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A SURVEY OF SARS-COV-2 GENETIC MATERIAL REDUCTION DURING A TRADITIONAL WASTEWATER TREATMENT TECHNOLOGY

ORSOLYA ADAMCSIK*[1], RENÁTA GERENCSÉR-BERTA[1], BORBÁLA OLÁHNÉ HORVÁTH[1], NIKOLETTA KOVÁCS[1], VIOLA SOMOGYI[2], ENDRE GÁBOR DOMOKOS[2], GÁBOR KEMENESI[3], ENDRE GÁBOR TÓTH[3], FERENC JAKAB[3], AND ILDIKÓ GALAMBOS[1]

[1]Soós Ernő Water Technology Research and Development Center, University of Pannonia, Zrínyi Miklós u. 18, Nagykanizsa, 8800 HUNGARY
[2]Research Centre for Biochemical, Environmental and Chemical Engineering, University of Pannonia, Egyetem u. 10, Veszprém, 8200 HUNGARY
[3]National Virology Laboratory, Szentágothai János Research Centre, University of Pécs, Ifjúság u. 20, Pécs, 7624 HUNGARY

The transmission of Severe Acute Respiratory Syndrome Coronavirus-2 in a community can be monitored by a wastewater-based epidemiological approach due to fecal shedding. Although sewage surveillance has gained a considerable amount of attention over the last 16 months, an indirect issue within the topic is whether traditional wastewater treatment technologies are sufficiently efficient to eliminate the genetic material of SARS-CoV-2. Samples were taken from the Wastewater Treatment Plant in Nagykanizsa before the virus was concentrated, nucleic acid extracted and SARS-CoV-2 detected by RT-qPCR (Quantitative reverse transcription PCR). The influent and primary treated samples tested positive, while after the secondary treatment, all the results were negative. Consequently, the activated sludge process proved to be efficient in terms of the removal of SARS-CoV-2.

Keywords: warning system, COVID-19, wastewater-based epidemiology

1. Introduction

During the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) since the start of the COVID-19 pandemic, wastewater-based epidemiology (WBE) has gained a considerable amount of attention as a surveillance system. Numerous research groups and health authorities are currently engaged in sewage monitoring worldwide. Apart from viruses, various compounds can be monitored, e.g. pharmaceuticals or illicit drugs, to indirectly monitor the behavior or habits of a defined population [1]. Furthermore, the approach allows anonymous, area-based monitoring in a community that is cost-, labor- and time-efficient compared to human nasopharyngeal swab test campaigns [2]. Moreover, fecal shedding can occur earlier than the onset of symptoms [3, 4], should any be expressed. While the ratio and role of asymptomatic and mild-symptomatic cases is debatable [5, 6], the WBE approach estimates the SARS-CoV-2 RNA concentration of the total community that is independent of patients' perception of well-being. Although WBE has its own limitations, sewage monitoring and human test campaigns can also support national and municipal decision-making. Considering the successful detection of SARS-CoV-2 from wastewater and during the consecutive steps of wastewater treatment, a considerable amount of aerosol formation has been observed. Moreover, since droplets are regarded as the most important transmission route of SARS-CoV-2, the working conditions at wastewater treatment plants (WWTP) should be addressed in terms of safety. According to the WHO [7], no special care is required other than the usual safety protocols at such plants since SARS-CoV-2 particles present in untreated and treated wastewater have been proven to be non-infectious. Another side interest within this topic is whether the sewage treatment technology is sufficiently effective to eliminate the SARS-CoV-2 virus particles detected in the influent to ensure the WWTP effluents are SARS-CoV-2-free, even if their infectivity has already been ruled out. The aim of this work is to assess the presence and concentration of SARS-CoV-2 RNA in untreated and treated wastewater samples.

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*Correspondence: adamcsik.orsolya@sooswrc.hu

Table 1: SARS-CoV-2 RNA detection from wastewater influent and effluent samples at Nagykanizsa WWTP.

Sample type	Date	Cq value (E-gene)
Influent	30/09/2020	+(35.69)
Influent	14/10/2020	+(33.88)
Influent	09/11/2020	+(32.39)
Influent	10/11/2020	+(33.70)
Influent	11/11/2020	+(34.27)
Influent	18/11/2020	+(36.67)
Influent	25/11/2020	+(37.03±0.11)
Effluent	25/11/2020	–
Influent	02/12/2020	+(33.70±0.50)
Effluent	02/12/2020	–

2. Materials and Methods

Sampling was conducted by Délzalai Víz és Csatornamű Zrt. at Nagykanizsa WWTP during the morning peak time. From May until December 2020, samples of influent and occasionally effluent as well were taken at various sampling frequencies. While in December, on three consecutive days, in addition to influent and effluent sampling points, primary and secondary treated samples were also checked. The pairs of influent and effluent samples were processed in duplicates, while to assess the efficacy of the WWTP, three repetitions were used over three consecutive days. 1 L grab samples were taken and placed in sterile bottles, transported to our laboratory at $4\,^{\circ}\mathrm{C}$ and immediately processed. The virus was concentrated and nucleic acid extracted according to Meleg et al. [8] with minor modifications. Concentrates were stored and transported at $-80\,^{\circ}\mathrm{C}$ to the National Virology Laboratory, University of Pécs for specific SARS-COV-2 detection by the quantitative reverse transcription polymerase chain reaction (RT-qPCR) technique, targeting the E and RdRp genes. Modular SARS-CoV-2 E-gene and Modular SARS-CoV-2 RdRp-gene detection kits (Roche, Germany) on the MyGo Pro real-time PCR instrument were employed. Quantification cycle values (Cq values) were used to compare the SARS-CoV-2 RNA concentration of the above-mentioned samples.

3. Results and Discussion

Sewage samples tested negative during the summer months of 2020 (data not shown), which is plausible considering the insignificant number of open cases nationwide [9] and the temperature of the sewage as one of the most important environmental factors [10]. At the end of September, as the second wave progressed, the concentration of SARS-CoV-2 RNA increased above the detection limit of the methods in the influent samples, while all effluent samples tested negative. During the autumn of 2020 - although the first positive result was registered, followed by fluctuating quantification cycles - all the results from influent samples (Table 1) were positive, while their effluent sample pairs all tested negative without exception. From this information alone, it can be concluded that the commonly used, complex wastewater treatment technology itself is sufficiently efficient to decrease the SARS-CoV-2 RNA load from wastewater without any additional steps, e.g. UV light treatment or surplus chemicals. Our results concerning the influent-effluent pairs are in good agreement with earlier findings by Haramoto et al. [11]

Table 2: SARS-CoV-2 RNA concentration at different stages of wastewater treatment at the Nagykanizsa WWTP (due to the rules of PCR amplification, higher quantification cycle values are linked to lower SARS-CoV-2 RNA concentrations)

Sample type	Date	Cq value (E gene)
Influent	08/12/2020	+(28.89)
	09/12/2020	+(34.06)
	10/12/2020	+(31.04)
Primary treated	08/12/2020	+(40.04)
	09/12/2020	+(35.43)
	10/12/2020	+(37.13)
Secondary treated	08/12/2020	–
	09/12/2020	–
	10/12/2020	–
Effluent	08/12/2020	–
	09/12/2020	–
	10/12/2020	–

By progressing one step further, samples were tested after primary and secondary treatment at the Nagykanizsa WWTP (Table 2). Over three consecutive days - while the influent samples tested positive, although the primary treated wastewater exhibited a slightly lower concentration of SARS-CoV-2 RNA - the results were still positive (manifesting in higher quantification cycles). From this reduction, it can be seen that the majority of the SARS-CoV-2 particles are not attached to the solid particles sedimented in this step. However, the duration of SARS-CoV-2 particles in wastewater is also an important factor [10] that can be partially responsible for the loss in RNA load compared to the raw influent. After the activated sludge process, the estimated duration of SARS-CoV-2 particles was longer than 8 hours and all samples tested negative. This loss of signal is reasonable considering the lengthy nature of the secondary treatment, the microbial activity together with the limited resistance of these viral particles to external agents [12] as well as the extreme sensitivity of the RNA. As in the case of the earlier tested influent-effluent pairs, all treated effluent samples yielded negative results. Over the last year, numerous other research groups have tested various WWTPs with partially different technological setups [11, 13–16] to that employed at Nagykanizsa WWTP. A general consensus has been reached in all these experiments regarding the negative results of all the effluent material flow (treated

effluent water and sludge line). Even though some studies yielded positive results for some of the secondary treated effluents [11,13] or secondary sludges [16], in these cases all the treated materials exiting the WWTP produced negative results without exception.

4. Conclusion

According to our findings, which are in good agreement with other published research on this topic, the common treatment of wastewater is capable of decreasing the SARS-CoV-2 RNA concentration below the complex detection limit of the multi-step measurement process. WWTPs are capable of minimizing the presence of coronavirus nucleic acid without having to add further steps to the traditional technology. All in all, the SARS-CoV-2 viral particles are particularly stable in aquatic environments, so the treated wastewater discharge and risks related to its reuse have not proved to be considerable.

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