Technologiezentrum Wasser, Karlsruhe TZW

DVGW

Development of molecular biological tools to monitor virus elimination in waste water reuse

C. Zawadsky*, M. Stieber*, B. Hambsch**, and A. Tiehm*

*Department Environmental Biotechnology, Water Technology Centre, Karlsruhe, Germany

**Department Microbiology, Water Technology Centre, Karlsruhe, Germany

Introduction

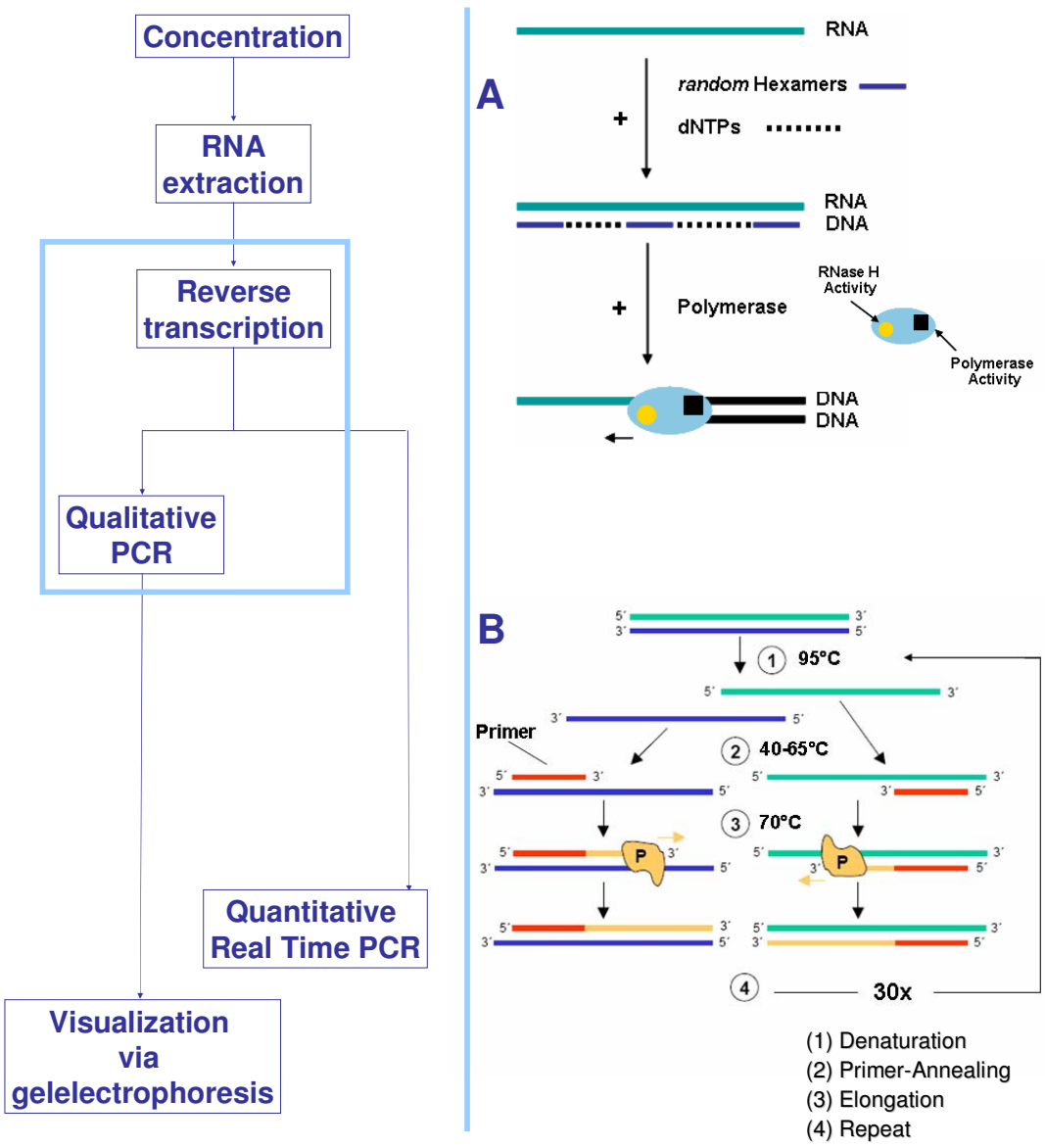
As part of the SMART Jordan Valley project (SMART, 2007), new integrated approaches for water management, aquifer recharge, and waste water reuse are developed. Decentralized membrane bioreactor technologies combined with consecutive subsoil conditioning are studied with respect to the removal of persistent organic pollutants (POPs) and pathogenic organisms.

Monitoring the elimination of pathogenic organisms is a pre-requisite for developing sustainable waste water reuse approaches. Since current cultivation methods are not available or extremely time consuming for viral pathogens, in our study molecular biological approaches are developed. In first experiments, MS2 bacteriophages were used as model organisms.

Material and Methods

After pre-concentration, RNA was extracted, and RNA concentrations were determined photometrically. cDNA was generated, and the resulting cDNA sample was applied to qualitative PCR. MS2 bacteriophages were detected according to Dreier *et al.* (2005).

MS2 bacteriophages were additionally detected by quantitative real time PCR. For this approach the standard curve method was applied.

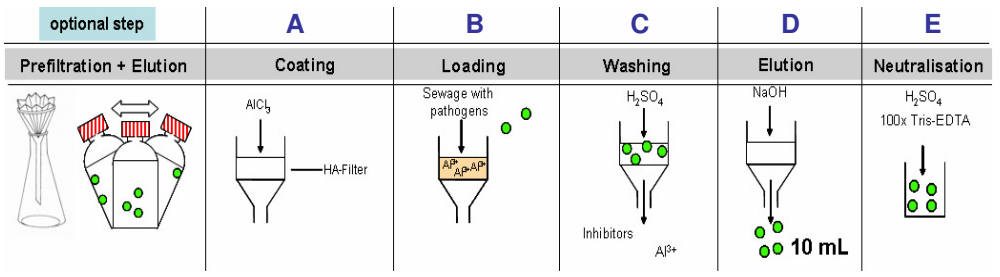


Virus detection via
(A) cDNA synthesis followed by
(B) Polymerase Chain Reaction (PCR).

Results and discussion

Modification of the cation-coated filter method.

The cation-coated filter method described for fresh- and tap water samples by Haramoto *et al.* (2005) was modified in order to detect MS2 bacteriophages in waste water treatment plant (WWTP) effluents. First tests were performed with 1L of WWTP effluent spiked with MS2-bacteriophages. Since the membrane filters instantly blocked, a pre-filtration step (folded filter) was added. Flasks were additionally rinsed with NaOH to recover phages adsorbed to the inside (Butot *et al.* 2007). The filtrate achieved by the cation-coated filter method was further concentrated in two additional steps to 20µL.



Cation-coated filter method.

Recovery of MS2 nucleic acids.

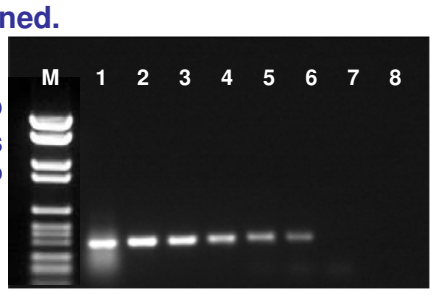
Dilution series of MS2 bacteriophages gained from pure cultures were prepared in drinking water (1L respectively). Samples were concentrated to 20µL with the advanced cation-coated filter method, and RNA was extracted. RNA concentrations of the input and of the eluted samples were determined photometrically. Recovery yields of MS2 phage nucleic acids between 76 and 93% were obtained.

pfu/sample	$9x10^4$	$2.25x10^4$	$5.63x10^3$	$1.41x10^3$	$3.51x10^2$	$8.78x10^1$
recovery [%]	88	90	93	89	76	89

Detection of MS2 bacteriophages in WWTP effluents.

WWTP effluents (1L respectively) were spiked with MS2 phages in different concentrations (plaque forming units, pfu) determined in cultivation tests. Samples were concentrated with the advanced cation-coated filter method and analyzed by PCR. A detection limit of 288 pfu/L was determined.

The method proved to be suitable for virus detection in WWTP effluents.



M – Marker
1 – positive control
2 – $3x10^5$
3 – $2x10^4$
4 – $1x10^3$
5 – $5x10^2$
6 – 288
7 – 144
8 – no template control

In addition, the quantitative real time PCR for the detection of MS2 bacteriophages in WWTP effluents was established.

Conclusions

The successful detection of the MS2 phage in waste water indicates that other viral contaminants also should be detectable. Both, qualitative and quantitative detection methods will be applied to monitor virus elimination in laboratory studies and in field samples taken at Jordan Valley waste water infiltration test sites.

References

Butot S., Putallaz T., Croquet C., Lamothe G., Meyer R., Joosten H. and Sánchez, G. (2007): Attachment of enteric viruses to bottles. *Applied and Environmental Microbiology*, 73(16), 5104-5110.

Dreier J., Störmer M., Kleesiek K. (2005). Use of bacteriophage MS2 as an internal control in viral reverse transcription-PCR assays. *Journal of Clinical Microbiology*, 43(9), 4551-4557.

Haramoto E., Katayama H., Oguma K. and Ohgaki S. (2005). Application of cation-coated filter method to detection of noroviruses, adenoviruses, and torque teno viruses in the Tamagawa river in Japan. *Applied and Environmental Microbiology*, 71(5), 2403-2411.

Acknowledgement

The authors gratefully acknowledge funding by the German Federal Ministry of Education and Research (02WM0803). The study is associated with the joint research project “Sustainable Management of Available Water Resources with Innovative Technologies (SMART: http://www.iwrm-smart.org/.)”.