



HAL
open science

Dimensional analysis and CFD simulations of microcarrier ‘just-suspended’ state in mesenchymal stromal cells bioreactors

Angélique Delafosse, Céline Loubière, Emmanuel Guedon, I. Chevalot, Dominique Toye, Eric Olmos

► To cite this version:

Angélique Delafosse, Céline Loubière, Emmanuel Guedon, I. Chevalot, Dominique Toye, et al.. Dimensional analysis and CFD simulations of microcarrier ‘just-suspended’ state in mesenchymal stromal cells bioreactors. *Chemical Engineering Science*, 2019, 203, pp.464-474. 10.1016/j.ces.2019.04.001 . hal-02151663

HAL Id: hal-02151663

<https://hal.science/hal-02151663>

Submitted on 22 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

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

C. Loubière^a, A. Delafosse^{b,c}, E. Guedon^a, I. Chevalot^a, D. Toye^{b,c}, E. Olmos^{a,*}

^a*Laboratoire Réactions et Génie des Procédés, Université de Lorraine, CNRS, LRGF, F-54000 Nancy, France*

^b*Laboratory of Chemical Engineering, Université de Liège, Allée de la Chimie 3, B6C, 4000 Liège, Belgium*

^c*Fonds de la Recherche Scientifique (FRS-FNRS), Rue d'Egémont 5, 1000 Bruxelles, Belgium*

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-

*Corresponding author

Email address: eric.olmos@univ-lorraine.fr (E. Olmos)

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

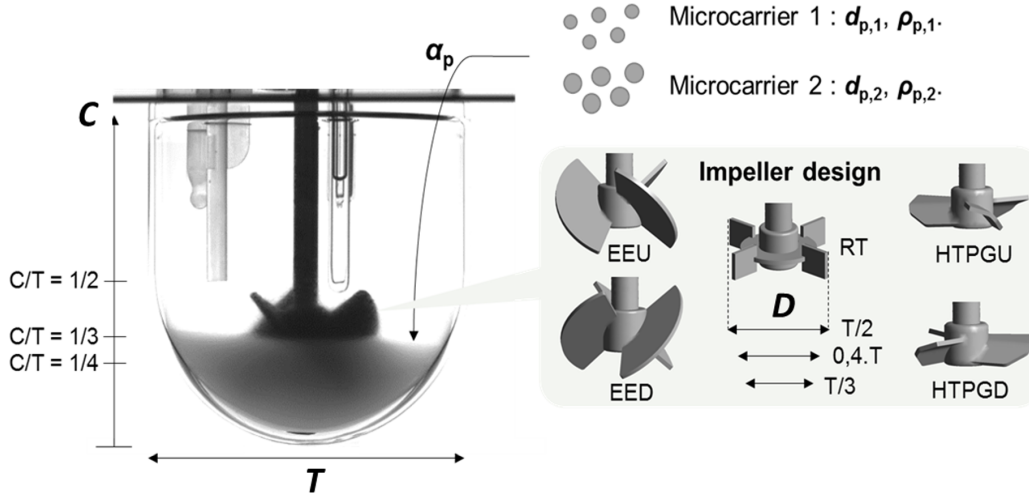


Figure 1: Variables used to define the operating conditions: the off-bottom clearance C and the impeller diameter D in function of the tank diameter T , the type of microcarrier with their specific diameters and density, the particle volume fraction α_p , and the impeller design with the Ear Elephant 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:

$$\pi_1 = \frac{N_{js} \cdot T^2 \cdot \rho_L}{\mu_L}, \quad \pi_2 = \frac{C}{T}, \quad \pi_3 = \frac{D}{T}, \quad \pi_4 = \frac{d_p}{T},$$

$$\pi_5 = \alpha_S, \quad \pi_6 = \frac{g \cdot T^3 \cdot \rho_L^2}{\mu_L^2}, \quad \pi_7 = \frac{\rho_p}{\rho_L}.$$

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

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

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

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

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

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

236 the last exponent values a_i , b_i and d_i . The objective of this optimization
237 consisted in minimizing the criterion $\omega = \sum_{i=0}^{n_{exp}} (N_{js}^{mod} - N_{js}^{exp})^2$ with N_{js}^{mod}
238 and N_{js}^{exp} were respectively the modeled and the experimental values of N_{js} .
239 Models were finally validated for 40 operating conditions chosen out of the
240 previous set of operating conditions defined for the the model solving, and
241 involving different impellers at different particle volume fractions between
242 1 and 19 %. An additional N_{js} data (Cytodex-1 at a volume fraction of
243 18.5 %) was recovered from the literature for the case of an Elephant Ear
244 impeller ($D/T = 1/2$ and $C/T = 1/3$) in down-pumping mode placed in
245 a 20 L hemispherical bioreactor, geometrically similar to the one used in
246 the present study [34]. Models were applied for these operating conditions
247 to verify their scalability. A last validation at the temperature of 37°C,
248 generally used in cell culture, was performed by comparing experimental N_{js}
249 values to the predicted ones at 20 and 37°C and for 4 different impellers.

250 3.3. Multiphase simulation by CFD

251 3.3.1. CFD simulations

252 CFD simulations were performed by using the commercial finite volume
253 solver ANSYS Fluent (ANSYS Inc., version 16.1). A granular Euler-Euler
254 RANS (Reynolds Averaged Navier-Stokes) approach was applied, considering
255 two interpenetrating phases. The continuity and momentum equations were
256 solved for each phase in steady-state by using the Moving Reference Frame
257 (MRF) approach to model the impeller rotation. The Reynolds stress tensor
258 $\overline{\tau}$ was modelled by the standard k- ϵ turbulence model, previously validated
259 for particles at the just-suspended state [15] and for microcarrier suspen-
260 sions [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.

$$\left\{ \begin{array}{l} \nabla \cdot (\alpha_L \rho_L \mathbf{v}_L) = \nabla(\gamma_L \nabla \alpha_L) \\ \nabla \cdot (\alpha_L \rho_L \mathbf{v}_L \mathbf{v}_L) = -\alpha_L \nabla p + \nabla \cdot \bar{\bar{\tau}}_L \\ \qquad \qquad \qquad + \alpha_L \rho_L \mathbf{g} + K_{LS}(\mathbf{v}_S - \mathbf{v}_L) \end{array} \right. \quad \begin{array}{l} (7) \\ (8) \end{array}$$

$$\left\{ \begin{array}{l} \nabla \cdot (\alpha_S \rho_S \mathbf{v}_S) = \nabla(\gamma_S \nabla \alpha_S) \\ \nabla \cdot (\alpha_S \rho_S \mathbf{v}_S \mathbf{v}_S) = -\alpha_S \nabla p - \nabla p_S + \nabla \cdot \bar{\bar{\tau}}_S \\ \qquad \qquad \qquad + \alpha_S \rho_S \mathbf{g} + K_{LS}(\mathbf{v}_L - \mathbf{v}_S) \end{array} \right. \quad \begin{array}{l} (9) \\ (10) \end{array}$$

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

277 spheres of equal diameter (0.63). However, the impact of the value of the
278 packing limit was negligible in our simulations as the maximal volume frac-
279 tions obtained remained lower than 0.3 approximately. It should be noticed
280 that microcarriers are also non-porous particles. Calculation domain was
281 discretized using between 230000 and 275000 tetrahedral meshes of around
282 5 mm, except in a zone in the vessel bottom, where the size was refined at
283 a maximum of 2 mm, as presented in Fig. 2 to allow a better description
284 of particle just-suspended state. This number of mesh cells was chosen as a
285 compromise between a reasonable calculation time and a sufficient accuracy
286 for the results analysis. To validate the choice of the $\approx 250k$ cells grid, cal-
287 culations were also performed for two cases using a finer grid of $\approx 1M$ cells.
288 First one (A) was impeller EED with $C/T = 0.5$; $D/T = 0.4$ and $\alpha = 12$
289 % and second one (B) was impeller HTPGD, $C/T = 0.5$, $D/T = 0.5$, α
290 = 4 %. While errors between experimental N_{js} measurements and 250k grid
291 calculations were respectively of 1.5 and 2.6 % for cases A and B, it reached
292 respectively 14.7 and 7.8 % with the 1M cells grid. Thus, the 250k cells grid
293 was finally used for the complete set of simulations.

294 The simulation was initialized, considering all particles quasi-settled ($\alpha_S =$
295 0.5) at the bottom of the bioreactor. Higher particle concentrations led to
296 divergence during the first iterations. Consequently, 0.5 was found to be a
297 good compromise, allowing particles settling during the first iterations and
298 avoiding divergence. Convergence of the simulations was supposed when
299 equation residuals were less than 10^{-5} and when liquid velocities and solid
300 volume fractions in the impeller vicinity were stabilized. This second val-
301 idation was necessary to ensure the stabilization of the N_{js} determination

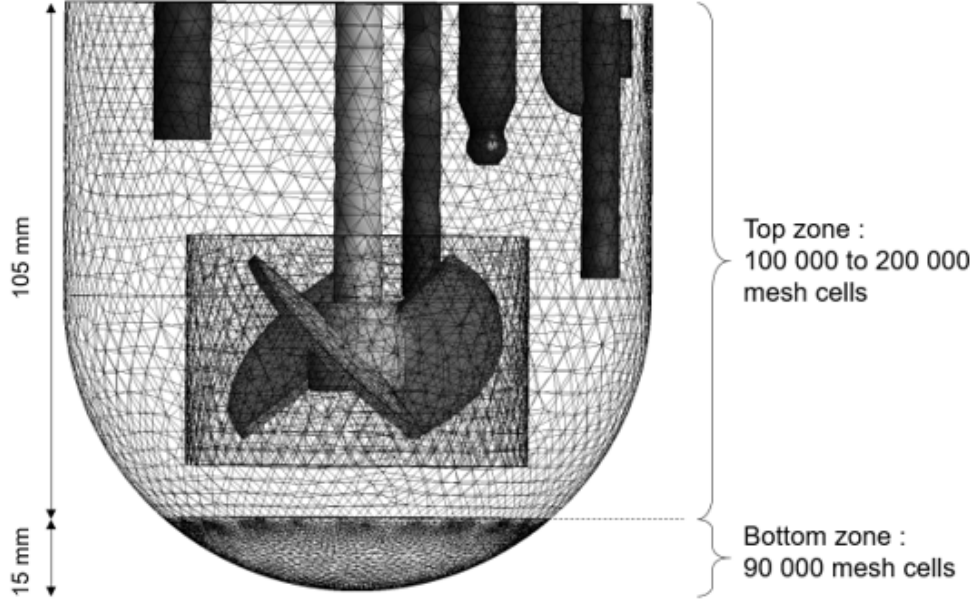


Figure 2: Mesh discretization of the vessel for the CFD simulations.

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).

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

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 *Unsuspected 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 valid 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)

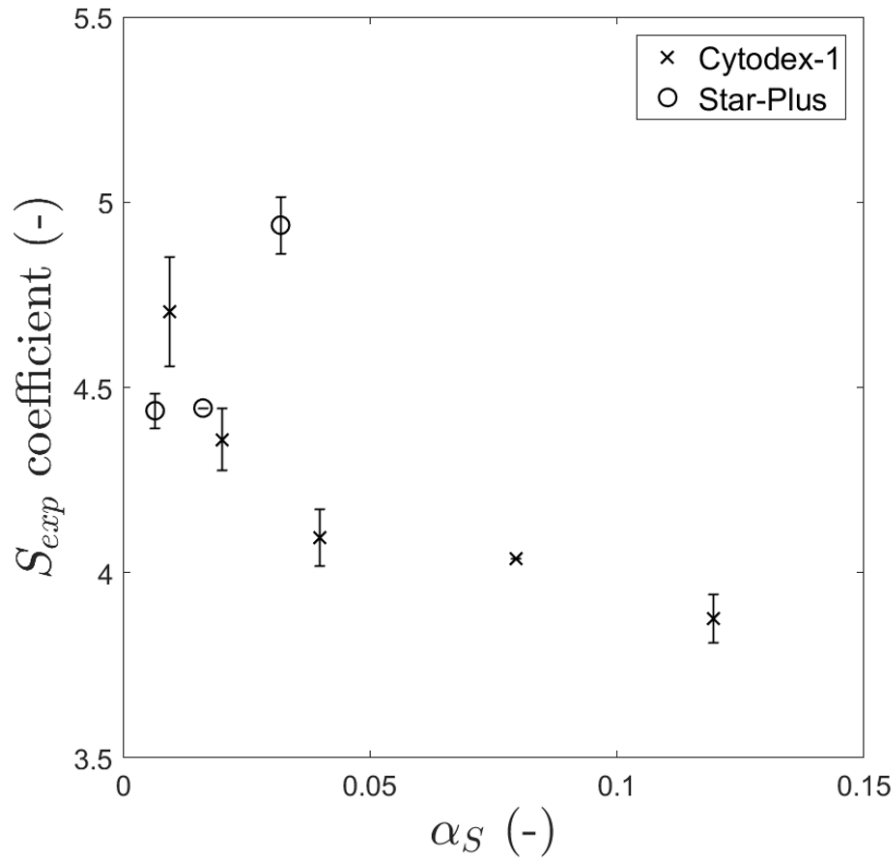


Figure 3: Evolution of the experimentally determined S coefficient from the Zwietering correlation with the microcarrier volume fraction in the bioreactor, for Cytodex-1 and Star-Plus.

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

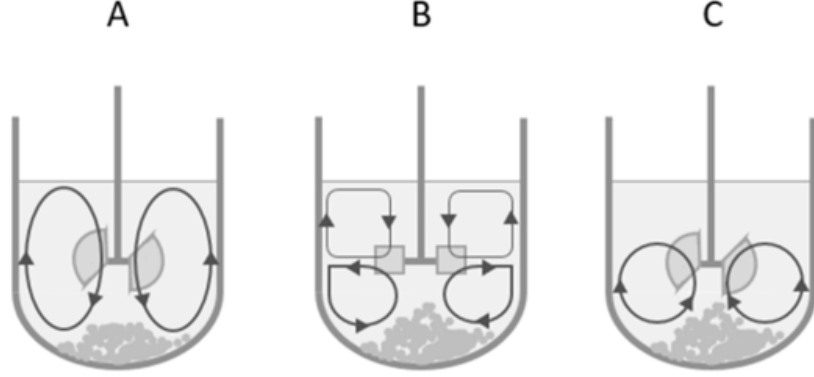


Figure 4: Flow patterns and particles bed shapes observed at agitation rates $N < N_{js}$ depending on mixing structures: axial flow with EED and HTPGD (A), radial flow with RT (B) and mixed-flow with EEU and HTPGU (C).

360 was that, despite it could be possible to determine a unique set of parame-
 361 ters 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 ($\times 10^{-3}$)	K_2	K_3	a_1	a_2	a_3	<i>Tot. α_S exponent</i>
(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

378 and the Rushton turbine at different C/T ratios and at different particle
 379 volume fractions. Results are presented in Fig. 5 for model 3. Models were
 380 globally validated but presented deviations for high particle volume fractions
 381 with the Elephant Ear impeller. The model establishment was based on
 382 data involving particle volume fractions up to 12 % and did not seem thus
 383 suitable for higher concentrations with this type of impeller. However, these
 384 high microcarrier volume fractions are seldom used in animal cell culture
 385 bioreactors. Considering the 95 configurations of the design of experiments
 386 and the 40 complementary measurements, mean relative errors under 10 %
 387 were obtained for the three models. Mean errors obtained with each model
 388 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 %

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

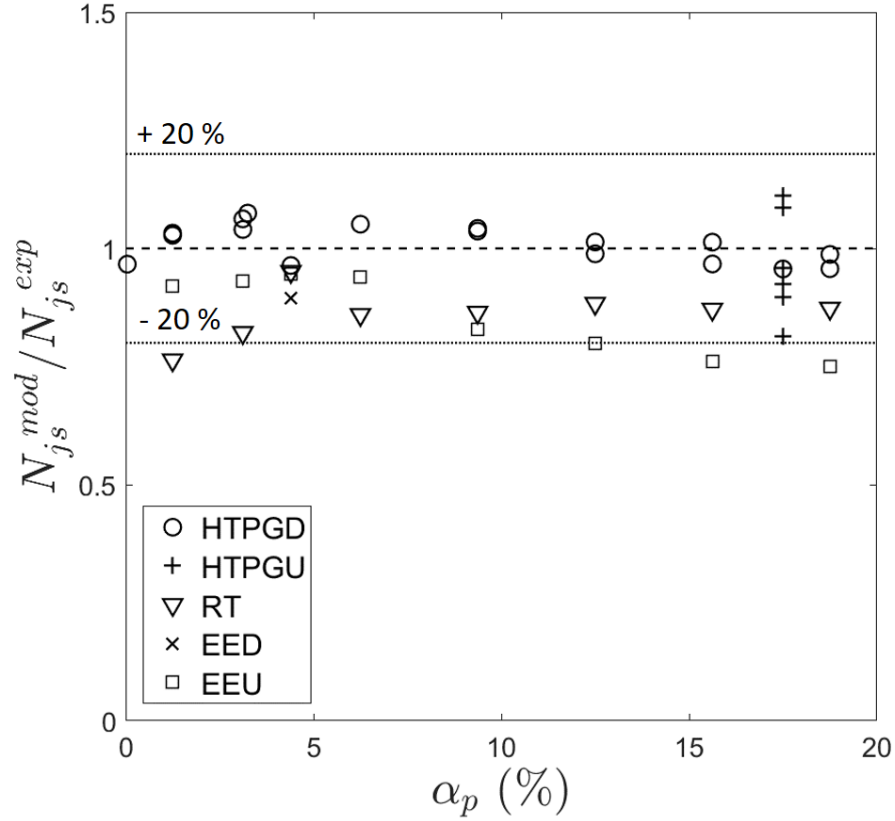


Figure 5: Comparison of the deviation between the experimental N_{js} and the N_{js} predicted by the model for operating conditions out of the design of experiment and model resolution.

392 temperature stability, fluid properties were admitted to be the same as water,
 393 with both density and viscosity dependent of the temperature. Results for
 394 four impeller designs are given in Table 4. All models presented mean devi-
 395 ations of less than 20 % between the experimental values and the predicted
 396 values, but model 2 (Eq. (15)) was found to be more robust than the others.
 397 Concerning the model scalability, results are presented in Table 5. In this
 398 case, the model 2 presented the highest deviation of 34 %. Consequently,
 399 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 scalabil-
401 ity, 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 %

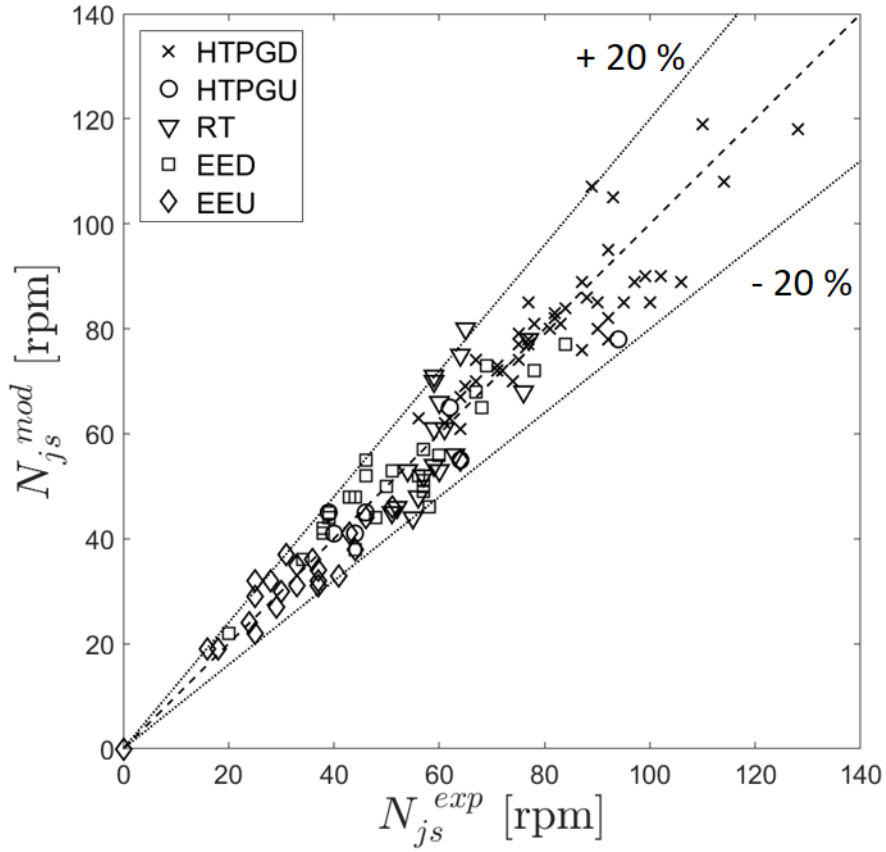


Figure 6: Comparison of experimental N_{js}^{exp} and modeled N_{js}^{mod} with the equation 16.

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.

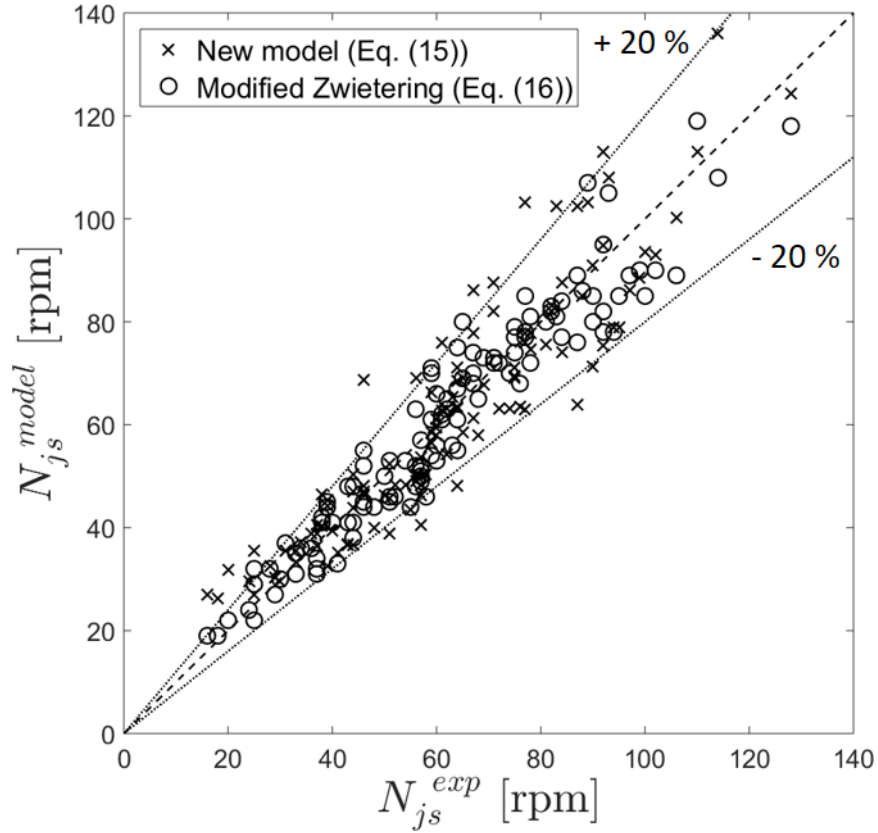


Figure 7: Comparison of modeled N_{js} and numerically adapted $N_{js,Zwietering}$

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

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

539 The CFD-based method to predict N_{js} was then applied on 20 oper-
540 ating conditions of the 1.12 L reactor described in the experimental ap-
541 proach part. The k- ϵ model adaptation was not necessary for this reac-
542 tor scale, displaying higher Reynolds number values of about 5 000, and
543 so the Standard Wall Function was used. This system may be still con-
544 sidered in transitional regime, but the Standard Wall Function appeared
545 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

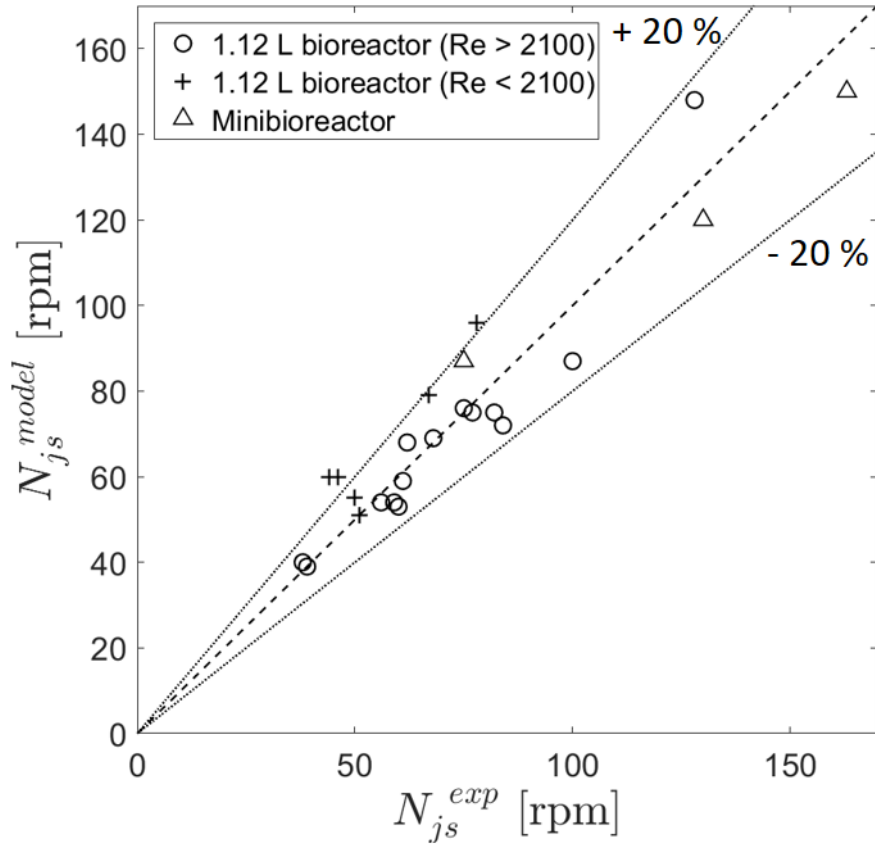


Figure 8: Comparison of experimental N_{js} and modeled N_{js} with the CFD-based method.

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 %.

577 **6. Acknowledgments**

578 The authors would like to thank the French Agence Nationale de la
579 Recherche (ANR) and the Interreg VA Grande Région program for their fi-
580 nancial support. They also acknowledge Fabrice Blanchard (LRGP, Nancy)
581 and Thierry Salmon (PEPs, Liège) for their technical contribution to this
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	$(\text{m}^2 \text{s}^{-1})$
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

586 **References**

- 587 [1] A. Stolzing, E. Jones, D. McGonagle, A. Scutt, Age-related changes
588 in human bone marrow-derived mesenchymal stem cells: Consequences
589 for cell therapies, *Mechanisms of Ageing and Development* 129 (2008)
590 163–173.
- 591 [2] J. Rowley, E. Abraham, A. Campbell, H. Brandwein, S. Oh, Meeting lot-
592 size challenges of manufacturing adherent cells for therapy, *BioProcess*
593 *International* 10 (2012) 7.
- 594 [3] A. L. Van Wezel, Growth of Cell-strains and Primary Cells on Micro-
595 carriers in Homogeneous Culture, *Nature* 216 (1967) 64–65.
- 596 [4] A. S. Simaria, S. Hassan, H. Varadaraju, J. Rowley, K. Warren,
597 P. Vanek, S. S. Farid, Allogeneic cell therapy bioprocess economics
598 and optimization: Single-use cell expansion technologies, *Biotechnology*
599 *and Bioengineering* 111 (2014) 69–83.
- 600 [5] T. Ma, A.-C. Tsai, Y. Liu, Biomanufacturing of human mesenchymal
601 stem cells in cell therapy: Influence of microenvironment on scalable
602 expansion in bioreactors, *Biochemical Engineering Journal* 108 (2016)
603 44–50.
- 604 [6] Q. A. Rafiq, K. M. Brosnan, K. Coopman, A. W. Nienow, C. J. He-
605 witt, Culture of human mesenchymal stem cells on microcarriers in a 5
606 l stirred-tank bioreactor, *Biotechnology Letters* 35 (2013) 1233–1245.
- 607 [7] T. Lawson, D. E. Kehoe, A. C. Schnitzler, P. J. Rapiejko, K. A. Der,
608 K. Philbrick, S. Punreddy, S. Rigby, R. Smith, Q. Feng, J. R. Murrell,

- 609 M. S. Rook, Process development for expansion of human mesenchymal
610 stromal cells in a 50 L single-use stirred tank bioreactor, *Biochemical*
611 *Engineering Journal* 120 (2017) 49–62.
- 612 [8] C. Ferrari, F. Balandras, E. Guedon, E. Olmos, I. Chevalot, A. Marc,
613 Limiting cell aggregation during mesenchymal stem cell expansion on
614 microcarriers, *Biotechnology Progress* 28 (2012) 780–787.
- 615 [9] M. S. Croughan, J.-F. Hamel, D. I. C. Wang, Hydrodynamic effects on
616 animal cells grown in microcarrier cultures, *Biotechnology and Bioengi-*
617 *neering* 29 (1987) 130–141.
- 618 [10] T. M. Maul, D. W. Chew, A. Nieponice, D. A. Vorp, Mechanical stim-
619 uli differentially control stem cell behavior: morphology, proliferation,
620 and differentiation, *Biomechanics and Modeling in Mechanobiology* 10
621 (2011) 939–953.
- 622 [11] T. R. Heathman, A. W. Nienow, Q. A. Rafiq, K. Coopman, B. Kara,
623 C. J. Hewitt, Agitation and aeration of stirred-bioreactors for the mi-
624 crocarrier culture of human mesenchymal stem cells and potential impli-
625 cations for large-scale bioprocess development, *Biochemical Engineering*
626 *Journal* 136 (2018) 9–17.
- 627 [12] C. J. Hewitt, K. Lee, A. W. Nienow, R. J. Thomas, M. Smith, C. R.
628 Thomas, Expansion of human mesenchymal stem cells on microcarriers,
629 *Biotechnology letters* 33 (2011) 2325–2335.
- 630 [13] R. S. Cherry, E. T. Papoutsakis, Physical mechanisms of cell damage in

- 631 microcarrier cell culture bioreactors, *Biotechnology and Bioengineering*
632 32 (1988) 1001–1014.
- 633 [14] A. C. Schnitzler, A. Verma, D. E. Kehoe, D. Jing, J. R. Murrell, K. A.
634 Der, M. Aysola, P. J. Rapiejko, S. Punreddy, M. S. Rook, *Bioprocessing*
635 *of human mesenchymal stem/stromal cells for therapeutic use: Current*
636 *technologies and challenges*, *Biochemical Engineering Journal* 108 (2016)
637 3–13.
- 638 [15] A. Tamburini, A. Brucato, A. Cipollina, G. Micale, M. Ciofalo, *CFD*
639 *predictions predictions of sufficient suspension conditions in solid-liquid*
640 *agitated tanks*, *International Journal of Nonlinear Sciences and Numer-*
641 *ical Simulation* 13 (2012) 427–443.
- 642 [16] T. N. Zwietering, *Suspending of solid particles in liquid by agitators*,
643 *Chemical Engineering Science* 8 (1958) 244–253.
- 644 [17] A. Nienow, *Suspension of solid particles in turbine agitated baffled*
645 *vessels*, *Chemical Engineering Science* 23 (1968) 1453–1459.
- 646 [18] S. Arvinth, P. G. Rao, T. Murugesan, *Critical impeller speed for solid*
647 *suspension in turbine agitated contactors*, *Bioprocess and Biosystems*
648 *Engineering* 14 (1996) 97–99.
- 649 [19] M. Kraume, *Mixing times in stirred suspensions*, *Chemical Engineering*
650 *& Technology* 15 (1992) 313–318.
- 651 [20] A. Delafosse, C. Loubière, S. Calvo, D. Toye, E. Olmos, *Solid-liquid*
652 *suspension of microcarriers in stirred tank bioreactor-experimental and*
653 *numerical analysis*, *Chemical Engineering Science* 180 (2018) 52–63.

- 654 [21] K. Raghava Rao, V. Rewatkar, J. Joshi, Critical impeller speed for
655 solid suspension in mechanically agitated contactors, *AIChE Journal* 34
656 (1988) 1332–1340.
- 657 [22] A. Tamburini, A. Cipollina, G. Micale, A. Brucato, M. Ciofalo, CFD
658 simulations of dense solid–liquid suspensions in baffled stirred tanks:
659 Prediction of suspension curves, *Chemical Engineering Journal* 178
660 (2011) 324–341.
- 661 [23] N. Kee, R. B. Tan, CFD simulation of solids suspension in mixing
662 vessels, *The Canadian Journal of Chemical Engineering* 80 (2002) 1–6.
- 663 [24] A. Mersmann, F. Werner, S. Maurer, K. Bartosch, Theoretical predic-
664 tion of the minimum stirrer speed in mechanically agitated suspensions,
665 *Chemical Engineering and Processing: Process Intensification* 37 (1998)
666 503–510.
- 667 [25] K. Wichterle, Conditions for suspension of solids in agitated vessels,
668 *Chemical Engineering Science* 43 (1988) 467–471.
- 669 [26] G. Baldi, R. Conti, E. Alaria, Complete suspension of particles in me-
670chanically agitated vessels, *Chemical Engineering Science* 33 (1978)
671 21–25.
- 672 [27] S. Ibrahim, A. W. Nienow, Suspension of Microcarriers for Cell Culture
673 with Axial Flow Impellers, *Chemical Engineering Research and Design*
674 82 (2004) 1082–1088.
- 675 [28] P. M. Armenante, E. U. Nagamine, Effect of low off-bottom impeller
676 clearance on the minimum agitation speed for complete suspension of

- 677 solids in stirred tanks, *Chemical Engineering Science* 53 (1998) 1757–
678 1775.
- 679 [29] M. Chudacek, Solids suspension behaviour in profiled bottom and flat
680 bottom mixing tanks, *Chemical Engineering Science* 40 (1985) 385–392.
- 681 [30] M. S. Croughan, J.-F. P. Hamel, D. I. C. Wang, Effects of microcarrier
682 concentration in animal cell culture, *Biotechnology and Bioengineering*
683 32 (1988) 975–982.
- 684 [31] Q. A. Rafiq, K. Coopman, A. W. Nienow, C. J. Hewitt, Systematic
685 microcarrier screening and agitated culture conditions improves human
686 mesenchymal stem cell yield in bioreactors, *Biotechnology journal* 11
687 (2016) 473–486.
- 688 [32] M.-L. Collignon, A. Delafosse, S. Calvo, C. Martin, A. Marc, D. Toye,
689 E. Olmos, Large-Eddy Simulations of microcarrier exposure to poten-
690 tially damaging eddies inside mini-bioreactors, *Biochemical Engineering*
691 *Journal* 108 (2016) 30–43.
- 692 [33] E. Olmos, K. Loubiere, C. Martin, G. Delaplace, A. Marc, Critical agi-
693 tation for microcarrier suspension in orbital shaken bioreactors: exper-
694 imental study and dimensional analysis, *Chemical Engineering Science*
695 122 (2015) 545–554.
- 696 [34] M.-L. Collignon, A. Delafosse, M. Crine, D. Toye, Axial impeller selec-
697 tion for anchorage dependent animal cell culture in stirred bioreactors:
698 methodology based on the impeller comparison at just-suspended speed
699 of rotation, *Chemical Engineering Science* 65 (2010) 5929–5941.

- 700 [35] S. Kaiser, V. Jossen, C. Schirmaier, D. Eibl, S. Brill, C. van den Bos,
701 R. Eibl, Fluid flow and cell proliferation of mesenchymal adipose-derived
702 stem cells in small-scale, stirred, single-use bioreactors, *Chemie Inge-*
703 *nieur Technik* 85 (2013) 95–102.
- 704 [36] C. Schirmaier, V. Jossen, S. C. Kaiser, F. Jüngerkes, S. Brill, A. Safavi-
705 Nab, A. Siehoff, C. Bos, D. Eibl, R. Eibl, Scale-up of adipose tissue-
706 derived mesenchymal stem cell production in stirred single-use bioreac-
707 tors under low-serum conditions, *Engineering in Life Sciences* 14 (2014)
708 292–303.
- 709 [37] V. Jossen, S. C. Kaiser, C. Schirmaier, J. Herrmann, A. Tappe, D. Eibl,
710 A. Siehoff, C. van den Bos, R. Eibl, Modification and qualification of a
711 stirred single-use bioreactor for the improved expansion of human mes-
712 enchymal stem cells at benchtop scale, *Pharmaceutical Bioprocessing* 2
713 (2014) 311–322.
- 714 [38] s. n. Fluent, *Ansys fluent theory guide 15.0*, Inc, Canonsburg, PA
715 (2013).
- 716 [39] P. M. Armenante, E. U. Nagamine, Effect of low off-bottom impeller
717 clearance on the minimum agitation speed for complete suspension of
718 solids in stirred tanks, *Chemical Engineering Science* 53 (1998) 1757–
719 1775.
- 720 [40] G. Montante, A. Brucato, K. Lee, M. Yianneskis, An experimental
721 study of double-to-single-loop transition in stirred vessels, *The Canadian*
722 *Journal of Chemical Engineering* 77 (1999) 649–659.

723 [41] G. Montante, K. Lee, A. Brucato, M. Yianneskis, Numerical simulations
724 of the dependency of flow pattern on impeller clearance in stirred vessels,
725 Chemical Engineering Science 56 (2001) 3751–3770.