub>, 2% O<sub>2</sub> and  
185 2.5%CO<sub>2</sub> gas mixture in the reactor (62, 6.7 and 1.75 mL/min, for H<sub>2</sub>, air and CO<sub>2</sub>). It  
186 allowed ensuring an H<sub>2</sub>-rich (H<sub>2</sub> being the less soluble gas) and safe environment controlling  
187 the O<sub>2</sub> % in the headspace below the explosive range (6% of air saturation at atmospheric  
188 pressure). The level of dissolved O<sub>2</sub> was controlled to meet the increasing biological demand  
189 while maintaining the level at 2% saturation in order to limit the reported inhibition of the

190 hydrogenases [23, 24] by increasing the ratio air/H<sub>2</sub> flow rate within the range of 6.7-25  
191 mL/min for air and 62-86 mL/min for H<sub>2</sub> (**Figure 2**). The duration of growth at atmospheric  
192 pressure was 15 h compared to 6 h with the reactor operating with the gas mix, illustrating the  
193 benefit of three independent spargers. Growth followed an exponential profile throughout the  
194 cultivation at atmospheric pressure with a maximum specific rate of  $0.11 \pm 0.01 \text{ h}^{-1}$ . At 15 h,  
195 inlet gas composition was 70% H<sub>2</sub>, 5% O<sub>2</sub> and 2.8% CO<sub>2</sub> (71, 26 and 2.8 mL/min for H<sub>2</sub>, air  
196 and CO<sub>2</sub>). When the inlet gas composition for O<sub>2</sub> reached the safety level (5%) defined to  
197 ensure a safe environment, the dissolved O<sub>2</sub> level was maintained by switching the strategy to  
198 a step-wise increase in total reactor pressure up to >3 bars. The growth profile was still  
199 exponential without any observed drop in specific rate ( $0.11 \pm 0.01 \text{ h}^{-1}$ ). A final pressure up  
200 to 4.2 bars was applied with a concomitant increase in H<sub>2</sub> (117 mL/min) and a decrease in O<sub>2</sub>  
201 level in the inlet gas (3.7%) in order to maintain the dissolved oxygen at a low level. The  
202 amount of CO<sub>2</sub> in the outlet gas was always found to be lower than in the inlet and never  
203 exceeded 1.6%. During this period, the specific growth was lower but constant at  $0.07 \pm 0.005$   
204  $\text{h}^{-1}$ . Cell viability was assessed by flow cytometry and did not show any fall in the percentage  
205 of PI unstained cells.

### 206 **Isopropanol production under autotrophic condition**

207 Isopropanol autotrophic production was tested in the designed bioreactor using an engineered  
208 strain Re2133/pEG7b [22]. Cultivation was initiated by sparging the three independent gases  
209 in order to reach 87% H<sub>2</sub>, 2% O<sub>2</sub> and 3%CO<sub>2</sub> mixture in the reactor (59, 6.7 and 2 mL/min  
210 respectively for H<sub>2</sub>, air and CO<sub>2</sub>) allowing an H<sub>2</sub>-rich, O<sub>2</sub>-low environment to be reached. The  
211 level of dissolved O<sub>2</sub> was monitored throughout by increasing the ratio air/H<sub>2</sub> flow rate and  
212 the overpressure in the bioreactor in order to fulfill the increasing microbial O<sub>2</sub> demand

213 **(Figure 3)**. The gas flow rates ranged between 59-200 mL/min for H<sub>2</sub>, 6.7 -75 mL/min for air  
214 and 2-6 mL/min for CO<sub>2</sub>. *C. necator* Re2133/pEG7b grew for 20 h at an average specific rate  
215 of 0.051±0.006 h<sup>-1</sup>. Growth slowly decreased to 0.023±0.001 h<sup>-1</sup> for the remaining period of  
216 the cultivation. The final biomass concentration reached at 85 h was 2.9 g L<sup>-1</sup>. Compared to  
217 the reference strain Re2133, the lower growth rate was likely due to inhibition by isopropanol  
218 and acetone as previously observed with the same strain with fructose (0.05 vs 0.17 h<sup>-1</sup>, for  
219 Re2133 and Re2133/pEG7b respectively) [22]. Isopropanol and acetone were produced  
220 concomitantly to reach maximum concentrations in the liquid phase of 3 and 0.65 g L<sup>-1</sup>  
221 respectively. Any isopropanol and acetone evaporated with the gas stream was trapped in  
222 water and quantified during the cultivation. Taking this evaporation into account, the maximal  
223 concentrations were equivalent to 3.5 and 0.9 g L<sup>-1</sup> of isopropanol and acetone, respectively.  
224 Reproducibility was assessed with a second independent cultivation and led to the production  
225 of 1.96 g L<sup>-1</sup> biomass, 2.1 g L<sup>-1</sup> isopropanol and 1 g L<sup>-1</sup> acetone. These performances were  
226 remarkable when compared to that obtained with fructose: Re2133/pEG7b was able to  
227 produce up to 2.2 g L<sup>-1</sup> isopropanol and 1 g L<sup>-1</sup> acetone in 88 h with fructose [22]. The overall  
228 specific production rate of isopropanol in *C. necator* Re2133/pEG7b from CO<sub>2</sub> was 0.013  
229 gg<sub>DCW</sub><sup>-1</sup>h<sup>-1</sup> (0.030 gg<sub>DCW</sub><sup>-1</sup>h<sup>-1</sup> with fructose) and peaked at 0.053 gg<sub>DCW</sub><sup>-1</sup>h<sup>-1</sup> with CO<sub>2</sub> (0.085  
230 gg<sub>DCW</sub><sup>-1</sup>h<sup>-1</sup> with fructose). Cell viability was assessed by flow cytometry after staining with PI  
231 during the cultivation. The percentage of PI unstained cells ranged from 82-98%, which was  
232 slightly lower cultivation of Re2133 in the pressurized bioreactor. Concomitantly with the  
233 reduction in the  $\mu_{\max}$ , this could be attributed to an inhibitory effect of isopropanol on  
234 Re2133/pEG7b.

235 The proof of concept of producing isopropanol from CO<sub>2</sub> with an engineered *C. necator* strain  
236 was previously demonstrated: 250 mg L<sup>-1</sup> isopropanol was obtained from 1 g L<sup>-1</sup> biomass  
237 using a classical bioreactor and commercialized gas mix containing H<sub>2</sub>, O<sub>2</sub> and CO<sub>2</sub> (60:2:10)  
238 [8]. It is clearly demonstrated here that designing a pressurized bioreactor with independent  
239 gas feeds significantly improved gas transfer to reach higher biomass and consequently higher  
240 isopropanol concentrations of up to 3.5 g L<sup>-1</sup>. The calculation of the carbon balance in this  
241 fermentation showed that 70-80% of the CO<sub>2</sub> was recovered into biomass, isopropanol and  
242 acetone. The yield of CO<sub>2</sub> converted into isopropanol was 0.13 Cmol Cmol<sup>-1</sup> in this study,  
243 slightly lower than 0.2 Cmol Cmol<sup>-1</sup> reached previously. [8]. However, strain Re2133/pEG7b  
244 used here constitutively expressed the isopropanol synthetic operon whereas Re2133/pEG23  
245 previously tested had an inducible isopropanol operon controlled by the arabinose promoter.  
246 Thus uncoupling of growth and production allowed the carbon flow to be better directed  
247 towards the isopropanol pathway, as reported with fructose [22].

## 248 **CONCLUSIONS**

249 The design of a dedicated gas bioreactor incorporating the independent management of the  
250 three gases, CO<sub>2</sub>, H<sub>2</sub> and Air, coupled with the use of high pressure has enabled a dramatic  
251 improvement in gas transfer in a small scale bioreactor. *C. necator* showed an ability to  
252 sustain a pressure of over 4 bars, leading to a higher biomass concentration and an  
253 isopropanol production of up to 3.5 g L<sup>-1</sup>. This is the first reported demonstration successfully  
254 producing compounds at the g L<sup>-1</sup> scale from engineered autotrophic bacteria and raises the  
255 prospect of the near-future development of CO<sub>2</sub>-based commercial bioprocesses.

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261

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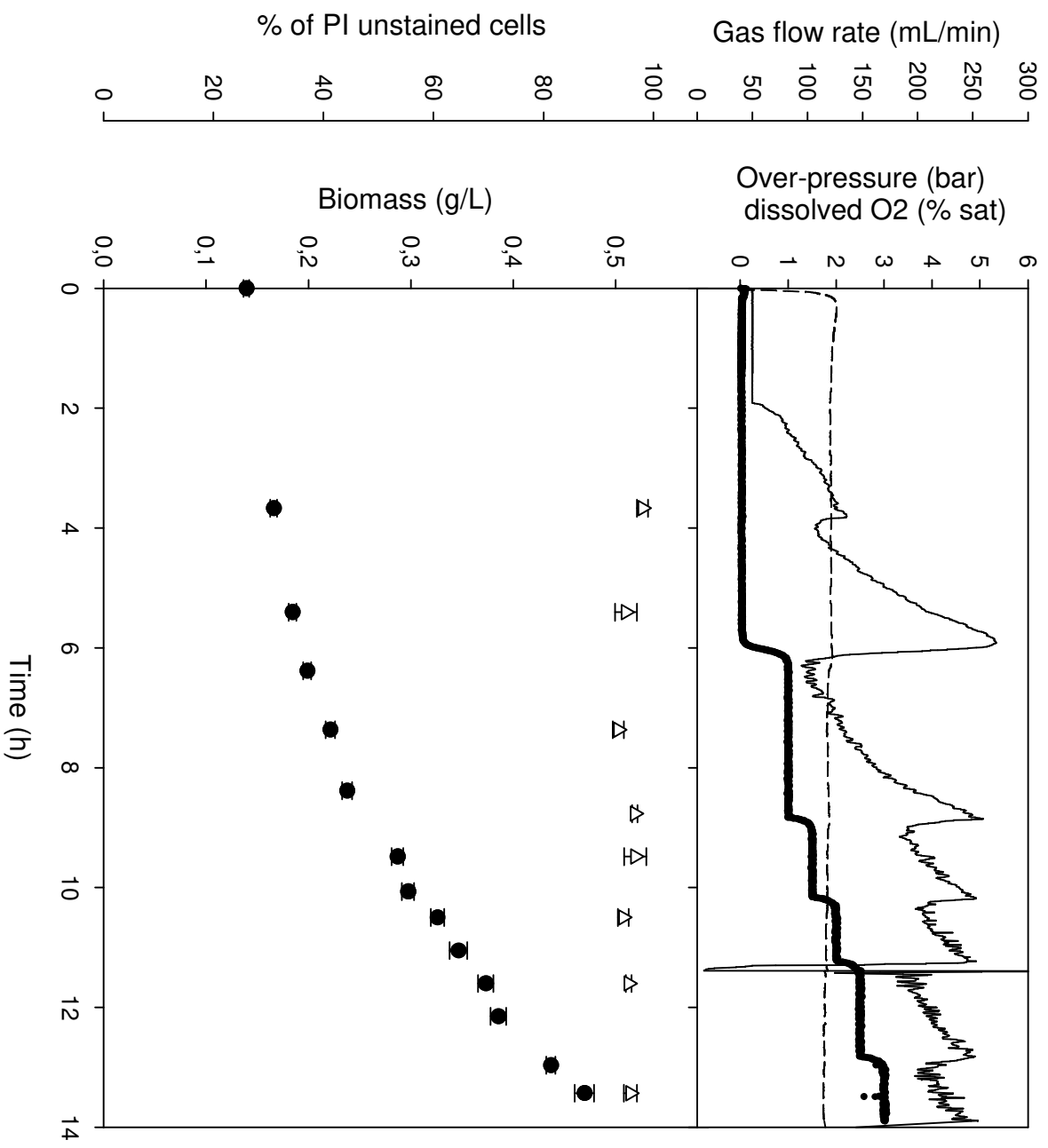
346 **Figure legends**

347 **Figure 1:** (A) Evolution of the dissolved O<sub>2</sub> level (dashed line), the total gas flow rate (thin  
348 black line) and the overpressure (thick black line) in the bioreactor. (B) Evolution of the  
349 biomass concentration (black circles) and cell viability (open triangles)

350 **Fig.2:** Autotrophic growth of *C. necator* Re2133 in the gas bioreactor modified with 3  
351 independent gas spargers. (A) Evolution of relative pressure (thick line) and partial pressure  
352 of dissolved oxygen (dashed line). (B) Evolution of the inlet gas composition for H<sub>2</sub> (solid  
353 thick line), O<sub>2</sub> (dashed line) and CO<sub>2</sub> (solid thin line) .(C) Evolution of biomass  
354 concentrations (●) and % of PI unstained cells (Δ).

355 **Fig.3:** Autotrophic isopropanol production of *C. necator* Re2133/pEG7b in the gas bioreactor  
356 modified with 3 independent gas spargers. (A) Evolution of the relative pressure (thick line)  
357 and the partial pressure of dissolved O<sub>2</sub> (dashed line). (B) Evolution of the inlet gas  
358 composition in H<sub>2</sub> (solid thick line), O<sub>2</sub> (dashed line) and CO<sub>2</sub> (solid thin line). (C) Evolution  
359 of biomass concentrations (●), % of PI unstained cells (Δ) and isopropanol (□), acetone (∇)  
360 concentrations in the bioreactor liquid phase. Total isopropanol (■) and acetone (▼)  
361 production were calculated taking amounts evaporated into account.

Figure 1



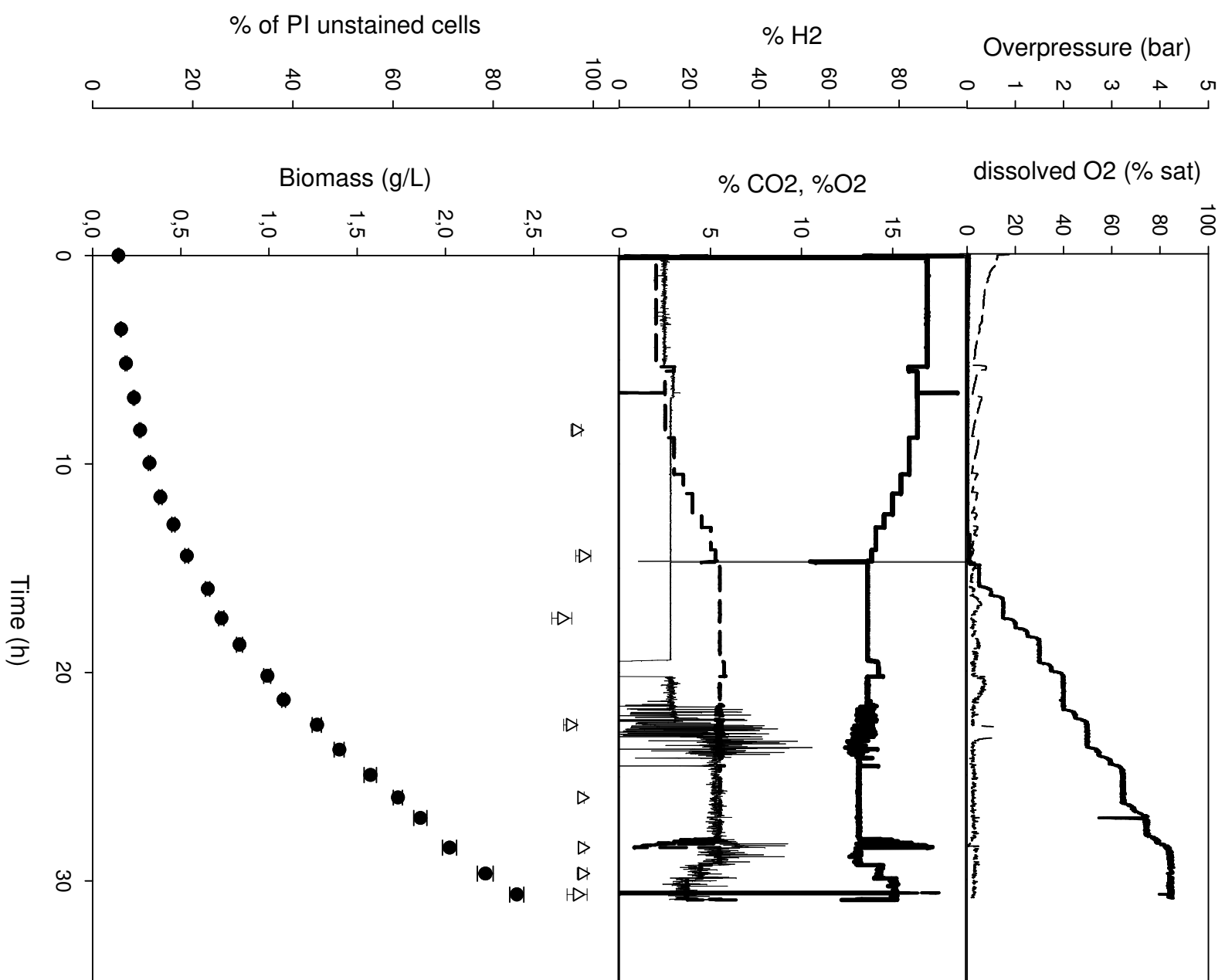


Figure 2

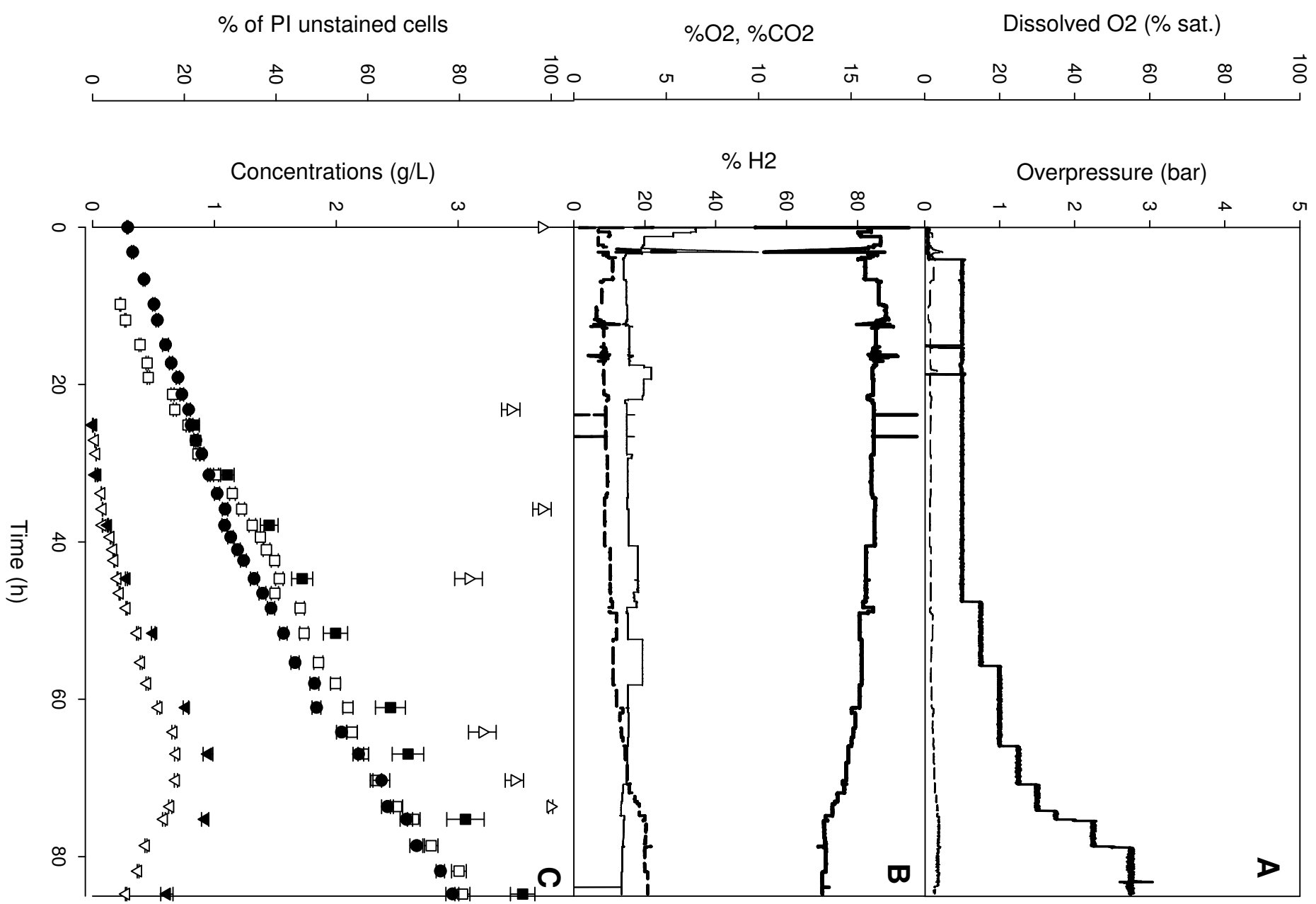


Figure 3