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Short communication

SARS-CoV-2 genetic material is removed during municipal wastewater treatment and is undetectable after advanced treatment

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HIGHLIGHTS
• First study to evaluate SARS-CoV-2 RNA throughout a potable reuse system.
• SARS-CoV-2 RNA was rarely detected after conventional wastewater processes.
• SARS-CoV-2 RNA was undetectable after advanced water treatment.
• Potable reuse is an unlikely transmission route for COVID-19.
• Water reuse practices are safe and effective, and protect public health.

ABSTRACT

The aim of this study was to establish whether SARS-CoV-2 genetic material is detectable after municipal wastewater treatment and to verify its expected removal from purified water that is reclaimed for potable reuse. Viral loads of SARS-CoV-2 (N1 and N2 genes) were monitored in raw influent wastewater (sewage) entering a water reclamation facility and in subsequent advanced treatment. Despite the large viral RNA load in raw sewage during peak COVID-19 outbreaks, substantial amounts of SARS-CoV-2 genetic material were removed during the conventional wastewater treatment process. Further, SARS-CoV-2 genetic material was undetectable after advanced purification. This confirms that potable reuse is resilient against high viral loads which are expected results given the advanced degree of wastewater and water treatment. Findings from this study may enhance public perception of the safety of potable water reuse; however, it should also be noted that studies to date worldwide indicate no evidence of SARS-CoV-2 transmission via water, and the CDC does not consider fecal waste or wastewaters as a source of exposure.

1. Introduction

Public education about potable reuse continues to be important to advance its practice in water stressed communities. With various studies reporting detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in wastewater (Ahmed et al., 2020; Crank et al., 2022), the virus that causes coronavirus disease 2019 (COVID-19), the potential for public concern regarding potable reuse was anticipated. In response, the reuse industry published various public communication materials highlighting the safeguards that protect public health from a variety of contaminants, including novel viruses (EPA, 2022).

It is well-established that COVID-19 is transmitted via respiratory droplets and person-to-person contact, with limited knowledge of disease caused via ingestion. The Centers for Disease Control and Prevention (CDC) does not consider fecal waste or waters as sources of exposure (Bullard et al., 2020; CDC, 2021) and SARS-CoV-2 is not classified as a waterborne pathogen since aquatic environments are not part of the virus' replication cycle and because there is no evidence of infection via water consumption (Cerrada-Romero et al., 2022; Jones et al., 2020). Still, high concentrations of SARS-CoV-2 RNA in raw wastewater around the world have raised public concerns about potential fecal-oral transmission.

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(Duvallet et al., 2022). Recent wastewater-based epidemiology (WBE) studies have focused on monitoring SARS-CoV-2 RNA in raw wastewater to inform public health actions. Such studies rarely surveyed concentrations beyond conventional wastewater treatment because evidence suggests COVID-19 is not transmitted through water and wastewater and because of the well-reasoned assumption that the virus would not be able to survive stringent advanced purification processes (Gerrity et al., 2022; Pecson et al., 2020).

The present study evaluated SARS-CoV-2 RNA concentrations throughout a potable reuse system comprised of a conventional wastewater treatment facility and an advanced water purification facility. To the authors’ knowledge, this is the first study to confirm the expected removal of SARS-CoV-2 RNA from reclaimed wastewater for potable reuse. Documented removal of the novel coronavirus provides relevant and useful information to the water industry. The findings from this study may promote public perception and trust for the potable reuse practice, in particular for removing viral pathogens, from drinking water supplies.

### 2. Methods

In this study, wastewater and water samples were collected from September 2020 to February 2022 from the Orange County Sanitation District (OC San) Reclamation Plant No. 1 (OC San P1) and the Orange County Water District (OCWD) Advanced Water Purification Facility (AWPF), both located in Fountain Valley, CA USA. The AWPF purifies OC San P1 secondary effluent for groundwater recharge for potable reuse (see graphical abstract). In total, 180 raw sewage samples, 17 chlorinated effluent samples (denoted here as microfiltration (MF) feed water), and 14 ultraviolet light-advanced oxidation process (UV-AOP) samples were collected (see supporting material for details).

Raw sewage composite samples (1 L) were collected using an automated composite sampler three times per week by OC San staff, and a 30 mL aliquot was delivered to Zymo Research Corporation in Irvine, CA for analysis. Direct extraction was performed on raw wastewater (0.5-5 mL) using Zymo Environ Water RNA kit (R2042), followed by RT-qPCR (CDC, 2022). AWPF MF feed water (31–60 L) and UV-AOP product water (303–470 L) were concentrated using a Rexeed-25 S ultrafilter (Innovaprep LLC, Drexel, MO) followed by secondary concentration using a Centricon® Plus-70 centrifugal filter unit (100 kDa), and preserved at −20 °C on-site at OCWD (Polanco et al., 2022; Sherchan et al., 2020). Samples from AWPF, consisting of feed and product water concentrates, were shipped to the Yuma Center of Excellence for Desert Agriculture (YCEDA) for analysis. All samples were processed for SARS-CoV-2 N1 and N2 genes using RT-qPCR as described by Betancourt et al., (Betancourt et al., 2021; CDC, 2022). The limit of detection and limit of quantitation were calculated as previously described (Betancourt et al., 2021). For non-detect samples, the limit of detection was calculated as a function of the total volume of water sampled (see Table 1). Spiked control samples were performed using φ6 phage (DSM No. 21518) to assess virus recovery (Table S.3).

Clinical incidence data were collected from the Orange County Health Care Agency dashboard ([Dataset] Orange County Health Care Agency, 2022). Data processing and statistical analyses were performed using Microsoft Excel (version 16.47.1, 2021) and Kaleidagraph (Synergy Software, version 4.5.4, 2018).

### 3. Results

SARS-CoV-2 concentrations in raw sewage ranged from non-detect (23 out of 180) to 5.1 × 10^7 gene copies per liter. Viral loads in sewage trended with the 7-day average of newly reported COVID-19 cases in Orange County, CA (Fig. 1A). This observation is consistent with a number of studies throughout the world in which virus concentrations in wastewater corroborated with community disease incidence (Abu Ali et al., 2021; Al-Faliti et al., 2022). Despite high concentrations of the virus in raw sewage, SARS-CoV-2 RNA was rarely detected (4 of 17) in chloraminated secondary effluent (MF feed) samples. Surveillance of SARS-CoV-2 genetic material through the AWPF showed that all UV-AOP samples (14 of 14) were non-detect for SARS-CoV-2 N1 and N2 genes (Fig. 1, Table 1).

### Table 1

SARS-CoV-2 RNA sampling summary.

| Sampling site                  | Date range       | No. of detections | Volume filtered | SARS-CoV-2 gene copies per liter (GC/L) | Method detection limit (GC/L) |
|-------------------------------|------------------|-------------------|-----------------|----------------------------------------|-------------------------------|
| Raw wastewater influent       | 9/2020 to 2/2022 | 157               | 0.5–5 mL        | N/A                                    | Non-detect 5.3 × 10^3 to 5.1 × 10^7 3.4 × 10^6 to 1.6 × 10^8 |
| MF feed (chloraminated secondary effluent) | 9/2020 to 2/2022 | 4/17              | 31–60 L        | 51 L                                   | Non-detect 1.1 × 10^7 to 1.6 × 10^3 1.1 × 10^5 to 1.4 × 10^7 |
| UV-AOP product water          | 12/2020 to 2/2022 | 0/14              | 303–470 L      | 414 L                                  | Non-detect 1.0 × 10^3 to 3.1 × 10^4 |

Notes: GC/L = gene copies per liter; N/A = Not applicable; MF = microfiltration; UV-AOP = ultraviolet advanced oxidation process.

![Fig. 1. COVID-19 incidence rates in Orange County, CA compared to SARS-CoV-2 RNA occurrence in study sample. A) COVID-19 seven day average daily positive case rate per 100 k individuals (data obtained from Orange County Health Care Agency and the California Department of Public Health). B) SARS-CoV-2 N1 concentrations observed for OC San P1 raw wastewater samples and SARS-CoV-2 N1 and N2 concentrations observed for OCWD AWPF MF feed and UV-AOP product water samples. Non-detect data for raw wastewater are not plotted. MF feed samples above the limit of quantitation are denoted with an (*); the remaining MF feed and UV-AOP product water results were below the limit of quantitation.](image-url)
4. Discussion

The concentration of SARS-CoV-2 N1 gene copies in raw wastewater influent entering OC San P1 align chronologically with the daily COVID-19 cases per capita in Orange County, CA (Fig. 1B). This finding further supports how recurrent outbreaks of COVID-19 in the community contribute to SAR-CoV-2 viral RNA loading into the local wastewater treatment facility (Duvall et al., 2022; McMahan et al., 2022). Despite the large viral RNA load during peak outbreaks, it is evident that a substantial amount of SARS-CoV-2 genetic material is removed during the conventional wastewater treatment process (Table 1), here indicated by the limited detections seen with chloraminated secondary effluent samples (MF feed). Prior work found that peak viral loads can be effectively dispersed (i.e. flattening the concentration distribution) during wastewater treatment due to engineered hydraulic conditions contributing to virus reduction (Gerrity et al., 2022).

OC San supplies the AWPF with non-disinfected secondary effluent water, where OCWDS adds chlorine to form chloramines prior to MF treatment to control membrane biofouling. Sampling MF feed after chlorine addition in this study therefore includes approximately 1 min of chloramine contact time which may contribute to the typically non-detectable occurrence of the SARS-CoV-2 N1 gene at this stage. Studies have shown that SARS-CoV-2 is highly susceptible to degradation by chlorination (Pecson et al., 2020; Ye et al., 2016). With additional chloramine contact time through MF (approximately 20 min-post MF equalization tanks), all SARS-CoV-2 virus particles, including genetic material, are likely destroyed. Beyond MF, virus particles and viral genetic material are not expected to persist through the reverse osmosis treatment barrier due to size exclusion (Adham et al., 1998; Black and Veatch, 2009) coupled with a significant dose of UV light during AOP treatment that far exceeds that required for pathogen disinfection (Pecson et al., 2020). This is further confirmed with all non-detect data observed for SARS-CoV-2 RNA in the present study after UV-AOP treatment.

The findings in this study demonstrate substantial removal of SARS-CoV-2 genetic material during wastewater treatment. Although SARS-CoV-2 RNA was rarely detected following secondary wastewater treatment, the study findings further show that the virus was undetectable after advanced purification. Advanced purification treatment trains incorporate multiple barriers such that potable reuse is resilient against peak viral loads, providing confidence in water reuse practices and ensuring protection of public health.

CRediT authorship contribution statement

Julio A. Polanco: Conceptualization, Formal Analysis, Writing – Original draft preparation, Visualization, Investigation, Methodology, Sarah Prasek: Resources, Writing – Review & Editing, Samuel Choi: Investigation, Formal Analysis, Validation, Writing – Review & Editing, Jana Safari: Methodology, Resources, Supervision, Validation, Bradley Schmitz: Methodology, Validation, Writing – Review & Editing, Resources, Megan H. Plumlee: Conceptualization, Supervision, Writing – Review & Editing.

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Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2015975.

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