Mixing efficiencies for the 2,000 L imPULSE Single-Use Mixer

Introduction

The 2,000 L Thermo Scientific™ imPULSE™ Single-Use Mixer (S.U.M.) provides innovative mixing for upstream and downstream processes. The unique design of the imPULSE S.U.M. produces more effective mixing at lower working volumes than other mixers currently on the market, and produces one-way flow mixing. The addition of the Touchscreen Console to the imPULSE S.U.M. adds real-time sensor visualization, data logging, and the ability to automate user-defined mixing recipes. To demonstrate the superior mixing capabilities for various applications and to determine optimal mixing parameters, four different mixing studies (Table 1) were performed with the 2,000 L imPULSE S.U.M. at various working volumes and mixing settings. The materials used in these studies were chosen to demonstrate the way buffers (NaCl solution), complex media (Gibco™ AGT™ medium), and viscous liquids (corn syrup) are mixed in the 2,000 L imPULSE S.U.M.

Materials and methods

A standard 2,000 L imPULSE S.U.M. was used with a standard 2,000 L Thermo Scientific™ imPULSE™ S.U.M. BioProcess Container (BPC) modified with sampling and probe ports at the bottom, middle, and top of the BPC.

The BPC was also equipped with a spray nozzle at the top, to which a pump was connected, creating a liquid recirculation loop. This pump was set to circulate water from the bottom of the BPC through the spray nozzle at a rate of 10 L/min during most of the mixing studies.

To test the wide range of applications of the 2,000 L imPULSE S.U.M., mixing studies were performed at various working volumes. The manufacturer-recommended maximum setting—the point where maximum agitation occurs without excessive splashing—at various working volumes was determined through visual inspection (Table 2).

Table 2. The manufacturer-recommended maximum setting determined for the working volumes of 10:1, 5:1, 2:1, and full volume.

Working volume	Manufacturer-recommended maximum setting (Hz)
10:1 (200 L)	0.66
5:1 (400 L)	0.9
2:1 (1,000 L)	2
Full volume (2,000 L)	2

Table 1. Mixing studies performed on the 2,000 L imPULSE S.U.M.

Mixing type	Mixing material	Concentration	Analytics
Liquid–liquid	NaCl solution	350 g/L NaCl in deionized (DI) H_2O	Conductivity
Liquid–solid	NaCl granules	1 M (final)	Conductivity, osmolality
Liquid–solid	AGT medium (granules)	1X (final)	Conductivity, osmolality, pH, glucose concentration, visual
Viscous liquid–liquid	Corn syrup with NaCl solution	80–88% corn syrup	Conductivity

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A drawing showing the dimensions of the 2,000 L imPULSE S.U.M. is shown in Figure 1.

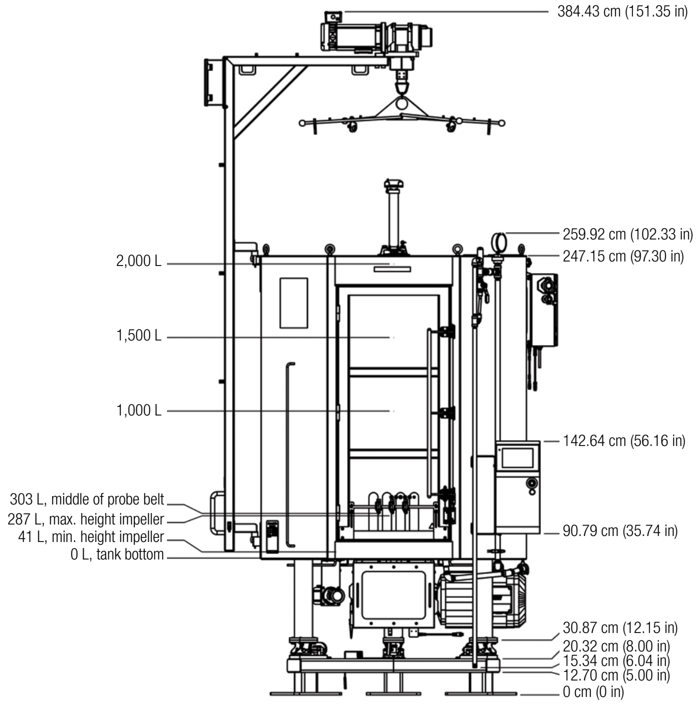

Figure 1. Drawing of the 2,000 L imPULSE S.U.M.

Liquid–liquid mixing: NaCl solution

Three conductivity probes were inserted into the BPC in the top, middle, and bottom ports. The imPULSE mixer was filled to four separate working volumes: 10:1, 5:1, 2:1, and full volume. At 10:1 and 5:1 working volumes, the mixing settings of 0.25, 0.5, and 0.75 Hz were tested in triplicate. 0.75 Hz is above the manufacturer-recommended maximum setting for the 10:1 volume; however, it was tested for comparative purposes. At 2:1 and full volume, the mixing settings of 0.25, 0.5, 0.75, 1, 1.5, and 2 Hz were tested in triplicate.

The predetermined agitation was set on the 2,000 L imPULSE S.U.M. and was allowed to mix for 2 minutes to allow mixing flow patterns to stabilize. A small volume of concentrated NaCl solution (350 g/L NaCl in DI H_2O) was pipetted into the mixer as close to the surface of the water as possible, over 3–4 seconds. Conductivity data were recorded for 5 minutes with a data logger. This process was repeated for each mixing setting at each volume in triplicate. An average T95 mixing time—when the measured value reaches 95% of the final stable value—was calculated as the average of the slowest-responding sensor among the test replicates for each test condition.

Liquid–solid mixing: NaCl granules

Three conductivity probes were inserted into the BPC in the bottom, middle, and top ports. The BPC was filled with DI water to 10:1, 5:1, and full volume. For each working volume, the manufacturer-recommended maximum setting was used (see Table 2).

The predetermined agitation was set on the 2,000 L imPULSE S.U.M. and was allowed to mix for 2 minutes. Enough NaCl was quickly added to the top of the mixer to make a 1 M NaCl solution. Samples were taken every minute for the first 20 minutes, every 5 minutes for the next 20 minutes, every 10 minutes for the next 20 minutes, and every minute for the last 10 minutes. Samples of 10 mL were taken from the bottom, middle, and top ports for full volume, and just the bottom sampling port for 10:1 and 5:1 working volumes. Conductivity was measured online, and osmolality was tested and recorded after samples were collected.

Liquid–solid mixing: AGT medium

Two pH probes were inserted into the BPC in the bottom and top sensor ports, and one conductivity probe inserted in the middle sensor port. A camera was attached to the top of the mixer to observe the time when foam and powder from the medium were completely in suspension. The BPC was filled with DI water to 90% of three working volumes: 10:1, 5:1, and full volume. At each working volume, the manufacturer-recommended maximum setting was used.

The mixer was turned on to the predetermined agitation, and the manufacturer-recommended amount of AGT medium was quickly added to the top of the mixer. Samples were taken over a 70-minute time span using the same method described in the previous section. Samples of 10 mL were taken from the bottom, middle, and top ports for full volume testing and just the bottom port for low working volumes. The pH and conductivity were measured online with the Touchscreen Console. The osmolality and glucose concentration of the samples were tested and recorded after samples were collected.

Viscous liquid–liquid mixing: corn syrup with NaCl solution

Conductivity probes were inserted into the top, middle, and bottom of the BPC. The BPC was filled initially with 2,000 L of 88% corn syrup/12% DI water (v/v). A solution of 350 g/L NaCl was made, and a 5 L mixture containing 12% of the salt solution and 88% corn syrup (v/v) was prepared. The recirculation pump was not present during this test. The agitation was set to 2 Hz, and the mixture of salt solution and corn syrup was quickly added. The viscosity of the 88% corn syrup solution in the imPULSE S.U.M. was measured with a viscometer. The change in conductivity was monitored for 15–30 minutes. This process was repeated in triplicate at 84% corn syrup and 80% corn syrup in the same BPC by draining a predetermined volume of the corn syrup solution and filling it back up to full volume with DI water until the final desired corn syrup percentage was achieved. The T95 mixing times were calculated using the method described in the prior liquid–liquid mixing section.

Results

Liquid–liquid mixing: NaCl solution

For liquid–liquid mixing of a salt solution, T95 mixing times were less than 4 minutes regardless of the working volume or agitation rate, as shown in Figure 2. At 2:1 and full volume, T95 mixing times decreased with increasing agitation. However, at the low working volumes of 5:1 and 10:1, already rapid mixing times were not always decreased with increased agitation.

From the data collected in this liquid–liquid study, it was determined that at lower working volumes the most aggressive agitation setting does not always correlate to the fastest T95 mixing time. Table 3 shows the optimal mixing setting to produce the fastest liquid–liquid mixing times.

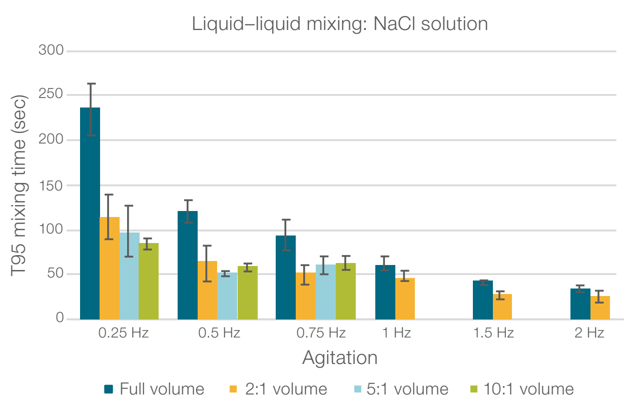

Figure 2. The average T95 mixing times (± 1 standard deviation) calculated for four working volumes at varying mixing speeds.

Table 3. The originally determined maximum agitation at all volumes tested and the optimal agitation to produce the fastest liquid–liquid mixing times.

Working volume	Manufacturer-recommended maximum setting (Hz)	Optimal liquid–liquid agitation (Hz)
10:1	0.66	0.5
5:1	0.9	0.5
2:1	2	2
Full volume	2	2

Liquid–solid mixing: NaCl granules

For liquid–solid mixing using salt granules, the conductivity was measured for three working volumes, as shown in Figure 3. The T95 mixing times by conductivity were 2 minutes at full volume and 5:1 volume, and 8 minutes at 10:1 volume. This mixing trend is different from the liquid–liquid mixing results observed previously, where T95 mixing times decrease with lower working volumes. It was observed during this mixing study that at the 10:1 working volume a portion of the salt granules temporarily sat at the bottom of the mixer; this resulted in a longer T95 mixing time. Similar results have been observed in the 50 L, 200 L, and 2,000 L Thermo Scientific™ HyPerforma™ S.U.M.s at low working volumes. Figure 4 shows the osmolality recorded for the tested working volumes. The T95 mixing time was reached in 2 minutes at full volume, 3 minutes at 5:1 volume, and 9 minutes at 10:1 working volume.

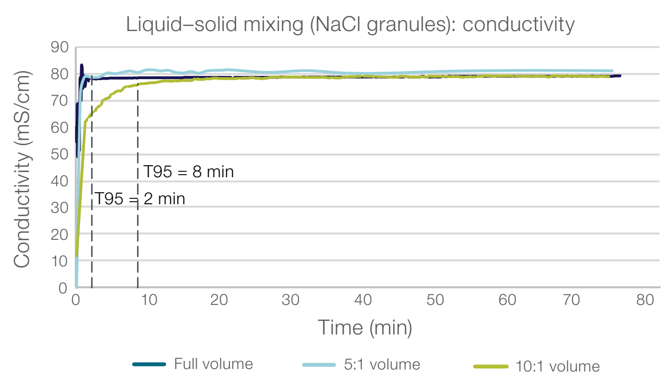

Figure 3. Conductivity data recorded at full volume, 5:1, and 10:1.

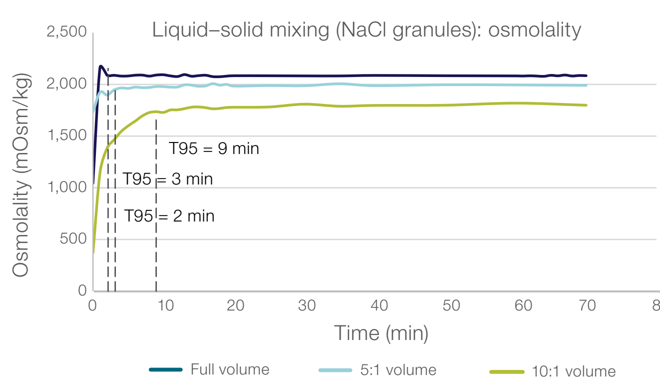

Figure 4. Osmolality recorded at full volume, 5:1, and 10:1.

Liquid–solid mixing: AGT medium

For liquid–solid mixing of representative AGT medium granules, the glucose and osmolality were measured offline after samples were collected. The results of the glucose concentration and osmolality are shown in Figure 5. At full volume, the glucose levels and osmolality had a T95 mixing time of 30 minutes. At 5:1 and 10:1 working volumes, the glucose levels and osmolalities had T95 mixing times of 1–3 minutes.

The conductivity T95 mixing times were 28 minutes at full volume, 2 minutes at 5:1 working volume, and 3 minutes at 10:1 working volume (Figure 6). At low working volumes there was a distinct upward shift in pH over time (Figure 7). It is likely that this shift is a result of CO_2 degassing due to the low working volume and rapid mixing. This shift is not ideal, because cell metabolism can be altered by changes in pH [1]. The pH shift can be corrected by the addition of acid or base. However, studies have shown that base or acid addition can be correlated with process variability and osmolality increase [2]. Generally, it is considered a best practice to avoid the addition of base or acid to cell culture media. During this study, the purpose was to show how rapidly a medium could be incorporated, and ideal conditions for cell culture medium preparation were not followed. More ideal conditions for mixing cell culture media are discussed in the conclusions and recommendations section.

The powder in this liquid–solid mixing study floated on top of the fluid, and the settling that was observed in the liquid–solid mixing with NaCl granules did not occur. Therefore, the lower working volumes in this liquid–solid mixing study had faster mixing times than full volume and those observed in the NaCl liquid–solid mixing study.

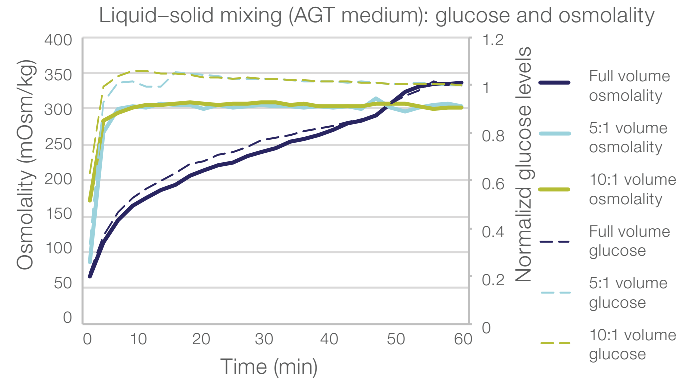

Figure 5. Glucose and osmolality recorded at full volume, 5:1, and 10:1.

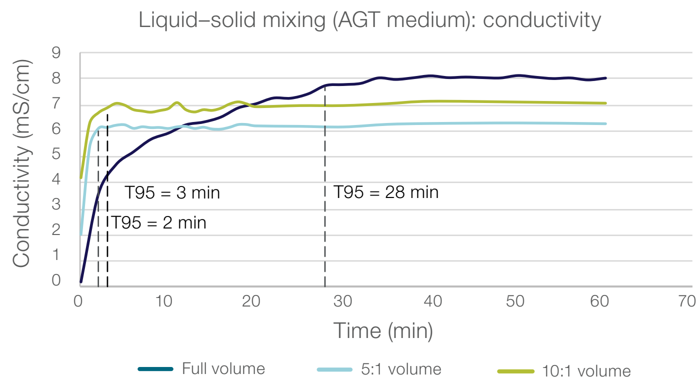

Figure 6. Conductivity data of the AGT medium recorded at full volume, 5:1, and 10:1.

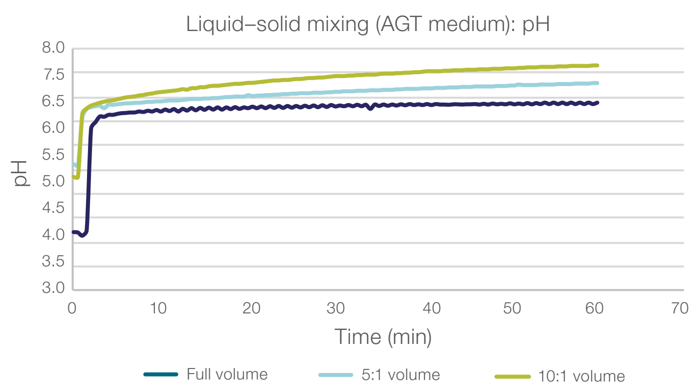

Figure 7. pH of the AGT medium recorded at full volume, 5:1, and 10:1.

Figure 8 shows photos taken at various times during the mixing study at full volume. After 46 minutes of mixing, it was observed that the powder was completely in suspension and foaming was diminished. This corresponds with the data in Figures 5 and 6 that show osmolality, conductivity, and glucose stabilizing in approximately the same time frame.

Figure 9 shows photos taken at various times during the mixing study at 5:1 working volume. After 2.5 minutes of mixing, it was observed that the powder was completely in suspension and foaming was diminished. This also corresponds with the data in Figures 5 and 6.

Figure 10 shows photos taken at various times during the mixing study at 10:1 volume. After 3 minutes of mixing, the powder was completely in suspension and foaming was diminished. This also corresponds with the data in Figures 5 and 6 for the 10:1 volume.

Viscous liquid–liquid mixing: corn syrup with NaCl solution

The measured viscosity of the corn syrup mixtures containing 80–88% corn syrup by approximate volume ranged from 336 cP to 881 cP, which is within the range of medium- to high-viscosity fluids seen in some applications. The conductivity data recorded are shown in Figure 11, with higher mixing times as viscosity increased. The average T95 mixing times were determined to be 4 minutes for 80% corn syrup (345 cP), 8 minutes for 84% corn syrup (521 cP), and 28 minutes for 88% corn syrup (861 cP). After the first two tests of the 88% corn syrup testing, significant degradation of the mixing flaps occurred, resulting in less than ideal mixing for the 88%, 84%, and 80% corn syrup.

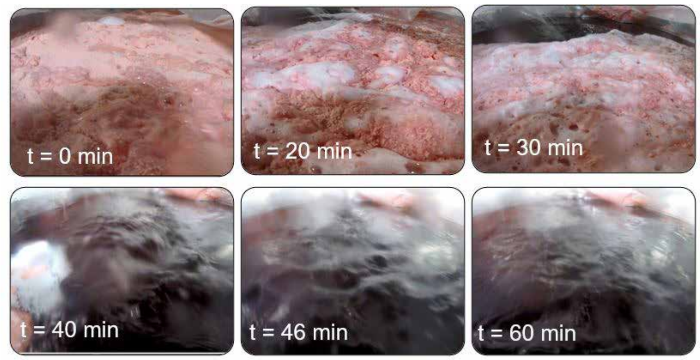

Figure 8. Photos of the fluid at the top of the mixer at various time points during the full-volume AGT medium mixing study.

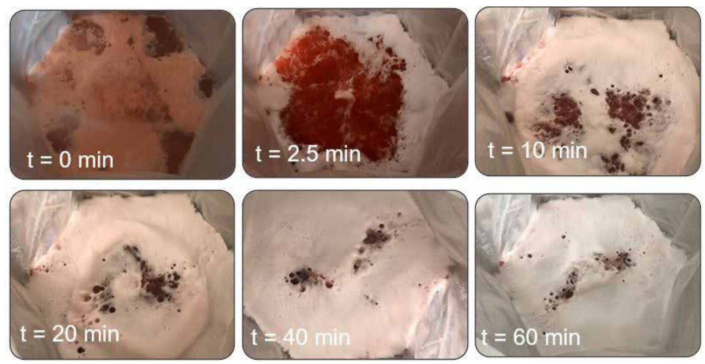

Figure 9. Photos of the fluid at the top of the mixer at various time points during the 5:1 volume AGT medium mixing study.

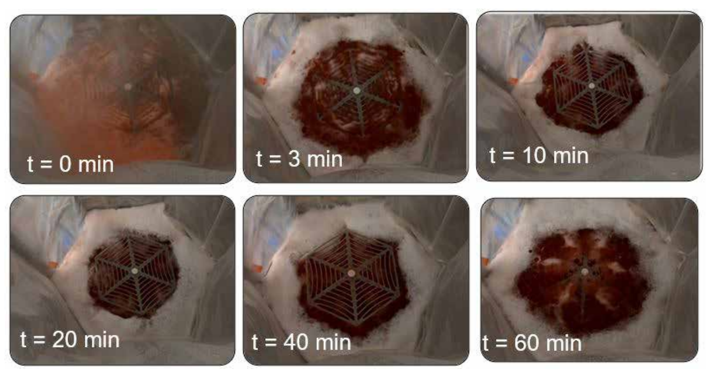

Figure 10. Photos of the fluid at the top of the mixer at various time points during the 10:1 volume AGT medium mixing study.

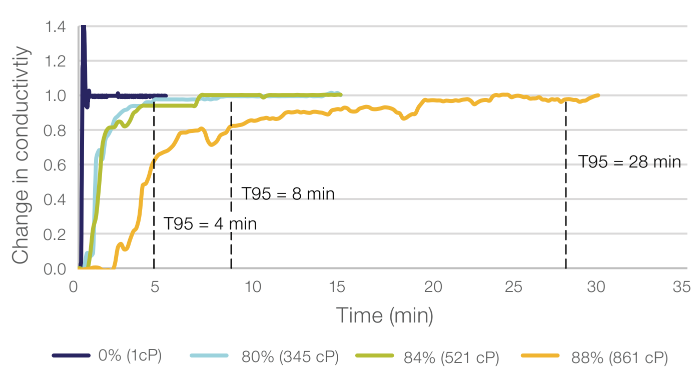

Figure 11. Conductivity data recorded during viscous liquid–liquid mixing with solutions of 0–88% corn syrup.

Conclusions and recommendations

It was found that the 2,000 L imPULSE S.U.M. exhibits robust and efficient mixing for volumes ranging from 10:1 to full volume at a wide range of mixing settings and with various mixing materials. It is capable of liquid–liquid mixing at any working volume in under 4 minutes, mixing floating solids at full volume in under an hour, and mixing highly viscous liquids in under 30 minutes. In general, at lower working volumes faster mixing was observed. However, mixing sinking solids was found to produce longer mixing times at lower working volumes due to settling. Table 4 shows when the solution reached a T95 mixing time based on the analytic used.

Table 4. Average times for mixing of various materials in the 2,000 L imPULSE S.U.M.

Mixing study	Mixing material	Working volume	Agitation (Hz)	Analytic	T95 mixing time (min)
Liquid–liquid	NaCl solution	10:1	0.5	Conductivity	1.0
		5:1	0.5	Conductivity	0.9
		2:1	2.0	Conductivity	0.4
		Full volume	2.0	Conductivity	0.6
Liquid–solid	NaCl granules	10:1	0.66	Conductivity	8.0
				Osmolality	9.0
		5:1	0.9	Conductivity	2.0
				Osmolality	3.0
		Full volume	2.0	Conductivity	2.0
				Osmolality	2.0
Liquid–solid	AGT medium	10:1	0.66	Conductivity	3.0
				Osmolality	3.0
				Glucose	1.0
				Visual inspection	3.0
		5:1	0.9	Conductivity	2.0
				Osmolality	2.0
				Glucose	2.0
				Visual inspection	2.5
		Full volume	2.0	Conductivity	28.0
				Osmolality	30.0
				Glucose	30.0
				Visual inspection	46
Viscous liquid	High-viscosity liquid (700–1,000 cP)	Full volume	2.0	Conductivity	28.0
	Medium-viscosity liquid (200–400 cP)	Full volume	2.0	Conductivity	8.0

For optimal mixing, we suggest filling the mixer, setting a desired agitation, and adding product incrementally. For liquid–solid mixing with floating solids, it is best practice that a recirculation loop is added to the imPULSE S.U.M. If a complex medium is being prepared at 5:1 working volume or lower, we recommend that the mixer not be set to the maximum setting possible at that working volume. Lowering the agitation rate, decreasing the overall processing time, and paying attention to pH drift will help ensure that the pH stays within range. Low working volumes with sinking solids can be problematic, and longer mixing times may be observed. For viscous liquids, the high-viscosity fluids can reduce the life of the agitator, and the conditions tested in this study should be considered worst-case. Material degradation of the mixing flaps was present throughout the viscous liquid–liquid mixing study. It is recommended when mixing highly viscous fluids (700–1,000 cP) to use lower agitation for shorter periods of time.

The 2,000 L imPULSE S.U.M. performs rapid mixing that can be used for a variety of applications with a wide variety of materials ranging from liquids, floating solids, sinking solids, and viscous fluids. The unique design of this mixer allows it to mix effectively at low working volumes. Due to the robust mixing, unique design, and addition of the Touchscreen Console, the 2,000 L imPULSE S.U.M. can meet the mixing requirements that are essential in today's competitive mixing industry.

References

1. Hagrot E (2011) Development of a culture system for modeling of pH effects in CHO cells. Student thesis. KTH School of Biotechnology, Department of Process Technology, Stockholm, Sweden.
2. Hoshan L, Jiang R, Moroney J et al. (2019) Effective bioreactor pH control using only sparging gases. *Biotechnol Prog* 35(1):e2743.