WHITE PAPER No. 106

1500PP Bottle (WM) Has the Same Bacteria Pelleting Efficiency as Smaller Bottles

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> 1500PP bottles (WM) isolate the same weight of bacterial pellet as 1.0 L bottles in relation to the culture volume
> 1500PP bottles (WM) allow for reduced procedure time compared to 1.0 L bottles
> 1500PP bottles (WM) therefore accelerate bacterial pelleting with the same efficiency as 1.0 L bottles and no minimum filling is required

Executive Summary

The harvest of biomass is an important step in each downstream process associated with bioprocesses, such as the preparation of DNA plasmids or the purification of recombinant protein. As it gains more and more interest in the research field, this isolation step tends to be scaled up to larger culture volume solutions such as high-volume flasks or fermenters. Here, we show that our Rotor R9A2 can hold 4 x 1500PP bottles (WM) and isolate the same weight of bacteria as the smaller 1.0 L bottle solutions.

Introduction

Nowadays, numerous applications are based on the cultivation of bacteria. First in dishes, then in flasks, and later in fermenters, bacterial research fields are constantly increasing the working volume to improve productivity and save processing time [1]. Rotors that can hold 1.0 L bottles or lower volume consumables usually serve as a reference for isolating bacterial cultures in benchtop or floor standing centrifuges. These setups sometimes require successive runs to process the complete batch volume, wasting precious time in the laboratory. Consequently, our 1500PP bottles (WM) allow the isolation of the same weight of bacterial pellet in fewer steps compared to lower volume solutions such as 1.0 L bottles.

Solution & Benefits

The Rotor R9A2 is uniquely designed to allow a capacity of 4 x 1500PP bottles (WM).

Available for both Centrifuge CR22N and Centrifuge CR30NX, this rotor already demonstrated a reduction in processing time for bottle filling, balancing, tight bottle closure, and rotor loading, to supernatant decanting, pellet recovery and finally bottle washing and autoclaving, by 32 % [2]. This White Paper focuses on the efficiency of the pelleting of bacteria in 1500PP bottles (WM) using the Rotor R9A2 in the Centrifuge CR22N. As the volume increases, it is important to prove that the harvesting efficiency is comparable to lower volume solutions such as 1.0 L bottles.

Experimental Design

Here we compared the pelleting potential of two bacterial culture conditions in different rotors and centrifuges (Figure 1). This setup compared the bacterial pellet weight isolated from 3.5 L of 16 hours (Lag phase) of bacterial culture and 3.5 L of 36 hours (Stationary phase) of bacterial culture. Each rotor contained 2 bottles (one filled with the 16 h and the other one with 36 h culture, respectively).

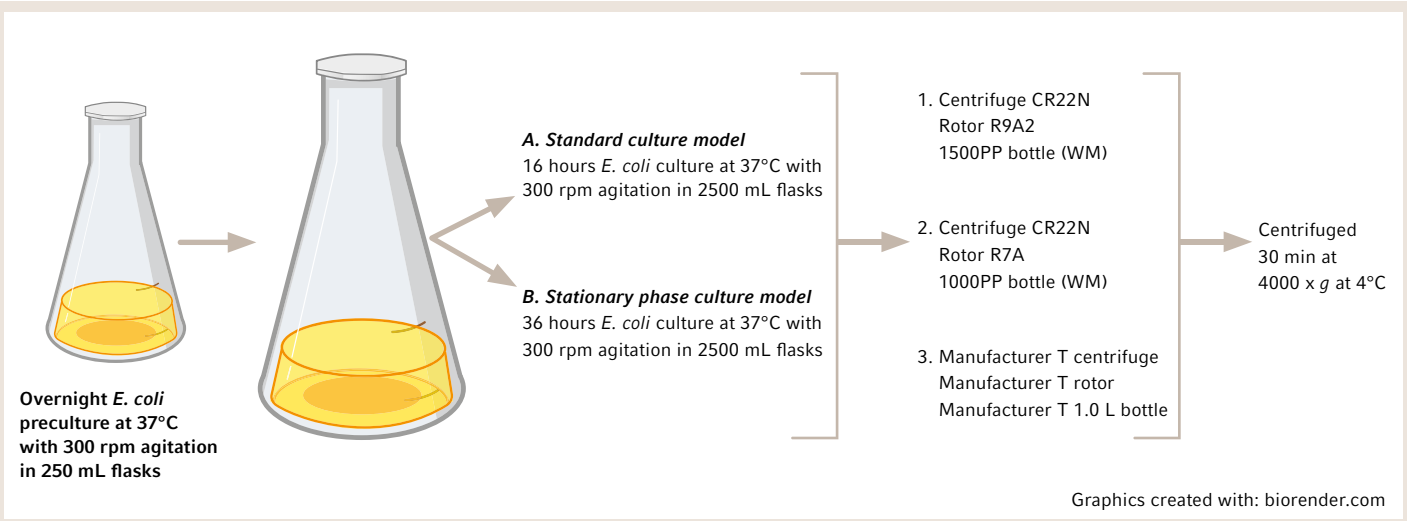

Figure 1: Workflow of the bacterial culture and harvesting solutions

Following centrifugation, all bottles showed a dense and noticeable pellet at their bottom (Figure 2.A). The supernatant was removed, and the pellet was firmly attached to the bottom of all bottles (Figure 2.B). The ratio of pellet weight to supernatant volume showed that every bottle was able to isolate the same weight of bacteria (Figure 2.C). Before the centrifugation and before discarding the supernatant, an aliquot was taken to measure the optical density of the culture solution. The optical density showed that after centrifugation, no bacteria remained in the supernatant as the values were comparable to the blank (original media – Figure 2.D).

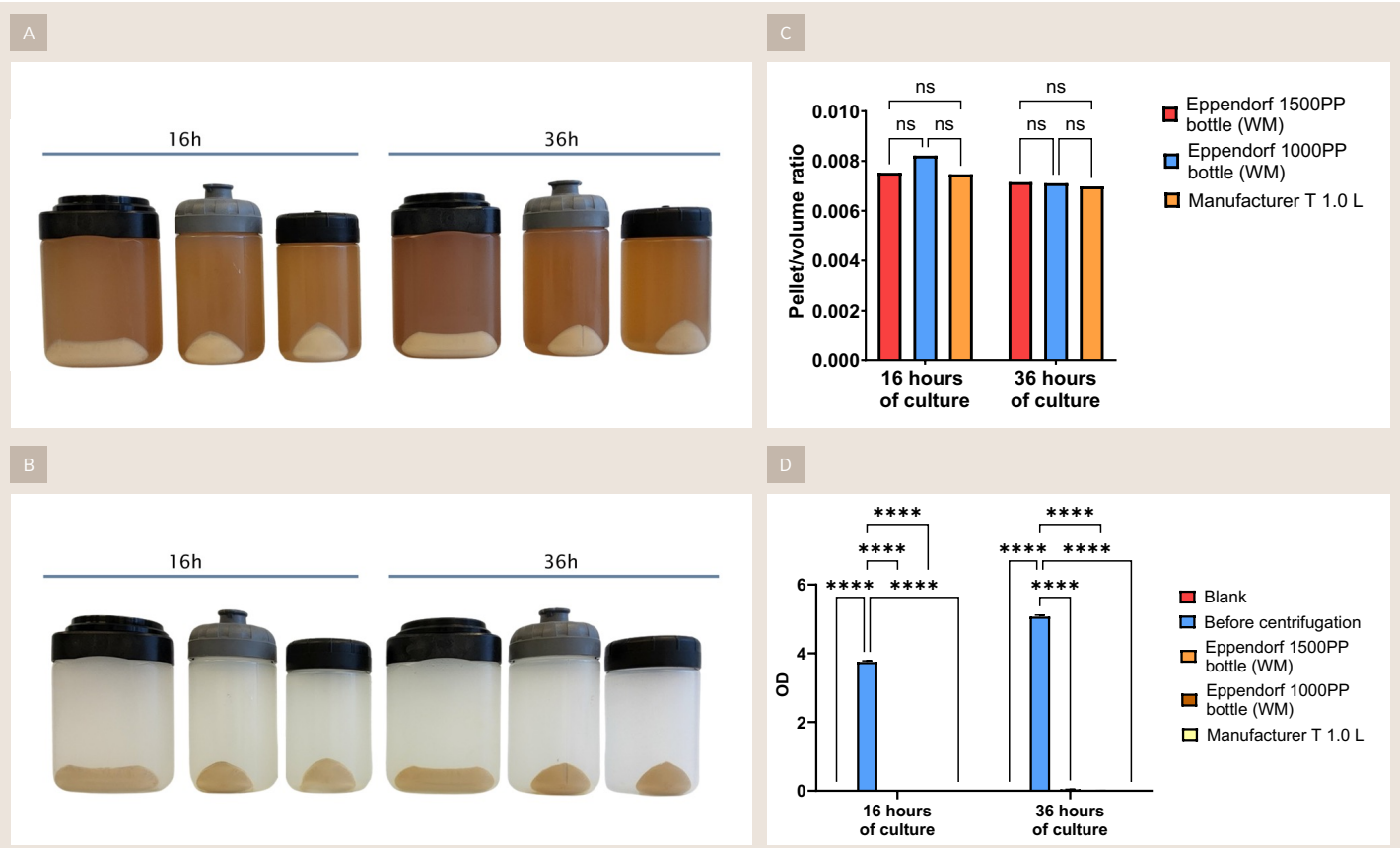

Figure 2: A) Bacterial pellet after centrifugation at 4,000 x *g* at 4°C for 30 min. B) Dry pellet after discarding supernatant. C) Pellet weight to volume ratio. D) Optical density measurements ns = non-significant; **** = $p \leq 0.0001$.

Summary

This experiment demonstrates that the 1500PP bottles (WM) can effectively isolate the same weight of bacteria in comparison to 1.0 L bottles. Therefore, researchers and laboratories can confidently choose the Centrifuge CR22N with the Rotor R9A2 and the 1500PP bottles (WM) to pellet their scaled-up bacterial cultures. Using these 1500PP bottles (WM) will ensure that the entire bacterial culture will be pelleted, in addition to saving time, as these 1500PP bottles (WM) require less time to process.

Literature

[1] Rasche, U. Bioreactors and Fermentors - Powerful Tools for Resolving Cultivation Bottlenecks. www.eppendorf.group/webinar-shaker-bioreactor.
[2] Eppendorf SE. Unique 4 x 1.5 L Capacity Rotor for High-Speed Centrifuges CR22N and CR30NX.

Eppendorf Ordering Information

Description	Manufacturer	Order no.
Centrifuge CR22N	Eppendorf	Inquire
Rotor R9A2	Eppendorf	5721 221 014
1500PP bottle (WM) assembly	Eppendorf	5721 411 035

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