SARTORIUS

Application Note #19

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# Rapid and Scalable Feed Preparation With Powdered Soy Hydrolysates or Yeastolates and Single-Use Magnetic Mixing Systems

## Executive Summary

This application note presents a new approach to increase the speed and consistency of feed preparation steps in bio manufacturing. The method combines ready to use feed formulations from SAFC®, with scalable, high efficiency, single-use mixing systems provided by Sartorius Stedim Biotech.

Two examples of feed preparation steps, 50X BD Yeastolate and 50X SE50MAF-UF Soy Hydrolysate, are presented for different volumes. The contained transfer of powdered feed formulations into the high torque single-use mixing system enables a rapid dissolution and dispersion of the feed powders in liquid for volumes of 50 L and 200 L. The performances of the single-use mixing system are characterized with quantitative (conductivity measurement) and qualitative (visual inspection) techniques. The proposed method and system provide seamless scale-up and consistent rapid feed mixing for process development and GMP manufacturing.

## Introduction

This application study presents the performances of a fully single-use mixing solution for the large scale preparation of two different feeds. The prepared feed are concentrated 50 times and can therefore be used for large volume media preparation using the appropriate dilution. The mixing technology selected for this application is Flexel® for Magnetic Mixer[1] with volumes of 50 L and 200 L. The magnetic coupling of the impeller with the Magnetic Mixer Drive Unit enables a rotation speed up to 300 rpm, providing a powerful mixing of the feed.

The first feed tested in this study was the 50X BD Yeastolate. This feed is formulated specifically for media used with insect cell culture and cell lines that utilize L-Glutamine. It is generally used at a final formulation of 4 g/L.

The second feed tested in this study was 50X SE50MAF-UF Soy Hydrolysate. This feed is an animal-component free feed utilized in cell culture and fermentation applications as a nitrogen source when non-animal components are required. It is generally used at a final concentration of 5 g/L for cell lines which do not need any glutamine addition like CHO-GS.

## Purpose of the Application Study

The purpose of this application study is to assess the performances of the Flexel® for Magnetic Mixer technology to dissolve the two feeds. The mixing times are determined by conductivity and visual inspection of the solution in the Flexel® Bag for Magnetic Mixer.

## Materials and Methods

The list of materials and equipments used for this application is:

1. Standard Flexel® Bags for Magnetic Mixer (50 L: FMB114867, 200 L: FMB114893).
2. Powder Transfer Bag System (15 L: FMA114008, 30 L: FMA114009).
3. Palletank® for Lev Mixer | Magnetic Mixer (50 L: FXC110820, 200 L: FXC110821).
4. Magnetic Mixer Drive Unit, 230 V, EU power cord (ref. LT-DU-006-EU).
5. Powder Bag holder (ref. FXA114344).
6. SAFC® feed:
   - 50X SE50MAF-UF Soy Hydrolysate (Product number: 58903C/2019219)
   - 50X BD Yeastolate (Product number: 58902C/2025050)
7. Conductivity sensor: WTW InoLab Cond 740i.
8. Floor scale: Sartorius IF S4 1500RR-1.

## Method Used:

1. The Flexel® Bag for Magnetic Mixer is placed into the Palletank® with conductivity and temperature sensors.
2. The bag is filled with deionised water to 80% of the nominal volume (water temperature: 20 °C).
3. The mixing speed is turned on and set up to the maximum speed of 300 rpm to optimize powders dispersion.
4. Feed powders are added slowly through the top port to ease the powder incorporation into the water
   - 50X BD Yeastolate
   - 50X SE50MAF-UF Soy Hydrolysate

The powders are incorporated in the Flexel® Bag for Magnetic Mixer using either:

- SAFC® bucket liner
- or Sartorius Stedim Biotech Powder Transfer Bag for a contained transfer to the mixing bag assembly

The actual concentrations of feed during the mixing phase are:

- 50X BD Yeastolate : 214 g/L (density = 1.068 kg/L)
- 50X SE50MAF-UF Soy Hydrolysate : 257 g/L (density = 1.078 kg/L)

5. Two mixing times are monitored from the addition of feed powders:
   5.1 "mixing time 1" is determined from the conductivity signal as follows:
   The "mixing time 1" corresponds to the time when 95% of the final value is reached and when all next measurements stay within a 5% tolerance.

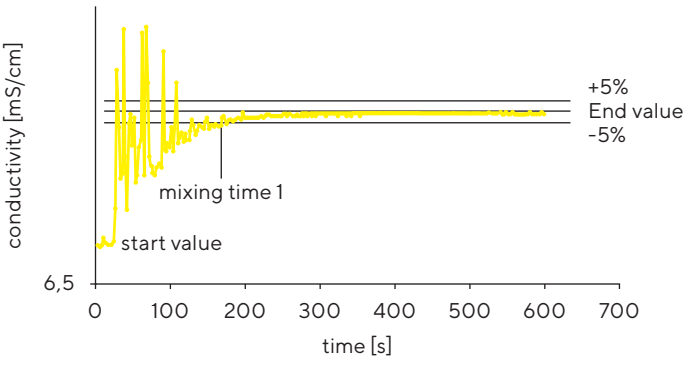


**Figure 1:** General principle of mixing time determination via conductivity

[1] This product uses Pall patended Magnetic Mixer technology. All information on patents can be found at Pall.com/patents.

5.2 “mixing time 2” is determined by a visual inspection. The “mixing time 2” corresponds to the time when all suspended particles are visually dissolved.


200 L Flexel® with Magnetic Mixer Technology


200 L Palletank® for Magnetic Mixer equipped with the Powder Transfer Bag System for the mixing trial

# Results and Discussions

**1. Mixing performances results**

50X BD Yeastolate and 50X SE50MAF-UF Soy Hydrolysate feed have been prepared in Flexel® Bag for Magnetic Mixer at 50 L and 200 L scales. Mixing time’s results are presented for each feed at the different scales.

**Mixing time results for 50X BD Yeastolate – 50 L**

For 50X BD Yeastolate preparation at 50 L, 10 kg of powder were added in less than 2 minutes in the mixing bag using SAFC® bucket liners. The feed powder was mixed in less than 8 minutes (visual check).

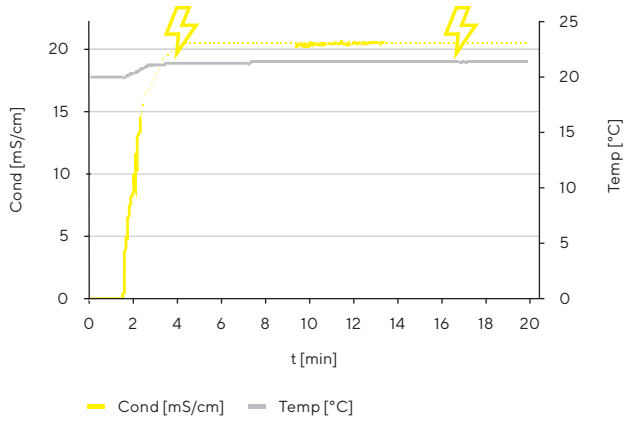


**Figure 2:** 50X BD Yeastolate preparation in 50 L Flexel® Bag for Magnetic Mixer

⚠ **Note:** conductivity values are not stable at 50 L due to the presence of air bubbles around the conductivity cell (strong vortex). A stable value of the conductivity could be observed only at lower impeller rotation speed.

### Mixing time results for 50X BD Yeastolate – 200 L

For the 50X BD Yeastolate preparation at 200 L, 40 kg of powder were added in less than 6 minutes into the mixing bag using two Powder Transfer Bags. The feed powder was mixed in 15 minutes according to conductivity and in 20 minutes according to visual check.

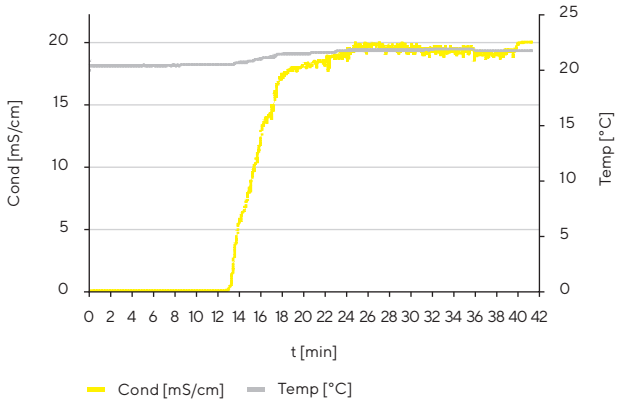


**Figure 3:** 50X BD Yeastolate preparation in 200 L Flexel® Bag for Magnetic Mixer

### Mixing time results for 50X SE50MAF-UF Soy Hydrolysate – 50 L

For the 50X SE50MAF-UF Soy Hydrolysate preparation at 50 L, 12.5 kg of powder were added in less than 5 minutes in the mixing bag using SAFC® bucket liners. The feed powder was mixed in less than 20 minutes (visual check).

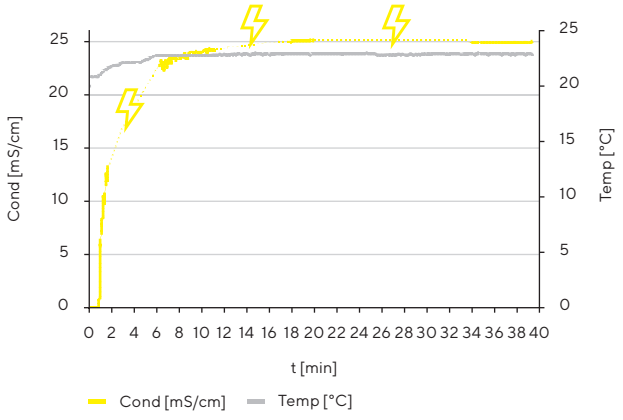


**Figure 4:** 50X SE50MAF-UF Soy Hydrolysate preparation in 50 L Flexel® Bag for Magnetic Mixer

⚠ **Note:** conductivity values are not stable at 50 L due to the presence of air bubbles around the conductivity cell (strong vortex). A stable value of the conductivity could be observed only at lower impeller rotation speed.

### Mixing time results for 50X SE50MAF-UF Soy Hydrolysate – 200 L

For the 50X SE50MAF-UF Soy Hydrolysate preparation at 200 L, 50 kg of powder were added in 15 minutes in the mixing bag using five Powder Transfer Bags. The feed powder was mixed in 30 minutes according to conductivity and 47 minutes according to visual check.

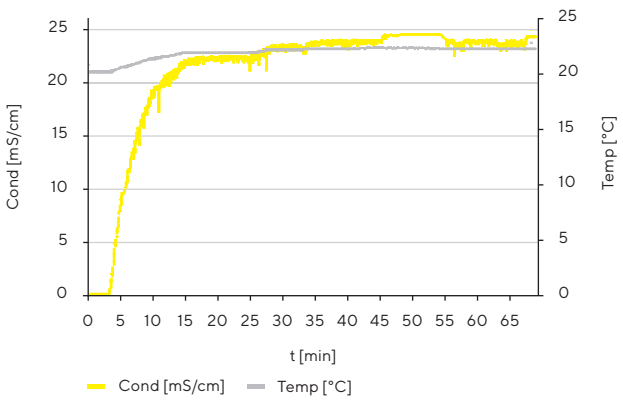


**Figure 5:** 50X SE50MAF-UF Soy Hydrolysate preparation in 200 L Flexel® Bag for Magnetic Mixer

### Mixing performances vs. feed type

The mixing time reported in the table represents only the time to dissolve the feed powders at the different scales. These mixing times include the transfer time of the multiple Sartorius Stedim Biotech Powder Transfer Bags System (for the 200 L scale) or SAFC® bucket liners (for the 50 L).

| Volume [L] | Control test | 50 | 200 |
|---|---|---|---|
| **Feeds powder** | | | |
| 50X BD Yeastolate | Conductivity Mixing time 1 | | < 15 min |
| | Visual inspection Mixing time 2 | < 8 min | < 20 min |
| 50X SE50MAF-UF Soy Hydrolysate | Conductivity Mixing time 1 | | < 30 min |
| | Visual inspection Mixing time 2 | < 20 min | < 47 min |

**Figure 6:** Overview on mixing times for the two powder feed dissolution

## General Comments

- A rapid dissolution was observed for both 50X SE50MAF-UF Soy Hydrolysate and 50X BD Yeastolate feed due to the strong mixing torque at 300 rpm.
- For the 50 L volume, the vortex at 300 rpm in the 50 L bag volume resulted in the generation of air bubbles that interfered with conductivity measurement with air trapped in the conductivity cell. The conductivity was not unwavering even though mixing was completed. A stable value of the conductivity could be observed only at lower impeller rotation speed. Therefore mixing time cannot be precisely defined using the conductivity signal. Mixing times were determined by visual inspection.
- For the 200 L volume, the conductivity of the solutions reaches a stable value. However, some fine particulates can still be visually observed in the solution. The agitation at 300 rpm was maintained until the particulates became visually totally dissolved. This visual control is facilitated by the large windows of the Palletank®.
- A 4× increase in feed preparation volume only leads to a 2× increase in the mixing times.
- The worst case feed preparation condition (200 L 50X SE50MAF-UF Soy Hydrolysate) with mixing of 50 kg of powder in 160 L of water resulted in a mixing time of only 47 min.

## Conclusions

- Large volume feed solutions are quick and easy to prepare using the combination of ready to use feed formulations and the high efficiency mixing of the Flexel® with Magnetic Mixer technology.
- Such high concentrated feed preparations are useful in large scale bioproduction. For instance, a 200 L feed preparation at 250 g/L used at 5 g/L in media formulations enables to work with a 10,000 L bioreactor.
- The contained processing conditions with the closed Powder Transfer Bag System docked onto the sterile Flexel® Bag for Magnetic Mixer are favourable to maintain low bioburden and to reduce to the minimum exposure of the operator to chemicals.
- The platform provides a single-use scalable feed preparation capability with a range of Flexel® Bags including volumes of 50 L, 100 L and 200 L.

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