Characterizing Mixing Dynamics within a Single-Use Stirred Tank Reactor (STR) System

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BIOPROCESSING

Abstract

The stirred tank reactor (STR) is the one of the most common types of bioreactor systems used for manufacturing operations within the biopharmaceutical industry. Maintaining homogeneous environments within STR systems is often critical for success across both upstream and downstream bioprocess applications. Environmental homogeneity supports thermal and chemical uniformity within process systems. Such uniformity can be essential for optimal cell culture growth, maximum process yields, and high overall product quality.

Inadequate mixing can lead to the formation of concentration and thermal gradients within STR systems. The emergence of these types of gradients have been shown to cause adverse process responses. The presence of gradients can produce extracellular conditions which are unfavorable for cell culture growth. Gradient formation may also result in decreased product stability and increased product aggregation. Such undesired responses have the potential to adversely affect both the overall titer and the critical quality attributes (CQAs) of the final product pool. The severity of such negative consequences suggests that mixing dynamics should be well understood and characterized throughout all relevant stages of upstream and downstream bioprocess development.

In this study, we characterized mixing dynamics within a benchtop STR bioreactor system using the pH tracer method. Results from this study demonstrated that upward axial flow patterns may be more efficient than downward axial flow patterns in regard to creating homogeneous bioreactor vessel conditions at a relatively low system power inputs (1.50 W/m^3). Additionally, when we evaluated mixing response times by overall system power input, it was determined that increased improvements in mixing time could not be detected at power inputs higher than 7.97 W/m^3.

Introduction

A thorough understanding of mixing dynamics within stirred tank reactors (STRs) is often necessary for effective bioprocess engineering. During bioprocess development, suitable mixing conditions for process operations are often defined either experimentally or through computational fluid dynamic (CFD) modeling. Defined mixing parameter setpoints, such as mixing time and agitation rate, are subsequently validated through confirmatory testing. Upon testing completion, validated parameter definitions may then be integrated into overall process designs. Adverse unintended consequences, such as the formation of localized concentration gradients, may occur if the mixing dynamics of STR systems are not appropriately characterized during the various stages of bioprocess development.[1,2,3]

Bioprocess development typically involves the volumetric scaling of processes from benchtop to production volumes. Such work is traditionally completed through bioprocess transfer and qualification projects; such workflows are commonly referred to as tech transfer initiatives. These tech transfer initiatives require the engineering of setpoint definitions for both volume-independent and volume-dependent process parameters. Definitions for both sets of parameters must be appropriate for the new bioreactor systems.

When engineering process setpoints for a tech transfer initiative, process definitions for volume-independent parameters are typically held constant across different volumetric scales. Examples of volume-independent parameters include temperature setpoint, glucose concentration, dissolved oxygen concentration, and pH setpoint. Conversely, setpoint definitions for volume-dependent parameters will likely change across different vessels and scales. Definitions for volume-dependent parameters are typically engineered through the conservation of a central criterion. Parameter definitions for each system are calculated so that the criterion value remains constant

across all process scales. Such a strategy helps to support process consistency across various volumetric scales and platforms.

Mixing parameters, such as agitation rate, mixing time, and working volume, are volume-dependent parameters. The most commonly used central criterion for scale-up or tech transfer of STR mixing processes is the average specific energy dissipation rate (P/ρ*V). When mixing solutions of similar densities, this factor is directly related to the volumetric power input of the system (P/V).[4]

The volumetric power input is influenced by the geometric dimensions of multiple vessel elements within STR systems. These factors should be considered during tech transfer initiatives. These design elements include the vessel aspect ratio (the ratio of the height of the liquid to the diameter of the liquid surface area), the ratio of impeller diameter to vessel diameter, the height of the impeller in relation the bottom of the vessel, and the overall impeller type and power number.[3,5]

When establishing parameters for mixing processes, the minimum and maximum acceptable volumetric power inputs for the system should be defined. These definitions can be influenced by process factors such as cellular aerobic requirements, culture shear tolerance, maximum allowable processing time, and product denaturation or aggregation kinetics.[6] The overall mixing dynamics of different STR systems can also impact these definitions.

Impeller type can have a large impact on the mixing dynamics of an STR system. Pitch blade impellers are a specific type of impeller which is commonly used within upstream and downstream bioprocessing operations. These impellers are widely utilized as they have been demonstrated to reduce the generation of hydrodynamic shear forces when compared to other types of impeller models. This attribute makes pitch blade impellers highly suitable for many cell culture bioprocessing systems.

Pitch blade impellers will drive either upward or downward pumping actions. This vertical mixing dynamic is described as axial flow. The upward or downward directionality of the axial flow will be determined by both the orientation of the impeller and the rotation directionality of the agitator shaft. The impeller may be right-handed (RH) or left-handed (LH), while the shaft may be rotated either clockwise (CW) or counterclockwise (CCW). The combination of these parameters will determine the ultimate directionality of axial flow within the system. The details of this relationship are described within **Figure 1**.[7]

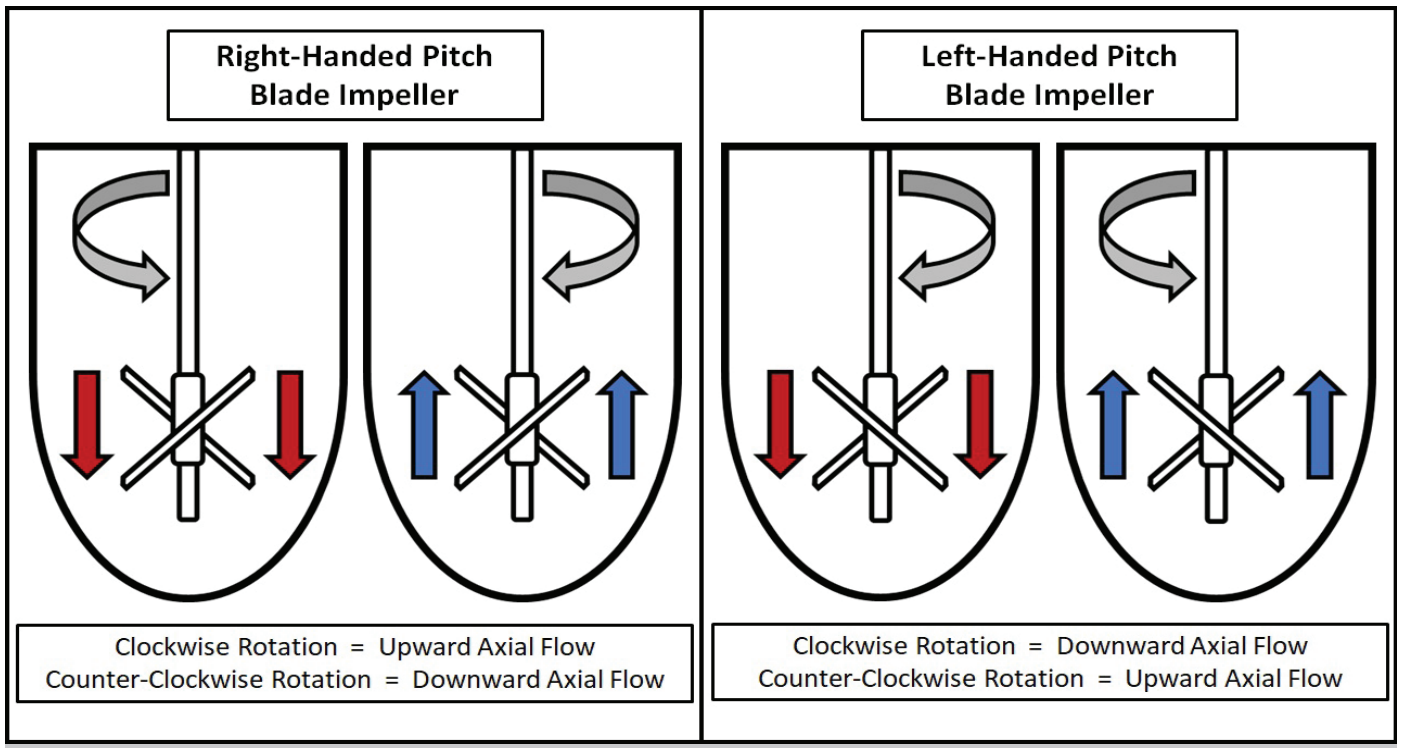


Figure 1: Relationship of pitch blade impeller orientation and rotational direction of agitator on axial flow pattern.

Mixing time is widely used as a metric for analyzing and validating the mixing dynamics within a stirred tank reactor.[8] Mixing time is generally defined as the time required to achieve a 90 to 95% homogeneous state within an STR system, at a specific power input. Assuming geometric and impeller similarity among scales, the correlation between mixing time (t_m) and vessel diameter (D) can be described using the formula shown in **Equation 1**.

$$t_m \propto D^{2/3} \quad \textbf{(Eq. 1)}$$

With this equation, it can be observed that mixing time increases proportionally with process scale.[9] This direct relationship suggests that scale down modeling for establishing mixing parameter definitions could potentially be a cost-effective and efficient method for developing larger volume bioreactor processes, such as during tech transfer initiatives.

Mixing time can be characterized experimentally using the pH tracer method. The pH tracer method is an invasive method which utilizes at least two pH sensors.[10] Traditionally, one pH sensor will be placed in the bottom of the system, while a second is positioned near the surface of the liquid. A high or low pH tracer signal is added to a buffer solution in the reactor. As the pH probes respond to the pH tracer addition, the difference between the two pH measurements is analyzed. The time required for each probe to reach signal stabilization is also often calculated. The pH tracer method is an elegant means of characterizing mixing dynamics within STR systems. The technique can be utilized to support numerous bioprocessing applications.

Within this study, we characterized mixing dynamics within a 5-L BIOne Single-Use Bioreactor (SUB) system using the pH tracer method. Both volumetric power input and axial flow direction were evaluated as study factors. Results demonstrated both the suitability of the pH tracer method for mixing dynamic characterization and the potential for both power input and axial flow direction to impact system homogeneity within a STR system.

Materials and Methods

A BIOne Single-Use Bioreactor (SUB) manufactured by Distek, Inc. was selected as the STR model for this study. This vessel is a benchtop STR system that is used for both upstream and downstream process modeling. The system also has utility as a vessel for small-volume biologics manufacturing. The BIOne SUB model evaluated during this study was a 5-L working volume model with a single right-handed pitch blade impeller. The bioreactor was operated using the BIOne 1250 Dual-Vessel Controller by Distek, Inc. (model number 2022-8122).

The working volume maintained during the study was 4800 mL. The model medium used during the testing was nonsterile 10% Phosphate Buffer Solution (PBS). The system temperature was maintained at a setpoint of 37°C +/- 1.0°C throughout testing.

Two pH sensors were used within the 5-L SUB during the study. The pH sensors were calibrated with pH 7.00 and pH 10.00 buffers prior to each study iteration. After calibrations were completed, pH Sensor 1 was positioned so that the frit of the probe was approximately one centimeter below the liquid surface. The second sensor, pH Sensor 2, was then installed so that the frit was near the bottom of the SUB system.

Prior to each study iteration, the vessel pH was confirmed to be stable between 7.40 – 7.70. After confirmation of pH, both the agitation rate and agitation direction were programmed based on the process definitions for the specific study trial. Trial parameters are described in Table 1. Once the defined agitation operational parameters were reached, a 10 mL volume of 1M Sodium Carbonate tracer solution (pH >11.0) was added to the system. Tracer addition was performed at the liquid surface of the system.

After the tracer addition, pH measurements from pH Sensor 1 and pH Sensor 2 were recorded every five seconds for a ninety second testing period. Data recorded were normalized using the formula shown in **Equation 2**.

$$Normalized\ pH\ at\ Time_X = \frac{(pH_{Time_x} - pH_{Time_0})}{(pH_{Time_{90}} - pH_{Time_0})} \times 100$$

(Eq. 2)

The normalized data were analyzed using a variable slope, sigmoidal curve regression (Graphpad Prism, Version 9.0). Using these data, the mixing homogeneity times were established for all experiential trials. Mixing homogeneity time was defined as the elapsed time required for both Probe 1 and Probe 2 to both reach the 95% homogeneity threshold. An overview of the overall experimental method for this study is shown in **Figure 2**.

Table 1: System Operational Parameters for Mixing Study Trials

Trial No.	Power Input (W/m³)	Agitation Rate (rpm)	Axial Flow Direction
A1, A2	1.50	100	Upwards
B1, B2,	1.50	100	Downwards
C1, C2	7.97	175	Upwards
D1, D2	7.97	175	Downwards
E1, E2	30.9	275	Upwards
F1, F2	30.9	275	Downwards
G1, G2	150	450	Upwards
H1, H2	150	450	Downwards

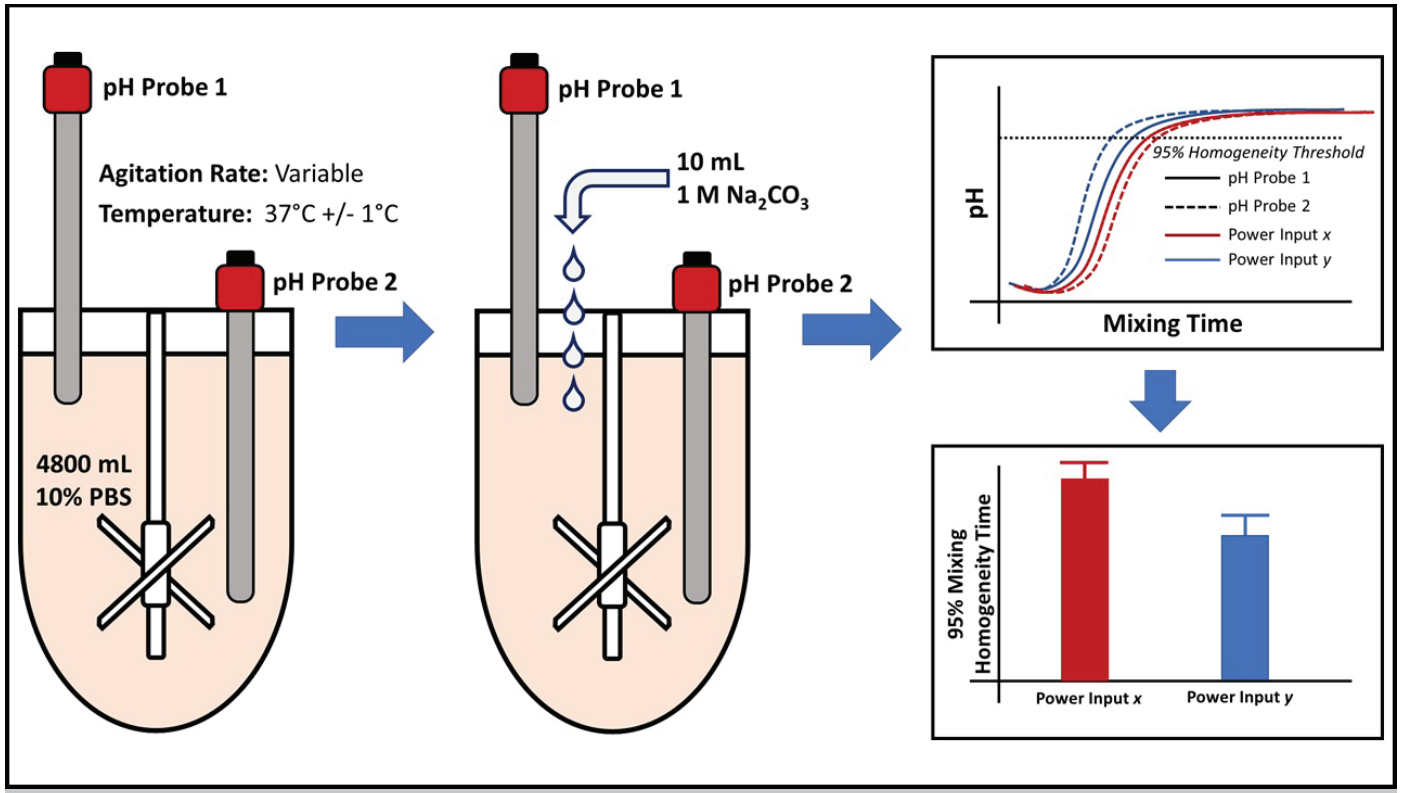


Figure 2: Overview of study design. Tracer solution (1 M Na_2CO_3) added to bioreactor medium (10% PBS) at defined operational conditions. Mixing Homogeneity Time defined as the time required for both pH Probe 1 and pH Probe 2 readings to reach 95% homogeneity standard.

Results and Discussion

Regression data from the mixing study trials are shown in **Figure 3**. Differences in mixing times for upward and downward axial flows can be observed across the 1.50 W/m³ testing conditions. Differences could not be visually observed across the other higher power input testing conditions. Further analysis of the mixing homogeneity times confirmed the visual trend of the regression models. The unpaired T-Test results demonstrated that the mixing times required for the upward and downward axial flow processes were statistically significantly different ($\alpha = 0.05$) at a power input of 1.50 W/m³, but not for power inputs greater than or equal to 7.97 W/m³. These results are shown in **Figure 4**.

The overall differences between the online signals of pH Probe 1 and pH Probe 2 during each of the trials are shown in **Figure 5**. The magnitude of the differences between the top and bottom pH probe signals for the 1.50 W/m³ conditions appear to be three to six times greater than differences observed across the other power input conditions. Additionally, it appears that extended mixing times were required to resolve the pH signal differences for the 1.50 W/m³ conditions, as compared to the durations required for pH signal disparity resolution across the higher agitation testing conditions.

These observations were supported by the results of a Tukey Multiple Comparison test. This test compared the 95% homogeneity mixing times by system power input for both upward and downward axial flow processes. Across both the upward and downward axial flow models, statistically significant differences in mixing homogeneity times were observed between the 1.50 W/m³ condition and other higher power input conditions. In contrast, no difference in mixing time differences were observed across the 7.97 W/m³, 30.9 W/m³, and 135 W/m³ conditions. These data are shown in **Figure 6**.

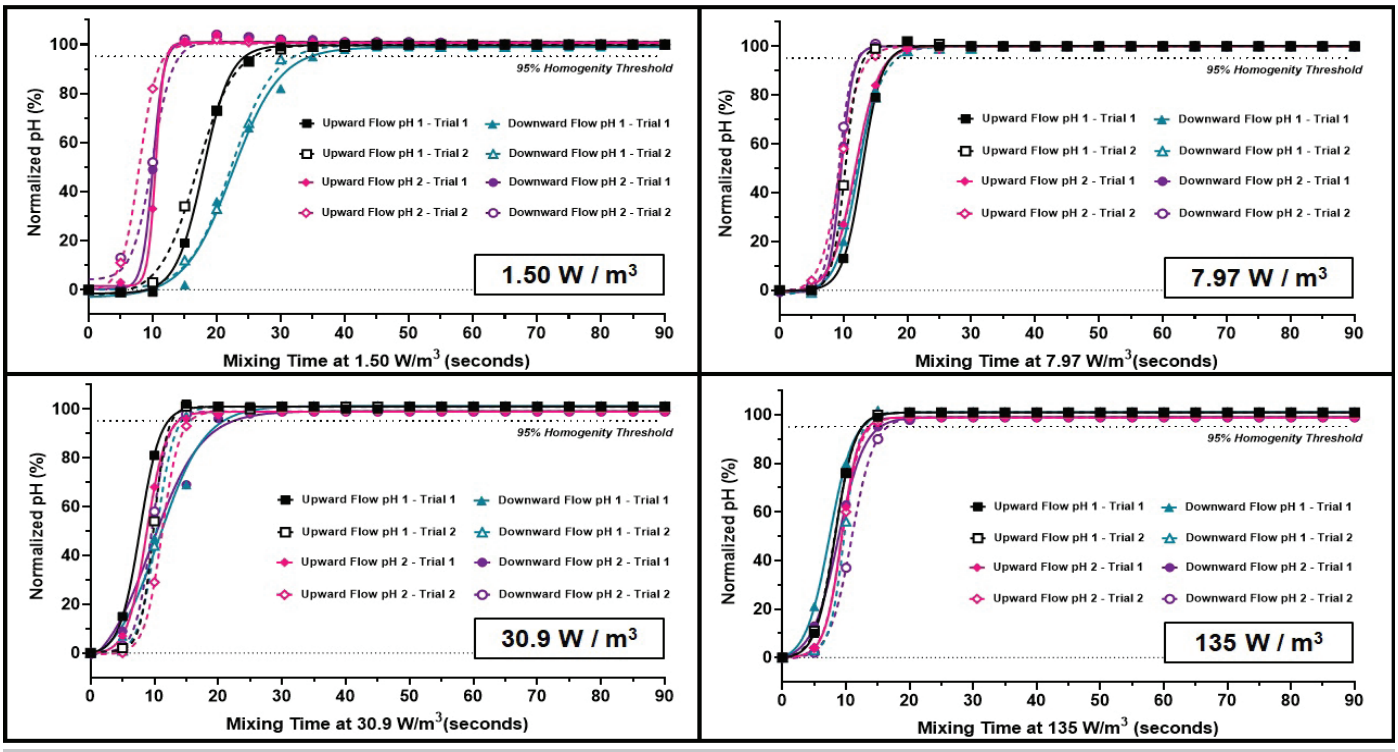


Figure 3: Non-Linear regression analyses of mixing study data. Differences in mixing time can be seen between upward and downward axial flows under 1.50 W/m3 testing conditions. No clear differences observed across higher power input conditions.

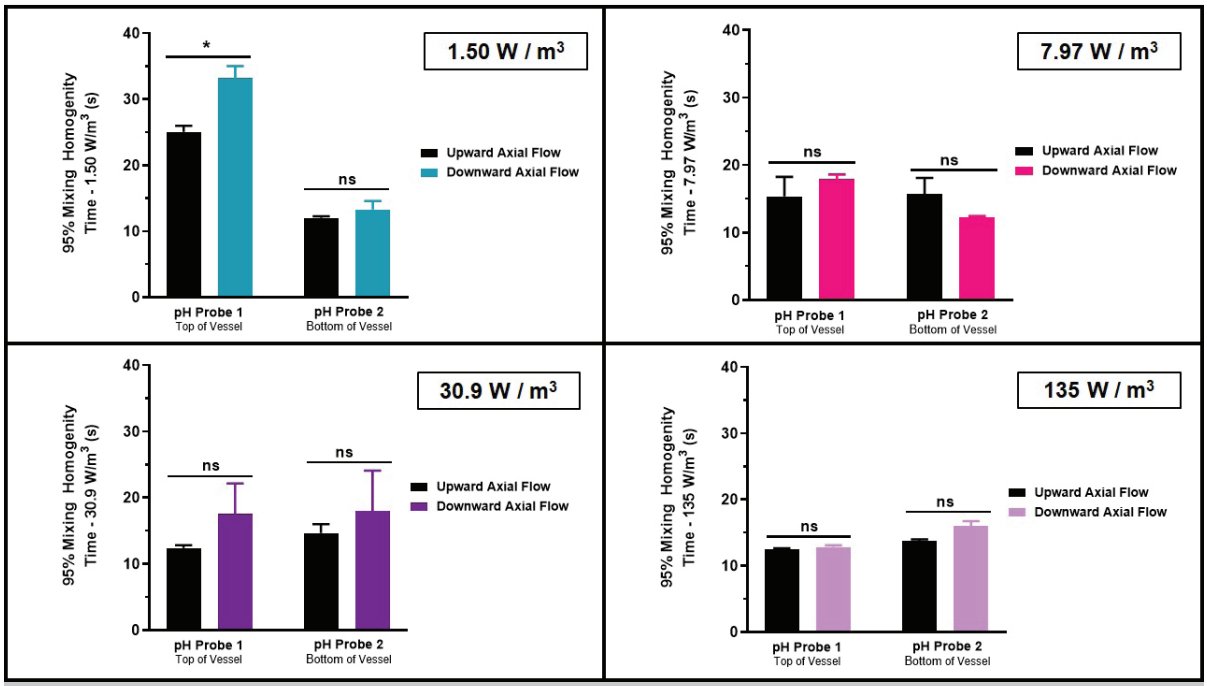


Figure 4: Comparison of mixing times for upward and downward axial flow systems at variable power inputs. Results demonstrated statistically significantly differences in mixing times for upward and downward flow patterns for 1.50 W/m3 condition. Data are mean values from n=2 replicates. Bars represent standard error. $\alpha = 0.05$.

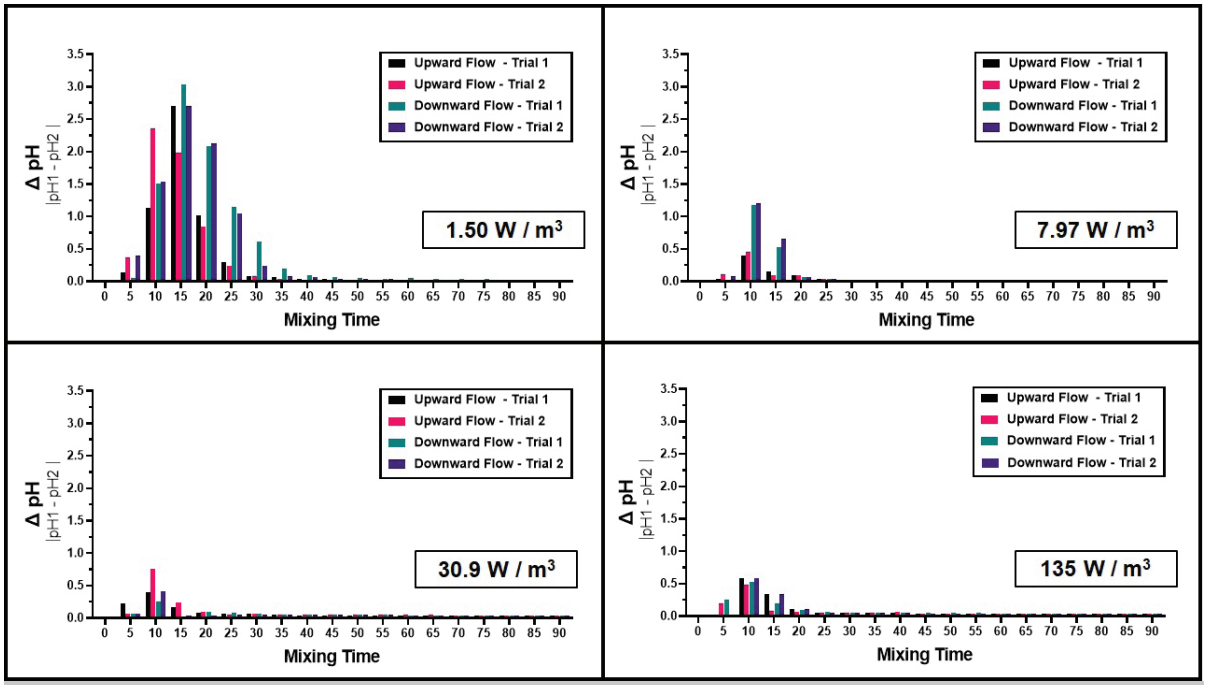


Figure 5: Online signal differences between pH 1 and pH 2 mixing. Magnitude of difference for 1.50 W/m3 condition appears 3 – 6x greater than differences in signal observed across higher power input conditions. Results also demonstrated a longer time is required to resolve pH prove signal disparity for 1.50 W/m3 conditions, as compared to higher power input conditions.

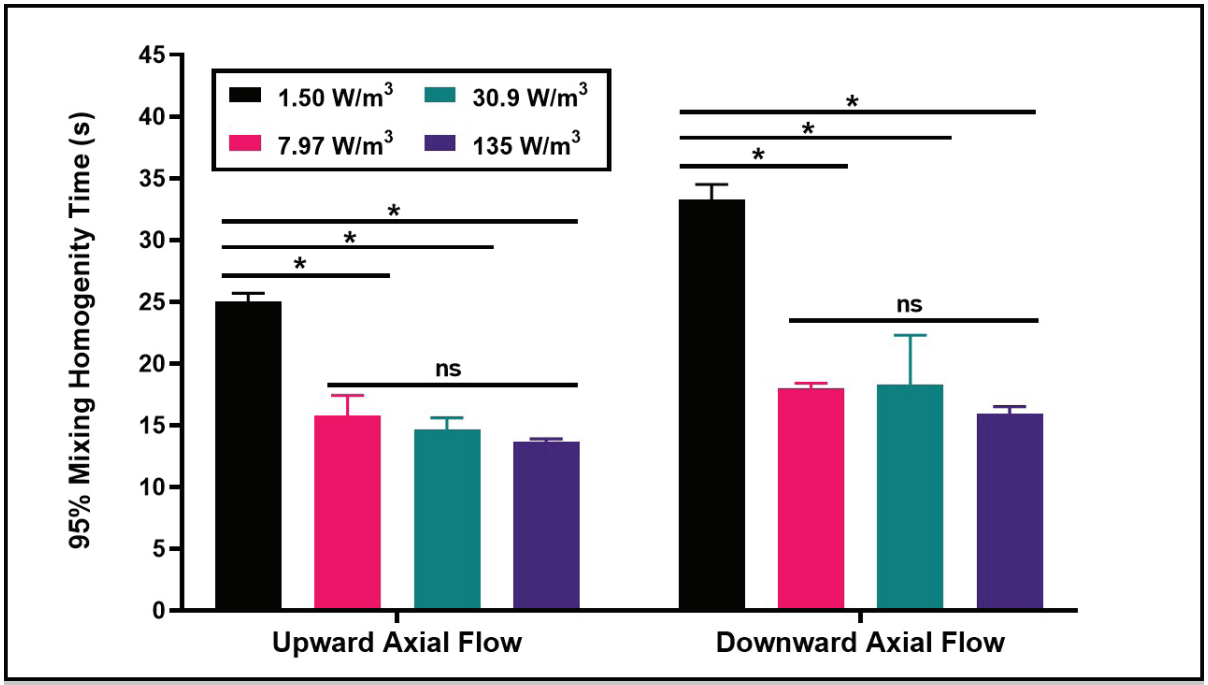
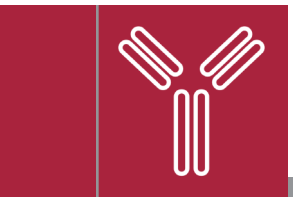


Figure 6: Results from Tukey Multiple Comparison test demonstrate significantly slower mixing times for 1.50 W/m³ conditions for both upward and downward axial flow processes. No significant differences observed across other higher power-input testing conditions. Data are mean values from n=2 replicates. Bars represent standard error. $\alpha = 0.05$

Conclusions

Thorough understanding of mixing dynamics within a stirred tank reactor can facilitate successful upstream and downstream bioprocess development and characterization. As demonstrated through this work, the pH tracer method can be a useful means of evaluating the effectiveness of mixing parameter definitions within a bioreactor system. Through this study, we demonstrated that both power input and axial flow direction have the potential to effect mixing dynamics and system homogeneity, particularly across low power-input processes.

Using similar methods, process scientists, engineers, and researchers may be able to better understand the mixing dynamics within their own STR bioprocessing system. Such knowledge may allow teams to more appropriately engineer definitions for volume-dependent mixing parameters, such as minimum mixing times and volumetric power inputs. Such improvements may help support optimal culture growth, greater processes yields, and higher overall product quality.



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