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Application Note

Determining Agitation Requirements for Microcarrier Processes: Method Development Using the Mobius® 50 L Single-Use Bioreactor

Mobius® Single-Use Bioreactors have been demonstrated to provide a scalable platform for optimal suspension cell culture performance[1]. However, not all cells can be grown in suspension. Adherent cells require a solid surface upon which to grow, and have traditionally been grown using 2D tissue culture flasks or roller bottles in which scale-up becomes cumbersome. Alternatively, adherent cells can be grown in 3D suspension culture if a solid surface, such as a microcarrier, is provided. In order to ensure successful cell culture, the microcarriers must be kept in suspension throughout the duration of the process. A methodology for determining the minimum agitation rate required to maintain adequate microcarrier suspension and thereby avoid microcarrier settling has been developed using collagen-coated SoloHill® microcarriers in the Mobius® 50 L Single-Use Bioreactor. We have demonstrated that by maintaining a constant agitation rate it is possible to maintain a similar suspension environment as the volume of the process is increased, as long as the microcarrier concentration remains constant. Additionally, we have employed the Zwietering correlation[2] to predict—and experimental analysis to verify—the agitation rates required to maintain suspension of different concentrations of microcarriers.

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Introduction

Microcarriers are commonly used when adherent cells, which require a solid surface in order to grow and proliferate, are cultivated in suspension. Cells commonly used with microcarriers include stem cells, Madin-Darby Canine Kidney (MDCK) and Vero cells used for the production of virus and/or virus-like particles for the manufacture of vaccines, or any other cell type not adapted for growth in suspension. One of the main challenges associated with microcarrier-based 3D cell culture processes is maintaining a two-phase suspension of microcarriers in the liquid bulk.

Generally, solid-liquid suspensions can be categorized into three qualitative states. On-bottom suspension (Figure 1A) is characterized by the complete motion of particles in the liquid bulk while some particles remain unsuspended at the bottom of the vessel. Off-bottom suspension (Figure 1B) is also characterized by the complete motion of particles in the liquid bulk but with no particles remaining on the bottom of the vessel for more than 1 to 2 seconds[2, 3]. The impeller speed that maintains this state of suspension is called the just suspended speed (N_{js}) and can be calculated using the Zwietering correlation[2]:

$$N_{js} = S\nu^{0.1}\left[\frac{g(\rho_s-\rho_l)}{\rho_l}\right]^{0.45} X^{0.13} d_p^{0.2} D^{-0.85}$$

S: Zwietering N_{js} constant*ν: kinematic viscosity (m^2/s)g: gravitational constant (m^2/s)ρ_s: solid density (kg/m^3)ρ_l: liquid density (kg/m^3)X: solids loading ((kg solids/kg liquid) × 100)d_p: particle diameter (m)D: impeller diameter (m)

*The Zwietering constant is a function of impeller and tank geometry. Empirical values can be found in literature.

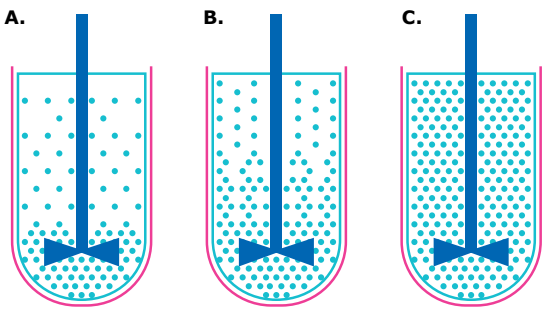

Figure 1.

States of solid suspension: (A) On-bottom, (B) Off-bottom or just suspended, and (C) Uniform suspension.

While no particles remain on the bottom at N_{js}, a gradient is observed with the solids concentration being higher at the bottom of the vessel. As the agitation rate increases, the gradient decreases until a uniform suspension (Figure 1C) is achieved such that the local solids concentration is equal to the average concentration throughout the entire liquid bulk. During microcarrier processes, it is desirable to maintain a homogeneous state of suspension to ensure a uniform growth environment throughout the entire vessel. However, since the sensitivity of cells to hydrodynamic shear forces can be exacerbated when cells are grown on microcarriers[4, 5], some microcarrier users develop their processes with agitation rates that are as low as possible. Balancing the need to keep the microcarriers adequately suspended and the bulk appropriately mixed while minimizing hydrodynamic shear can be challenging.

There are a wide variety of commercially available microcarriers, which span a range of sizes, densities and porosities. While the breadth of microcarrier options provide significant advantages to process scientists and engineers, it also creates challenges since, a priori, it is unrealistic to expect that a standard set of operating parameters would be applicable to every microcarrier/cell combination. Therefore, it is essential that an approach for the optimization of microcarrier-based cell culture processes be developed taking into account the unique properties of the platform of interest. The final process should be built upon knowledge developed around the basic performance characteristics of the microcarrier chosen in the context of the stirred tank bioreactor implemented. While the Zwietering correlation has been used to help predict agitation requirements in traditional stainless steel bioreactors, it cannot be applied directly to the vast majority of single-use systems because empirical Zwietering constants[2] do not exist for the unique geometries of these vessels. The work presented herein describes an approach for defining agitation requirements of a microcarrier-based cell culture process using the Mobius® 50 L Single-Use Bioreactor and collagen-coated microcarriers as a model system. The principles and approach discussed are applicable to any microcarrier/stirred-tank bioreactor system.

Method and Results

Collagen-coated microcarriers were employed to characterize suspension in the Mobius® 50 L Single-Use Bioreactor. This microcarrier was chosen because its size, density and composition are representative of the majority of commercially available microcarriers.

Studies were carried out in mock media (phosphate buffered saline containing 2 g/L Pluronic® F-68). This is a representative suspension buffer selected to mimic the density, viscosity, and surface tension of cell culture media.

Physical characteristics of collagen-coated microcarriers

Average Density (g/cm^3)	1.026
Average Diameter (µm)	170
Bead Composition	Solid polystyrene

Table 1A.

Physical properties of mock media and cell culture media containing 10% Fetal Bovine Serum (FBS)

	Mock Media	Cell Culture Media/10% FBS
Density (g/mL)	1.004	1.006
Viscosity (cP)	1.24	1.13
Surface Tension (dynes/cm^2)	42.51	44.87

Table 1B.

Understanding Flow Patterns in the Mobius® 50 L Single-Use Bioreactor

An initial qualitative study was performed in order to understand the flow and microcarrier settling patterns in the bioreactor. Most importantly, this work also served to identify appropriate sampling locations for subsequent quantitative studies. In order to more readily visualize settling patterns, a low concentration of microcarriers pre-stained with trypan blue was added to 10 L of mock media in the Mobius® 50 L Single-Use Bioreactor. The impeller was then started, and microcarrier settling was observed at increasing agitation rates in the bioreactor. Figure 2 shows the resulting settling patterns.

Very low agitation rates were not sufficient to maintain suspension such that all of the microcarriers remain settled or pooled at the bottom of the vessel (Figure 2A). As the impeller speed was increased, complete motion of some of the microcarriers in the liquid bulk was observed, however a population of microcarriers remained on the bottom of the vessel (Figure 2B). Finally, by increasing the impeller speed to N_{js}, off-bottom suspension was achieved (Figure 2C).

Quantifying Collagen-Coated Microcarrier Suspension

It is difficult to define a minimal agitation rate qualitatively because it is rarely possible to clearly visualize microcarrier movement throughout an entire vessel. The remainder of this study outlines the method developed to quantitatively map the microcarrier suspension gradients for the range of practical agitation rates in the Mobius® 50 L Single-Use Bioreactor as the first step towards defining an appropriate operating window for agitation.

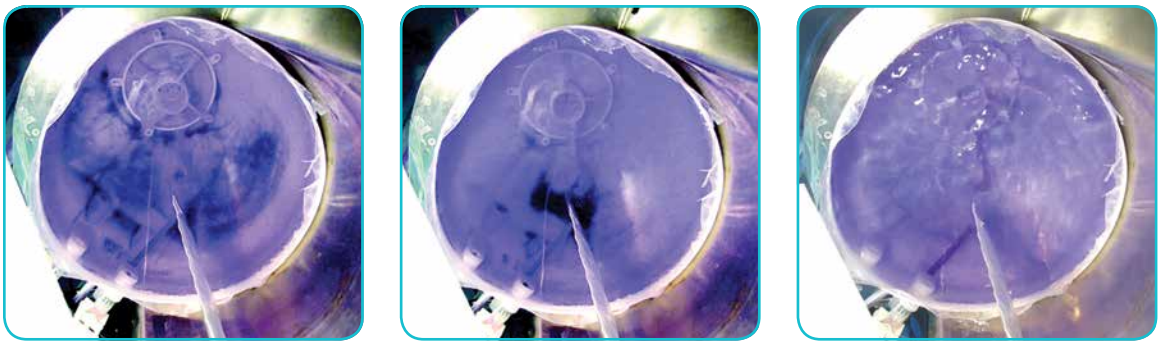

Figure 2.

Top-down view of the Mobius® 50 L Single-Use Bioreactor base. Suspension was characterized for a range of agitation rates (photos taken after steady state mixing was achieved).

A full characterization of microcarrier suspension gradients was performed using collagen-coated microcarriers at a concentration of 7 g/L.

Gradients were quantified over a range of agitation rates for 10, 25 and 50 L working volumes by taking single-point samples from the bioreactor at the locations shown in Figure 3. For each condition, the bioreactor was allowed to reach steady state mixing before 10 mL samples were collected using a serological pipette. The concentration of microcarriers in suspension at each location was determined after filtration of the samples through cell strainers (Fisher Scientific), and calculating the mass of microcarriers collected.

Visual observation at the 10 L working volume enabled identification of the minimum agitation required for suspension (referred to as N_{min}) as well as N_{js} at 30 RPM and 67 RPM, respectively. Thus, the qualitative states of suspension can be characterized for the full range of practical agitation rates:

- < 30 RPM: No suspension
- 30 to 67 RPM: On-bottom mixing
- > 67 RPM: Off-bottom mixing

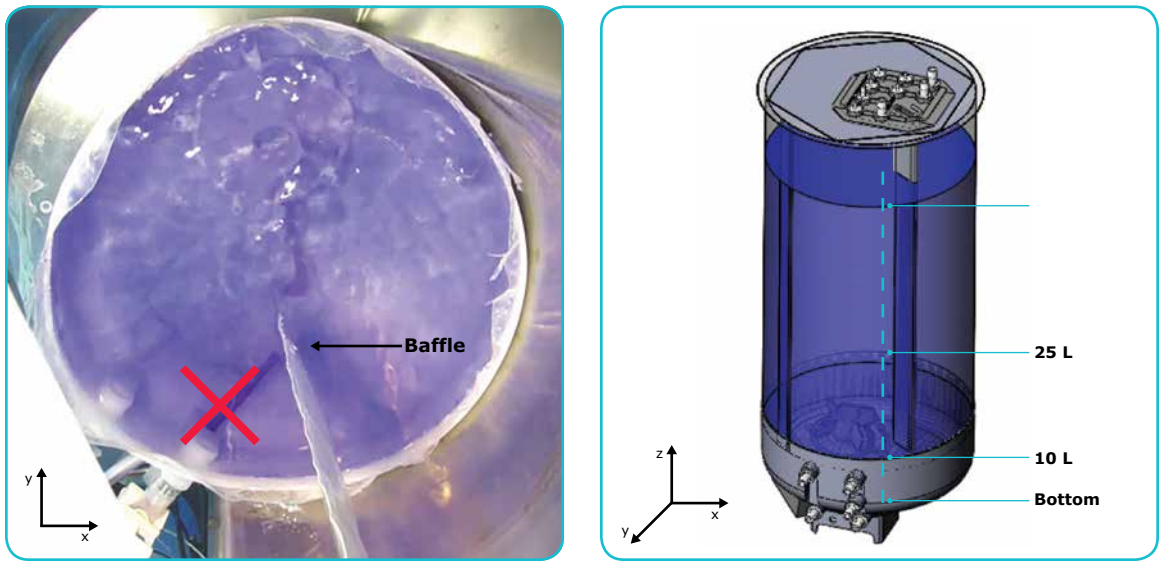

Figure 3.

Sampling locations in the Mobius® 50 L Single-Use Bioreactor. 10 mL samples were taken from the bottom, 10, 25 and 50 L heights at the location marked by the "X". This corresponds to the site where the majority of settling was observed in the study shown in Figure 2.

Figure 4A shows the microcarrier concentration gradients, or ΔC (where $\Delta C = C_{bottom} - C_{top}$), quantified at the 10 L working volume. As expected, increasing the agitation rate resulted in a decrease in the concentration gradient until a homogeneous suspension (ΔC=0) was achieved at approximately 115 RPM (referred to as N_h). Additionally, these data show that there was a critical agitation rate between N_{min} and N_{js} (the on-bottom mixing range) at which ΔC decreased sharply approaching zero. At rates between 30 and 50 RPM, suspension was achieved, but a large gradient existed, such that the majority of microcarriers remained settled on the bottom of the vessel. Sixty RPM was the critical agitation rate at which the microcarrier gradient was drastically reduced, and although there was a small population of settled microcarriers, ΔC was close to zero, indicating a nearly homogeneous suspension environment. This critical agitation rate, referred to as N_c for the remainder of this study, allows for quantitative characterization of concentration gradients for the full range of practical agitation rates:

- 30 to 60 RPM: Large gradient
- 60 to 115 RPM: Minimal gradient
- > 115 RPM: No gradient

Traditionally, N_{js} is used as the minimum agitation requirement for suspension of solids in a stirred-tank system. However, the results of this study suggest that it may be more practical to use the newly identified agitation rate, N_c. While there is a qualitative difference between the states of suspension at these two rates (off-bottom at N_{js} and on-bottom at N_c), there is no significant quantitative difference (ΔC is comparable). The use of N_c as the minimum agitation requirement can widen the operating space for a given process, making it easier to balance shear effects.

Figures 4B and 4C show that the measured gradients were similar for corresponding agitation rates when the working volume was increased to 25 and 50 L, respectively. These data suggest that maintaining a constant agitation rate will result in similar suspension as the volume is increased, as long as the microcarrier concentration remains constant.

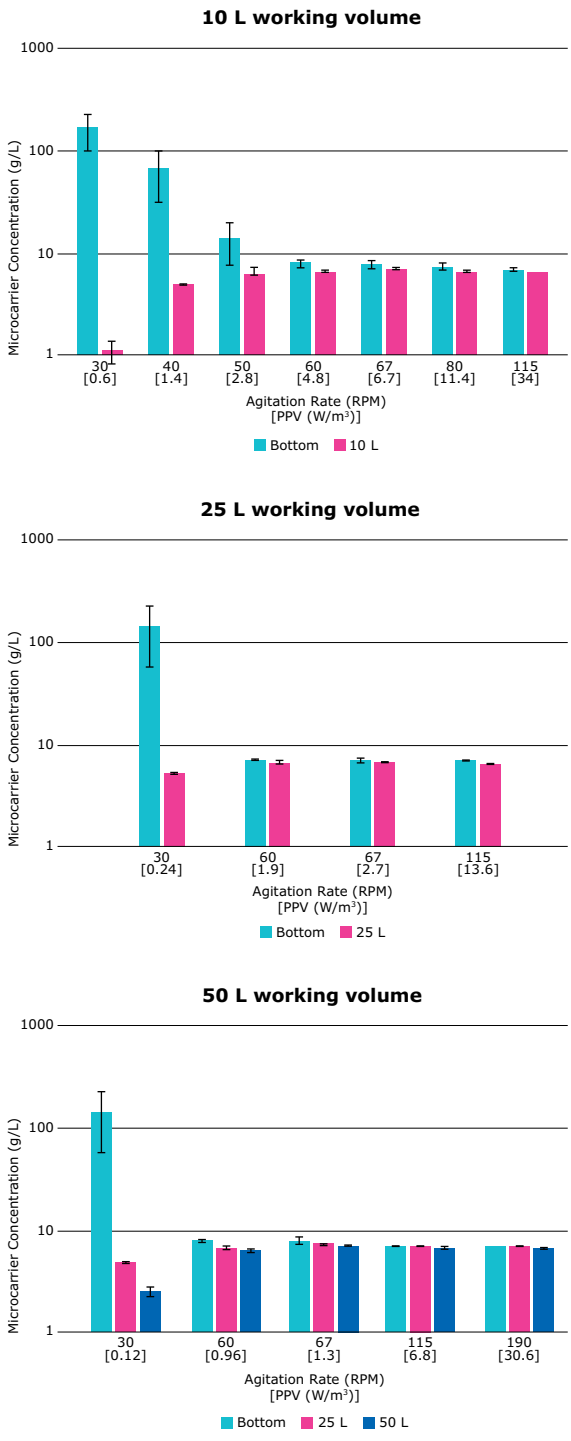

Figure 4.

Microcarrier concentration gradients of 7 g/L collagen-coated microcarriers in the Mobius® 50 L Single-Use bioreactor at 10 L (A), 25 L (B), and 50 L (C) working volumes. The values shown represent the mean of triplicate determinations, along with the standard deviation.

Using the Zwietering Correlation

While the Zwietering correlation alone cannot be used to directly predict agitation requirements for a given system, it can be used in conjunction with empirical determinations to make predictions across varying concentrations of a specific type of microcarrier in a single vessel.

$$N_{js} = S\nu^{0.1}\left[\frac{g(\rho_s-\rho_l)}{\rho_l}\right]^{0.45} X^{0.13} d_p^{\,0.2} D^{-0.85}$$

When the only parameter that changes is the system solids loading, i.e., the microcarrier concentration, all of the variables in the Zwietering correlation remain constant, with the exception of the just suspended speed (N_{js}) and the solid:liquid mass ratio of the system (X). Therefore, if an agitation requirement for one microcarrier concentration is known (e.g., experimentally determined), a ratio can be taken to calculate the agitation speed for other concentrations of microcarriers:

$$\frac{N_{js2} = S\nu^{0.1}\left[\frac{g(\rho_s-\rho_l)}{\rho_l}\right]^{0.45} X_2^{\,0.13} d_p^{\,0.2} D^{-0.85}}{N_{js1} = S\nu^{0.1}\left[\frac{g(\rho_s-\rho_l)}{\rho_l}\right]^{0.45} X_1^{\,0.13} d_p^{\,0.2} D^{-0.85}}$$

The rest of the terms cancel out:

$$\frac{N_{js2} = X_2^{\,0.13}}{N_{js1} = X_1^{\,0.13}}$$

$$N_{js2} = N_{js1} \cdot \left[\frac{X_2}{X_1}\right]^{0.13}$$

This equation was used along with the results from the 7 g/L collagen-coated suspension studies to calculate the key rates for anticipated ranges of likely microcarrier concentrations in the Mobius® 50 L Single-Use Bioreactor (Figure 5).

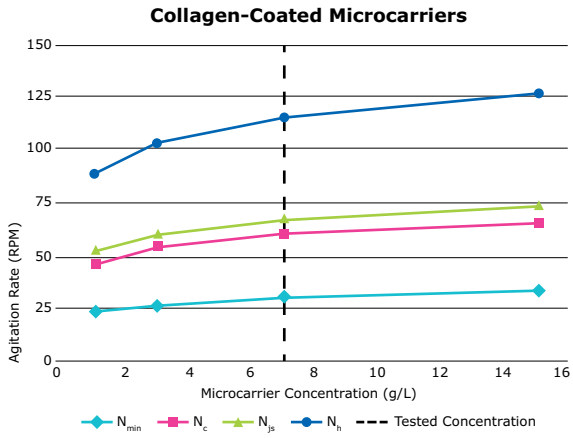

Figure 5.

Key agitation rates for collagen-coated microcarrier concentrations in the Mobius® 50 L Single-Use Bioreactor.

To confirm the validity of using the Zwietering correlation approach to predict agitation requirements, suspension studies were performed using additional concentrations of microcarriers. Figure 6 shows the microcarrier suspension results when using 15 g/L collagen coated microcarriers at the key agitation rates calculated from the experimental data developed at a 7 g/L concentration (Table 2).

Key Agitation Rate	Experimentally Determined for 7 g/L Collagen-coated	Calculated for 15 g/L Collagen-coated
N_{min}	30	33
N_c	60	66
N_{js}	67	74
N_h	115	127

Table 2.

The Zwietering equation was used with empirical data to calculate key agitation rates for 15 g/L collagen-coated microcarriers in the Mobius® 50 L Single-Use Bioreactor.

As shown in Figure 6, the Zwietering correlation was used to accurately predict key agitation rates (N_{min}, N_c, N_{js} and N_h) for 15 g/L collagen-coated microcarriers in the Mobius® 50 L Single-Use Bioreactor. The microcarrier gradients at 15 g/L match those at 7 g/L, confirming that the Zwietering correlation can be used to accurately predict suspension requirements for varying concentrations of microcarriers.

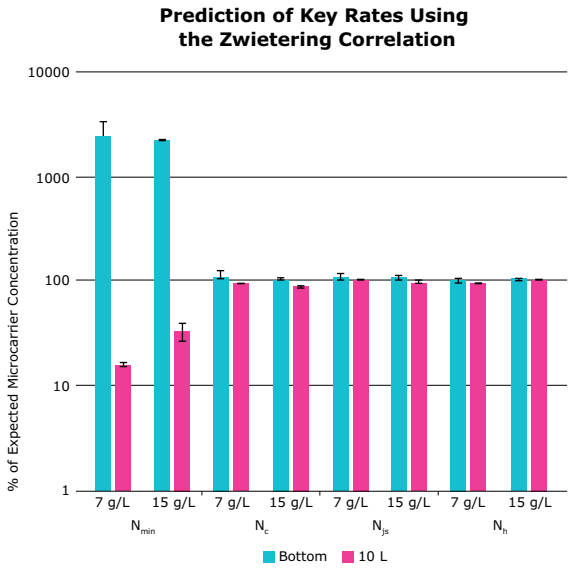

Figure 6.

Microcarrier concentration gradients of 7 g/L and 15 g/L collagen-coated microcarriers in the Mobius® 50 L Single-Use Bioreactor at the 10 L working volume. The values shown represent the mean of triplicate determinations, along with the standard deviation.

Conclusions

The wide breadth of available microcarrier options for adherent cells necessitate that a specific set of operating parameters be generated for each cell/microcarrier/vessel combination. This study outlines a general approach for characterizing qualitative states of suspension and quantitative concentration gradients as a starting point to define an appropriate process operating window. The method outlined in this study can be used to generate a characterization map of microcarrier suspension and key agitation rates for a microcarrier/stirred-tank bioreactor system (Figure 7).

Qualitative characterization of states of suspension and quantitative characterization of microcarrier gradients can be used to identify the following key agitation rates as a starting point to defining an appropriate operating window:

- N_{min}: The minimum agitation rate necessary for complete motion of at least one microcarrier in the liquid bulk
- N_c: The agitation rate at which the microcarrier gradient is drastically reduced, and increasing agitation has little effect on decreasing the gradient
- N_{js}: The minimum agitation rate necessary to maintain complete motion of all microcarriers in the liquid bulk
- N_h: The minimum agitation rate necessary to maintain a homogeneous state of suspension in the liquid bulk

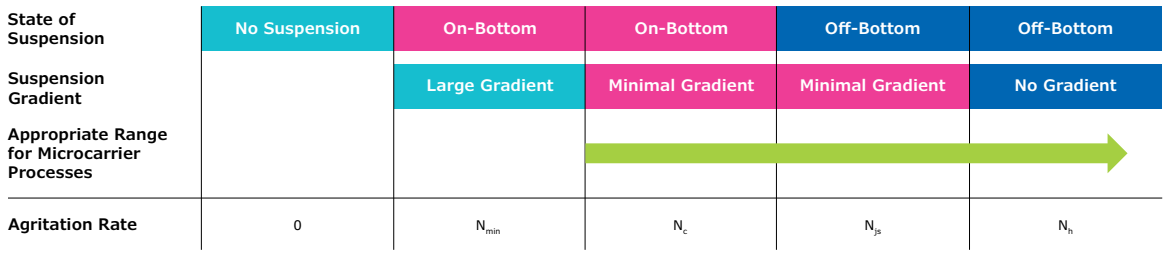

Figure 7.

General map for characterizing microcarrier suspension in a stirred-tank bioreactor, where qualitative states of suspension and quantitative gradients are defined for the key agitation rates: N_{min}, N_c, N_{js}, and N_h.

Conclusions (continued)

While the actual impeller speeds required for achieving these key agitation rates will be unique for every microcarrier/stirred-tank bioreactor combination, this study has outlined an approach to minimize the amount of experimental characterization needed.

Additionally, this study has identified a novel suspension state, N_c, that, while qualitatively different than N_{js}, is quantitatively comparable. The use of this lower agitation rate at N_c may open the operating window for microcarrier processes.

Working volume: The key agitation rates, states of suspension, and quantitative concentration gradients remain constant over the range of working volumes in the Mobius® 50 L Single-Use Bioreactor.

Therefore, a constant agitation rate can be defined for microcarrier suspension for scale-up or -down in liquid volume.

Microcarrier concentration: The general characterization map shown in Figure 7 only needs to be generated experimentally for one microcarrier concentration. The Zwietering correlation can then be used to predict the agitation requirements for varying concentrations. However, each microcarrier requires its own characterization.

The methods outlined in this study can help to define the lower limit of the agitation process window for microcarrier culture in stirred-tank bioreactor systems. Further experimentation is necessary to find an optimal operating window to balance microcarrier suspension with a multitude of other process considerations, such as shear effects, liquid-liquid mixing, and increasing agitation requirements as cells grow on the microcarrier surface.

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