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Dimensional analysis and CFD simulations of microcarrier 'just-suspended' state in Mesenchymal Stromal Cells bioreactors

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Abstract

Large-scale Mesenchymal stem / stromal cells culture uses 3D culture systems involving spherical solid particles, called microcarriers. Cells adhere on these spheres, which are then set in suspension in stirred tank bioreactors. This work was more particularly focused on the determination of the critical impeller agitation rate N_{js} , allowing complete beads suspension. It is indeed generally assumed that this value is a good compromise between sufficient nutrients homogenization, mass transfer and minimization of hydromechanical stress encountered by the cells. However, no robust correlation predicting N_{js} in the case of microcarriers can be found in literature. To fill this lack, a set of various operating conditions was carried out, dealing with geometrical variables and two different microcarriers, and N_{js} were experimentally de-

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terminated for 140 conditions. An empirical correlation was established and a dimensional analysis was performed, showing that the impact of the particle concentration on N_{js} was function of the impeller design. Moreover, two dimensionless numbers characterizing the number of particle and an Archimede number applied on the particle cloud were found to better describe the impact of particle diameter and density on N_{js} . Simultaneously, a strategy based on Computational Fluid Dynamics simulations was conducted in order to predict N_{js} and was validated with the N_{js} experimental values.

Keywords: microcarrier suspension, bioreactor, critical just-suspended agitation rate, Computational Fluid Dynamics (CFD), dimensional analysis

1. Introduction

Today, still growing attention is paid to mesenchymal stem / stromal cell (MSC) culture for applications in regenerative medicine, with 843 MSC-based clinical completed or ongoing trials identified (www.clinicaltrials.gov, May 2018). Nevertheless, these cells are present in limited quantities in the human body, and this number decreases with the age of the donor [1]. An *ex-vivo* expansion phase is thus necessary to get sufficient cells quantities to meet the medical needs which are generally estimated between 10^9 and 10^{12} cells/lot [2]. As some other cell sources, MSCs are anchorage-dependent, namely they need to adhere on a surface to proliferate. Thus, MSCs are typically cultivated in static mode in 2D monolayer systems such as planar culture flasks. However, these devices hold some significant disadvantages for expansion process scale-up such as the storage of numerous flasks in incubators, the difficulty of operating conditions control, as well as the operational

15 costs and the contamination risks.

16 To overcome these limits, cultures are now transposed on microcarriers,
17 consisting of spheres of about 200 μm diameter with a density close to water
18 one [3]. Once cells adhere on the surface of these particles, they are suspended
19 in a bioreactor filled with the culture medium by mechanical agitation. The
20 specific adherence surface available for cell expansion is greatly enhanced in
21 comparison with 2D static culture, and the addition of monitoring / control
22 loop is made easier, in accordance with GMP specifications. Moreover, mix-
23 ing allows a better culture homogenization, limiting O_2 , pH and nutrients
24 gradients, enhancing mass transfer and available liquid-solid interfacial area
25 maximization. It has been demonstrated that growth on microcarriers allows
26 a higher number of cells recovered by lot, despite requiring more efforts and
27 investments in R&D for a company currently using T-flasks to change to
28 microcarrier based cultures [4, 5]. Recently, microcarrier MSC cultures have
29 been successfully conducted at a few liter-scale [6, 7].

30 However, while a too low agitation would lead to microcarriers settling
31 in the bottom of the vessel and aggregation [8], a too high agitation may
32 generate damages to the cells [9], induce their differentiation [10] or lead to
33 a failure of the cells to attach efficiently to the particles [11]. These damages
34 may result from three separate mechanisms [12]: collisions with other mi-
35 crocarriers, collisions with bioreactor internals (wall, probes, other immersed
36 equipment, and particularly the impeller) and interactions with turbulent
37 eddies whose sizes are close to microcarriers one [13]. In that case, these
38 authors showed that turbulent eddies led to direct shear damages on the mi-
39 crocarriers either by making them rotate at the Kolmogorov velocity scale,

40 or entrap them in counter-rotating eddies. One reasonable strategy to get a
 41 sufficient mixing performance and minimization of hydromechanical stress is
 42 to operate at the impeller just-suspended agitation rate N_{js} allowing com-
 43 plete bead suspension. This means that bioreactor hydrodynamics have to be
 44 therefore intensively studied to guarantee cells viability and allow a process
 45 scale-up [14], requiring reliable model and numerical tools. Many methods
 46 were established to estimate N_{js} , and some of them were well-developed in
 47 [15]. They were classified in 3 categories: (i) 'empirical methods' with direct
 48 methods like the Zwietering visual method [16, 17, 18] stating that when
 49 N_{js} is reached, particles do not stay longer than 1 or 2 seconds in the vessel
 50 bottom, and indirect methods like the cloud height method [19], the parti-
 51 cle concentration determination using light attenuation technique [20] or the
 52 power number method [21], (ii) 'numerical methods' based on data recovered
 53 from CFD simulations [22, 23], and (iii) finally 'theoretical methods'. In the
 54 last case, investigations were mainly based on the assumption of an equilib-
 55 rium between particle settling and the off-bottom lifting [24, 25, 26] and led
 56 to models derived from experimental results, according to limited ranges of
 57 geometries and particle properties. The most commonly used was developed
 58 by Zwietering [16] and is presented in Eq. (1).

$$N_{js} = S \cdot \nu_L^{0.1} \cdot \left(\frac{g \cdot (\rho_p - \rho_L)}{\rho_L} \right)^{0.45} \cdot X^{0.13} \cdot d_p^{0.2} \cdot D^{-0.85} \quad (1)$$

59 Where X is the particle mass fraction and S is a dimensionless parameter
 60 related to the geometry of the vessel and the impeller, via the ratio of the
 61 impeller diameter on the tank diameter D/T , the ratio of the off-bottom
 62 clearance on the tank diameter C/T and the impeller design. With these

63 dimensionless parameters, S is independent of the scale of the system but
64 not of the system geometry. However, this correlation was established and
65 validated for particles that were significantly denser than microcarriers, like
66 sand or glass beads. It was shown by [27] that the calculation of the S
67 coefficients using Eq. (1), in the same operational conditions, could present
68 up to 50 % overestimation for microcarriers. This difference was attributed to
69 the low density difference between the solid and the liquid phases (around 40
70 kg m⁻³) in the case of microcarriers, which facilitates the lifting of particles
71 from the tank bottom.

72 Complementary studies were conducted on the determination of N_{js} and
73 its dependence on geometrical, physical and operational variables like, among
74 others, the effects of the low off-bottom impeller clearance or the bottom
75 shape on flow patterns evolution [28, 29], the impacts of the solid volume
76 fraction incorporated in the reactor [30], or in the specific case of micro-
77 carriers, the type of microcarriers used [31] and the impeller design leading
78 to different possible agitation modes [32]. Recently, microcarrier suspension
79 were also characterized in orbital shakers and led to the establishment of a
80 new correlation predicting N_{js} . A dimensional analysis provided the most
81 appropriate geometrical characteristics for cell cultures [33]. However, de-
82 spite all of these efforts, no robust correlation predicting N_{js} can be found
83 in the literature for microcarrier suspension in stirred tank bioreactors.

84 To fill this lack, the present study was focused on the numerical prediction
85 of N_{js} in the specific case of microcarriers. To do that, the critical agitation
86 rate N_{js} was experimentally determined for 140 operating conditions in the
87 case of a hemispherical-bottom shape bioreactor. A dimensionless equation

88 was then established using these data. Simultaneously, a strategy based on
89 Computational Fluid Dynamics (CFD) simulations was conducted in order
90 to predict N_{js} for specific operating conditions of the cell culture. In this
91 study, the CFD simulations of particles suspension were validated using the
92 collected experimental data. As a complementary approach to experiments,
93 these preliminary CFD simulations were more particularly motivated by mid-
94 term perspectives of numerical applications, such as (*i*) study of the impact of
95 culture system on the spatial distribution of the particles, (*ii*) the simulation
96 of particle ‘lifelines’ within the bioreactor and particle-particle interactions
97 or (*iii*) the definition of *a priori* scale-up rules.

98 2. Experimental approach

99 2.1. Design of experiment

100 The bioreactor investigated was a hemispherical-bottom transparent glass
101 vessel equipped with three probes (dissolved oxygen, pH and temperature)
102 on the head plate. It was filled with Dulbecco’s Phosphate Buffered Saline
103 solution (DPBS) for a working volume of 1.12 L, at which the liquid height
104 corresponded to the vessel diameter $H = T = 0.12$ m. The temperature
105 was controlled and regulated at 20°C, at which the liquid properties were
106 considered to be the same as water, and a baffle was added to the pH probe in
107 order to avoid vortex formation during the agitation. Operating conditions
108 were chosen to cover a wide range of values for five parameters related to
109 microcarriers, impeller, and design characteristics (Fig. 1 ; see supplementary
110 material 1).

111 Firstly, the impeller geometry impact was investigated by changing the

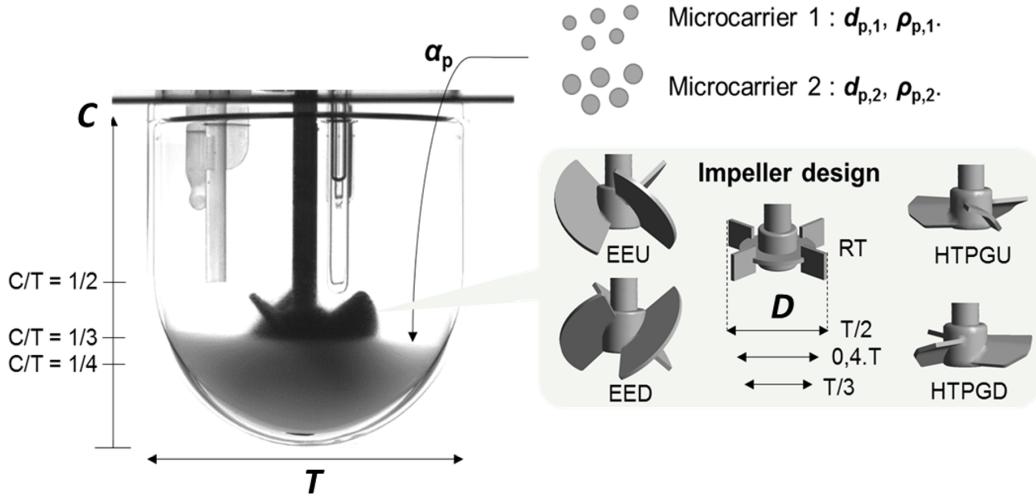


Figure 1: Variables used to define the operating conditions: the off-bottom clearance C and the impeller diameter D in function of the tank diameter T , the type of microcarrier with their specific diameters and density, the particle volume fraction α_p , and the impeller design with the Ear Elephant in up-pumping (EEU) and down-pumping (EED) modes, the HTPG impeller up-pumping (HTPGU) and down-pumping (HTPGD) and the Rushton turbine (RT).

112 impeller design and two geometrical ratios: D/T , defining the impeller di-
 113 ameter on the vessel diameter, and C/T , defining the off-bottom clearance
 114 on the vessel diameter ratio. More specifically, three impeller designs were
 115 studied: a radial four-blade Rushton Turbine (RT), an axial 3-blade impeller
 116 (HTPG) and an Elephant Ear impeller (EE). Both axial impellers were stud-
 117 ied in up-pumping and down-pumping modes, allowing at least five agitation
 118 types. Each impeller was available at three D/T ratios: 0.33, 0.4 and 0.5,
 119 and placed according to three possible C/T ratios: 0.5, 0.33 and 0.25. In this
 120 study, the off-bottom clearance C was considered as the distance between the
 121 vessel bottom and the bottom side of the impeller hub.

122 Secondly, microcarrier properties impact on N_{js} was determined by vary-
 123 ing the particle volume fraction α_S and the type of microcarrier used. Two
 124 different microcarriers were investigated: the Cytodex-1 (GE Healthcare)
 125 composed of a cross-linked dextran matrix with positively charged N, N-
 126 diethylaminoethyl groups, and the Star-Plus (Pall SoloHill) composed of a
 127 polystyrene core and featuring a surface chemistry with a net positive charge.
 128 Cytodex-1 microcarriers swell when put in solution. Their swelling factor,
 129 and thus their diameter and density, depend on the salt concentration of the
 130 solution. After spending a night in a phosphate buffer saline (DPBS) solu-
 131 tion, the diameter distributions were established from microscope pictures
 132 and their density was estimated from their swelling factor and the density
 133 of the dry microcarriers (see [20] for details). The same procedure was also
 134 applied to Star-Plus microcarriers. The physical properties assumed for all
 135 the study were the experimental data given in Table 1. For comparison, the
 136 values provided by the manufacturer were also reported. Five microcarrier
 137 volume fractions ranging from 1 to 12 % were investigated, corresponding to
 138 the concentrations classically used during cell cultures.

Table 1: Microcarrier physical properties: manufacturer and experimental data.

Microcarrier	Manufacturer data			Experimental data			
	ρ_P kg m ⁻³	d_{50} μm	d_{5-95} μm	ρ_P kg m ⁻³	d_{50} μm	d_{5-95} μm	d_{mean} μm
Cytodex-1	1030	190	150-250	1018	170	140-200	180
Star-Plus	1020-1030	N/A	125-212	1020	150	120-170	160

139 *2.2. Experimental suspension criterion and N_{js} visual determination*

140 The vessel was placed in an aquarium with a mirror added below, per-
141 mitting a better visualization and N_{js} was visually established by particle
142 movements at the bottom of the vessel using the protocol of Zwietering cri-
143 terion [20]. For each N_{js} determination, two measurements strategies were
144 performed. For the first one, all particles were settled in the vessel bottom
145 with no agitation followed by a slow increase of the agitation rate until just-
146 suspended state $N_{js, up}$. For the second one, agitation rate was progressively
147 reduced from complete suspension to just-suspended state $N_{js, down}$. For all
148 operating conditions, it was noted that these two agitation rate values re-
149 mained significantly closed from one to each other (mostly a difference of two
150 RPM). Thus, $N_{js, up}$ will be used as N_{js} in the rest of the study to mimic the
151 N_{js} investigation during cultures. 95 operating conditions were used for the
152 model resolution. The model was then validated using 45 other operating
153 conditions.

154 **3. Particle suspension modelling**

155 *3.1. Zwietering correlation validity*

156 First, the N_{js} experimentally collected were used to verify the Zwietering
157 correlation given in Eq. 1, by calculating the experimental S coefficients
158 which are a function of the impeller design, D/T and C/T ratios and the
159 vessel bottom shape. It was also assumed that S is independent of other pa-
160 rameters, such as the solid volume fraction α_S or the particle physical prop-
161 erties ρ_p and d_p . Since not all S coefficients of the experimental conditions
162 presented in the present study were available in the literature, particularly

163 with a hemispherical vessel bottom shape, experimental S can not be di-
164 rectly compared to their theoretical values. However, if it was assumed that
165 they are not dependent of the particle volume fraction and particles diam-
166 eter and density, S should stay constant while only these parameters were
167 varied. This was verified for two different microcarriers at particle volume
168 fractions between 1 % and 12 %. If significant variations were observed be-
169 tween the different experimental values, it would deny the model proposed
170 by Zwietering (Eq. 1) for microcarrier suspension.

171 *3.2. Dimensional analysis*

172 The targeted variable N_{js} was determined according to the 9 following
173 physical parameters:

- 174 • Geometrical parameters including T the tank diameter, D the impeller
175 diameter and C the off-bottom clearance.
- 176 • Material properties of the liquid phase (density ρ_L and dynamic vis-
177 cosity μ_L) and of the solid phase (density ρ_p , diameter d_p and volume
178 fraction α_S).
- 179 • Gravity acceleration g .

180 These parameters were considered independent and involved 3 fundamen-
181 tal dimensions, namely mass, length and time. According to the Vachy-
182 Buckingham theorem, these 10 variables may be related by a relationship
183 between 7 dimensionless numbers. The following dimensionless ratios π_i were
184 thus constructed:

$$\begin{aligned}\pi_1 &= \frac{N_{js} \cdot T^2 \cdot \rho_L}{\mu_L}, & \pi_2 &= \frac{C}{T}, & \pi_3 &= \frac{D}{T}, & \pi_4 &= \frac{d_p}{T}, \\ \pi_5 &= \alpha_S, & \pi_6 &= \frac{g \cdot T^3 \cdot \rho_L^2}{\mu_L^2}, & \pi_7 &= \frac{\rho_p}{\rho_L}.\end{aligned}$$

185 By looking for their physical meaning, they were adapted into the 6 fol-
 186 lowing dimensionless ratios:

- 187 • Geometrical ratios: $\frac{C}{T}$, $\frac{D}{T}$ and $\frac{d_p}{D}$.
- 188 • Reynolds number: $\frac{\rho_L \cdot N_{js} \cdot D^2}{\mu_L}$.
- 189 • Solid volume fraction: α_S .
- 190 • Liquid viscosity ratio $\mu^* = \frac{\mu_L}{(\rho_p - \rho_L) \cdot g^{0.5} \cdot T^{1.5}}$.

191 The last parameter μ^* was adapted from Olmos *et al.* [33], obtained from
 192 the dimensional analysis established for microcarrier suspension in orbital
 193 shaken bioreactors. A second set of dimensionless parameters was proposed
 194 with the following ratios:

- 195 • Geometrical ratios: $\frac{C}{T}$ and $\frac{D}{T}$.
- 196 • Reynolds number: $\frac{\rho_L \cdot N_{js} \cdot D^2}{\mu_L}$.
- 197 • Solid volume fraction: α_S .
- 198 • Archimede number of the particle cloud: $\frac{g \cdot \alpha_S \cdot T^3 \cdot \rho_L \cdot (\rho_p - \rho_L)}{\mu_L^2}$.
- 199 • Dimensionless number related to the particle number: $\left[\alpha_S \cdot \left(\frac{T}{d_p} \right) \right]^3$.

200 On the basis of the experimental data, three monomial models were build
 201 and the most robust N_{js} model was determined. The first one, given in Eq.
 202 (2) expressed N_{js} directly as a function of α_S , C , D and d_p , whereas the two
 203 others presented in Eqs. (3) and (4) involved the two set of dimensionless
 204 ratios, with the targeted variable N_{js} through the Reynolds number Re_{js} .

205 Model 1

$$N_{js} = K_1 \cdot \alpha_S^{a_1} \cdot C^{b_1} \cdot D^{c_1} \cdot d_p^{d_1} \quad (2)$$

206 Model 2

$$Re_{js} = K_2 \cdot \alpha_S^{a_2} \cdot \left(\frac{C}{T} \right)^{b_2} \cdot \left(\frac{D}{T} \right)^{c_2} \cdot \left(\frac{d_p}{D} \right)^{d_2} \cdot \left(\frac{\mu_L}{(\rho_p - \rho_L) \cdot g^{0.5} \cdot T^{1.5}} \right)^{e_2} \quad (3)$$

207 Model 3

$$Re_{js} = K_3 \cdot \alpha_S^{a_3} \cdot \left(\frac{C}{T} \right)^{b_3} \cdot \left(\frac{D}{T} \right)^{c_3} \cdot \left[\alpha_S \cdot \left(\frac{T}{d_p} \right)^3 \right]^{d_3} \cdot \left(\frac{g \cdot \alpha_S \cdot T^3 \cdot \rho_L \cdot (\rho_p - \rho_L)}{\mu_L^2} \right)^{e_3} \quad (4)$$

208 In Eqs. (2) - (4), K_i are constants characterizing the impeller design and
 209 a_i , b_i , c_i , d_i and e_i are the exponents to which the parameters or dimensionless
 210 numbers were raised to. It is important to emphasize that in this study d_p , ρ_p
 211 and the liquid properties, ρ_L and μ_L , were specific to microcarriers and chosen
 212 in the industrial context of animal cell culture. Hence, d_p and ρ_p belonged
 213 to a narrow range of values, with a density close to the liquid phase similar
 214 to water. The phase density difference was found very sensitive in the model

resolution, caused by very low values and inaccuracies. So, $(\rho_p - \rho_L)$ was not included in the first model (Eq. (2)), and e_i exponents for model 2 and 3 (Eqs. (3) and (4)) were not possible to determine by experimental data fit. Consequently, another strategy was adopted to calculate these values. It was indeed demonstrated by Mersmann *et al.* that particle suspensions occurred at constant (P/V) ratios for systems with geometrical similarities [24]. The model proposed should thus respect this assumption to be consistent, so e_i coefficients were calculated in order to keep constant the $(P/V)_{js}$ ratio defined in Eq. (5).

$$\left(\frac{P}{V}\right)_{js} = \frac{N_p(N = N_{js}) \cdot \rho_L \cdot N_{js}^3 \cdot D^5}{V} \quad (5)$$

In the present study, Reynolds numbers were calculated between 1000 and 12000. Without reliable N_p measurements in a such small volume, N_p was nevertheless considered steady in this flow regime. A constant $(P/V)_{js}$ would lead to the equation (6).

$$\frac{N_{js}^3 \cdot D^5}{T^3} \propto 1 \quad (6)$$

In order to reduce the number of exponents to be solved, (D/T) exponent determination was based on the literature. In one hand, the Zwietering model (Eq. (1)) proposed a value of -0.85, admitting that the model constant was also function of the impeller diameter. In the other hand, Nienow *et al.* extracted the impeller variable from the constant and obtained a final exponent of -2.21 [17]. The present models were thus solved for different values comprised between these two bounds. They were solved by using an optimization algorithm (non-linear, GRC, Excel, Microsoft) in order to get

the last exponent values a_i , b_i and d_i . The objective of this optimization consisted in minimizing the criterion $\omega = \sum_{i=0}^{n_{exp}} (N_{js}^{mod} - N_{js}^{exp})^2$ with N_{js}^{mod} and N_{js}^{exp} were respectively the modeled and the experimental values of N_{js} . Models were finally validated for 40 operating conditions chosen out of the previous set of operating conditions defined for the the model solving, and involving different impellers at different particle volume fractions between 1 and 19 %. An additional N_{js} data (Cytodex-1 at a volume fraction of 18.5 %) was recovered from the literature for the case of an Elephant Ear impeller ($D/T = 1/2$ and $C/T = 1/3$) in down-pumping mode placed in a 20 L hemispherical bioreactor, geometrically similar to the one used in the present study [34]. Models were applied for these operating conditions to verify their scalability. A last validation at the temperature of 37°C, generally used in cell culture, was performed by comparing experimental N_{js} values to the predicted ones at 20 and 37°C and for 4 different impellers.

3.3. Multiphase simulation by CFD

3.3.1. CFD simulations

CFD simulations were performed by using the commercial finite volume solver ANSYS Fluent (ANSYS Inc., version 16.1). A granular Euler-Euler RANS (Reynolds Averaged Navier-Stokes) approach was applied, considering two interpenetrating phases. The continuity and momentum equations were solved for each phase in steady-state by using the Moving Reference Frame (MRF) approach to model the impeller rotation. The Reynolds stress tensor $\bar{\tau}$ was modelled by the standard k- ε turbulence model, previously validated for particles at the just-suspended state [15] and for microcarrier suspensions [35, 36, 37], despite Reynolds numbers related to a transitional regime.

261 Among other set of models, Delafosse *et al.* demonstrated that this model
 262 led to the most robust results [20]. Two dominant fluid/solid interactions
 263 were considered with the drag and the turbulent dispersion forces. These
 264 two conservation equations were developed in Eqs. (7) and (8) for the liquid
 265 phase, and Eqs. (9) and (10) for the solid phase.

$$\left\{ \begin{array}{l} \nabla \cdot (\alpha_L \rho_L \mathbf{v}_L) = \nabla(\gamma_L \nabla \alpha_L) \\ \nabla \cdot (\alpha_L \rho_L \mathbf{v}_L \mathbf{v}_L) = -\alpha_L \nabla p + \nabla \cdot \bar{\tau}_L \end{array} \right. \quad (7)$$

$$+ \alpha_L \rho_L \mathbf{g} + K_{LS}(\mathbf{v}_S - \mathbf{v}_L) \quad (8)$$

$$\left\{ \begin{array}{l} \nabla \cdot (\alpha_S \rho_S \mathbf{v}_S) = \nabla(\gamma_S \nabla \alpha_S) \\ \nabla \cdot (\alpha_S \rho_S \mathbf{v}_S \mathbf{v}_S) = -\alpha_S \nabla p - \nabla p_S + \nabla \cdot \bar{\tau}_S \end{array} \right. \quad (9)$$

$$+ \alpha_S \rho_S \mathbf{g} + K_{LS}(\mathbf{v}_L - \mathbf{v}_S) \quad (10)$$

266 Where \mathbf{v} was the velocity of the corresponding phase, p the pressure
 267 shared by all phases and \mathbf{g} the acceleration due to gravity. The interphase
 268 drag force involved the interphase momentum exchange coefficient K_{LS} , mod-
 269 elled by the Huilin-Gidaspow equations (ANSYS Fluent theory guide [38]).
 270 The diffusion-in-VOF equation was used to model the turbulent dispersion
 271 and was included in the mass conservation equation through $\nabla(\gamma_L \nabla \alpha_L)$ and
 272 $\nabla(\gamma_S \nabla \alpha_S)$ terms.

273 Cytodex-1 particles were chosen as reference particles for the simulations,
 274 defined by the experimental mean diameter and density. Based on experi-
 275 mental observations of bed height, the value of the packing limit was esti-
 276 mated to 0.6, which was a value close to the solid fraction of randomly packed

spheres of equal diameter (0.63). However, the impact of the value of the packing limit was negligible in our simulations as the maximal volume fractions obtained remained lower than 0.3 approximately. It should be noticed that microcarriers are also non-porous particles. Calculation domain was discretized using between 230000 and 275000 tetrahedral meshes of around 5 mm, except in a zone in the vessel bottom, where the size was refined at a maximum of 2 mm, as presented in Fig. 2 to allow a better description of particle just-suspended state. This number of mesh cells was chosen as a compromise between a reasonable calculation time and a sufficient accuracy for the results analysis. To validate the choice of the $\approx 250k$ cells grid, calculations were also performed for two cases using a finer grid of $\approx 1M$ cells. First one (A) was impeller EED with $C/T = 0.5$; $D/T = 0.4$ and $\alpha = 12\%$ and second one (B) was impeller HTPGD, $C/T = 0.5$, $D/T = 0.5$, $\alpha = 4\%$. While errors between experimental N_{js} measurements and 250k grid calculations were respectively of 1.5 and 2.6 % for cases A and B, it reached respectively 14.7 and 7.8 % with the 1M cells grid. Thus, the 250k cells grid was finally used for the complete set of simulations.

The simulation was initialized, considering all particles quasi-settled ($\alpha_s = 0.5$) at the bottom of the bioreactor. Higher particle concentrations led to divergence during the first iterations. Consequently, 0.5 was found to be a good compromise, allowing particles settling during the first iterations and avoiding divergence. Convergence of the simulations was supposed when equation residuals were less than 10^{-5} and when liquid velocities and solid volume fractions in the impeller vicinity were stabilized. This second validation was necessary to ensure the stabilization of the N_{js} determination

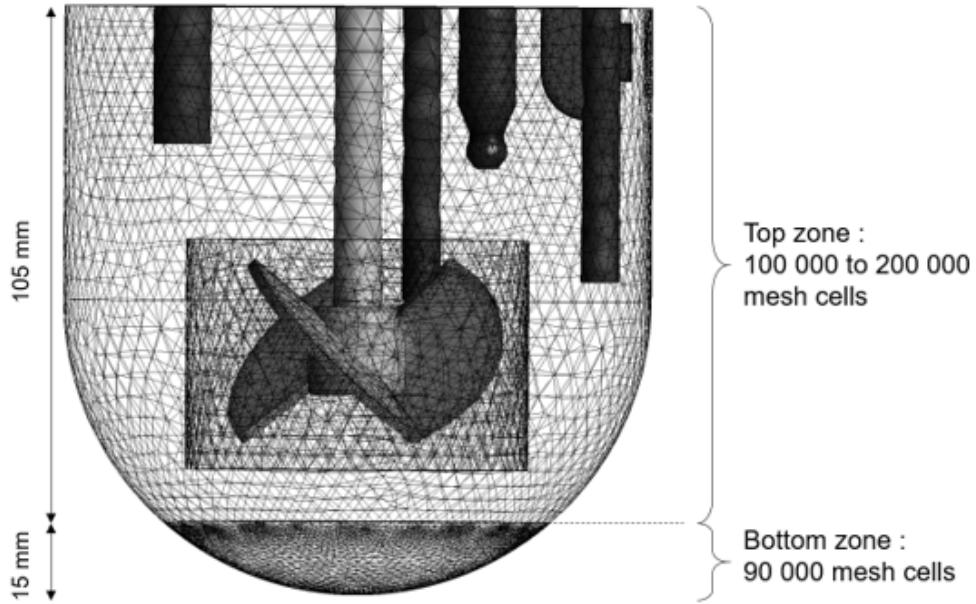


Figure 2: Mesh discretization of the vessel for the CFD simulations.

302 criterion presented in the next following part.

303 *3.3.2. N_{js} determination strategy by using CFD*

304 To model the subjective experimental determination of N_{js} , two simula-
305 tion outputs were proposed.

306 The first one Packed Fraction PF related the volume of packed particles
307 to the total volume of particles. To determine the value of PF , particles
308 were supposed packed if their local volume fraction was between $\alpha_{ref} = 0.55$
309 and the packing limit 0.6 (Eq. 11).

$$306 \quad PF = \frac{V_p (\alpha_p > \alpha_{ref})}{V_{p, tot}} \quad (11)$$

310 The second one, Still Fraction SF (Eq. 12), related the volume of ‘quasi-
 311 motionless’ particles on the total volume of particles. It was based on the
 312 determination of the local particle residence time τ_p in mesh cells in the
 313 bottom of the system. If this value was smaller than a reference residence
 314 time based on a time of 1 second to pass through a cube with 2 mm sides,
 315 then particles were considered to be almost motionless in the mesh cell.
 316 Experimentally, last microcarriers to be lifted tended to stay closely together
 317 by forming a little cone with a slow circular movement. SF reflected this
 318 phenomenon and returns the percentage of particles in this situation.

$$SF = \frac{V_P (\tau_p < \tau_{ref})}{V_{p, tot}} \quad (12)$$

319 As considered by Tamburini *et al.* [22] with the development of their CFD
 320 method *Unsuspended Solids Criterion*, the results were dependent of the grid
 321 and the volume discretization. A coarse grid could lead to uncertainties and
 322 to underestimations of N_{js} values. In the present study, the finer meshing in
 323 the bottom zone permitted to limit this effect.

324 Using these two criteria, a CFD-based strategy was developed to reach
 325 the value of N_{js} by a progressive decrease of the agitation rate N until a
 326 predefined critical value of PF or SF was reached. Preliminary simulations
 327 were performed in a 200 mL minibioreactor with the experimental data recov-
 328 ered from Collignon *et al.* works [32]. The strategy consisted in preliminary
 329 validation in a scale-down geometry. Hence, this step allowed to validate the
 330 chosen models and to define the critical values of the two parameters defin-
 331 ing the just-suspended state of particles: PF^{crit} and SF^{crit} . After this first
 332 validation, the method was transferred to the geometry of the 1.12 L reactor

333 of this study. The approach was validated for various geometrical conditions
 334 and particle concentrations in the case of the HTPG and the Ear Elephant
 335 impellers in down-pumping mode. Then, the method was applied at one or
 336 two operating conditions of the Rushton turbine and the HTPG and Ear
 337 Elephant impellers in up-pumping mode.

338 4. Results and discussions

339 4.1. Suspension modelling

340 First, S coefficients of the Zwietering correlation presented in Eq. (1)
 341 were calculated from 8 experimental N_{js} measurements, by reorganizing Eq.
 342 (1) into Eq. (13). They all shared the same impeller conditions: an HTPG
 343 impeller in down-pumping mode, at a D/T ratio of 0.5 and a C/T ratio of
 344 0.5. These S values are presented in function of the microcarrier volume
 345 fraction α_S in Fig. 3. On the contrary to what was commonly admitted, it
 346 can be noted that, in the case of microcarriers, S depended on the value of
 347 α_S with a 27 % deviation between the lowest and the highest values of S .
 348 Thus, exponents of the Zwietering correlation should be revisited, especially
 349 concerning the terms involving the microcarrier properties.

$$S = \frac{N_{js}}{\nu_L^{0.1} \cdot \left(\frac{g \cdot (\rho_p - \rho_L)}{\rho_L} \right)^{0.45} \cdot X^{0.13} \cdot d_p^{0.2} \cdot D^{-0.85}} \quad (13)$$

350 The five impellers could be classified in three types, according to the
 351 flow patterns induced and microcarrier bed shapes. With increasing agita-
 352 tion rates, the bed progressively evolved from a planar-surface volume to a
 353 cone shape for both radial flow (RT) and mixed-flow (EEU and HTPGU)

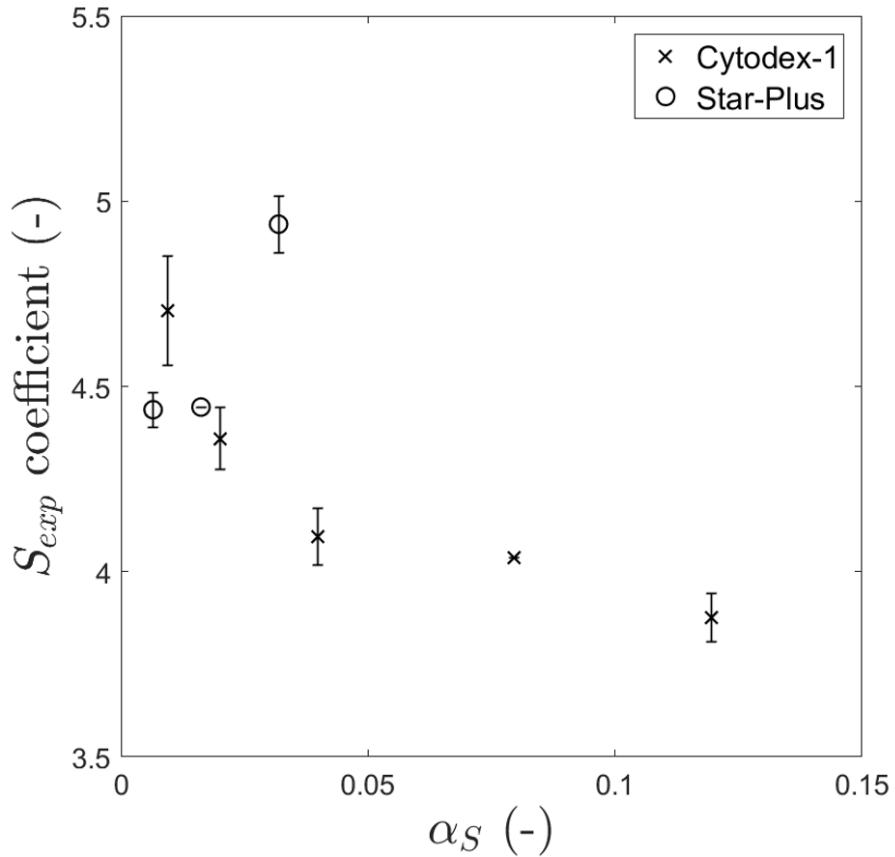


Figure 3: Evolution of the experimentally determined S coefficient from the Zwietering correlation with the microcarrier volume fraction in the bioreactor, for Cytodex-1 and Star-Plus.

354 impellers, as presented in Fig. 4 (B and C). Particles were pushed inward
 355 and driven by the impeller. With axial flow (EED and HTPGD) the bed was
 356 a peakless cone as shown in Fig. 4 (A). In this case, particles were pushed
 357 down and suspended after sliding along the side wall. Thus, these three flow
 358 patterns involved different suspensions phenomena, which were taken into
 359 account for the model establishment. The consequence on parameter fitting

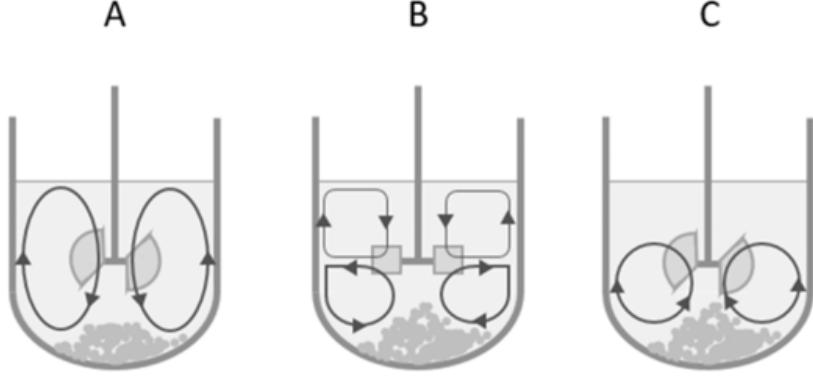


Figure 4: Flow patterns and particles bed shapes observed at agitation rates $N < N_{js}$ depending on mixing structures: axial flow with EED and HTPGD (A), radial flow with RT (B) and mixed-flow with EEU and HTPGU (C).

360 was that, despite it could be possible to determine a unique set of parameters
 361 b_i , c_i and d_i for the three flow patterns, the exponents related to particle
 362 volume fraction had to be specific to each of them. The model constants K_i
 363 logically depended on impeller geometry. Best fitted models are given in Eqs.
 364 (14) - (16) and the parameters K_i given in Table 2, were only dependent on
 365 the impeller design. The coefficients a_1 , a_2 and a_3 displayed three values ac-
 366 cording to the flow pattern and are given in table 2. a_1 and a_2 were the only
 367 exponents involving α_S . Considering Model 3 (Eq. (16)), α_S was involved
 368 in three terms, with $\alpha_S^{0.3}$, $\alpha_S^{0.4/3}$ and $\alpha_S^{14/45}$, consequently, a total exponent
 369 *Tot. α_S exponent* was calculated as *Tot. α_S exponent* = $a_3 + 0.4/3 + 14/45$.

370 Model 1

$$N_{js} = K_1 \cdot \alpha_S^{a_1} \cdot C^{1/3} \cdot D^{-1} \cdot d_p^{-0.4} \quad (14)$$

³⁷¹ Model 2

$$Re_{js} = K_2 \cdot \alpha_S^{a_2} \cdot \left(\frac{C}{T} \right)^{1/3} \cdot \left(\frac{D}{T} \right)^{0.6} \cdot \left(\frac{d_p}{D} \right)^{-0.4} \cdot \left(\frac{\mu_L}{(\rho_p - \rho_L) \cdot g^{0.5} \cdot T^{1.5}} \right)^{-28/75} \quad (15)$$

³⁷² Model 3

$$Re_{js} = K_3 \cdot \alpha_S^{a_3} \cdot \left(\frac{C}{T} \right)^{1/3} \cdot \left(\frac{D}{T} \right)^1 \cdot \left[\alpha_S \cdot \left(\frac{T}{d_p} \right)^3 \right]^{0.4/3} \cdot \left(\frac{g \cdot \alpha_S \cdot T^3 \cdot \rho_L \cdot (\rho_p - \rho_L)}{\mu_L^2} \right)^{14/45} \quad (16)$$

Table 2: K_i and a_i parameters of the three models predicting N_{js} , according to the impeller design.

Flow pattern	Impeller	K_1	K_2	K_3	a_1	a_2	a_3	$Tot. \alpha_S$
(A)	HTPGD	7.9	8.2	2.4	0.07	0.07	-0.37	0.07
(A)	EED	4.9	5.0	1.5	0.07	0.07	-0.37	0.07
(B)	RT	6.3	6.4	2.0	0.1	0.1	-0.34	0.1
(C)	HTPGU	6.7	6.8	2.0	0.2	0.2	-0.24	0.2
(C)	EEU	4.9	5.1	1.5	0.2	0.2	-0.24	0.2

³⁷³ These three models were applied on 40 operating configurations out of
³⁷⁴ the operating conditions defined for the models solving. The experimental
³⁷⁵ measurements were performed by three different operators to include the
³⁷⁶ potential subjective N_{js} determination, and involved the HTPG impeller
³⁷⁷ in down-pumping mode, the Elephant Ear impeller in up-pumping mode

and the Rushton turbine at different C/T ratios and at different particle volume fractions. Results are presented in Fig. 5 for model 3. Models were globally validated but presented deviations for high particle volume fractions with the Elephant Ear impeller. The model establishment was based on data involving particle volume fractions up to 12 % and did not seem thus suitable for higher concentrations with this type of impeller. However, these high microcarrier volume fractions are seldom used in animal cell culture bioreactors. Considering the 95 configurations of the design of experiments and the 40 complementary measurements, mean relative errors under 10 % were obtained for the three models. Mean errors obtained with each model are presented for the different impeller designs in Table 3.

Table 3: Mean errors between N_{js} predicted by the models and experimental values at $T = 20^\circ\text{C}$, according to the impeller design and the model used.

	Model		
	1	2	3
HTPGD	6.0 %	6.0 %	5.8 %
EED	8.6 %	8.4 %	8.6 %
RT	11.1 %	12.8 %	11.5 %
HTPGU	9.3 %	8.9 %	8.9 %
EEU	12.3 %	13.2 %	12.5 %
Mean error	8.7 %	9.1 %	8.7 %

In order to determine the most robust model between the three proposed, the validity of each model was verified at the cell culture working temperature of 37°C and for a scale-up application in a 20 L vessel. Concerning the

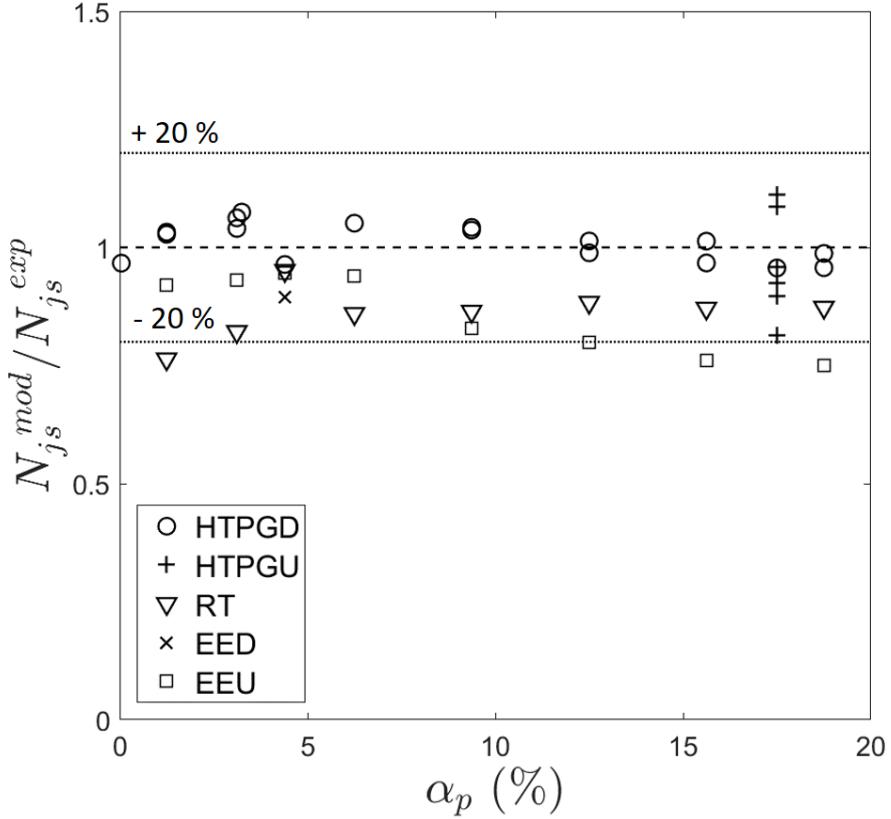


Figure 5: Comparison of the deviation between the experimental N_{js} and the N_{js} predicted by the model for operating conditions out of the design of experiment and model resolution.

temperature stability, fluid properties were admitted to be the same as water, with both density and viscosity dependent of the temperature. Results for four impeller designs are given in Table 4. All models presented mean deviations of less than 20 % between the experimental values and the predicted values, but model 2 (Eq. (15)) was found to be more robust than the others. Concerning the model scalability, results are presented in Table 5. In this case, the model 2 presented the highest deviation of 34 %. Consequently, model 1 (Eq. (14)) and model 3 (Eq. (16)) were found to be more suitable.

400 Regarding the mean relative error, the temperature validity, the scalability,
 401 and the physical meanings of each parameters, model 3 (Eq. (16)) was
 402 considered to be the best compromise. The comparison between the pre-
 403 dicted values N_{js}^{mod} and the experimental values N_{js}^{exp} is reported in Fig. 6
 404 for particle volume fractions under 12 %. A Student's t-test was performed
 405 with a statistical significance chosen at 0.05. Due to the distinct values of K_3
 406 and a_3 coefficients according to the impeller design, t-tests were applied sep-
 407 arately for each impeller design. For each of them, a regression analysis led
 408 to p -values of less than 0.01, indicating that all parameters of the model were
 409 statistically significant. This model will be used for the following discussion
 410 on the impact of each parameter, characterizing the operating conditions, on
 411 N_{js} . For the sake of clarity, K_3 and a_3 will be respectively renamed K and
 412 a .

Table 4: Mean errors between N_{js} predicted by the models and experimental values, at 37°C according to the impeller design and the model used.

	Model		
	1	2	3
HTPGD	18.5 %	0.5 %	15.1 %
EED	22.5 %	6.1 %	20 %
RT	15.1 %	7.2 %	11.3 %
EEU	19.1 %	0.9 %	15.3 %
Mean error	18.8 %	3.7 %	15.4 %

Table 5: Mean errors between N_{js} predicted by the models and experimental values at 20 rpm, in a 20 L vessel equipped with a EE down-pumping impeller, according to the impeller design and the model used.

Model	N_{js}^{mod} (rpm)	Deviation
1	21	5 %
2	27	34 %
3	22	11.2 %

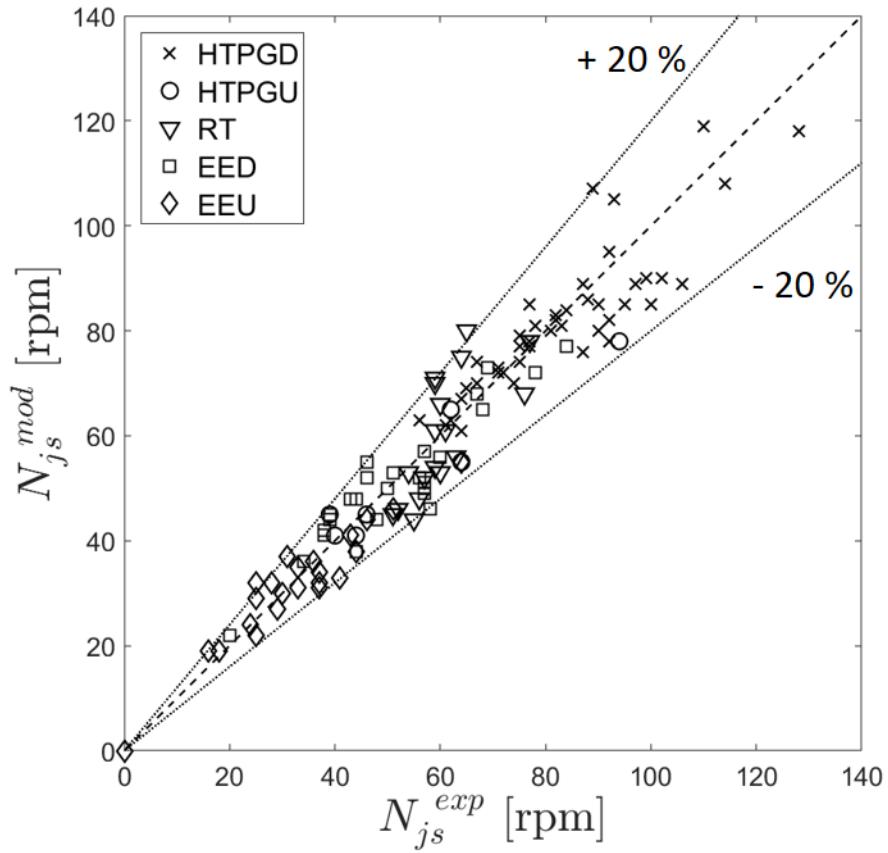


Figure 6: Comparison of experimental N_{js}^{exp} and modeled N_{js}^{mod} with the equation 16.

413 4.2. Effect of the impeller geometry on N_{js}

414 First, the off-bottom impeller clearance C was raised to the power of $1/3$
415 which was in accordance with literature data, stating that N_{js} become lower
416 as the C/T ratio was reduced [17, 26]. In this study, the lowest value of C/T
417 was 0.25, so it was admitted that flow patterns respected a 'double-eight'
418 regime in the case of radial impellers. There was no pattern modification
419 with the decrease of C/T [39, 40, 41].

420 Concerning the effect of the impeller diameter, it was found that N_{js} was
421 proportional to D^{-1} , with the three models (Eqs. (14) to (16)). This trend
422 was also in accordance with the literature [16, 17, 18]. A larger impeller
423 presents a higher surface in contact with the continuous phase, and thus
424 delivers a higher discharge stream, which facilitates the solids suspension.
425 Moreover, our results showed that the impact of D/T ratio was much more
426 significant than that of C/T , which is in agreement with previous works [18].
427 However, the -1 coefficient identified in our work was significantly lower than
428 the one proposed by Nienow *et al.* [17], with a value of -2.21, or the -3.45
429 estimated by Arvinth *et al.* [18].

430 The last geometrical parameter considered in the models was the impeller
431 shape, independently of its size. Depending on this shape, different model
432 parameters were then determined for the constant K and the exponent a
433 of particle volume fraction α_S to improve model precision. This choice was
434 justified by the macroscopic structures of flow, promoting particle suspen-
435 sion either by particle aspiration or particle down-pushing according to the
436 impeller design. In order to compare the different impellers used, both α_S
437 and K effects on N_{js} were taken into account, and the $K.\alpha_S^a$ product was

438 calculated for each impeller design. Products values are given in Table 6.

Table 6: Impact of the impeller design on N_{js} and classification.

Impeller design	$K.\alpha_S^a$		
	$\alpha_S=4\%$	$\alpha_S=8\%$	$\alpha_S=12\%$
HTPGD	8.1	6.3	5.4
RT	5.0	3.9	3.3
EED	5.9	4.7	4.0
HTPGU	4.3	3.7	3.3
EEU	3.3	2.6	2.6

439 These values showed that the radial Rushton turbine and the impellers
440 operating in mixed-flow regime displayed lower values, reflecting that it re-
441 quired less energy to lift particles by pushing them inward and aspirating
442 them through the impeller direction than pushing them outward and making
443 them slide along the wall, as it was represented in Fig. 4. Impellers could
444 thus be classified, according to their efficiency to minimize N_{js} : the Elephant
445 Ear impeller displayed lower N_{js} than the other impeller, especially at high
446 solid concentrations and in up-pumping mode, whereas the HTPG impeller
447 in down-pumping mode presented the highest value for N_{js} . But for cell cul-
448 ture applications, the impeller design has to be carefully chosen as displaying
449 a lower N_{js} does not necessarily imply a minimization of hydromechanical
450 stress induced. It was indeed shown by Collignon *et al.* that the Elephant
451 impeller Ear displayed a lower N_{js} , but also higher Energy Dissipation / Cir-
452 culation function than the Rushton turbine and the Marine propellers at the
453 same power input indicating a higher exposition frequency of microcarriers

454 to mechanical stresses with the Elephant Ear impeller [32]. For the investiga-
455 tion of an optimal impeller design, a parameter involving frequency exposure
456 to hydromechanical stress should thus be taken into account, in addition to
457 the N_{js} minimization.

458 *4.3. Effect of the microcarrier concentration on N_{js}*

459 As it was previously described and confirmed by the CFD, the particle
460 concentration term was found to be a function of the impeller design in the
461 $\alpha_S^{a_3}$ term, where a_3 displayed at least three values according to the agitation
462 mode. An increase of N_{js} was observed with the solid volume fraction. The
463 exponents obtained after the model resolution were close to the one proposed
464 in the Zwietering correlation, where the solid mass fraction was raised to 0.13
465 (Eq. (1)). For microcarrier density, it corresponded to a solid volume fraction
466 raised to 0.12. In the present study, the three values proposed by the new
467 model were 0.07, 0.1 and 0.2, leading to a mean value of 0.12. The new
468 model was thus broadly in accordance with the Zwietering correlation, but
469 provided more precision with distinct values for each agitation mode.

470 *4.4. Effect of microcarrier properties*

471 Regarding only the particle diameter d_p effect on N_{js} , a negative value of
472 -0.4 was found, which was in contradiction with correlations in the literature,
473 presenting positive values [16, 17, 18]. In the present study, a dimensionless
474 quantity, proportional to the number of particles was proposed instead of
475 the d_p/D ratio, and was found to be raised to a positive value of 0.4/3.
476 Hence, for a given particle concentration, larger beads led to lower number
477 of particles, and thus to lower total interface area between the liquid and

478 solid phases. For the case of microcarrier, more input power was required
 479 to suspend or avoid settling of a higher number of particles, despite their
 480 smaller diameter. Concerning particle density, it was involved through an
 481 Archimede number with a length scale characterizing the microcarrier cloud.
 482 As expected, denser particles required more input power, and thus a higher
 483 agitation rate to be fully suspended.

484 *4.5. Comparison with the Zwietering correlation*

485 In order to compare this new model (Eq. 16) with the Zwietering correla-
 486 tion, the S coefficient of the Eq. (1) has to be determined for each geometrical
 487 configuration of the study. To do that, experimental Zwietering coefficients
 488 S_{exp} were calculated from the experimental N_{js} measurements. The objec-
 489 tive was then to gather all configurations varying the particle concentration
 490 or the microcarriers used, but displaying the same geometrical configuration,
 491 and determine a common S value. Because of the dispersed operating condi-
 492 tions, the majority of geometrical configurations had only one or two points.
 493 So, based on the results of this work, it was assumed that $N_{js} \propto C^{1/3}$, per-
 494 mitting to divide all measurements in 15 groups according to their common
 495 D/T value and impeller design. The ratio N_{js} was then expressed in function
 496 of the modified Zwietering function $f_{Zwietering, m}$ presented in Eqs. (17) and
 497 (18), inspired from a modified Zwietering correlation with $C^{1/3}$ extracted
 498 from the S coefficient (Eq. (17)). This $f_{Zwietering, m}$ term should be linear to
 499 N_{js} , with a slope corresponding to the common S_{calc} value of a given group.
 500 The S_{calc} values obtained after linear regressions are presented in Table 7.

$$N_{js, Zwietering\ calc} = S_{calc} \cdot f_{Zwietering, m} \quad (17)$$

$$f_{Zwietering, m} = \nu_L^{0.1} \cdot \left(\frac{g \cdot (\rho_p - \rho_L)}{\rho_L} \right)^{0.45} \cdot X^{0.13} \cdot d_p^{0.2} \cdot D^{-0.85} \cdot C^{1/3} \quad (18)$$

Table 7: Adapted Zwietering coefficients S_{calc} values according to the impeller design and the D/T ratio.

Design	D/T		
	0.33	0.4	0.5
EED	7.1	7.1	6.3
HTPGD	11.8	11.3	11.1
RT	7.6	7.4	8.4
EEU	5.2	4.6	4.4
HTPGU	7.5	6.0	5.3

501

502 A comparison of this modified Zwietering model (Eq. (17)) with the new
 503 model (Eq. (16)) was finally performed and is represented in Fig. 7. The
 504 new model led to a relative error of 8.8 % with 96 % of points under 20 %
 505 deviation, whereas the adapted Zwietering model provided a relative error of
 506 12.9 %, with 18 % of the absolute deviations over 20 % and leading up to 69
 507 % deviation. The new model proposed thus better N_{js} prediction. Moreover,
 508 contrary to the Zwietering correlation, the constant K of the new model was
 509 only dependent of the impeller design. As animal cell cultures use media
 510 with water-like viscosities, no significant viscosity changes were considered
 511 in this model. Thus, higher model deviations may be expected for higher
 512 viscosity liquids.

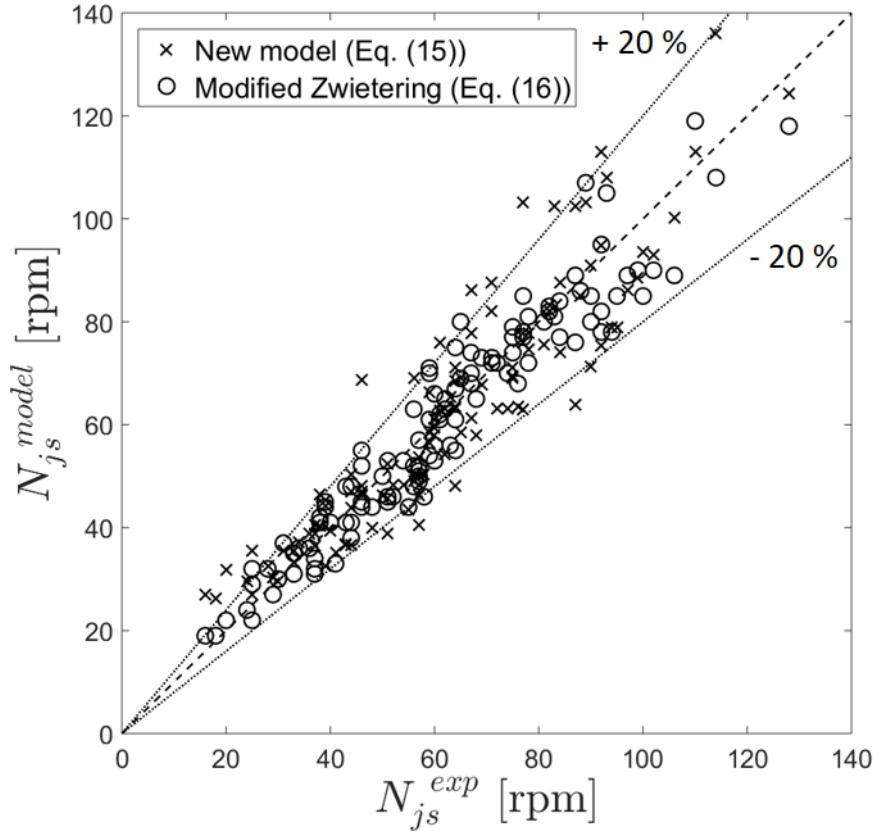


Figure 7: Comparison of modeled N_{js} and numerically adapted $N_{js,Zwietering}$

513 4.6. CFD-based method to predict N_{js}

514 Preliminary simulations were run to determine a suitable criterion to
 515 predict N_{js} . To do that, both *PF* and *SF* criteria (Eq. (11) and (12)) were
 516 calculated on the 200 mL minibioreactor geometry with 4 different impeller
 517 designs (EEU, EED, RT and Marine propeller in up-pumping mode). At
 518 the just-suspended state, the Reynolds numbers were comprised between 1
 519 000 and 1 800, traducing a transitional regime. At the just-suspended state,
 520 using a standard-wall function for the near-wall turbulence modelling, values

of y^+ below 15 were obtained which was not in the recommended validity range ($y^+ > 15$). Thus, the k- ϵ Scalable Wall Function was instead used. Simulations run at the experimental critical agitation rate N_{js}^{exp} provided SF values comprised between 0.08 % and 0.13 % and null values for PF . The SF criterion was thus used for the study with a critical value SF^{crit} fixed at 0.10 %. Predicted critical agitation rates N_{js}^{simul} were then determined by looking for the agitation rate presented $SF = SF^{crit}$. Results are presented in Fig. 8 and led to a relative error of 9 %. This choice of criterion differed from the one of Tamburini *et al.* [22]. For glass bead suspensions, they found that a solid volume fraction based criterion presented better results than a one based on particle velocity. This second criterion was developed assuming that only particles presenting velocities equal to zero were considered to be unsuspended, unlike the SF criterion of the present study, in which particles with a very low residence time were considered to be unsuspended. This was in accordance with microcarriers suspension observations. Probably due to a density close to the liquid phase, the last particles to be suspended, as defined by the Zwietering criterion, were not totally motionless when staying in the bottom of the reactor.

The CFD-based method to predict N_{js} was then applied on 20 operating conditions of the 1.12 L reactor described in the experimental approach part. The k- ϵ model adaptation was not necessary for this reactor scale, displaying higher Reynolds number values of about 5 000, and so the Standard Wall Function was used. This system may be still considered in transitional regime, but the Standard Wall Function appeared to correctly solve the turbulent equations. As previously, SF^{crit} value was

546 firstly determined from SF values obtained from simulations run at the ex-
547 perimental critical agitation rate N_{js}^{exp} . In this case, SF^{crit} was found at
548 0.20 %. This percentage indicated a quasi-motionless microcarrier volume
549 of around 0.2 cm^3 , which seemed to be representative of the experimen-
550 tal observations. N_{js}^{simul} were finally determined according to SF^{crit} and
551 compared to their corresponding experimental data. Comparison of the ex-
552 perimental and the predicted critical agitation rates are presented in Fig.
553 8 and displayed a mean relative error of 9 %. Higher deviations were ob-
554 served with configurations presenting the lowest Reynolds number values, less
555 than 2 100, at which the chosen turbulent models seemed to be inadequate.
556 By removing these configurations, a lower mean relative error of 9 % was
557 obtained in this restricted domain.

558 5. Conclusion

559 A new empirical correlation was established to predict N_{js} for microcar-
560 riers and was found to provide a better accuracy than the most commonly
561 used Zwietering correlation. Another advantage displayed by the new model
562 concerned the constant of the correlation. The traditional Zwietering corre-
563 lation proposed a constant S function of the impeller design and both D/T
564 and C/T ratios, which may be difficult to find in the literature. In the present
565 study, the constants K were only dependent of the impeller design.

566 A dimensional analysis provided two other remarkable information. First,
567 the influence of the particle volume fraction α_S seemed to be a function of
568 the regime applied by the impeller. The determination of the constants
569 K permitted to classify impellers, showing that the up-pumping mode led

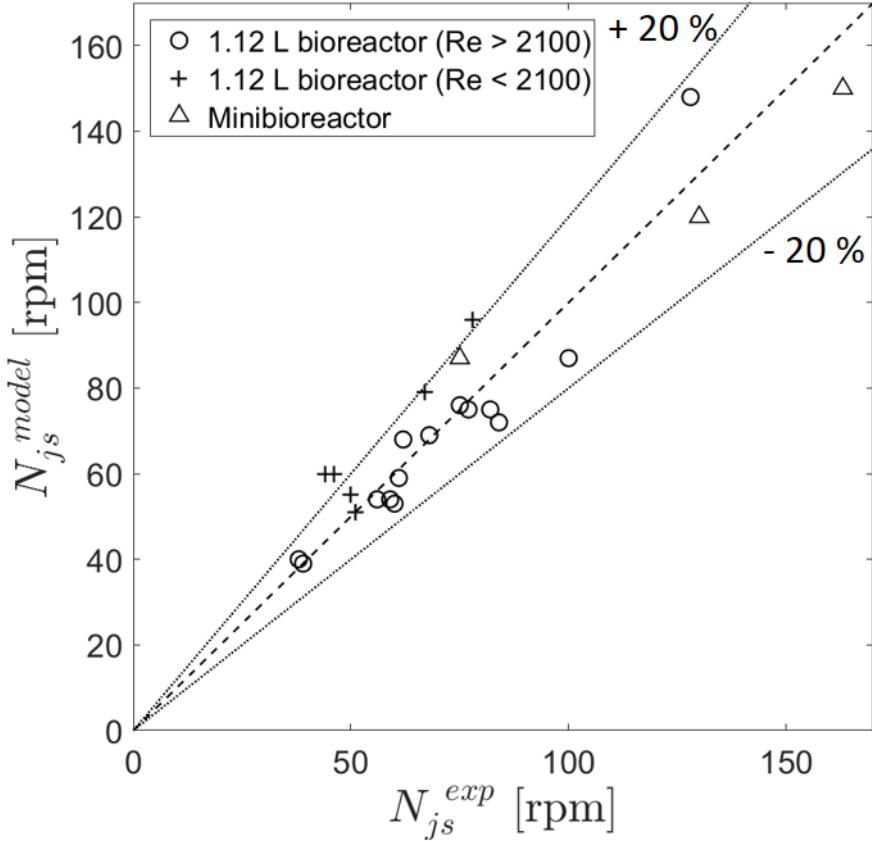


Figure 8: Comparison of experimental N_{js} and modeled N_{js} with the CFD-based method.

570 to lower N_{js} values. Secondly, in the case of microcarriers, the number of
 571 particles was found to better suit than the d_p/D ratio.

572 Finally, a CFD-based method was developed to numerically predict N_{js} .
 573 The models chosen and the method were validated according to two different
 574 working volumes, with 17 configurations for the specific vessel of this study.
 575 The particle residence time in the bottom was found to be the best criterion
 576 for microcarriers, with a critical value fixed at SF^{crit} of 0.20 %.

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582 work.

⁵⁸³ **Nomenclature**

Variables

N	Impeller agitation rate	(s^{-1})
S	Zwietering coefficient (Eq. (1))	(-)
g	Gravity constant	(m s^{-2})
X	Mass concentration of particles	(%)
d	Diameter	(m)
D	Impeller diameter	(m)
C	Off-bottom clearance	(m)
T	Tank diameter	(m)
H	Tank height	(m)
V	Working volume	(m^3)
P	Global power input	(W)
⁵⁸⁴ $K_{1,2,3}$	Model impeller constants	(-)
$a_{1,2,3}$	Model constants	(-)
$b_{1,2,3}$	Model constants	(-)
$c_{1,2,3}$	Model constants	(-)
$d_{1,2,3}$	Model constants	(-)
PF	Packed fraction criterion (Eq. 11)	(-)
SF	Still fraction criterion (Eq. 12)	(-)
n_{exp}	Number of experiments	(-)

Greek letters

α	Volume fraction	(-)
μ	Dynamic viscosity	(Pa s)
ω	Optimization criterion (-)	

ν	Kinematic viscosity	(m ² s ⁻¹)
ρ	Density	(kg m ⁻³)
τ	Residence time	(s)

Dimensionless number

Ar	Archimede number	(-)
Re	Reynolds number	(-)
Fr	Froude number	(-)
Np	Newton number	(-)

Subscripts

585

js	Just-suspended state
up	Determination by N increase
down	Determination by N decrease
L	Liquid phase
m	Mixture
p	Particle
S	Solid phase
50	Median
5-95	Confidence interval
mean	Mean
mod	Modeled
exp	Experimental
crit	critical

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