Mixing Operations for 50 L to 2000 L Single-Use Mixer: Liquid-Liquid Mixing Characterization and Slurry Suspension

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ABSTRACT
Mixers are employed throughout all parts of bioprocessing. Single-use mixers (S.U.M.) are commonly used for both upstream and downstream operations. Upstream operations can include media formulation and hydration, media holding, and startup filtration. Downstream operations can include product storage, viral inactivation, buffer preparation, and slurry preparation. S.U.M. offers various advantages when compared to fixed mixing parameters and control are key elements for successful mixing operations particularly in the transition from non-single-use platform to single-use platforms. This study presents the automation of simple mixing procedures using the Thermo Scientific™ HyPerforma™ S.U.M. with Touchscreen Console. Also reported is the selection method for scalable mixing parameters allowing for several operation types across all single-use mixer sizes.

The following applications are demonstrated in this study. Characterization of scalable mixing regime parameters by comparing input power per volume and T95 blend time criteria. Slurry suspension in preparation for chromatography column packing using the 100 L S.U.M. Minimum speed for complete slurry suspension is identified as well as quantification of resin damage post mix. This work demonstrates best practices for mixing in bioprocessing unit operations including use of the Touchscreen Console.

INTRODUCTION
Mixing is a unit operation heavily used throughout all steps in bioprocessing. Establishing a convenient mixing gradient is the primary function of the HyPerforma S.U.M. Optimal operating parameters are critical for success in scaling and are best practice for mixing. Often when mixers are considered RPM is the only parameter considered when operating and scaling mixing to various sizes.

Gradients can be measured using various sensing methods including RTDs, pH and conductivity meters, metabolite analyzers, etc. Gradients are dissipated by inputting power from a motor into the liquid via an impeller. The amount of power input to the liquid by a motor is characterized by the Power Input per Volume (PIV) equation:

\[ PIV = \frac{P}{V} \]

Where \( P \) is impeller type or power number, \( N \) is fluid density, \( V \) is speed of the impeller, \( d \) is impeller size, and \( V \) is the volume of the mixer. The power number \( N \) is a constant unique to the impeller and can be considered similar to a drag coefficient. We assume a power number for all impellers investigated to be 2.1. The PIV equation is an extremely valuable test particularly when scaling vessel size and volume. Scaling by PIV provides normalized mixing performance regardless reactor characteristics.

Thermo Scientific hyperforma S.U.M. with Touchscreen Console offers the following functionality within the bioproduction workflow including:

- Automated BioProcess Container air fill
- Aeration control
- Sterile media inlet tank
- Sterile media inlet filtration with sterile filter monitoring
- Automated base fill
- Automated harvest
- pH monitoring
- Conductivity monitoring
- Saline filtration control using bauxa addition
- Control parameter monitoring
- Pump control: up to 4 pumps
- Global bauxa addition feature inhibit by pH or conductivity process values
- Pneumatic pinch valves for fill and harvest lines
- Temperature monitoring and control
- Mass or volume monitoring
- Data historian to monitor all modules and output through USB or Profibus connection
- User adjustable alarms and interlocks
- Additional analog auxiliary ports for additional transmitters

Applications of the Hyperforma Mixer with Touchscreen panel include:

- Media buffer hydration
- Sterile media holding tank
- Automated pH and conductivity shifts
- Automated fill and harvest
- Vial inactivation
- Slurry suspension
- Product pooling
- Waste containment
- Bulk storage and filling
- Robust data collection via historian

Rein preparation for column packing is an audacious time consuming task typically performed in a stainless vessel. Often just cleaning stainless vessels consumes the majority of the operators day. Substituting the stainless mixer with Thermo-Fisher Hyperforma S.U.M allows end users to efficiently mix and store resin at the desired concentration while eliminating costly clean up time. This presentation demonstrates first scalability of the S.U.M. and second the ability of the Hyperforma S.U.M. to sufficiently mix resin at multiple concentrations and volumes in a 100 L S.U.M.

MATERIALS AND METHODS
S.U.M. Mixing Characterization
To test mixer capabilities liquid-liquid mixing was performed on the 50, 100, 150 L, 1000 L, and 2000 L S.U.M. at each full volume, half volume and 5:1 volume. Gradients were formed by manually adding a bolus of concentrated NaCl and measured using conductivity sensors placed at the top, middle, and bottom of each vessel. PIV of each vessel was evaluated at max speed, 100 W/m \(^3\) and 20 W/m \(^3\). Maximum operating speeds for all vessel sizes is 350 RPM.

S.U.M. Slurry Mixing
Testing was performed on the 100 L S.U.M. to identify minimum power required to keep slurry in suspension in 30%, 50% and 70% concentration. Evaluation of slurry mixing at 20 L, 50 L and 100 L volumes at each respective concentration was also considered. Evaluation consisted of operating the mixer at 15, 20, 30, 40, and 50 W/m \(^3\) power input at each volume and concentration.

Mixing evaluation consisted sampling at the top, middle, and bottom of the vessel and measuring for conformity. After the mixing regime had been established 50 mL samples were taken and centrifuged for 10 minutes at 10000 rpm. Upon centrifugation, samples were observed for turbidity of fine particles and damage to the slurry was measured by microscopy and centrifugation, samples were observed for turbidity of fine particles and damage to the slurry was measured by microscopy.

Results demonstrate effectiveness of the S.U.M. in scaling based on power input across a wide range of volumes and mixer sizes.

CONCLUSIONS
Hyperforma mixers are designed to eliminate gradients in vessels. Correctly operating mixers can maximize mixing performance increasing overall operator efficiency in scaling. Results demonstrate effectiveness of the S.U.M. in scaling and volume and using standard equipment and operating procedures. Operation in the 20 to 30 W/m \(^3\) range provides sufficient mixing without damaging resin.

REFERENCES

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