Fast Monitoring of Bacteriophage Production During Fermentation Using the Agilent Bio-Monolith HPLC Column

Application Note

Biopharmaceutical

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Abstract

Bacteriophages, viruses that infect bacteria, were discovered at the beginning of the twentieth century. Today, phages are being used as antibacterial agents, in phage display screening, in the development of phage delivery vaccines, as targeted gene therapy delivery systems, and for specific bacteria typing. In order to use phages in these applications, they are commonly purified by liquid chromatography and must be free of all impurities. A purification and concentration process was recently developed using an ion exchange monolithic column [1]. After propagation and lysis of their host, phages are released into the growth medium. Along with the phages, other host components are released into the growth media, one of them being the host genomic DNA (gDNA), which poses a problem because it soon starts to degrade and form smaller fragments. When purifying phages with ion-exchange chromatography, it is crucial that the medium containing the phages does not contain any fragmented DNA. Free DNA fragments can interfere in the *in vitro* phage applications, phage display, and bacteria typing, and can be toxic in the therapeutic phage applications, vaccine production, and gene delivery. The Agilent Bio-Monolith DEAE column (weak anion exchange) can be used to monitor the fermentation process and to evaluate the amount of degraded gDNA throughout the purification process.

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Introduction

The Agilent Bio-Monolith DEAE HPLC columns demonstrate the utility of monolithic column assays for monitoring the bacteriophage fermentation process. The rapid and high-quality analytical separations offered by the Bio-Monolith DEAE column enables control of the fermentation and efficient purification of phage particles. Monitoring the concentration (or peak height of gDNA) in the phage bacterial suspension with the Bio-Monolith DEAE column enables the quick determination of the optimal fermentation endpoint. Once the gDNA concentration reaches a maximum, the fermentation process is stopped and the sample is ready for purification by ion exchange chromatography.

Experimental

HPLC Conditions

Column	Agilent Bio-Monolith Column DEAE (5.2 mm id × 4.95 mm) p/n 5069-3636
Mobile phase A	125 mM phosphate buffer, pH 7.0
Mobile phase B	125 mM phosphate buffer + 1M NaCl, pH 7.0
Gradient	Linear gradient: 100% buffer A (2.5 min); 0 to 100% buffer B (10 min); 100% buffer A (2 min)
Detection	UV at 280 nm
Flow rate	1 mL/min
HPLC system	A high-pressure gradient HPLC system, Agilent 1200

Agilent Bio-Monolith column (left), Bio-Monolith column housing, and monolith disc (right).

Results and Discussion

Laura-Bertani medium was inoculated with *E. coli* after 16 minutes in a lab-scale bioreactor. Phage T4 was added at 70 minutes and allowed to propagate. Figure 1a represents the monitoring of the fermentation process with conventional sensors (pO2 and pH electrode), which gives a very general view of the process. Using the Bio-Monolith DEAE column (Figure 1b), one can observe an important change in the composition of the fermentation broth in the first 36 minutes, whereas the conventional sensors show just a minor pH drop. The decreasing height of peak 1 in Figure 1b represents a decrease in the original media components that results in an increasing concentration of *E. coli* cells. The ability to see this decrease (increase of *E. coli*) allows for optimal addition of phage to increase final phage yields.

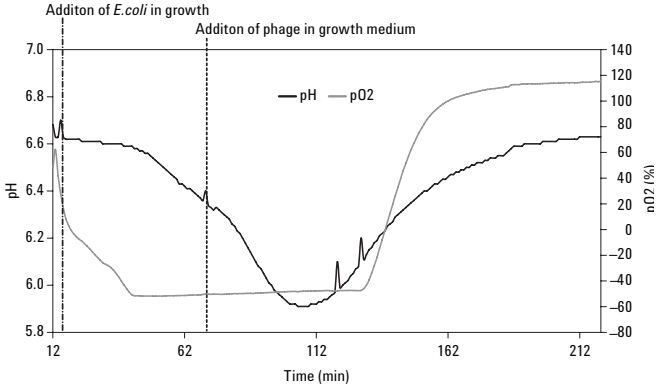

Figure 1a. Monitoring of phage T4 growth in the bioreactor. Laura-Bertani medium was inoculated with E. coli at 16 min and phage T4 was added at 70 min.

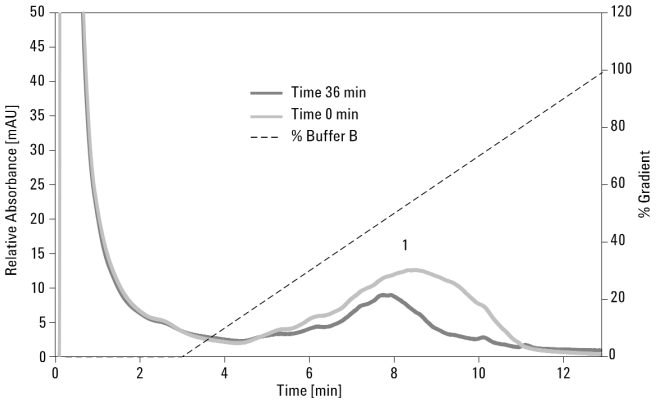

Figure1b. Chromatographic profile of starting media suspension and 36 minutes after inoculation (1) with E. coli. *The peak is decreasing as the media is being used up by the* E. coli.

Upon addition of the phages at 70 minutes, cell lysis began to take place and phages were released from host cells. Phage propagation and gDNA concentrations were monitored using the Bio-Monolith DEAE clumn as shown in Figures 2a and 2b. Along with the intact phages, gDNA was also released from cells (Figure 2a, peak 2). Over time, more and more host cells were lysed, the number of phages increased (Figure 2a, peak 1), and gDNA concentrations increased (Figure 2a, peak 2). As phage and gDNA concentrations increased, other impurities (media, cells) that coelute with the phages were decreasing over time (Figure 2b, peak 1). The decreasing concentration of these coeluting impurities greatly simplifies the purification steps after fermentation. Phages were identified in the fraction containing peak 1 (Figure 2b) by plaque assay. However, the concentration was below the HPLC limit of detection and there was no identifiable increase in peak 1 (Figure 2b). Towards the late stages of the fermentation, between 158 and 191 minutes, the released gDNA began to degrade into DNA fragments

(Figure 2b, peak 3). As stated earlier, the degraded gDNA cannot be removed by ion exchange purification. So, in this case, stopping the fermentation process at 158 minutes would yield a highly pure, final phage sample after ion exchange purification, virtually free of gDNA fragments.

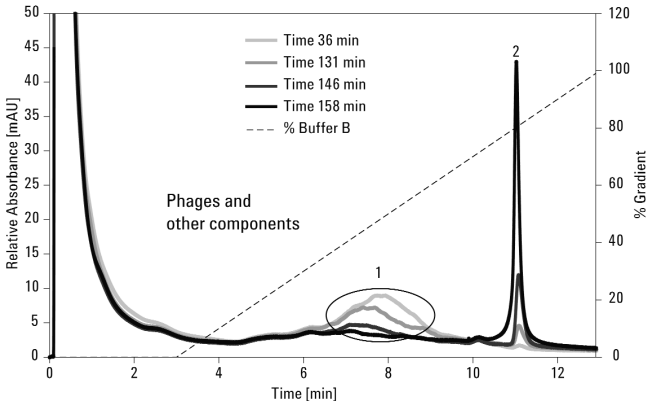

Figure 2a. Peaks representing phages, cells, and media suspension (1) and gDNA (2).

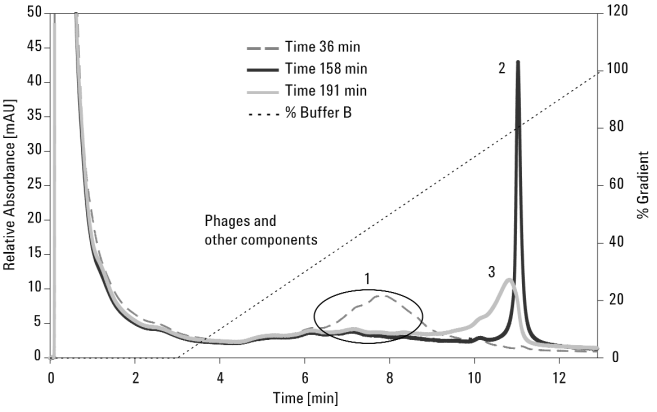

Figure 2b. A peak representing phages, impurities (1), gDNA (2), and fragmented gDNA (3).

Conclusions

The Agilent Bio-Monolith DEAE column can be effectively used to monitor phage propagation throughout fermentation. Based on the process monitoring obtained with the column, the fermentation process can be terminated at the optimal point for phage purification. These methods can be used throughout early development of the fermentation process and later for process monitoring and quality control purposes.

Reference

1. F. Smrekar, M. Ciringer, M. Peterka, A. Podgornik, and A. Štrancar, *J. Chromatogr. B,* 861(2008) 177–180

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