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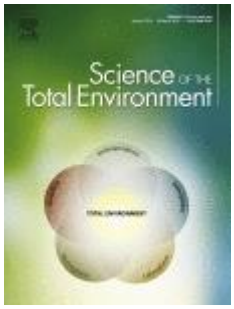

Bias of library preparation for virome characterization in untreated and treated wastewaters

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Highlights

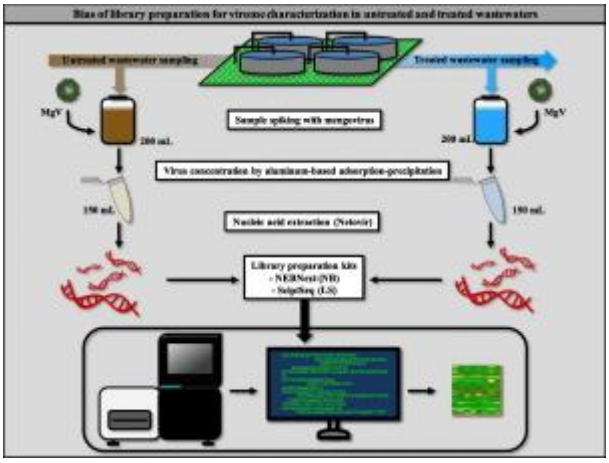
- Work-flow procedure for the characterization of virome in wastewaters
- Sequencing libraries lead to different virome profiles.
- Not all proposed viral indicators correlate with the presence of enteric viruses.

Abstract

The use of metagenomics for virome characterization and its implementation for wastewater analyses, including wastewater-based epidemiology, has increased in the last years. However, the lack of standardized methods can led to highly different results. The aim of this work was to analyze virome profiles in upstream and downstream wastewater samples collected from four wastewater treatment plants (WWTPs) using two different library preparation kits. Viral particles were enriched from wastewater concentrates using a filtration and nuclease digestion procedure prior to total nucleic acid (NA) extraction. Sequencing was performed using the ScriptSeq v2 RNA-Seq (LS) and the NEBNext Ultra II RNA (NB) library preparation kits. Cleaned reads and contigs were annotated using a curated *in-house* database composed by reads assigned to viruses at NCBI. Significant differences in viral families and in the ratio of detection were shown between the two library kits used. The use of LS library showed *Virgaviridae*, *Microviridae* and *Siphoviridae* as the most abundant families; while *Ackermannviridae* and *Helleviridae* were highly represented within the NB library. Additionally, the two sequencing libraries produced outcomes that differed in the

detection of viral indicators. These results highlighted the importance of library selection for studying viruses in untreated and treated wastewater. Our results underline the need for further studies to elucidate the influence of sequencing procedures in virome profiles in wastewater matrices in order to improve the knowledge of the virome in the water environment.

Graphical abstract



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