Medium preparation for single-use fermentation

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Overview

Microbial fermentation is conventionally performed in stainless steel vessels. However, such equipment requires extensive cleaning and qualification between runs. In addition, reusable equipment includes an inherent cross-contamination risk.

To overcome many of the challenges associated with stainless steel vessels, single-use fermentors offer an increasingly viable alternative. With single-use fermentors, less time is spent on equipment preparation and qualification prior to start-up as well as routine maintenance and requalification of the equipment (1). In addition, all process components that have been in contact with the process material can be discarded after use, eliminating the cross-contamination risk between fermentation runs.

Here, we describe how medium preparation and liquid management can be performed for an *E. coli* fermentation process in single-use workflows (2).

Fig 1. XDR-500 MO fermentor system.

Protocol

Preparation of medium for a 350 L (starting volume) fermentation in the Xcellerex™ XDR-500 MO fermentation system (Fig 1), equipped with a disposable XDA 500 L fermentor bag was performed using the Xcellerex XDM/XDUO 500 L Mixing System. Medium components are listed in Table 1. Culture medium was separated into base medium (components 1–10), small-volume additives (components 11–16), feed solution (component 17), and chemicals for consumption (components 18–20). Setup of the fermentor system is shown in Figure 2.

Table 1. Medium components used for the 350 L *E. coli* fermentation process

Base medium		
1	Bacto™ yeast extract	5 g/L
2	Difco™ Select Soytone	10 g/L
3	$(NH_4)_2SO_4$	2 g/L
4	KH_2PO_4	3.09 g/L
5	K_2HPO_4	10 g/L
6	$C_6H_5O_7Na_3 \times 2H_2O$	0.8 g/L
7	NH_4Cl	0.4 g/L
8	Na_2SO_4	1.6 g/L
9	Glycerol (98%)	10 g/L
10	Thiamine hydrochloride	0.06 g/L
Small-volume additives		
11	$MgSO_4 \cdot 7H_2O$	1 g/L
12	Kanamycin monosulfate	0.05 g/L
13	Trace A	0.67 g/L
14	Trace B	0.67 g/L
15	Vitamins	0.60 g/L
16	IPTG	0.5 mM
Feed solution		
17	Glycerol (98%)	60% (w/w)
Chemicals for consumption		
18	Ortophosphoric acid, 2 M	pH regulation, acid
19	Ammonia, 23%	pH regulation, base
20	Antifoam 204	50 mL

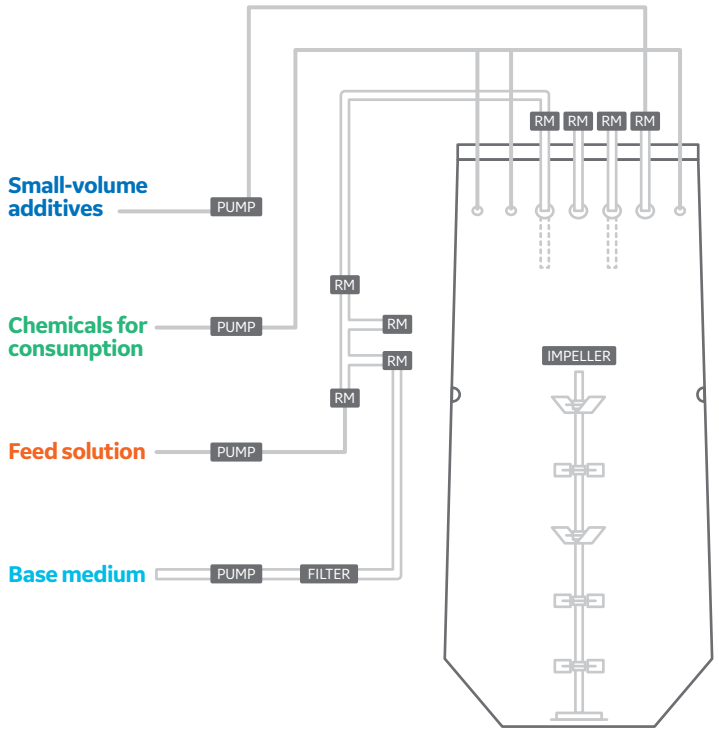

Fig 2. Fermentation liquid management diagram. RM = ReadyMate aseptic connector.

Base medium was prepared by dissolving components 1 and 2 in 110 L distilled water (heated to between 45°C and 50°C to facilitate solubilization) in a 500 L single-use XDM mixer bag. The volume was adjusted to 300 L with distilled water, components 3 to 10 were added to the mixer bag, and the components were mixed at 150 rpm. The bottom drain line from the mixer consumable was connected to the inlet port of a presterilized 20 inch ULTA™ HC 0.2 µm filter. An in-line pressure sensor was included between the mixer and the filter. A single-use manifold with either 4- or 6-ports (4 or 6RMT) with aseptic ReadyMate™ (RM) connectors can be used to connect a medium sterilization filter to the 1/2" liquid addition port at the top of the fermentor bag, using RMRM tubing jumpers. The filter inlet pressure was monitored using a SciLog™ pressure monitoring unit. After taring the weight indicator on the X-Station portable XDR control console, a stand alone Watson-Marlow™ 620Bp pump was used for filling, the mixer bag was rinsed with H_2O, and starting fermentor volume was adjusted to 350 L. The filter capsule was clamped and disconnected from the fermentor bag after completion of the medium addition process.

Small-volume additives were added separately, as sterile solutions of component 11–16. Isopropyl-ß-D-thio-galactopyranosidase (IPTG) for culture induction was dissolved in H_2O and sterile filtered using bottle-top 0.2 µm filtration units (VWR). A 4RMT or 6RMT manifold can be connected to one of the feed lines on the disposable fermentor bag to simplify addition of the solutions.

Feed solution (60% glycerol) was sterile filtered using a 5 inch ULTA HC 0.2 µm filter into a 200 L ReadyCircuit™ disposable 3D bag. Feed additions to the fermentor bag were performed from the 3D bag placed on a floor scale, Mettler-Toledo ID3sRTx. Feed addition was regulated by a predefined set-point table in the X-Station portable XDR control console.

Chemicals for consumption included acid and base for pH regulation and antifoam agent. Antifoam was autoclaved before use and kept in a glass bottle equipped with a 1/8 inch C-Flex® pump tubing connected through an external pump used for addition of the antifoam agent. After adding medium to the fermentor bag, approximately 100 mL antifoam agent was added. Further additions were done during the cultivation when needed to control foaming. Acid solution was kept in a 10 L disposable pillow bag and base solution in a 25 L plastic container with dip tube. Little or no acid addition was expected to be required during the fermentation process.

Concluding remarks

Single-use technologies bring speed and flexibility to process development and biomanufacturing. To make full use of the benefits of such technologies, planning and preparation is essential, especially during the early stages of implementation. Liquid management represents a fundamental process management task, and its proper implementation is particularly relevant to single-use fermentation processes because of their newness in contrast to the long-standing operational methods associated with stainless and autoclavable fermentors.

This application note covers key elements associated with medium preparation and process liquid management, for a large-scale single-use fermentation process (Fig 3). Among those key elements are:

- Number and volume of liquid components.
- Staging and layout of appropriate liquid containers.
- Sterilization methods associated with various liquids.
- Process liquid flow path connectivity.

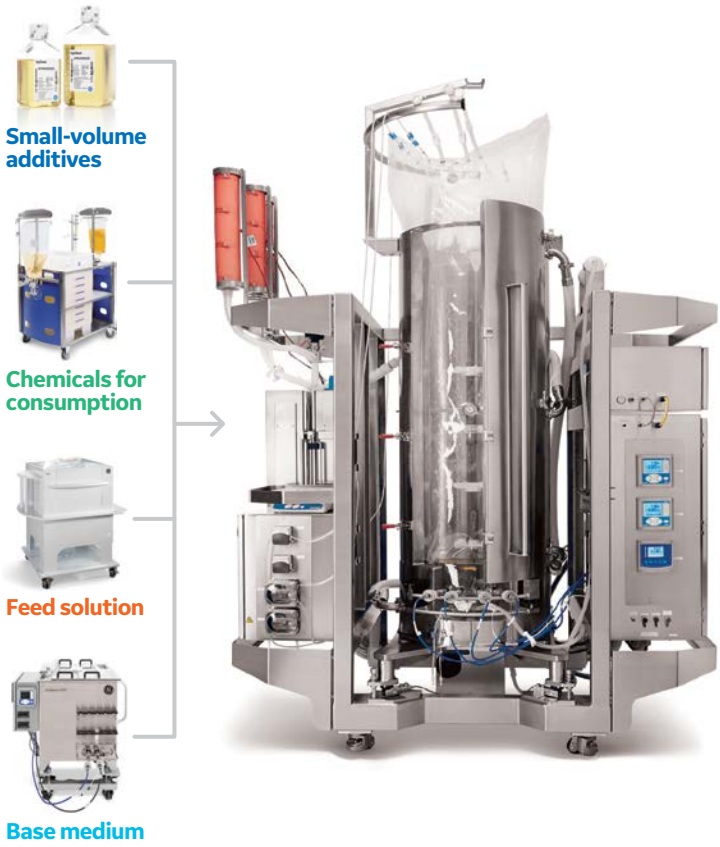

Fig 3. Single-use fermentation medium preparation equipment and supplies.

The note's format as a protocol is intended as a guide to facilitate the adoption of practical and effective liquid management practices when employing single-use technologies. Specific procedural guidance, from the presented protocol, should be selected and applied with respect to your own process.

References

1. White paper: Process economy and production capacity using single-use versus stainless steel fermentation equipment. GE Healthcare, 29143348, Edition AB (2015).

2. Application note: Performance of single-use Xcellerex XDR-500 MO and XDR-50 MO stirred-tank fermentor systems. GE Healthcare, 29253229, Edition AA (2016).

Ordering information

Product	Description	Product code
ULTA HC 0.2 µm filter	20 in	12410098
ULTA HC 0.2 µm filter	5 in	12410095
ReadyCircuit disposable 3D bag assembly	200 L	12410208
ReadyCircuit disposable pillow bag assembly	10 L	12410222
XDA-500 fermentor bag	500 L	888-2-0713-C
XDM-500 Plus	500 L	888-0156-C
4RMT manifold	Jumper 0.5 × 6 in CF1pk	12410175
RMRM tubing	Jumper 0.5 × 3 ft CF1pk	12410121

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