MethodsX 1 (2014) 197–206

Contents lists available at ScienceDirect

MethodsX

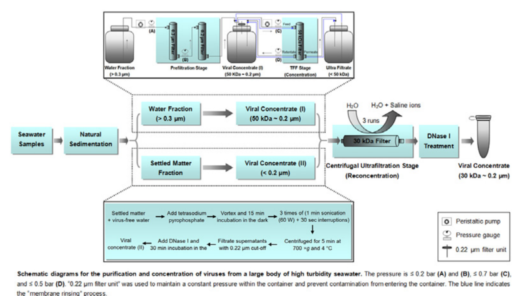
journal homepage: www.elsevier.com/locate/mex

Efficient purification and concentration of viruses from a large body of high turbidity seawater

Guowei Sun [a,b], Jinzhou Xiao [a,b], Hongming Wang [a,b], Chaowen Gong [a,b], Yingjie Pan [a,b], Shuling Yan [c], Yongjie Wang [a,b,*]

[a] *Laboratory of Quality and Safety Risk Assessment for Aquatic Products on Storage & Preservation (Shanghai), Ministry of Agriculture, China*
[b] *College of Food Science and Technology, Shanghai Ocean University, Shanghai, China*
[c] *Institute of Biochemistry and Molecular Cell Biology, University of Göttingen, Germany*

GRAPHICAL ABSTRACT



Schematic diagrams for the purification and concentration of viruses from a large body of high turbidity seawater. The pressure is ≤ 0.2 bar **(A)** and **(B)**, ≤ 0.7 bar **(C)**, and ≤ 0.5 bar **(D)**. "0.22 μm filter unit" was used to maintain a constant pressure within the container and prevent contamination from entering the container. The blue line indicates the "membrane rinsing" process.

ABSTRACT

Marine viruses are the most abundant entities in the ocean and play crucial roles in the marine ecological system. However, understanding of viral diversity on large scale depends on efficient and reliable viral purification and concentration techniques. Here, we report on developing an efficient method to purify and concentrate viruses from large body of high turbidity seawater. The developed method characterizes with high viral recovery efficiency, high concentration factor, high viral particle densities and high-throughput, and is reliable for viral concentration from high turbidity seawater. Recovered viral particles were used directly for subsequent analysis

* Corresponding author at: College of Food Science and Technology, Shanghai Ocean University, Shanghai, China. Tel.: +86 21 61900505.
E-mail address: yjwang@shou.edu.cn (Y. Wang).

http://dx.doi.org/10.1016/j.mex.2014.09.001
2215-0161/© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/3.0/).

by epifluorescence microscopy, transmission electron microscopy and metagenomic sequencing. Three points are essential for this method:

- The sampled seawater (>150 L) was initially divided into two parts, water fraction and settled matter fraction, after natural sedimentation.
- Both viruses in the water fraction concentrated by tangential flow filtration (TFF) and viruses isolated from the settled matter fraction were considered as the whole viral community in high turbidity seawater.
- The viral concentrates were re-concentrated by using centrifugal filter device in order to obtain high density of viral particles.

© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/3.0/).

ARTICLE INFO

Method name: Concentration of marine viruses

Keywords: Marine viruses, Concentration, Tangential flow filtration, Ultrafiltration, High turbidity seawater

Article history: Received 9 June 2014; Accepted 12 September 2014; Available online 16 September 2014

Method details

Sample preparation

More than 300 L of subsurface water (2 m depth), characterized by highly suspended matter contents (approximately 3.4 g L^{-1} (wet weight)), was sampled from Yangshan Deep-Water Port, South East Shanghai, China. Among them, 500 mL was fixed *in situ* by adding 0.02 μm filtered formalin (37–40% (w/v) formaldehyde solution) (Sangon, Shanghai, China) to 2% (v/v) final concentration for enumeration of viruses in the original water sample. The collected water samples were kept on ice and delivered to the laboratory as quickly as possible after finishing sampling.

Enumeration of viruses in original seawater sample

In order to assess the recovery efficiency of the method established in this study for purification and concentration of marine viruses from high turbidity seawater, viral particles in the original seawater samples (Table 1 and Fig. 1A) were initially determined by epifluorescence microscopy after SYBR Green I staining according to the procedure described in [1,2]. All enumeration of viral particles in this study was performed by using the identical protocols.

Concentration of viruses from the high turbidity seawater

The procedures for purification and concentration of virus particles from water samples are outlined in Graphical Abstract. The major procedures are as followed:

1. *Sedimentation by natural gravity*. 150 L of seawater samples were maintained in the dark for 12 h at 4 °C. After sedimentation, the samples were divided into two parts: water and settled matter fractions. The water fraction (approximately 150 L) was subsequently subjected to viral concentration, and the settled matter (approximately 509.8 g) was stored at −80 °C before viruses were isolated and concentrated.
2. *Removal of all particles or cells larger than 0.2 μm*. The water fraction (approximately 150 L) was successively filtered through 0.3 and 0.2 μm pore-sized filters in a stainless steel filter holder, a high performance and throughput filtration system (Millipore, MA, USA) equipped with the reusable cartridge filter with a large surface area, under a low entry pressure (<0.2 bar) driven by a peristaltic pump (Millipore, MA, USA). Afterwards, the "viral fraction" seawater (the filtrate, approximately 150 L) was obtained. The number of viral particles in the filtrate (Table 1 and Fig. 1B) was determined by epifluorescence microscopy after SYBR Green I staining.
3. *TFF concentration*. The "viral fraction" seawater (approximately 150 L) was subsequently concentrated by using a large-scale TFF system with 50 kDa cut-off tangential flow filter (Millipore, MA, USA) (see Graphical Abstract). The intake pressure driven by the peristaltic pump was below 10 p.s.i. (approximately 0.7 bar) to protect viruses from being destroyed, resulting in loss of virus

Download English Version:

https://daneshyari.com/en/article/2058712

Download Persian Version:

https://daneshyari.com/article/2058712

Daneshyari.com