

# $\mu$ MAX of *Saccharomyces Cerevisiae*: So Often Used, So Seldom Put into Perspective

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## Original Article

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## **TITLE:**

**$\mu_{\text{MAX}}$  of *Saccharomyces cerevisiae*: so often used, so seldom put into perspective**

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## **ABSTRACT:**

The maximum specific growth rate of a microbe in a given growth condition is of primary relevance for biological research and bioprocess development. In the case of the unicellular yeast *Saccharomyces cerevisiae*, this physiological parameter is routinely calculated in (almost) every laboratory, but this procedure conceals several challenges that are often neglected in scientific works, which might lead to misinterpretation of the reported data and of phenomena. We present here several pitfalls involved in  $\mu_{\text{MAX}}$  calculation and interpretation, which was achieved through comparative analyses of: 1) the use of different methodologies for determining cell concentration, 2) different calibration procedures to

correlate indirect (e.g. absorbance) to direct (e.g. dry cell mass) cell concentration measurements, 3) different statistical methods for determining the significance of  $\mu_{\text{MAX}}$  differences, 4) the influence of culture media composition, and 5) the influence of the cultivation system (e.g. microplate, shake-flask or bioreactor). It becomes clear that each of these factors has a great influence on  $\mu_{\text{MAX}}$  calculation and interpretation. We also present a case study involving three yeast strains and three different carbon sources, illustrating that opposite conclusions can be drawn in a screening procedure, if proper caution is not taken during data generation and analysis. Last but not least, we conclude this work with a series of recommendations that we believe could make experimental planning, data generation,  $\mu_{\text{MAX}}$  calculation and interpretation more meaningful and scientifically sound, contributing to the improvement of yeast research and of microbiology in general.

**KEYWORDS:** cell concentration, microbial growth, *Saccharomyces cerevisiae*, specific growth rate, yeast physiology,  $\mu_{\text{MAX}}$

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The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Not applicable.

## **Consent to participate**

Not applicable.

## **Consent for publication**

Not applicable.

## **Availability of data and material**

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## **Code availability**

Not applicable.

## **Authors' contributions**

**Carla Inês Soares Rodrigues:** Data collection and analysis, original draft preparation.

**Bianca Eli Della-Bianca:** Data analysis, original draft preparation. **Andreas K. Gombert:** Study conception and design, data analysis, draft review and editing, supervision.

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# 1 Introduction

2 Growth of a microbial population is not the increase in size of the individual cells, but  
3 rather the increase in total cell number, total cell mass or even total cell volume in the  
4 population (Wheals and Lord 1992). Determining the rate at which a microbial population  
5 grows is one of the main interests of the fundamental microbiologist, as well as presumably  
6 the most important piece of information in an industrial bioprocess. This aspect is captured in  
7 a parameter referred to as the specific growth rate, most commonly represented by the Greek  
8 letter  $\mu$  (Doran 2012; Clarke 2013; Liu 2016; Stanbury et al. 2017). Cell growth is an  
9 autocatalytic reaction, meaning that the catalyst itself is a product of the reaction (Doran  
10 2012). Hence, the cell (or biomass) specific growth rate, rather than a simple growth rate, is  
11 the most appropriate parameter to describe microbial growth. Mathematically,

$$\mu = \frac{1}{X} * \frac{dX}{dt} \quad \mathbf{1}$$

12 where  $X$  = cell concentration (e.g. in cells/volume or dry cell mass/volume) and  $t$  is the  
13 reaction time (e.g. in hours).

14 From equation **1**, it can be observed that  $\mu$  is similar to the kinetic constant of a 1<sup>st</sup>-  
15 order chemical reaction and has dimensions of time<sup>-1</sup>. Other formulations for rates, such as  
16 total or volumetric rates, are scale-dependent and do not directly reflect catalyst performance.

17 The exponential growth phase (EGP) occurs very often both in research and in  
18 applied cases, and is typically the longest phase of a conventional batch cultivation. During  
19 the EGP, cells encounter neither any nutrient limitation nor any inhibition. The population  
20 then grows at the maximum possible rate (the maximum specific growth rate,  $\mu_{MAX}$ ) under the  
21 applied conditions, until one nutrient becomes growth-limiting or some compound achieves  
22 inhibitory concentrations. The term “balanced growth” is often used to describe the  
23 physiology of cells during the EGP, since the cell composition typically does not change,  
24 although the composition of the nutrient medium is constantly changing (Campbell 1957).

25            Instead of  $\mu$ , some professionals prefer to use the doubling (or generation) time ( $t_G$ ) to  
26 quantify the rate of microbial growth.  $t_G$  is the time required for the microbial population to  
27 double its size (e.g. in terms of cell number or dry cell mass). The two parameters are  
28 intrinsically related by the following equation:

$$\mu = \frac{\ln 2}{t_G} \quad 2$$

29

30 We will here only use  $\mu$  for all our analyses and discussions.

31             $\mu$  cannot be directly measured. Nevertheless, measurements of cell concentration at a  
32 minimum of two time points allow for the estimation/calculation of this parameter.

33            There are several methods to determine cell concentration, including direct cell count,  
34 dry cell mass, particle count and colony forming units, among other direct off-line methods  
35 (Sonnleitner et al. 1992). Moreover, cell concentration is usually assessed by light-scattering  
36 measurements, such as those performed with the use of a spectrophotometer, a ubiquitous  
37 laboratory piece of equipment. Other terms used to designate this type of measurement are  
38 optical density (OD), turbidity, and absorbance. However, the results of such an indirect  
39 analysis need to be calibrated against a direct method, and this requires some caution.  
40 Calibration should be performed under a particular condition and applied to this circumstance  
41 only. Otherwise, the correlation could be compromised. Even analyses performed with cells  
42 from a single cultivation but collected at different growth phases represent a source of error  
43 due to inadequate calibration. The possibly different cell morphologies in each growth phase  
44 affect deviation of light and compromise the translation of the indirectly assessed cell  
45 concentrations into real cell concentrations.

46            Further options for indirect determination of cell concentration rely on the  
47 measurement of a cell component, for instance protein or DNA. In this case, calibration is  
48 also necessary and, as discussed above, care should be taken in the sense that cell composition  
49 during growth might differ from the one employed during the calibration procedure.

50 Furthermore, although online methods centered on turbidity, permittivity (Harris et al. 1987),  
51 or fluorescence can as well be used to assess cell concentration, as yet they have not  
52 substituted the above mentioned off-line methods, which require sampling.

53 Another relevant aspect for the analysis of microbial growth is the cell cycle, which  
54 for yeast comprises the phases G1, S, G2, and M (Juanes 2017). Individual cells in a  
55 population are in different phases of the cell cycle, meaning they are not synchronised  
56 (Cooper 2019). Thus, for a sample withdrawn from a cultivation, be it a microtiter plate or a  
57 million-liter-scale bioreactor, the measured cell concentration involves billions of cells at  
58 different stages of the cell cycle.

59 Fermentation Technology and/or Bioprocess Engineering textbooks usually do not  
60 provide a discussion on how cell concentration measurements affect the calculation of the  
61 specific growth rate. In one case, it is even stated that "During balanced growth, the net  
62 specific growth rate determined from either cell number or cell mass would be the same"  
63 (Shuler and Kargi 2002; Liu 2016). Stanbury et al. (2017) present the specific growth rate  
64 without any connection to cell concentration determination methods. In one exception, Clarke  
65 (Clarke 2013) points out that " $\mu_{MAX}$  can vary significantly depending on the method used to  
66 measure the cell concentration". This author also mentions that, in the case of the budding  
67 yeast *S. cerevisiae*, there might be a difference in  $\mu_{MAX}$  calculated from cell mass and cell  
68 number. This is because, in the beginning of the EGP, yeast cells tend to present many buds  
69 (small cells) of lower mass than fully grown cells, which are counted by direct cell counting  
70 methods. The opposite is observed towards the end of the EGP, when the budding rate  
71 decreases.

72 There are basically two different approaches to calculate  $\mu_{MAX}$  from cell concentration  
73 measurements. One of them is based on a first adjustment of a growth model to data from an  
74 entire batch cultivation, including all growth phases (lag, log, de-acceleration and stationary).  
75 Frequently used models include the logistic model, the Gompertz and the Richards models,  
76 among others (Pylvänäinen 2005). The second method consists of the integration of equation

77 **1** under the assumption that in the EGP  $\mu$  is constant and equal to  $\mu_{MAX}$ . While early  
78 researchers used a  $\log_2$  or  $\log_{10}$  transformation to linearize this equation (Clarke 2013),  
79 nowadays, the use of the natural logarithm is common practice:

$$\ln X = \ln X_0 + \mu_{MAX} * t \quad \mathbf{3}$$

80 where  $X_0$  = cell concentration at the beginning of the EGP, corresponding to  $t = 0$ .

81 This transformation allows us to calculate  $\mu_{MAX}$  by plotting  $\ln(X)$  values along time  
82 and taking the slope of the linear region as  $\mu_{MAX}$ . This procedure also results in the  
83 identification of the duration of the EGP. Due to the use of the natural logarithm,  $\mu_{MAX}$   
84 represents the number of “*e*-fold” generations in a given time point  $t$ , or the exponential  
85 increase of biomass by a factor of  $e$  (Manhart and Shakhnovich 2018). We will restrict our  
86 analysis and discussion here to this approach, because it is by far the most frequently  
87 employed in the context of yeast research.

88  $\mu_{MAX}$  is also a key parameter in kinetic models used in biological research and in  
89 bioprocess development. In its simplest form, it appears in the Monod equation that relates  
90  $\mu_{MAX}$  to the limiting substrate concentration  $S$ :

$$\mu = \mu_{MAX} * \frac{S}{S + K_S} \quad \mathbf{4}$$

91  $\mu_{MAX}$  has also been termed the Malthusian parameter and used as a proxy for fitness  
92 by part of the scientific community, mainly those involved in population genetics or  
93 experimental evolution studies (Lenski et al. 1991).

94 For the sake of completeness, it should be mentioned that there are methods to  
95 calculate  $\mu_{MAX}$  using continuous cultivation data (Jannasch and Egli 1993) and methods that  
96 take substrate and product concentrations into account (Oner et al. 1986). We will not discuss  
97 them here.

98 Finally, it is important to mention that not only the analytical method used to  
99 determine cell concentration influences  $\mu_{MAX}$  calculations, but also other factors such as the



100 cultivation system. Potvin et al (Potvin et al. 1997) compared  $\mu_{MAX}$  values obtained for  
101 *Lactobacillus plantarum* cells grown in an automated plate reader, in shake-flasks and in a  
102 bioreactor, otherwise under similar conditions. Bioreactor cultivations led to higher  $\mu_{MAX}$   
103 values as compared to shake-flask cultivations, which the authors attributed to external pH  
104 control in bioreactors. These authors also showed that the  $\mu_{MAX}$  calculated from direct  
105 absorbance measurements in an automated plate reader, without sample dilution, differed  
106 from the values obtained with samples from shake-flask cultivations that were diluted prior to  
107 the absorbance measurements. Although these observations seem obvious, this matter has  
108 only been given proper attention in few published works.

109 In the only report we identified involving yeast, Stevenson et al. (2016) evaluated the  
110 relationship between optical density and cell counts both in *Escherichia coli* and  
111 *Saccharomyces cerevisiae* cultures with respect to particle size and shape, refractive index,  
112 cultivation volume, spectrophotometer model, cell growth phase, among others. The authors  
113 concluded that the cell size effect on the calibration between OD and cell counts was stronger  
114 in bacteria than in yeast. This is because the size of the bacterial cells is closer to the  
115 wavelength of light (600 nm) used in the OD measurements. In this sense, the bigger size of  
116 yeast cells makes them more suitable than bacteria for the application of light scattering  
117 techniques at 600 nm or similar wavelengths. Moreover, they demonstrated that the difference  
118 between the refractive index of the medium and that of the cells influences the calibration  
119 curve. This has implications for yeast research, since sugars commonly used in yeast media,  
120 such as sucrose, change the refractive index of the medium significantly.

121 This context motivated us to investigate how different cell concentration  
122 determination methods, statistical analyses, cultivation systems, and also culture media  
123 influence  $\mu_{MAX}$  calculations during yeast cultivations performed with different strains,  
124 including wild isolates, laboratory and industrial ones.

## 125 **Material and Methods**

### 126 **Yeast strains and preservation**

127 Eight *S. cerevisiae* strains from indigenous, industrial or laboratory origin were used  
128 in this work (**Table 1**). Stock cultures were prepared by growing cells until stationary phase in  
129 500-ml Erlenmeyer flasks containing 100 ml YPD (1% yeast extract, 2% peptone, and 2%  
130 glucose) medium. 20% (v/v, final concentration) sterile glycerol was added and 1-ml aliquots  
131 were stored in 2-ml cryogenic vials in an ultra-freezer (ColdLab, Piracicaba, Brazil) at -80 °C  
132 until further use.

### 133 **Cultivation media**

134 Yeast cultivations were carried out using either a defined medium (Verduyn et al.  
135 1992), the composition of which altered depending on the cultivation system (**Table 2**), or a  
136 complex medium (YPD). Microplate cultivations were performed using both media, whilst  
137 shake-flask and bioreactor cultivations were restricted to the defined medium. When needed,  
138 urea was used as the sole nitrogen source in replacement for ammonium sulphate, to avoid  
139 drastic changes in the broth's pH caused by proton release during ammonium consumption.  
140 Glucose was added as carbon and energy source to all cultivation media, unless otherwise  
141 stated. Each medium was sterilised either by autoclaving some of its components at 121 °C  
142 for 20 min or by filtration through 0.22-µm pore membranes. Glucose, vitamin and trace  
143 element solutions were always filter-sterilised to avoid Maillard reactions or thermal  
144 decomposition of the components.

### 145 **Cultivations**

#### 146 *Microplate cultivations*

147 All eight strains were cultivated in 96-well microplates (CELLSTAR® flat bottom, mfr. No.  
148 655151 - Greiner bio-one, Kremsmunster, Austria) using the plate reader Tecan Infinite M200  
149 Pro. Initially, cells from the -80 °C stock were streaked onto solid YPD medium (with 2%

150 agar) and incubated at 30 °C (502 Incubator, FANEM, São Paulo, Brazil) for 48 h. Cells from  
151 a single colony were then transferred to a 50-ml centrifuge tube filled with 3 ml of either a  
152 defined medium or a complex medium, constituting the inoculum. The inoculum was placed  
153 in a shaker incubator (Innova 4430, New Brunswick Scientific, Edison, USA) operating at  
154 200 rpm and 30 °C for 24 h. An aliquot of each tube's content, enough to make 1 ml of a cell  
155 suspension with absorbance at 600 nm equal to 1, was then collected. The aliquot was  
156 centrifuged at 974 g for 5 min (MIKRO200 centrifuge, Hettich, Tuttlingen, Germany), the  
157 supernatant discarded and the pellet washed with 1 ml of fresh culture medium. This washing  
158 procedure was performed twice. Next, 10 µl of the cell suspension was transferred to one well  
159 of a microplate that had already been filled with 90 µl of the same culture medium used for  
160 inoculum growth. Once all the desired wells were filled with both medium and cell  
161 suspension, the microplate was sealed with PCR sealing film (AMPLISeal™ - Greiner bio-  
162 one, Kremsmunster, Austria). The cultivation was carried out in quintuplicate (5 wells on the  
163 same plate) at 30 °C with an orbital agitation amplitude of 3.5 mm and frequency of 198.4  
164 rpm. Absorbance at 600 nm wavelength and 9 nm bandwidth was measured every 15 min  
165 during 24 h.

166 **Table 1**

167 **Table 2**

### 168 *Shake-Flask cultivations*

169 Shake-flask cultivations were performed with strains CEN.PK113-7D, PE-2, JP1,  
170 UFMG-CM-Y257, and UFMG-CM-Y259. First, each inoculum was prepared by transferring  
171 cells from one colony of each of the five strains into 500-ml baffled Erlenmeyer flasks  
172 containing 100 ml synthetic medium. The inoculum was incubated in a shaker (Innova 4430,  
173 New Brunswick Scientific, Edison, USA) at 30 °C and 200 rpm for 24 h. Then, sufficient cell  
174 suspension to begin the cultivation with an absorbance at 600 nm of 0.2 was centrifuged at  
175 2153 g for 5 min (NT810 centrifuge, Nova Técnica, Piracicaba, Brazil). The supernatant was  
176 discarded, cells were washed twice and the cell pellet was resuspended in 1 ml synthetic

177 medium. This cell suspension was transferred to another Erlenmeyer flask containing fresh  
178 synthetic medium.

179 Samples of the cultivation broth were collected hourly and their absorbance at 600  
180 nm measured in a spectrophotometer (Genesys 20, Thermo Fisher Scientific, Massachusetts,  
181 USA). Sample pH was read using a pHmeter (DM21, Digimed, São Paulo, Brazil). The  
182 cultivations were stopped when the cells reached the stationary phase of growth.

### 183 ***Bioreactor batch cultivations***

184 To prepare the inoculum for bioreactor cultivation, the content of one cryogenic vial  
185 was transferred to a 500-ml Erlenmeyer flask containing 100 ml of medium, which was  
186 prepared as described for shake-flask cultivations. The pH of this pre-inoculum medium was  
187 adjusted to 6.0 by addition of 2 mol l<sup>-1</sup> KOH. Cells were propagated at 30 °C in a shaker  
188 (Certomat BS-1, Braun Biotech International, Berlin, Germany) under a stirring speed of 200  
189 rpm. After 24 h, 1 ml of the pre-inoculum was directly transferred to another shake-flask  
190 filled with fresh inoculum medium. Following a second round of growth in a shaker, an  
191 aliquot sufficient to start the batch cultivation with an absorbance of 0.2 at 600 nm was  
192 collected, centrifuged at 3500 g for 3 min, and the pellet resuspended in fresh cultivation  
193 medium. Afterwards, the cell suspension was transferred to a 2-l bioreactor (Applikon  
194 Biotechnology B.V., Delft, The Netherlands), making up an initial working volume of 1.2 l.

195 Cells were cultivated at 30 °C and 800 rpm until a decrease in the CO<sub>2</sub> molar fraction  
196 in the off-gas was observed. Aeration in the bioreactor occurred with compressed air at 0.5 l  
197 min<sup>-1</sup> flow rate injected through a mass flow controller (Model 58505, Brooks Instrument  
198 B.V., Hatfield, USA). The pH of the medium was adjusted to 5.0 and kept constant by  
199 automatic addition of 0.5 mol L<sup>-1</sup> KOH solution. Whenever needed, a 10% (v/v) antifoam C  
200 emulsion (Sigma-Aldrich, Missouri, USA) was added manually to the broth. Samples of the  
201 broth were withdrawn approximately every hour to have their dry mass and absorbance  
202 measured. Dry cell mass was determined according to (Olsson and Nielsen 1997), except that  
203 the membranes were dried in an oven at 70 °C for 48 h. The result was expressed in g<sub>DM</sub> l<sup>-1</sup>.

204 Absorbance was measured at 600 nm in a spectrophotometer (LibraS11, Biochrom,  
205 Cambridge, United Kingdom).

### 206 **Calculation of the maximum specific growth rates and statistical comparisons**

207 All calculations for statistical comparisons were performed with either GraphPad  
208 Prism 8 (San Diego, USA) or Microsoft Excel 365 (Redmond, USA). The maximum specific  
209 growth rate ( $\mu_{MAX}$ ) was obtained by plotting the natural logarithm of  $Abs_{600}$  (or dry cell mass)  
210 values against time and then fitting a linear regression model to the data within the  
211 exponential growth phase, the slope of which corresponds to the  $\mu_{MAX}$ .

212 Using Microsoft Excel, data from independent replicates were analyzed separately,  
213 each one yielding a  $\mu_{MAX}$  value of its own fitted by the least-squares regression method. The  
214 average and the standard deviation of these  $\mu_{MAX}$  values were then calculated (**Fig. 1, Method**  
215 **A**). Significant changes in  $\mu_{MAX}$  were evaluated using t-tests with 95% and 99% confidence  
216 levels. On the other hand, using GraphPad Prism, data from independent replicates of each  
217 experiment were analyzed together, generating one single  $\mu_{MAX}$  value from one regression  
218 line also fitted by the least-squares method. This procedure also generated the standard error  
219 of the slope (**Fig. 1, Method B**). Significant changes in  $\mu_{MAX}$  were evaluated using F-tests  
220 with 95% and 99% confidence levels.

221 **Fig. 1**

## 222 **Results & Discussion**

### 223 **Calculated $\mu_{MAX}$ values depend on the cell concentration determination and on the** 224 **calibration with a direct method**

225 In spite of being an indirect method for the determination of cell concentration,  
226 Absorbance (Abs) measurements are commonly used during yeast cultivations. Researchers  
227 frequently use these measurements to directly calculate  $\mu_{MAX}$ , by plotting  $\ln(Abs)$  values  
228 against time, identifying the EGP as the linear region, performing a linear regression with the

229 corresponding data and taking the slope as  $\mu_{MAX}$ . In other cases, authors report the calibration  
230 equation used to convert the Abs data into real cell concentrations, without mentioning how  
231 (or under which conditions) it was obtained. Calibration can be performed in different ways  
232 and these might influence the calculation of  $\mu_{MAX}$ . To illustrate this, let us consider the cell  
233 concentration data points  $X_1$  and  $X_2$  obtained at two time points during the EGP ( $t_1$  and  $t_2$ );  
234 from these data,  $\mu_{MAX}$  can be calculated as:

$$\mu_{MAX} = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \quad \mathbf{5}$$

235 Taking a linear relation (calibration) between Abs measurements and a direct cell  
236 concentration (X) method, as follows:

$$X = a * Abs + b \quad \mathbf{6}$$

237 and substituting equation **6** into **5**, results in:

$$\mu_{MAX} = \frac{\ln(a * X_2 + b) - \ln(a * X_1 + b)}{t_2 - t_1} \quad \mathbf{7}$$

238 It is clear from equation **7** that only if the linear coefficient (intercept)  $b = 0$ ,  $\mu_{MAX}$  calculated  
239 from Abs and true cell concentration measurements will be the same.

240 In our experience at least,  $b$  is usually different from zero. We demonstrate this here  
241 with  $\mu_{MAX}$  calculations from data obtained during bioreactor cultivations of three different  
242 yeast strains on glucose, namely CEN.PK113-7D, UFMG-CM-Y259, JP1 (**Table 3**). Samples  
243 taken throughout the cultivation had their absorbances measured after proper dilution and  
244 their cell concentration determined by a direct method (dry cell mass).  $\mu_{MAX}$  was calculated  
245 using four different approaches: 1) directly from Abs data; 2) directly from dry cell mass data;  
246 3) from calculated dry cell mass values obtained using a calibration equation established  
247 between the Abs and the dry cell mass data, including all data points in the cultivation; 4)  
248 from calculated dry cell mass values obtained using a calibration equation established

249 between the Abs and the dry cell mass data, including only data points in the EGP (as  
250 identified from the dry cell mass data used for calibration).

251 **Table 3**

252 Remarkably,  $\mu_{MAX}$  values calculated based on approach 1 were in the range of 25 to  
253 50% higher than those calculated from dry cell mass data (approach 2). Because the latter  
254 approach is based on a direct assessment of cell concentration, widely considered as an  
255 accurate analytical method (as long as the appropriate amount of biomass is weighed on the  
256 filtration membrane or in the centrifuge tube, (Olsson and Nielsen 1997)), we took this  $\mu_{MAX}$   
257 value as the reference.

258 On the other hand,  $\mu_{MAX}$  values calculated using approaches 3 or 4 were much closer  
259 to the reference  $\mu_{MAX}$  value. In the case of the 3<sup>rd</sup> approach, which includes data points from  
260 the lag, EGP and de-acceleration growth phases in the calibration procedure, the calculated  
261  $\mu_{MAX}$  values differed at most 10% from the reference  $\mu_{MAX}$  value, even when the calibration  
262 had been established with data from a different strain (see supplementary material).  
263 Nevertheless, it should be noted that other approaches, such as a modified version of approach  
264 3 to force the linear regression to an intercept of zero, or the establishment of a calibration  
265 curve between Abs and dry cell mass using the final data point in the cultivation only, lead to  
266 the same results as those obtained using approach 1. This latter option would have a very  
267 practical implication, since it could allow for the use of shake-flask cultivations monitored by  
268 absorbance measurements (which require small sample volumes) along the whole cultivation,  
269 accompanied by dry cell mass determination (which requires larger sample volumes) in the  
270 final sample only.

271 **Errors associated to  $\mu_{MAX}$  values depend on the regression method and may alter**  
272 **statistical outcomes**

273 Experiments in scientific research are often carried out in replicates, so that statistical  
274 comparisons can be performed. It is of interest, for instance, to verify how the  $\mu_{MAX}$  of a given  
275 strain compares to that of another strain under the same conditions, or to the  $\mu_{MAX}$  of the same

276 strain under different conditions. The error associated to the calculated  $\mu_{\text{MAX}}$  value is therefore  
277 critical, since it is the basis for statistical comparisons. One approach to determine the  
278 absolute error that affects  $\mu_{\text{MAX}}$  was proposed by Borzani (Borzani 1980, 1994), and it  
279 depends on both the relative error of the cell concentration measurements and the duration of  
280 the experiment. This methodology was not used here since often researchers do not know the  
281 relative error of the cell concentration measurement itself, given that cell concentration is  
282 usually measured only once at each time point.

283 Also, we would like to stress that time-series data are not independent, meaning that  
284 the value of one data point depends on the value of previous data points. And, strictly  
285 speaking, linear regression could not be used when data are not independent (McDonald  
286 2014). However, data from microbial growth curves have historically been treated as being  
287 independent. This is due to the assumption that “Whether one point is above or below the line  
288 is a matter of chance, and does not influence whether another point is above or below the  
289 line” (Motulsky 2020). Hence, we also proceeded this way in this work.

290 Using Abs values from exponential growth of strain CAT-1 in microplates, two  
291 methods for statistical comparison of  $\mu_{\text{MAX}}$  on defined and complex media were evaluated  
292 (**Table 4, Supplementary Material**). Although the final  $\mu_{\text{MAX}}$  values obtained from both  
293 methods were the same, each was linked to distinct deviation/error values representing the  
294 scattering of the same data.

295 Another analysis we carried out was the removal of outliers, since this is a common  
296 procedure adopted by scientists in research. After visual inspection, some data appeared much  
297 more distant to the regression lines than the others, with no apparent reason. The removal of  
298 outliers based on an informal, visual approach is not recommended; thus the ROUT (Robust  
299 regression followed by Outlier identification) method was used. This is an automatic routine,  
300 based only on the distance of the point from the robust best-fit curve (Motulsky and Brown  
301 2006). We evaluated all data points again in GraphPad Prism software using the ROUT  
302 method, set up to eliminate outliers with a coefficient  $Q = 1\%$  (Motulsky and Brown 2006).



303 We then calculated  $\mu_{\text{MAX}}$  with the remaining data points by Method B (**Table 4**,  
304 **Supplementary Material**). As expected, different  $\mu_{\text{MAX}}$  values were calculated and their  
305 standard errors were lower than the ones obtained by Method B without removal of outliers.

#### 306 **Table 4**

307 Next, we performed statistical comparisons of the data from **Table 4** to check if the  
308 methods would yield the same results. Method A required a t-test to compare the averages  
309 from different treatments (in this case, the two cultivation media) and define whether their  
310 difference was statistically significant or not. A two-tailed, pooled t-test was chosen because  
311 we assumed that both populations were independent and normally distributed, their variances  
312 were unknown but equal, and the sample sizes were small ( $n = 5$  for each data set)  
313 (Montgomery and Runger 2011). Method B, on the other hand, relied on an F-test, which is  
314 equivalent to an Analysis of Covariance (ANCOVA). The F-value is based on the residual  
315 sum-of-squares of both the common and the pooled regressions, the number of regressions  
316 tested, and the degrees of freedom of the pooled regression (details in (Zar 2010) and  
317 **Supplementary Material**). For both methods, the null hypothesis was  $H_0: \mu_{\text{MAX},1} = \mu_{\text{MAX},2}$ ,  
318 and the alternative hypothesis was  $H_1: \mu_{\text{MAX},1} \neq \mu_{\text{MAX},2}$ . If the calculated p-value was less  
319 than the significance level  $\alpha$  (0.05 or 0.01), we would reject the null hypothesis and the  $\mu_{\text{max}}$   
320 from the two cultivation media could be considered different at the significance level used  
321 (**Table 5**).

#### 322 **Table 5**

323 Depending on the method and the significance level applied, the outcomes of the  
324 comparison diverged, as shown by the resulting p-values. At  $\alpha = 0.01$ , both methods A and B  
325 (with the complete data set) agreed in that the  $\mu_{\text{MAX}}$  values of strain CAT-1 in defined or  
326 complex media are not statistically different from each other. However, at  $\alpha = 0.05$  the  
327 methods disagreed. A different result was observed for strain *S. cerevisiae* UFMG-CM-Y259.  
328 At  $\alpha = 0.05$  both methods resulted in a significant difference between defined and complex  
329 media, whereas that was not the case at  $\alpha = 0.01$ . Other strains were also tested, but the same

330 conclusions were achieved from both methods and significance levels (**Supplementary**  
331 **Material**). After the removal of outliers, Method B resulted in completely different  
332 conclusions at both  $\alpha$  for strain CAT-1, when compared to the same method using all data  
333 points.

334 Even though Method A is widely used due to its simplicity and straightforwardness, it  
335 may not be the best way to calculate the error associated to  $\mu_{\text{MAX}}$  values. Each replicate  $\mu_{\text{MAX}}$ ,  
336 once calculated independently, already has its own error associated to the fitness of the  
337 regression line itself. But these errors are not taken into account by Method A as they are  
338 simply not calculated, differently from Method B. Additionally, we showed that the removal  
339 of outliers was decisive for the results. One can easily see that the comparison between  $\mu_{\text{MAX}}$   
340 values calculated using distinct methods is extremely discouraged. First, because results from  
341 statistical comparisons are always to be taken with caution. Second, poorly described statistics  
342 in microbial physiology papers makes it difficult to understand how data were obtained and  
343 even more difficult to know whether interlaboratory comparisons can be performed.

#### 344 **Influence of the type of medium on $\mu_{\text{MAX}}$ calculations**

345 Researchers often report  $\mu_{\text{MAX}}$  values of a yeast strain on a given carbon and energy  
346 source, such as glucose. However, whether this carbon source is provided in a synthetic  
347 defined medium or in a complex undefined medium will influence the growth rate of a  
348 microbial population. In principle,  $\mu_{\text{MAX}}$  values should be higher in the latter environment,  
349 because cells benefit from compounds that can be taken up directly from the medium, instead  
350 of having to synthesize them from metabolic intermediates at the expense of energy. To verify  
351 to which extent  $\mu_{\text{MAX}}$  values vary between these two types of media, we evaluated this  
352 physiological parameter for eight different *S. cerevisiae* strains cultivated in microplates (**Fig.**  
353 **2**).

354 **Fig. 2**

355 Overall, the  $\mu_{\text{MAX}}$  values were higher for a given strain in YPD medium than in  
356 defined Verduyn medium, as expected. Nevertheless, the level to which this occurs varies  
357 among strains (**Table 6**), and, for a few cases, the difference between the pair of  $\mu_{\text{MAX}}$  values  
358 was not significant at 95% or higher confidence level. The complex/defined  $\mu_{\text{MAX}}$  ratio ranged  
359 from 1.12 to 2.33, which is quite remarkable, considering that all strains belong to the same  
360 species and that both media employed here are commonly used in experimental research. We  
361 were not able to identify any trend in these data, e.g. whether the haploid CEN.PK113-7D  
362 strain would present a different behavior than the diploid ones, or whether industrial strains  
363 (CAT-1, JP-1, PE-2) would behave differently than the laboratory, the baker's or the wild  
364 isolates. This indicates that these results are probably related to cell morphology, which  
365 strongly influence Abs measurements (Stevenson et al. 2016), rather than to cells' metabolism  
366 or physiology, once again highlighting the importance of taking great care when calculating  
367 and/or interpreting  $\mu_{\text{MAX}}$  values from such indirect, light-scattering-based methods.

368 Although complex and defined media must contain all the essential nutrients for cell  
369 growth, Abelovska and colleagues (Abelovska et al. 2007) demonstrated that the amount of  
370 some compounds can vary up to 20 fold from one sort to another. These authors compared the  
371 elemental composition of complex (2% peptone, 1% yeast extract) and minimal media (yeast  
372 nitrogen base), and detected lower levels of important enzyme cofactors such as magnesium  
373 and manganese in the complex medium. However, for the cofactors iron and zinc, as well as  
374 for sodium and potassium ions, which are crucial elements in the generation of  
375 electrochemical potential across the cell membrane (Madigan et al. 2012), the results turned  
376 out to be the opposite.

## 377 **Table 6**

### 378 **Influence of the cultivation system on $\mu_{\text{MAX}}$ calculations**

379 We assessed how the cultivation system affects the calculation of  $\mu_{\text{MAX}}$  by comparing  
380 the calculated values obtained from microplate, shake flask, and bioreactor cultivations of  
381 three *S. cerevisiae* strains (**Fig. 3**). The calculations were performed considering the Abs

382 values of distinct samples from the EGP as described in the Material and Methods section  
383 (**Fig. 1, Method B**). For any particular strain, the three systems led to different  $\mu_{\text{MAX}}$  values,  
384 with the lowest values always being achieved using microplate cultivations. This is consistent  
385 with our expectations, and has been observed before with bacteria (Potvin et al. 1997). Cells  
386 are exposed to different growth conditions in the three systems, leading to varying oxygen  
387 availabilities and pH values. This per se should lead to different physiologies.

388         However, the measuring peculiarities of each system also contribute to the observed  
389 differences in  $\mu_{\text{MAX}}$ . While in microplates the absorbance is usually measured without prior  
390 dilution of the cell broth, in the other two setups, dilution is performed to assure the measured  
391 Abs values fall within the limits of proportionality with cell number or dry cell mass  
392 (Madigan et al. 2012). The real Abs is then calculated by multiplying the measured value by  
393 the dilution factor. Begot and co-workers (Begot et al. 1996) evaluated the growth of several  
394 *Listeria monocytogenes* strains in both microplate and bioreactor systems, and showed that  
395 the range of proportionality between Abs and bacterial population (CFU/mL) depended on the  
396 apparatus used to measure Abs, which adds even more complexity and demands prior  
397 knowledge on the particular piece of equipment used.

398         In the case of the results shown here, the spectrophotometer used for measuring the  
399 absorbance during shake-flask cultivations was different from the one used for the bioreactor  
400 cultivations (see Material and Methods section for specifications), as these experiments were  
401 performed in different laboratories. Thus, one should also take the contribution of changing  
402 the equipment into account, when interpreting these data. As an example of how different  
403 spectrophotometers can affect the measurements, Koch (Koch 1970) demonstrated that the  
404 standard curves of apparent absorbance versus bacterial dry mass concentrations vary among  
405 different instruments under a selected range of wavelengths and aperture widths. By apparent  
406 absorbance the author refers to the absorbance measured in non-ideal turbidimeters. Because  
407 the absorbance represents the logarithmic difference between the light transmitted by the light

408 source and the light received by the detector, the slit width plays an important role in  
409 quantifying this parameter, and so does the wavelength (Stevenson et al. 2016).

410 **Fig. 3**

411 **A practical example on how to misinterpret  $\mu_{MAX}$  values**

412 To further illustrate the importance of taking proper care while reporting or  
413 interpreting  $\mu_{MAX}$  data, we calculated this parameter for some *S. cerevisiae* strains during  
414 cultivations in a defined medium containing a carbon and energy source other than glucose,  
415 namely sucrose or fructose. These  $\mu_{MAX}$  values were then compared to the glucose data, both  
416 for microplate and shake-flask cultivations. As an example, a researcher could be interested in  
417 verifying on which of the three sugars yeast would grow with the highest  $\mu_{MAX}$ , or one could  
418 be interested in screening several yeast strains for fructophilic behavior, which is a desirable  
419 feature in the wine industry, for instance, to overcome challenges with stuck fermentations  
420 (Bauer and Pretorius; Berthels et al. 2004; Tronchoni et al. 2009).

421 The results obtained in microplates do not necessarily corroborate those obtained in  
422 shake-flask cultivations (**Fig. 4**). For instance, the UFMG-CM-Y259 strain displayed faster  
423 growth on sucrose in the microscale system, compared to its growth on either of the hexoses.  
424 In shake-flask cultivations, however it grew with a smaller  $\mu_{max}$  on sucrose, again compared to  
425 growth on glucose or fructose. The CEN.PK113-7D strain also displayed a higher  $\mu_{MAX}$  on  
426 sucrose in microplate cultivations, but no significant difference was observed in the  $\mu_{MAX}$   
427 values on the three substrates during shake-flask cultivations.

428 When considering growth on fructose, in comparison to glucose only, the UFMG-  
429 CM-Y257 strain showed higher  $\mu_{MAX}$  on glucose for cultivations using microplates, whereas  
430 equivalent growth rates on both substrates were observed during shake-flask cultivations. The  
431 opposite was observed for the JP1 strain. Resolving the mechanisms underlying such different  
432 behaviors is beyond the scope of this work. Here, the importance relies on the fact that one  
433 could easily miss the cultivation system-dependency of  $\mu_{MAX}$  in *S. cerevisiae*, if a careful  
434 evaluation of the reported methodologies was not performed. In fact, in a typical scientific

435 study, more than one cultivation system is seldom employed. In spite of this, comparisons  
436 with literature data are often reported, without properly highlighting the differences in the  
437 experimental setup between the evaluated studies, which frequently leads to misinterpretation.

438

439 **Fig. 4**

## 440 **Final remarks**

441 Determining the maximum specific growth rate is routine in any microbiology  
442 laboratory, be it in industry or academia. The several different methods available for this  
443 purpose, however, add up to challenge this task. Most frequently, researchers report the  $\mu_{\text{MAX}}$   
444 values they calculate in a comparative manner, either with external publications or with those  
445 within their research group. The challenge of these comparative analyses is to assure that the  
446 evaluated cultivations and analytical procedures have been executed in the exact same way,  
447 and with proper caution. We demonstrated here, through a series of examples, the  
448 implications on  $\mu_{\text{MAX}}$  calculations when distinct cultivations setups or analytical  
449 methodologies are employed. We, therefore, would like to draw the attention of our fellow  
450 microbiologists to the following:

451 1) Avoid calculations of  $\mu_{\text{MAX}}$  directly from Abs measurements. First convert the Abs  
452 data to real cell concentration values using a pre-established calibration equation, obtained  
453 under identical cultivation conditions, and only then calculate  $\mu_{\text{MAX}}$ . This calibration equation  
454 can be established using data from an entire batch cultivation, but ideally only data points in  
455 the EGP should be used to avoid any eventual artifacts introduced by cell morphology  
456 changes.

457 2) When methodologies other than obtaining  $\mu_{\text{MAX}}$  directly from Abs measurements  
458 are not an option, one should never think of the calculated values as absolute. Comparisons  
459 with data reported in different works should thus be avoided.

460 3) Always make comparisons of your own calculated  $\mu_{\text{MAX}}$  values with caution and  
461 explicitly report the conditions used by other authors or under which other experiments in the  
462 same lab were carried out.

463 4) Do not overstate findings related to  $\mu_{\text{MAX}}$ , since its value can vary with any  
464 cultivation detail that is different, such as the geometry of the cultivation vessel, contaminants  
465 present in chemicals used to formulate media, rotation radius of the shaker incubator, method  
466 used to determine the cell concentration, etc.

467 5) Decide on a statistical method to use for comparisons between your own  $\mu_{\text{MAX}}$  data  
468 and explicitly describe it. Report p-values rather than simply stating the statistical conclusion  
469 (Valentin Amrhein 2019).

470 6) Describe all calculations in detail, even if they are quite obvious to some.  
471 Supplementary material in research articles or data repositories could be used for this purpose.  
472 This will make comparisons easier, more meaningful and scientifically more sound.

## 473 **Supplementary material**

474 **Fig. S1** Illustration of  $\mu_{\text{Max}}$  calculation, using the eight different methods described in Table  
475 S1, for *S. cerevisiae* strains CEN.PK113-7D, JP1, and UFMG-CM-Y259 cultivated in aerobic  
476 batch bioreactors with glucose as sole carbon and energy source.

477 **Table S1** Absorbance and cell concentration data for *S. cerevisiae* CEN.PK113-7D, JP1, and  
478 UFMG-CM-Y259 grown on glucose in aerobic batch bioreactors. Experiments were carried  
479 out in duplicate. Experimental data is highlighted in green.

480 **Table S2** Maximum specific growth rate, calculated using different calibration approaches, of  
481 *S. cerevisiae* CEN.PK113-7D, JP1, and UFMG-CM-Y259 during growth on glucose in  
482 aerobic batch bioreactors.

483 **Table S3** Maximum specific growth rate ( $\mu_{\text{MAX}}$ ) for eight different *S. cerevisiae* strains grown  
484 on either defined or complex medium supplemented with glucose as sole carbon and energy  
485 source, using microplate as cultivation system. Experiments were carried out in five

486 replicates, and for each replicate one  $\mu_{\text{MAX}}$  was calculated from Abs600 data within the  
487 exponential growth phase (EGP).

488 **Table S4** Comparative statistical analysis, based on method A<sup>a</sup>, of the maximum specific  
489 growth rates showed in Table S3.

490 **Table S5** Comparative statistical analysis, based on method B<sup>1</sup>, of the maximum specific  
491 growth rates ( $\mu_{\text{MAX}}$ ) of different *S. cerevisiae* strains grown on either defined or complex  
492 medium supplemented with glucose as sole carbon and energy source, using microplate as  
493 cultivation system. Experiments were performed in five replicates. One single  $\mu_{\text{MAX}}$  was  
494 calculated from Abs600 data from all replicates.

495 **Table S8** Raw Abs600 data from the exponential phase of growth of *S. cerevisiae* CAT-1  
496 cultivated on either defined or complex medium supplemented with glucose as sole carbon  
497 and energy source, using microplate as cultivation system.

498 **Table S7** Calculations for testing for significant differences among slopes for the *S.*  
499 *cerevisiae* strain CAT-1.

500 **Table S8** Summary of the statistical outcome of the F-test for the *S. cerevisiae* strain CAT-1.

501 **Table S9** Raw Abs600 data from the exponential phase of growth of *S. cerevisiae* CAT-1  
502 cultivated on either defined or complex medium supplemented with glucose as sole carbon  
503 and energy source, using microplate as cultivation system. The crossed out data represent the  
504 outliers identified using ROUT option on GraphPad Prism software with  $Q = 1\%$ .

505 **Table S10** Calculations for testing for significant differences among slopes for the *S.*  
506 *cerevisiae* strain CAT-1 after removal of outliers.

507 **Table S11** Summary of the statistical outcome of the F-test for the *S. cerevisiae* strain CAT-1  
508 after removal of outliers.

509 **Table S12** Comparative statistical analysis, based on method B<sup>1</sup>, of the maximum specific  
510 growth rates displayed by *S. cerevisiae* CEN.PK113-7D, JP1, and UFMG-CM-Y259 during  
511 growth on synthetic medium supplemented with glucose as sole carbon and energy source,  
512 using either microplate, shake-flask, or bioreactor as cultivation system.



513 **Table S13** Comparative statistical analysis, based on method B<sup>1</sup>, of the maximum specific  
514 growth rates displayed by *S. cerevisiae* CEN.PK113-7D, JP1, PE-2, UFMG-CM-Y257, and  
515 UFMG-CM-Y259 during growth on synthetic medium supplemented with glucose, fructose,  
516 or sucrose as sole carbon and energy source, using either microplate or shake-flask as  
517 cultivation system.

## 518 **References**

519 Abelovska L, Bujdos M, Kubova J, et al (2007) Comparison of element levels in  
520 minimal and complex yeast media. *Can J Microbiol.*  
521 <https://doi.org/10.1139/W07-012>

522 Basso LC, De Amorim H V., De Oliveira AJ, Lopes ML (2008) Yeast selection for  
523 fuel ethanol production in Brazil. In: *FEMS Yeast Research*

524 Bauer EF, Pretorius LS Yeast Stress Response and Fermentation Efficiency: How to  
525 Survive the Making of Wine-A Review

526 Beato FB, Bergdahl B, Rosa CA, et al (2016) Physiology of *Saccharomyces*  
527 *cerevisiae* strains isolated from Brazilian biomes: New insights into biodiversity  
528 and industrial applications. *FEMS Yeast Res.*  
529 <https://doi.org/10.1093/femsyr/fow076>

530 Begot C, Desnier I, Daudin JD, et al (1996) Recommendations for calculating growth  
531 parameters by optical density measurements. *J Microbiol Methods* 25:225–232.  
532 [https://doi.org/10.1016/0167-7012\(95\)00090-9](https://doi.org/10.1016/0167-7012(95)00090-9)

533 Berthels NJ, Cordero Otero RR, Bauer FF, et al (2004) Discrepancy in glucose and  
534 fructose utilisation during fermentation by *Saccharomyces cerevisiae* wine yeast  
535 strains. *FEMS Yeast Res.* <https://doi.org/10.1016/j.femsyr.2004.02.005>

536 Borzani W (1994) A general equation for the evaluation of the error that affects the  
537 value of the maximum specific growth rate. *World J Microbiol Biotechnol*

538 10:475–476. <https://doi.org/10.1007/BF00144476>

539 Borzani W (1980) Evaluation of the error that affects the value of the maximum  
540 specific growth rate. *J Ferment Technol* 58:299–300

541 Campbell A (1957) Synchronization of cell division. *Bacteriol Rev* 21:263–272

542 Clarke KG (2013) Microbial kinetics during batch, continuous and fed-batch  
543 processes. In: *Bioprocess Engineering*, 1st Editio. Woodhead Publishing  
544 Limited, pp 97–146

545 Cooper S (2019) The synchronization manifesto: a critique of whole-culture  
546 synchronization. *FEBS J* 286:4650–4656. <https://doi.org/10.1111/febs.15050>

547 Da Silva Filho EA, De Melo HF, Antunes DF, et al (2005) Isolation by genetic and  
548 physiological characteristics of a fuel-ethanol fermentative *Saccharomyces*  
549 *cerevisiae* strain with potential for genetic manipulation. *J Ind Microbiol*  
550 *Biotechnol.* <https://doi.org/10.1007/s10295-005-0027-6>

551 Della-bianca B, Gombert AK (2013) Stress tolerance and growth physiology of yeast  
552 strains from the Brazilian fuel ethanol industry. 1083–1095.  
553 <https://doi.org/10.1007/s10482-013-0030-2>

554 Doran P (2012) Homogeneous Reactions. In: *Bioprocess Engineering Principles*, 2nd  
555 Editio. Academic Press Inc., pp 599–701

556 Harris CM, Todd RW, Bungard SJ, et al (1987) Dielectric permittivity of microbial  
557 suspensions at radio frequencies: a novel method for the real-time estimation of  
558 microbial biomass. *Enzyme Microb Technol* 9:181–186.  
559 [https://doi.org/10.1016/0141-0229\(87\)90075-5](https://doi.org/10.1016/0141-0229(87)90075-5)

560 Jannasch HW, Egli T (1993) Microbial growth kinetics: a historical perspective.  
561 *Antonie Van Leeuwenhoek* 63:213–224. <https://doi.org/10.1007/BF00871219>

562 Juanes MA (2017) Methods of Synchronization of Yeast Cells for the Analysis of Cell

563 Cycle Progression. In: Monje-Casas F, Queralt E (eds) The Mitotic Exit  
564 Network: Methods and Protocols. Springer New York, New York, NY, pp 19–34

565 Koch AL (1970) Turbidity measurements of bacterial cultures in some available  
566 commercial instruments. *Anal Biochem* 38:252–259.  
567 [https://doi.org/10.1016/0003-2697\(70\)90174-0](https://doi.org/10.1016/0003-2697(70)90174-0)

568 Lenski RE, Rose MR, Simpson SC, Tadler SC (1991) Long-Term Experimental  
569 Evolution in *Escherichia coli* . I . Adaptation and Divergence During 2 , 000  
570 Generations. *Am Nat* 138:1315–1341

571 Liu S (2016) How Cells Grow. In: *Bioprocess Engineering*, 2nd edn. Elsevier, pp  
572 629–697

573 Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of*  
574 *Microorganisms*, 13th edn. Benjamin Cummings, San Francisco

575 Manhart M, Shakhnovich EI (2018) Growth tradeoffs produce complex microbial  
576 communities on a single limiting resource. *Nat Commun* 9:.  
577 <https://doi.org/10.1038/s41467-018-05703-6>

578 McDonald JH (2014) *Handbook of biological Statistics*, 3rd edn. Spark House  
579 Publishing, Baltimore

580 Montgomery DC, Runger GC (2011) *Applied Statistics and Probability for Engineers*,  
581 5th edn. John Wiley and Sons Inc.

582 Motulsky HJ (2020) Analysis checklist: Simple linear regression. In: *GraphPad Curve*  
583 *Fitting Guid.* [http://www.graphpad.com/guides/prism/8/curve-](http://www.graphpad.com/guides/prism/8/curve-fitting/reg_analysischeck_linearreg.htm)  
584 [fitting/reg\\_analysischeck\\_linearreg.htm](http://www.graphpad.com/guides/prism/8/curve-fitting/reg_analysischeck_linearreg.htm). Accessed 5 Oct 2020

585 Motulsky HJ, Brown RE (2006) Detecting outliers when fitting data with nonlinear  
586 regression - A new method based on robust nonlinear regression and the false  
587 discovery rate. *BMC Bioinformatics* 7:1–20. <https://doi.org/10.1186/1471-2105->

588 7-123

589 Olsson L, Nielsen J (1997) On-line and in situ monitoring of biomass in submerged  
590 cultivations

591 Oner MD, Erickson LE, Yang SS (1986) Analysis of exponential growth data for  
592 yoghurt cultures. *Biotechnol Bioeng* 28:895–901.  
593 <https://doi.org/10.1002/bit.260280617>

594 Potvin J, Fonchy E, Conway J, Champagne CP (1997) An automatic turbidimetric  
595 method to screen yeast extracts as fermentation nutrient ingredients. *J Microbiol*  
596 *Methods* 29:153–160. [https://doi.org/10.1016/S0167-7012\(97\)00032-8](https://doi.org/10.1016/S0167-7012(97)00032-8)

597 Pylvänäinen I (2005) A parametric approach to yeast growth curve estimation and  
598 standardization. Chalmers University of Technology and Goteborg University

599 Shuler ML, Kargi F (2002) *Bioprocess Engineering Basic Concepts*, 2nd edn. Prentice  
600 Hall PTR, Upper Saddle River, NJ.

601 Sonnleitner B, Locher G, Fiechter A (1992) Biomass determination. *J Biotechnol*  
602 25:5–22. [https://doi.org/10.1016/0168-1656\(92\)90107-K](https://doi.org/10.1016/0168-1656(92)90107-K)

603 Stanbury PF, Whitaker A, Hall SJ (2017) Microbial growth kinetics. In: *Principles of*  
604 *Fermentation Technology*. Elsevier Ltd, pp 21–74

605 Stevenson K, McVey AF, Clark IBN, et al (2016) General calibration of microbial  
606 growth in microplate readers. *Sci Rep* 6:. <https://doi.org/10.1038/srep38828>

607 Tronchoni J, Gamero A, Arroyo-López FN, et al (2009) Differences in the glucose  
608 and fructose consumption profiles in diverse *Saccharomyces* wine species and  
609 their hybrids during grape juice fermentation. *Int J Food Microbiol.*  
610 <https://doi.org/10.1016/j.ijfoodmicro.2009.07.004>

611 Valentin Amrhein SG (2019) Retire statistical significance. *Nature* 567:305–307

612 van Dijken J., Bauer J, Brambilla L, et al (2000) An interlaboratory comparison of

613 physiological and genetic properties of four *Saccharomyces cerevisiae* strains.  
614 *Enzyme Microb Technol* 26:706–714. <https://doi.org/10.1016/S0141->  
615 0229(00)00162-9

616 Verduyn C, Postma E, Scheffers WA, van Dijken JP (1992) Effect of Benzoic Acid  
617 on Metabolic Fluxes in Yeasts: *Yeast* 8:501–517.  
618 <https://doi.org/10.1007/BF00270792>

619 Wheals AE, Lord PG (1992) Clonal heterogeneity in specific growth rate of  
620 *Saccharomyces cerevisiae* cells. *Cell Prolif* 25:217–223. <https://doi.org/j.1365->  
621 2184.1992.tb01396.x

622 Zar JH (2010) *Biostatistical Analysis*, 5th edn. Pearson Prentice-Hall, Upper Saddle  
623 River, NJ.

624

**Table 1** Yeast strains used in this work.

Strain designation	Group	Ploidy	Precedence	References
CEN.PK113-7D	Laboratory	n	Dr. Peter Kötter (University of Frankfurt, Germany)	(van Dijken et al. 2000)
Fleischmann	Industrial (baking)	2n	Dr. L. C. Basso (USP, Brazil)	(Della-bianca and Gombert 2013)
PE-2	Industrial (fuel ethanol)	2n	Dr. L. C. Basso (USP, Brazil)	(Basso et al. 2008)
CAT-1	Industrial (fuel ethanol)	2n	Dr. L. C. Basso (USP, Brazil)	(Basso et al. 2008)
JP1	Industrial (fuel ethanol)	2n	Dr. M. A. de Morais Jr (UFPE, Brazil)	(Da Silva Filho et al. 2005)
UFMG-CM-Y257	Indigenous <sup>a</sup>	2n	Dr. C. A. Rosa (UFMG, Brazil)	(Beato et al. 2016)
UFMG-CM-Y259	Indigenous <sup>a</sup>	2n	Dr. C. A. Rosa (UFMG, Brazil)	(Beato et al. 2016)
UFMG-CM-Y267	Indigenous <sup>b</sup>	2n	Dr. C. A. Rosa (UFMG, Brazil)	(Beato et al. 2016)

<sup>a</sup>Originally from barks of *Quercus rubra*, located within the Brazilian Atlantic Forest biome.

<sup>b</sup>Originally from barks of *Tapira guaianenses*, located within the Brazilian Cerrado biome.

**Table 1** Composition of the cultivation media used in this work.

Cultivation Medium	Components	Composition (g l <sup>-1</sup> )	Cultivation System
Complex (YPD)	Yeast Extract	10.0	Microplate
	Peptone	20.0	
	Glucose	10.0	
Defined Adapted from (Verduyn et al. 1992)	K <sub>2</sub> SO <sub>4</sub>	6.6	Microplate
	CH <sub>4</sub> N <sub>2</sub> O	2.3	
	KH <sub>2</sub> PO <sub>4</sub>	3.3	
	MgSO <sub>4</sub> .7H <sub>2</sub> O	0.5	Shake-flask
	Trace Elements solution	1.0	
	Vitamins solution	1.0	
	Glucose	10.0	
Defined (Verduyn et al. 1992)	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	5.0	Bioreactor
	KH <sub>2</sub> PO <sub>4</sub>	3.0	
	MgSO <sub>4</sub> .7H <sub>2</sub> O	0.5	
	Trace Elements solution	1.0	
	Vitamins solution	1.0	
	Glucose	20.0	

**Table 3**  $\mu_{MAX}$  values calculated using four different approaches for three *S. cerevisiae* strains cultivated in aerobic bioreactors with glucose as sole carbon and energy source.

Approach	CEN.PK113-7D		JP1		UFMG-CM-Y259	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	0.440	0.415	0.423	0.398	0.413	0.452
<u>2</u>	<u>0.315</u>	<u>0.336</u>	<u>0.293</u>	<u>0.260</u>	<u>0.296</u>	<u>0.289</u>
3	0.327	0.327	0.281	0.286	0.315	0.322
4	0.325	0.331	0.330	0.270	0.316	0.304

Approaches: 1) Directly from Abs data; 2) directly from dry cell mass data; 3) from calculated dry cell mass values obtained using a calibration equation established between the Abs and the dry cell mass data, including all data points in the cultivation; 4) from calculated dry cell mass values obtained using a calibration equation established between the Abs and the dry cell mass data, including only data points in the EGP.



**Table 4** Maximum specific growth rates ( $\mu_{\text{MAX}}$ ) for strains *S. cerevisiae* CAT-1 and UFMG-CM-Y259 grown in microplates in two cultivation media, calculated using two different regression methods\*.

Medium	Method A			Method B (all data)			Method B (without outliers)		
	$\mu_{\text{MAX}}$	SD	n	$\mu_{\text{MAX}}$	SE	n	$\mu_{\text{MAX}}$	SE	n
	<b>CAT-1</b>								
Defined	0.2588	0.0171	5	0.2588	0.0131	40	0.2516	0.0039	32
Complex	0.3221	0.0525	5	0.3221	0.0900	20	0.3436	0.0460	16
	<b>UFMG-CM-Y259</b>								
Defined	0.2500	0.0068	5	0.2500	0.0069	40		N.A.	
Complex	0.2808	0.0253	5	0.2808	0.0081	30		N.A.	

\*described in the Methods section. SD is the standard deviation; SE is the standard error of the slope; n is the number of observations.

N.A. = not available. For this case, outliers were not identified.

**Table 5** Statistical comparison of  $\mu_{\text{MAX}}$  values for strains *S. cerevisiae* CAT-1 and UFMG-CM-Y259 grown on defined or complex media, using data from Table 4.

	Test statistic	p-value	Conclusion ( $\alpha = 0.05$ )	Conclusion ( $\alpha = 0.01$ )
	<b>CAT-1</b>			
<b>Method A</b>	2.5632 <sup>a</sup>	0.0335	different $\mu_{\text{MAX}}$	same $\mu_{\text{MAX}}$
<b>Method B (all data)</b>	1.1016 <sup>b</sup>	0.3178	same $\mu_{\text{MAX}}$	same $\mu_{\text{MAX}}$
<b>Method B (without outliers)</b>	9.9324 <sup>b</sup>	0.0029	different $\mu_{\text{MAX}}$	different $\mu_{\text{MAX}}$
	<b>UFMG-CM-Y259</b>			
<b>Method A</b>	2.6294 <sup>a</sup>	0.0302	different $\mu_{\text{MAX}}$	same $\mu_{\text{MAX}}$
<b>Method B (all data)</b>	7.4850 <sup>b</sup>	0.008	different $\mu_{\text{MAX}}$	different $\mu_{\text{MAX}}$

<sup>a</sup> t-test; <sup>b</sup> F-test

**Table 6** Ratio between  $\mu_{MAX}$  of different *S. cerevisiae* strains in a complex medium (YPD) and in a defined medium with glucose as sole carbon and energy source.

STRAIN →	CAT-1	CEN.PK113-7D	Fleischmann	JP1	PE-2	UFMG-CM-Y257	UFMG-CM-Y259	UFMG-CM-Y267
<b>Ratio</b>	1.24	1.73	1.41	2.33	1.69	1.16	1.12	1.91

**Fig. 1** Methods used for calculating and comparing the slope of regression lines ( $\mu_{\max}$ ). Method A yields an average  $\mu_{\max}$  and a standard deviation while Method B yields a unique  $\mu_{\max}$  and a standard error.

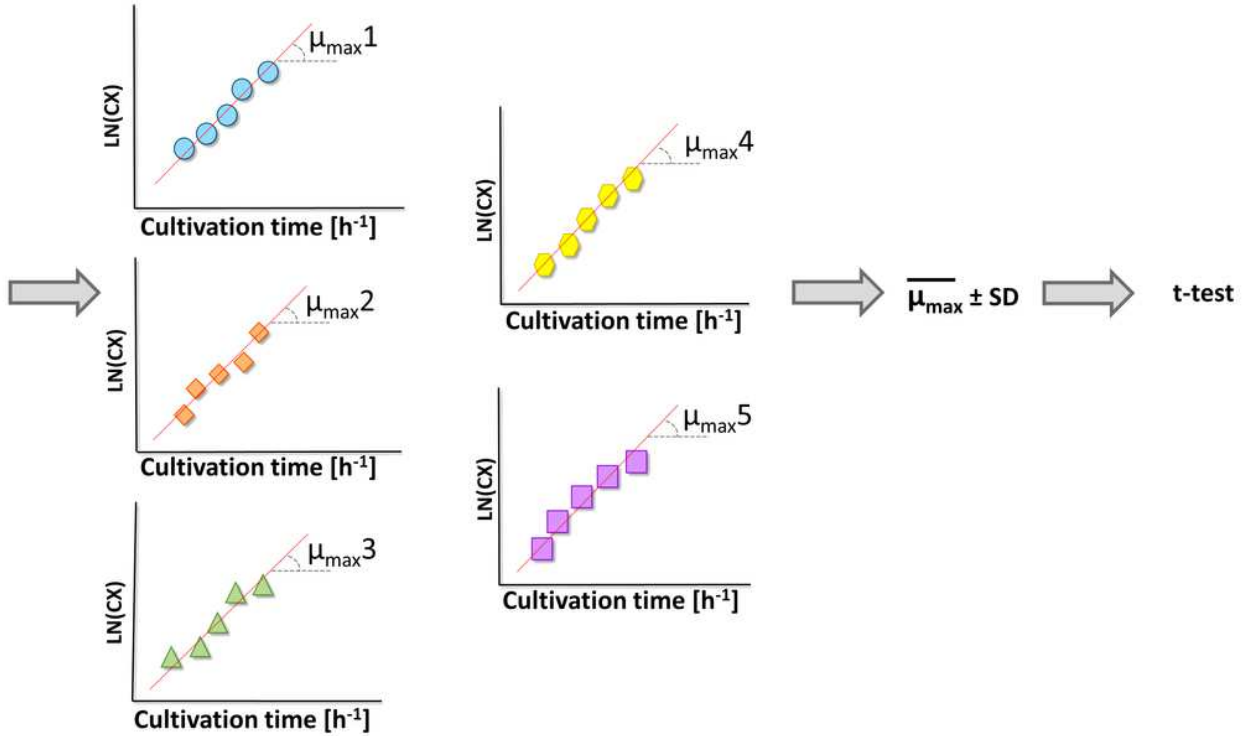
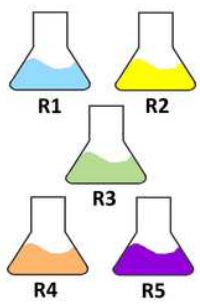
**Fig. 2** Maximum specific growth rates ( $\mu_{\text{MAX}}$ ) of strains grown in microplates in two cultivation media, calculated using two different regression methods (A and B). Depending on the statistical method and the significance level used, distinct conclusions can be drawn. \* represent the p-value at which a significant difference between the treatments were observed; ns ( $p > 0.05$ ); \* ( $p \leq 0.05$ ); \*\* ( $p \leq 0.01$ ); \*\*\* ( $p \leq 0.001$ ); \*\*\*\* ( $p \leq 0.0001$ )

**Fig. 3** Maximum specific growth rates ( $\mu_{\max}$ ) for three *S. cerevisiae* strains grown in a defined medium in three different cultivation systems. Data from different systems were used to calculate and statistically compare  $\mu_{\text{MAX}}$  values using Method B and GraphPad Prism software. This yielded a p-value  $\leq 0.0001$  (\*\*\*\*) for all strains

**Fig. 4** Maximum specific growth rates ( $\mu_{\text{MAX}}$ ) of *S. cerevisiae* strains grown in microplates or in shake-flasks in a defined medium supplemented with sucrose, glucose or fructose as sole carbon and energy source, calculated by Method B

# Figures

## Method A



## Method B

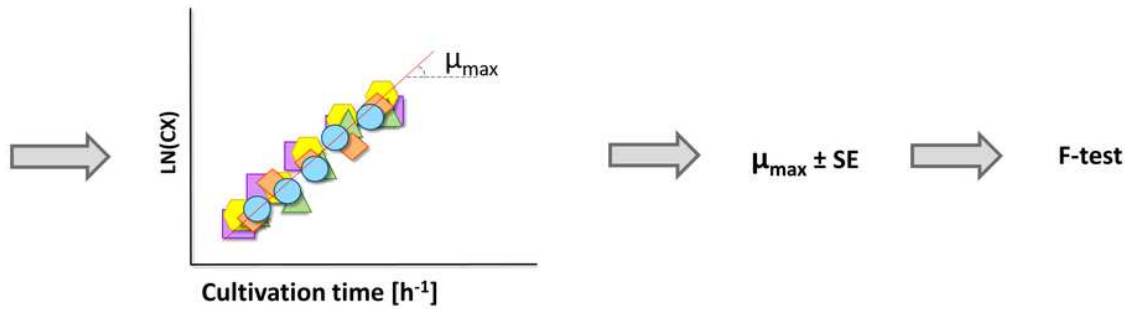
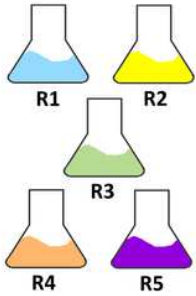
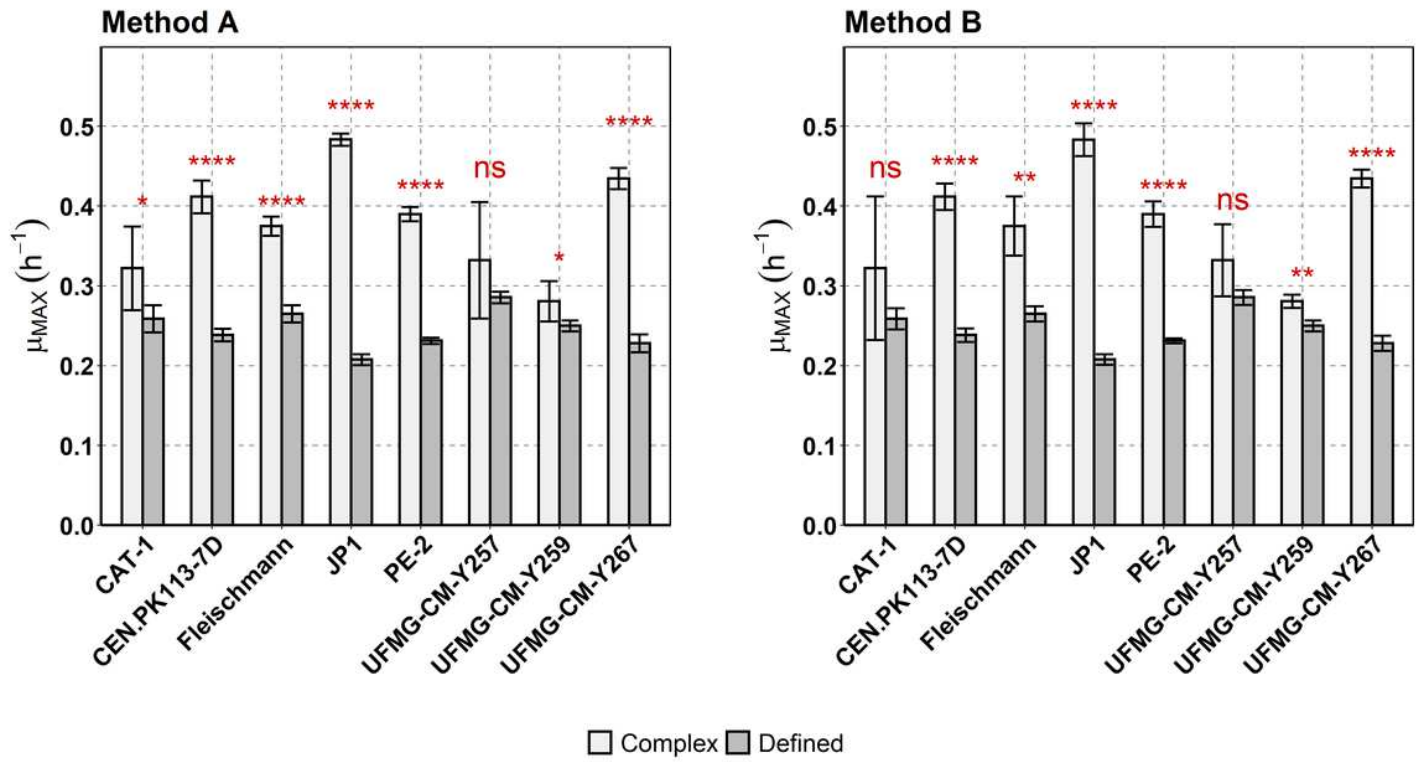


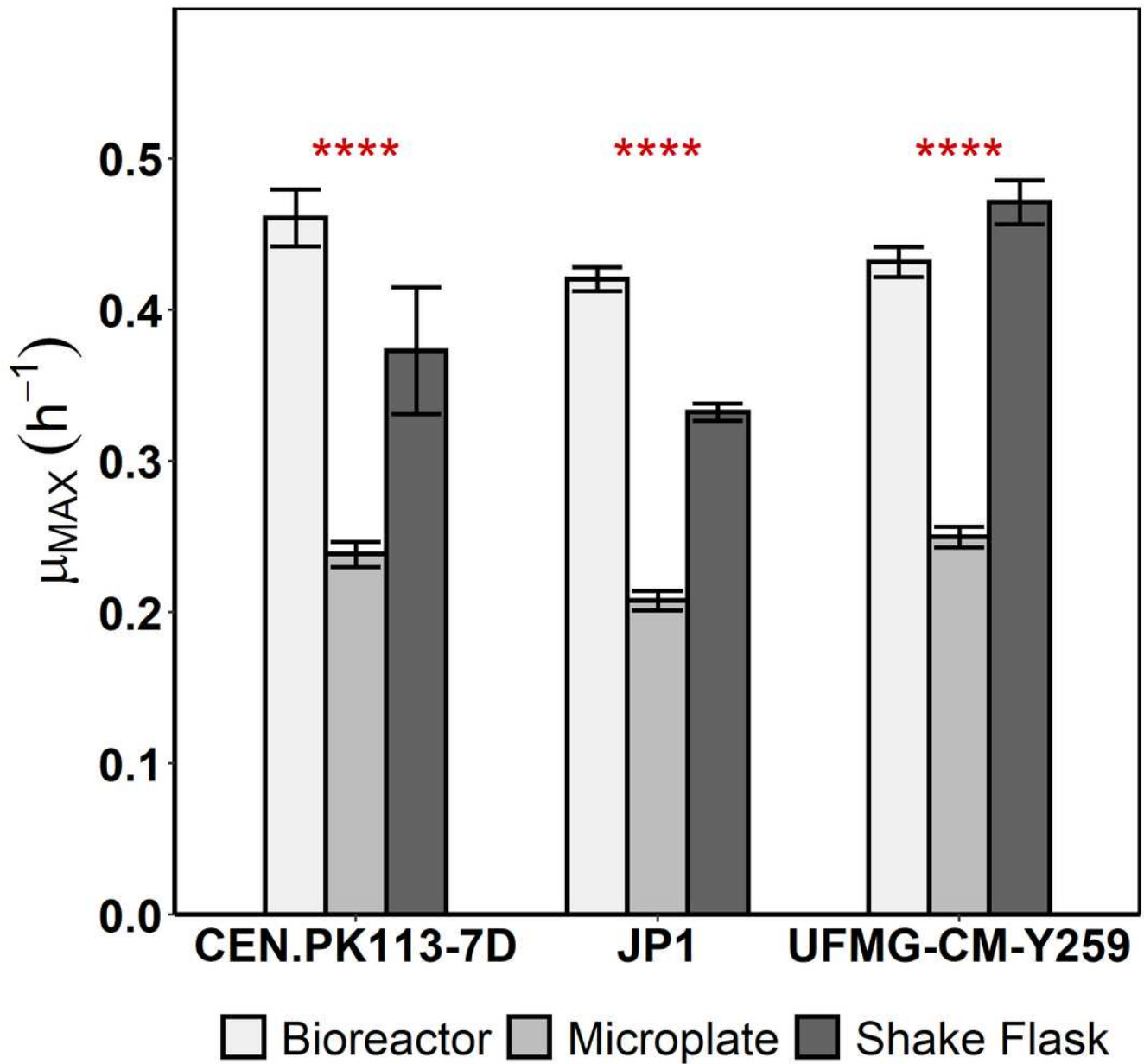
Figure 1

Methods used for calculating and comparing the slope of regression lines ( $\mu_{\max}$ ). Method A yields an average  $\mu_{\max}$  and a standard deviation while Method B yields a unique  $\mu_{\max}$  and a standard error.



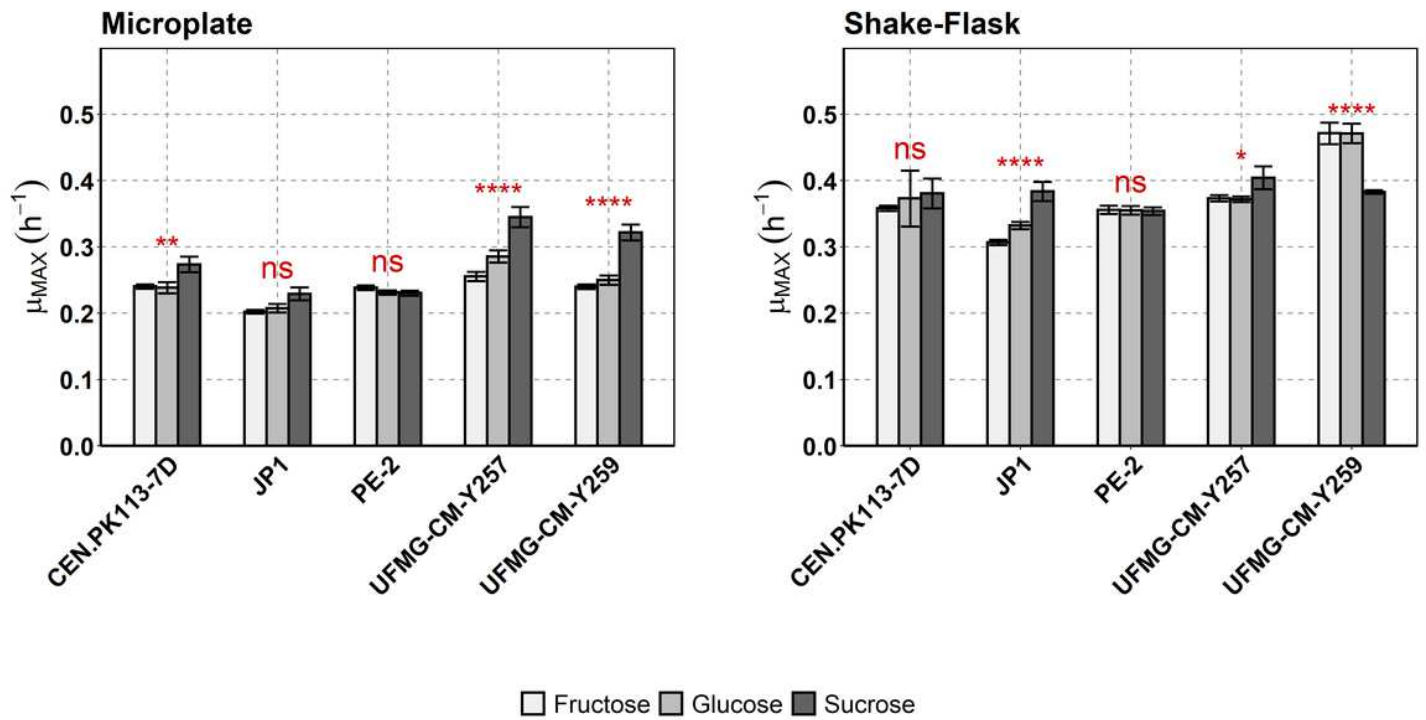
**Figure 2**

Maximum specific growth rates ( $\mu_{MAX}$ ) of strains grown in microplates in two cultivation media, calculated using two different regression methods (A and B). Depending on the statistical method and the significance level used, distinct conclusions can be drawn. \* represent the p-value at which a significant difference between the treatments were observed; ns ( $p > 0.05$ ); \* ( $p \leq 0.05$ ); \*\* ( $p \leq 0.01$ ); \*\*\* ( $p \leq 0.001$ ); \*\*\*\* ( $p \leq 0.0001$ )



**Figure 3**

Maximum specific growth rates ( $\mu_{MAX}$ ) for three *S. cerevisiae* strains grown in a defined medium in three different cultivation systems. Data from different systems were used to calculate and statistically compare  $\mu_{MAX}$  values using Method B and GraphPad Prism software. This yielded a p-value  $\leq 0.0001$  (\*\*\*\*) for all strains



**Figure 4**

Maximum specific growth rates ( $\mu_{MAX}$ ) of *S. cerevisiae* strains grown in microplates or in shake-flasks in a defined medium supplemented with sucrose, glucose or fructose as sole carbon and energy source, calculated by Method B

## Supplementary Files

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- [Supplementarymaterial.docx](#)