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White Paper

A Comparative Analysis of Mixing Characterization Methods in Stirred Tanks

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Summary

A homogenous environment and a rapid response to changing conditions are key requirements for a bioreactor. While, there are many effective methods of measuring mixing performance, there is no single, standard method in the bioprocessing industry. It is important to choose a method that will provide the highest quality data and enable effective cross-system comparison of results. To support the development of the Mobius® iFlex Bioreactor portfolio, four commonly used methods for measuring mixing time were tested:

- Conductivity sensor data
- pH sensor data
- Phenolphthalein (pH indicator) colorimetry
- Iodine decomposition colorimetry

All four methods were benchmarked by accuracy, repeatability, safety, and ease of use. In a 50 L vessel, at high power densities (100 W/m^3), all methods were within 2 seconds of reported mixing time from one another, however this difference increased to 8 seconds at low power operation (10 W/m^3). Colorimetric approaches reported lower mixing times at mid to high power, with phenolphthalein colorimetry reporting the lowest mixing time at all power densities.

Sensor lag showed little impact on measured mixing time, only having an effect outside of standard deviation in high power conductivity mixing studies. Larger liquid tracer volumes did have an impact on measured mixing times, resulting in longer reported mixing times for the conductivity and iodine-based methods. The effects of tracer volume were stronger for sensor-based methods, causing a similar relative change to measured mixing time at both 50 and 500 L process scales.

No method performed the best on all qualitative or quantitative results for mixing time. Therefore, the two best performing methods, quantitative pH sensor and qualitative phenolphthalein colorimetry, were combined to provide visual feedback and data-driven assessment of mixing time for bioreactors performance characterization.

Introduction

Fast, effective mixing is a key functionality of bioreactors, ensuring a homogenous environment for cell growth and allowing for rapid response to changing cell culture conditions. This study investigates the four most widely used methods for measuring mixing time, as reported by several major bioreactor manufacturers. The key criteria for comparing mixing methods include:

- Results: A side-by-side comparison of the qualitative vs. quantitative mixing data collected by each method.
- Accuracy: How data collected with one method compares to data collected with others.
- Repeatability: Data quality and reproducibility of each method, and how many times and how quickly each method can be repeated within the same solution.
- Ease of use: Scalability, logistical needs, and material hazard concerns of each method.

Methods

Sensor Lag Study

To understand the response time of pH and conductivity sensors, an initial sensor lag study was performed. Sensor time constants Tau (τ) were found by inducing a step change in operating conditions and recording the sensor readout. Tau is measured as the time between the step change and the readout reaching 63.2% of the final value[1].

Both pH and conductivity sensor methods utilized a Mettler Toledo M800 transmitter and ABB RVG200 data logger to collect data at 1 second intervals. Conductivity sensors were Mettler Toledo InPro 7100i, and pH sensors were Mettler Toledo 405-DPAS-SC.

Four pH and four conductivity sensors were tested in both increasing and decreasing changes by quickly swapping the sensors between 50 mL tubes containing pH and conductivity standards, respectively: pH standards of 4.01 and 7.00 were used for pH sensors, and conductivity standards of 1.41 mS/cm and 12.8 mS/cm were used for conductivity sensors. All standard solutions were at room temperature. A total of 24 τ values were measured and averaged for each sensor type to obtain a final value.

Mixing time data for the two sensor based methods was then corrected using Equation 1, where: τ is the sensor time constant, X_0 the reading at the start of the experiment, t the time since the start of the experiment, X the reading at time t, and X_{LC} is the corrected reading at time t_1.

$$X_{LC} = \frac{(X - X_0)}{1 - e^{-\frac{t}{\tau}}} + X_0 \qquad \text{Eq.1}$$

Mixing characterization method benchmarking

Four methods of measuring mixing time in a bioreactor were compared using a clear 50 L acrylic vessel with a 1.6:1 liquid height to diameter ratio. The prototype used a bottom mounted, magnetically driven impeller (N_p = 3.6) and a flexible X-shaped baffle which was installed to mimic the single-use assembly design in the Mobius® iFlex Bioreactor portfolio. All four mixing methods were tested at room temperature at 50 L volume, and at three power densities (10, 50, 100 W/m^3). A brief overview of the four tested mixing methods is shown in **Table 1.**

Table 1. Overview of all four mixing methods (RO = reverse osmosis)

Type	Method	Method	Addition Volume
Sensor	Conductivity	Increase in RO water conductivity	1.5 mL/L of 4M NaCl
	pH	Increase and decrease in RO water pH	0.04 mL/L of 5M HCl or NaOH
Colori-metry	Phenolphthalein	Color change of 1ppm phenolphthalein solution from transparent pink to clear	0.04 mL/L of 5M NaOH (0.04 mL/L 5 M HCl to return to pink)
	Iodine	Color change of 80 ppm KI, 40 ppm I_2, and 50 ppm soluble starch in RO water from dark blue to colorless	4 mL/L of 0.1M $Na_2S_2O_3$

Sensor-Based Methods

Two pH and two conductivity sensors were used in each test, one located below the minimum fill line and the other approximately halfway up the tank. Mixing time was assessed at both points using a t95% system, considering the tank to be fully mixed when the change in sensor reading stabilized within 5% of the final measured change in value. Mixing time was calculated for both sensors, then averaged per run, with four runs completed for each method at each power density. Mixing times were corrected using τ values. Mixing times were also measured in a 500 L vessel of geometrically similar design to the 50 L acrylic tank to assess scalability of these methods.

In conductivity mixing studies, the salinity of reverse osmosis (RO) water is increased via addition of a concentrated salt (NaCl) solution. In this method, 1.5 mL of a 4 M NaCl solution was added per liter of total tank volume to induce a salinity change of approximately 300 µS/cm.

In pH mixing studies, the pH of RO water is raised and lowered repeatedly using concentrated acid (HCl) and base (NaOH) solutions. In this method, the starting solution is adjusted to an initial pH of 4.0, after which alternate additions of 0.04mL of 5 M NaOH and HCl are added per liter of total tank volume to alternate the pH between approximately 4.0 and 8.5.

Colorimetric Methods

The colorimetric methods were performed in the same clear acrylic test tank with a solid white background and strong lighting for maximum color visibility. Mixing time was measured as the time required to change from a colored solution to a colorless solution, with video recording taken as evidence. Each method was run in triplicate. Mixing time values were averaged for each power density at which they were collected to report the final mixing time.

In phenolphthalein mixing studies, the pH of the mixing solution was raised and lowered with concentrated acid and base to change the color of a phenolphthalein pH indicator. In this method, the tank is filled with a

solution of 1 ppm phenolphthalein before alternate additions of 0.04 mL of 5 M HCl and NaOH respectively are added per liter of total tank volume to induce the color change; this study was executed in the same solution of the pH mixing studies. The color transitions between a transparent pink at high pH and clear at low pH, with mixing time only recorded in the pink-to-clear direction.

Iodine mixing studies use the decomposition of elemental iodine into sodium iodide to change the color of the solution from dark blue to colorless. In this method, the tank is filled with a solution of 80 ppm KI, 40 ppm I_2, and 50 ppm soluble starch in RO water, before 4 mL of a 0.1 M sodium thiosulfate solution is added per liter of tank volume to decompose the iodine. The color changes from dark blue to clear[1].

Effects of Liquid Addition Volume

Since it was found that the volume of liquid tracer added during a mixing study can have a strong impact on mixing time in colorimetric methods, a modified pH method was performed at 50 L and 500 L scale, to determine the effect of the addition volume on mixing time[2]. In these studies, the liquid additions were diluted from the original methods, to match the larger volume additions of the liquid tracer added during the conductivity method; this resulted in liquid addition for the pH method increasing from 0.04 mL/L to 1.5 mL/L. Mixing time was calculated for both sensors (top and mid-tank), then averaged per run, with four runs completed at each power density (10, 50, 100 W/m^3), at full working volume. Mixing times were corrected using τ values.

To determine the effect of liquid addition volume on colorimetric studies, a modified phenolphthalein study was tested at 50 L scale, adding 4 mL/L of tracer to match the volume addition of the iodine-based method. Due to concerns about the volume of iodine waste that would be created performing the iodine test at 500 L, the volume effects on the phenolphthalein colorimetric method were only tested at the 50 L volume.

Results

Sensor Lag Effects

Understanding the impact of sensor lag for each of the sensors was a key first step in assessing mixing method performance. In total, four pH and four conductivity sensors were tested, with 3 trials each in increasing and decreasing conductivity or pH conditions, for a total N of 6. All sensors tested had sensor time constants under 10 seconds, with a standard deviation less than 15%. The average measured time constants and standard deviations for both sensor types are shown in **Table 2**. Conductivity sensors showed a slightly higher average time constant, with greater variability than the pH sensors.

Table 2. Sensor time constant data

Sensor Type	Avg. Time Constant and Standard Deviation (τ, sec)	Max. Time Change (sec)
Conductivity	8.9 ±1.3	0.5 ±0.1
pH	6.8 ±0.6	N/A

For both sensor types, sensor lag had minimal impact on the reported mixing time. The largest impact was observed at high power, where the corrected mixing time was lower by approximately half a second for the conductivity sensor. As seen in **Figure 1**, the effect of sensor lag was negligible at all other points. There was no difference between the raw and lag-corrected pH mixing time data.

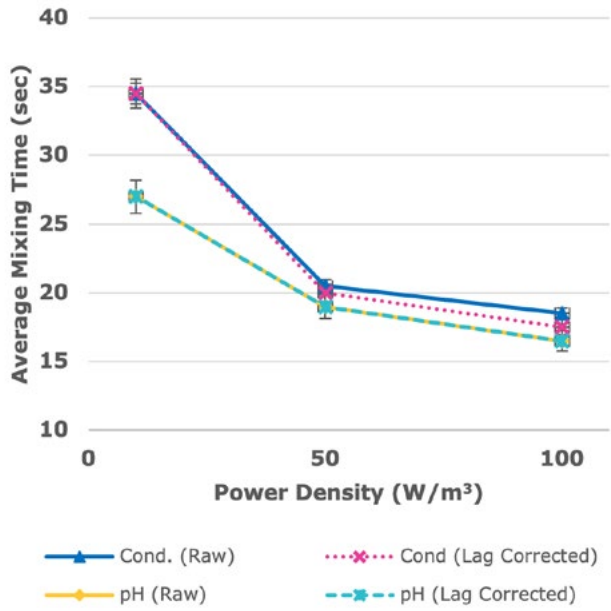

Figure 1. Average mixing time vs. power density for raw and lag-corrected sensor data. Error bars represent standard deviation of N=4 runs.

Mixing Method Comparison

A direct comparison of the four mixing methods provided insight into how to compare and interpret reported results. As shown in **Figure 2**, all methods showed similar performance at high power mixing. All performance curves aligned, with less than 2 seconds difference between methods. However, in low power mixing, the results differed significantly.

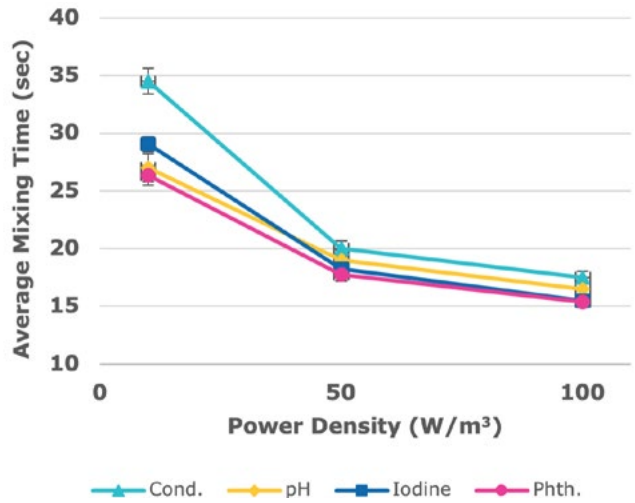

Figure 2. Average mixing time vs. power density for all mixing methods. Error bars represent standard deviation of N=3 runs for the colorimetric methods, and N=4 runs for the sensor-based methods.

The lowest overall average mixing times measured, shown in **Table 3**, were reported by the colorimetric methods. The phenolphthalein method reported the fastest mixing times across all power densities, with the iodine method reporting equivalently low mixing times at high power. The conductivity method reported slowest mixing time at all power densities, especially at low power mixing.

Table 3. Mixing performance overview

Type	Method	Average Mixing Time and Standard Deviation (sec)	
		100 W/m³	10 W/m³
Sensor	Conductivity	17.5 ±0.5	34.5 ±1.5
	pH	16.5 ±1.1	27.0 ±1.2
Colorimetry	Phenolphthalein	15.4 ±0.1	26.4 ±1.7
	Iodine	15.5 ±0.1	29.1 ±0.7

All methods gave effective results, with highly comparable reported mixing times at power densities of 50 and 100 W/m³. However, some procedural issues did arise during testing. For example, the iodine testing method as written resulted in a brown initial solution instead of blue, but still transitioned to a clear solution after the addition of the sodium thiosulfate tracer[1]. In addition, improper tank cleaning between trials impacted the results of the pH sensor and both colorimetric methods, slowing or even preventing appropriate changes in pH or color. While none of these issues had a major impact on data quality when properly accounted for, they did have an impact on the repeatability of each method.

Repeatability

An ideal mixing characterization method should allow the ability to determine mixing times several times in the same solution, without the need to replacing the whole volume, especially when large tanks are tested. Literature research and active use of all four mixing methods provided insight into how many times each test could be performed concurrently.

The conductivity method can be repeated up to 20 times, at which point the additional volume of the added salt solution changes the total volume of solution enough to see a noticeable impact on mixing time.[3] The pH method can be repeated up to 16 times, at which point the tank can begin to show buffering effects, slowing the acid/base reaction, and affecting the measured mixing time.

As for the colorimetric methods, the pink-to-clear method can be repeated approximately 8 times, at which point the solution begins to show buffering effects, and the iodine method can only be performed one time per preparation due to the decomposition reaction involved, requiring the full volume of solution to be replaced between trials[3]. Due to the toxicity of the iodine solution and iodine salts, the tank also needs to be fully drained into secure waste disposal and cleaned between iodine trials.

Effects of Liquid Addition Volume

Analyzing dilution effects confirmed that the volume of the liquid added during the study plays a key role in measured mixing time regardless of the method or process scale. As shown in **Figure 3**, both the pH and phenolphthalein methods reported similar mixing times to the conductivity and iodine methods respectively when diluted to the same volume of tracer addition. The sensor and colorimetry data shows that reported mixing time, particularly at lower power, is impacted by the volume of the liquid addition used. This result was confirmed by the qualitative colorimetry data, where visual mixing progress for the dilute phenolphthalein test was slower with the diluted tracer.

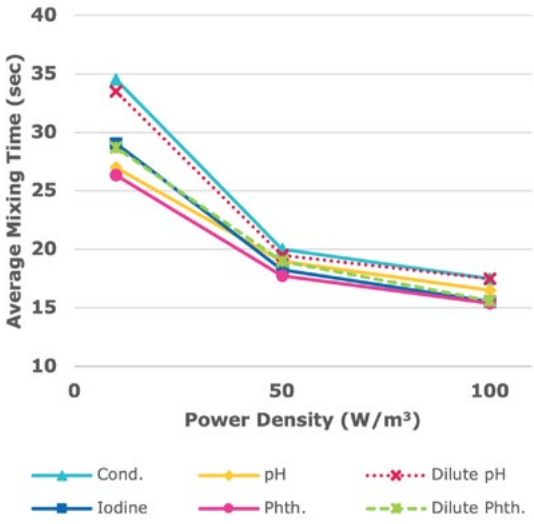

Figure 3. Average mixing time vs. power density at 50 L scale for standard and diluted mixing methods. Error bars represent standard deviation of N=3 runs for the colorimetric methods, and N=4 runs for the sensor-based methods.

The effect of increasing the volume of the tracer solution impacted the sensor-based metrics more strongly than the colorimetric methods. While increasing the volume of the tracer solution resulted in longer reported mixing times for both sensor and colorimetric methods particularly at low power, the diluted pH sensor test reported an even slower mixing time than the diluted phenolphthalein test despite the diluted phenolphthalein test using a larger volume tracer solution comparatively.

The test data at 500 L, shown in **Figure 4**, also confirmed two hypotheses gathered from the 50 L results. First, the 500 L test confirmed the relative performance difference between pH and conductivity methods observed in the 50 L system. Second, this larger scale testing confirmed that addition volume has an equivalent impact on reported mixing time, independent of scale.

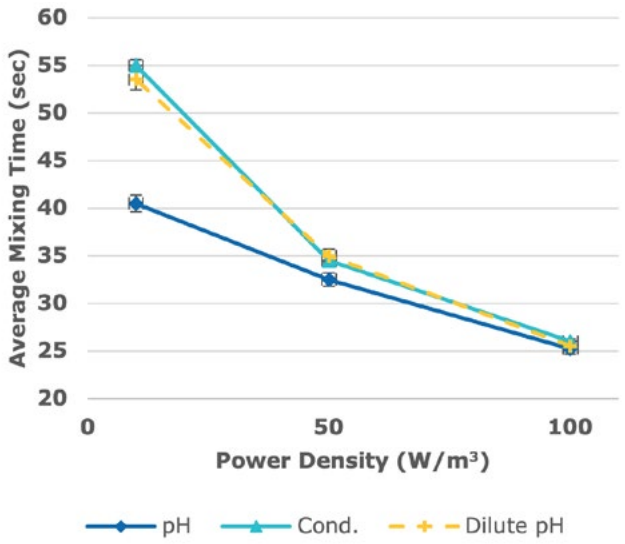

Figure 4. Average mixing time vs. power density at 500 L scale for standard and diluted mixing methods. Error bars represent standard deviation of N=4 runs.

Conclusion

All four mixing characterization methods were successfully benchmarked for accuracy, repeatability, safety and ease of use and the advantages and disadvantages of each method were identified throughout this study. For sensor-based methods (pH and conductivity), there were concerns that the sensor response time could potentially impact the viability of the method; however, it was demonstrated that the sensor lag effect was negligible for the sensors used in this study, with lag corrected mixing time deviating by less than one second from the raw mixing time across all powers and scales tested.

While no one method performed the best in all evaluated metrics, some methods had more advantages than others. For example, the iodine method did provide the second lowest reported mixing time among the methods tested. However, the material handling concerns of iodine waste limited the scales and frequency at which this method could be used. Additionally, while the conductivity method was the safest, it reported the longest mixing times, due to the large volume of tracer required to obtain enough increase in conductivity to accurately measure a t95% change. Tracer volume for the conductivity method is limited by the saturation concentration of NaCl, where no alternative salts are more soluble to lower the addition volume to match the pH method. The results of the dilution studies suggest that lowering the volume of liquid additions by using more concentrated solutions will lead to generally faster mixing performance during bioreactor operation.

Table 4. Comparison of results obtained for all four mixing methods. Cyan boxes show the best results, yellow shows acceptable results, and pink shows sub-optimal results. All methods demonstrated comparable accuracy.

Type	Method	Max. Repeats	Impact of tracer volume	Material Safety
Sensor	Conductivity	20x	Largest impact	Salt (NaCl)
	pH	16x	Smallest Addition	Concentrated Acid/Base
Colori-metry	Phenolphthalein	8x	Smallest addition	Concentrated Acid/Base
	Iodine	1x	Some impact	I2 Iodide Salts

The phenolphthalein colorimetry and pH sensor methods both provided low and consistent mixing times, performing the best in their respective categories. Phenolphthalein provided the strongest color clarity and visual feedback regarding mixing homogeneity, but there were concerns about the color change occurring before the acid and base reaction was fully mixed or completed. The pH method provided clarity as to exactly when the reaction had completed, but only at select points within the tank. Because these two methods share the same underlying chemistry, an acid-base reaction, it is possible to combine them into one tandem method to optimize the effectiveness of the mixing study results.

The combined pH/phenolphthalein approach utilizes sensor data to provide thorough, quantitative mixing data, while confirming the qualitative efficacy of mixing by observing the patterns visible from the pH indicator color shift. Sensor data can be collected in both increasing and decreasing pH conditions, while colorimetry data is collected in the increasing direction only, from pink to clear, to yield the most effective mixing pattern observations. This combined approach is used for measuring the mixing time of Mobius® iFlex Bioreactors due to their accuracy and safety.

References

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3. Bauer, I., et al., 2020. Recommendations for process engineering characterisation of single-use bioreactors and mixing systems by using experimental methods. 2nd ed. Frankfurt: DECHEMA Biotechnologie Expert Group Single-Use Technology, pp.33-45.

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