Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
SARS-CoV-2 removal by mix matrix membrane: A novel application of artificial neural network based simulation in MATLAB for evaluating wastewater reuse risks

Sasan Zahmatkesh\textsuperscript{a,b,*}, Yousof Rezakhani\textsuperscript{c}, Abdoulmohammad Gholamzadeh Chofreh\textsuperscript{d}, Melika Karimian\textsuperscript{e}, Chongqing Wang\textsuperscript{f}, Iman Ghodrati\textsuperscript{g}, Mudassir Hasan\textsuperscript{h}, Mika Sillanpaa\textsuperscript{i,j,k}, Hitesh Panchal\textsuperscript{l}, Ramsha Khan\textsuperscript{m}

\textsuperscript{a} Department of Chemical Engineering, University of Science and Technology of Mazandaran, P.O. Box 48518-78195, Behshahr, Iran
\textsuperscript{b} Tecnologico de Monterrey, Escuela de Ingeniería y Ciencias, Puebla, Mexico
\textsuperscript{c} Department of Civil Engineering, Pardis Branch, Islamic Azad University, Pardis, Iran
\textsuperscript{d} Sustainable Process Integration Laboratory, SPIL, NETME Centre, Faculty of Mechanical Engineering, Brno University of Technology, VUT Brno, Technická 2896/2, 616 00, Brno, Czech Republic
\textsuperscript{e} Faculty of Civil Engineering, Architecture and Urban Planning, University of Eyvanekey, Iran
\textsuperscript{f} School of Chemical Engineering, Zhengzhou University, Zhengzhou, 450001, China
\textsuperscript{g} Faculty of Science and Technology, School of Applied Physics, University Kebangsaan Malaysia, 43600, Bangi, Selanger, Malaysia
\textsuperscript{h} International Research Centre of Nanotechnology for Himalayan Sustainability (IRCNHS), Shoolini University, Solan, 173212, Himachal Pradesh, India
\textsuperscript{i} Department of Chemical Engineering, College of Engineering, King Khalid University, Abha, 61411, Saudi Arabia
\textsuperscript{j} Mechanical Engineering Department, Government Engineering College, Patan, Gujarat, India
\textsuperscript{k} Department of Chemical Engineering, Institute of Technology, Shri Ramswaroop Memorial University, Barebanki, 225003, UP, India

HIGHLIGHTS

- A PC-Ag-NP MMM removes SARS-CoV-2 with a higher efficiency than a PC-HMO MMM
- Models for ultrafiltration membrane treatment based on mixed matrix membrane
- SARS-CoV-2 reduction using artificial neural networks (ANNs)
- Nanoparticles can be used effectively in wastewater

ABSTRACT

The COVID-19 outbreak led to the discovery of SARS-CoV-2 in sewage; thus, wastewater treatment plants (WWTPs) could have the virus in their effluent. However, whether SARS-CoV-2 is eradicated by sewage treatment is virtually unknown. Specifically, the objectives of this study include (i) determining whether a mixed...
1. Introduction

Various activities can cause municipal wastewater, including bathing, toileting, washing clothes, etc (Chuah et al., 2022). Salons, wood mills, and other commercial establishments can generate industrial wastewater (Zahmatkesh and Pirouzi, 2020). Wastewater contains 99% water, and 1 percent is an additive that must be considered because it can pose a health risk (Sun et al., 2019). This information appears on the Institute of Agriculture and Natural Resources profile page accessed in 2022a). Even if RNA is detected for SARS-CoV-2, infectious viral particles are unclear whether it poses a transmission risk (Zahmatkesh et al., 2022a). SARS-CoV-2 virus after patients had disposed of it from their stool (WWTP) in various countries detected raw wastewater containing the SARS-CoV-2 virus (Patriarca et al., 2017).

According to a cohort study, COVID-19 may have been transmitted via wastewater in an environment with poor sanitation (Zahmatkesh et al., 2022b). The reduction of the possibility of infection threats from SARS-CoV-2 in untreated municipal water must be known to be able to control and minimize its potential (Zahmatkesh et al., 2022a). The threat of infection risk from SARS-CoV-2 is unknown in untreated wastewater (Jones et al., 2020; Lim et al., 2021). Past studies have concentrated on enteric viruses with non-enveloped capsids, which contain protein capsids. The SARS-CoV-2 virus is enveloped and protected from external agents by phospholipid bilayer membranes surrounding it. Due to the vulnerability of enveloped viruses, wastewater treatment processes reduce enveloped viruses more efficiently than non-enveloped viruses (Mohapatra et al., 2021).

The capability of mixed matrixed membrane (MMMs) to reduce SARS-CoV-2 was evaluated using a neural network approach to artificial intelligence. The effect of PC-HMO and PC-Ag-NP MMMs on SARS-CoV-2 was therefore investigated in secondary WWTP effluent. Untreated wastewater containing SARS-CoV-2 should be treated to reduce the possibility of ecological risks. These findings can assist in decreasing the prevalence of SARS-CoV-2.

2. The collection and processing of samples

From April to May 2022, 100 samples covering moderate and low-risk periods were collected at Shariati Hospital in Mashhad, Iran (Fig. 1). In these facilities, wastewater potentially associated with infected individuals was handled. Samples of COVID-19 were collected from hospital sewage systems and the surrounding environment. Comprehensive cleaning of wastewater from wastewater systems, streams, rivers, and lakes was followed by a collection of samples of the environment. Throughout the study, sewage tests were primarily conducted in one district: the secondary hospital WWTP of Shariati Hospital was used to collect samples in the Torghabeh district. As a result, specimens were transported to the analysis facility within 8 h of their collection as part of a cold-chain transportation protocol (Fig. 2).

2.1. RNA extraction and RT-qPCR

After concentrating samples in a laboratory, RNA was extracted using an ELISA kit (Pishitzeb, Tehran, Iran) following the package guidelines. Following that, real-time quantitative PCR (RT-qPCR) was carried out using primer and probe sets targeting the RBD2 and ORF1ab genes (Table 1). To prepare standard RNA samples, RT-droplet digital polymerase chain reaction (RT-ddPCR) was carried out at 10-fold sequential diluted concentrations of SARS-CoV-2 at 107–101 particles per milliliter (PFU/mL).

3. Fabricate membrane

The synthesis of a membrane is described in detail using the casting solution. To dissolve the appropriate substances in N-methyl-2-pyrrolidone (NMP), including polycarbonate (PC), glycerol, cellulose acetate matrixed membrane (MMM) is able to remove SARS-CoV-2 (polycarbonate (PC)-hydrous manganese oxide (HMO) and PC-silver nanoparticles (Ag-NP)), (ii) comparing filtration performance among different secondary treatment processes, and (iii) evaluating whether artificial neural networks (ANNs) can be employed as performance indicators to reduce SARS-CoV-2 in the treatment of sewage. At Shariati Hospital in Mashhad, Iran, secondary treatment effluent during the outbreak of COVID-19 was collected from a WWTP. There were two PC-Ag-NP and PC-HMO processes at the WWTP targeted. RT-qPCR was employed to detect the presence of SARS-CoV-2 in sewage fractions. For the purposes of determining SARS-CoV-2 prevalence rates in the treated effluent, 10 L of effluent specimens were collected in middle-risk and low-risk treatment MMMs. For PC-HMO, the log reduction value (LRV) for SARS-CoV-2 was 1.3–1 log10 for moderate risk and 0.96–1 log10 for low risk, whereas for PC-Ag-NP, the LRV was 0.99–1.3 log10 for moderate risk and 0.94–0.98 log10 for low risk. MMMs demonstrated the most robust absorption performance during the sampling period, with the least significant LRV recorded in PC-Ag-NP and PC-HMO at 0.94 log10 and 0.96 log10, respectively.

Acknowledgments

This research was supported by the Islamic Azad University of Mashhad, Iran (Grant No. 890373).

Keywords:
Mix matrix membrane
SARS-CoV-2
Wastewater treatment
Artificial neural network

Chemosphere 310 (2023) 136837
2
(CA), HMO, or Ag NPs, the appropriate materials were mixed in NMP. This paper describes an experiment using PC-HMO or PC-Ag-NP depending on the ratio of inorganic nanoparticles in the casting solution \( y = 0.15 \). Under a pressure of 0.2 bar, the pure water flux could reach 415 L m\(^{-2}\) h\(^{-1}\) with the membrane size of 250 mm \( \times \) 260 mm \( \times \) 8 mm. The membrane had a porosity of 100 nm and a mechanical strength of 1 bar (Zahmatkesh et al., 2022a).

During the preparation of an inorganic nanoparticle suspension, PC, CA, HMO, and Ag Ng were dried at 99 °C for 18 h. They were stirred for 8.30 h at 39 °C. Sonication of a predetermined amount of PC suspension was conducted in NMP for 30 min to dissolve and agitate it for 18 h at 39.9 °C. Once the polymer suspension was prepared, insoluble components, such as glycol and carboxymethylcellulose, were gradually added to the nanoparticle suspension. A mechanical stirrer was applied to agitate the suspension at 40 °C for 24 h. This was until the ideal consistency was achieved after mixing the latter suspension with the nanoparticle suspension. A uniform and flawless glass plate could only be achieved by casting the glass on a cooled container; therefore, the casting solution had to be cooled to ambient temperature. Water had to be added to the mix in order for it to coagulate. It was necessary to keep the membrane intact by placing it in a deionized water bath for 48 h after completion and peeling it off the glass plate. A daily membrane change was conducted to remove residual NMP. After drying the membranes at room temperature for 24 h, they were coded according to their HMO and Ag NP loadings.

### 3.1. Virus concentration

Using hospital wastewater collected at secondary effluent WWTPs, Alamin et al. (2022) demonstrated that SARS-CoV-2 concentrations can be determined by centrifuging at 2500 g for 2.5 min at 3.5 °C (Alamin et al., 2022). Following the centrifugation, PEG precipitation was performed to concentrate the liquid fraction. The specimens were then maintained overnight at 4 °C with an 8% PEG8000 solution and a 1 M NaCl solution. The pellets were suspended in 250L phosphate buffer solution after centrifugation at 5500g for 25 min at 3.5 °C. The virus concentration was first achieved using an ultrafiltration membrane device within 24 h of sampling. In preparation for the filtration of samples, 250 mL of 3% FBS solution was circulated on the membrane unit. A 10.1 ppm concentration of antifoam A was added to an elution buffer that included Tween 59.9 (0.14%), sodium polyphosphate (79.9 mg/L), and antifoam A (10.1 ppm) for eluting virus concentrate left in membrane units after filtering 10 L of wastewater extract. The PEG precipitation method was used to concentrate the recovered extract (approximately 100–250 mL). RNA was extracted from the concentrated samples after they had been kept at −70 °C for several days. A viral RNA ELISA kits (Pishpaz Teb, Tehran, Iran) was used to extract virus RNA from 150 L of concentrate.

### 4. Analyzing and designing experiments based on artificial intelligence

The treatment of effluents with membranes has been demonstrated to be one of the most sustainable and environmentally friendly methods. ANN solutions are offered in the literature to promote membranes that are able to handle diverse effluents. Although these technologies are state-of-the-art and environmentally friendly, there are still some challenges to overcome.

According to the widely accepted artificial intelligence approach, a neural network is modeled as a biological neuron and classified as a computational intelligence model. These models facilitate effective and accurate process descriptions by analyzing processes’ nonlinear connections. Through computer algorithms and statistics, artificial neural networks (ANNs) simulate human brain function. To establish synaptic weighted values, functional parts are connected to artificial neurons through synapses. A neuron sends an output after receiving input from another. As a result, the next neuron’s input characteristics are determined by the input parameters of the last neuron. An algorithm that analyzes training data and identifies relationships between data points simulates, predicts, and optimizes the performance of a system.

A model based on ANNs was extracted to better understand membrane mechanisms. PC-HMOs and PC-Ag-NPs used in wastewater treatment are predicted by machine learning algorithms.

#### 4.1. Approach based on artificial neural networks

Prior to the development of computers, ANNs were designed by merging neurons derived from living organisms and combining them with parallel processors. According to Fig. 3, neuron models are characterized by weighted inputs, bias terms, and transfer functions that illustrate how these variables are transformed into outputs. Data collected from experiments and the field directly informs ANNs, which is a significant advantage. Modeling complex challenges using them provides a fundamentally different perspective than conventional approaches. An artificial neural network can provide a practical solution to a wide range of civil engineering problems involving numerical, characterized, and linear functions. In civil and chemical engineering, ANNs are used to plan construction projects, estimate damage, predict the impact of disasters, characterize the impact of compaction on construction models, predict river flows, and enhance structures. A software coding approach must be implemented into the ANN algorithm to guarantee its quality for many years. People, as well as machine-learning neural networks, learn from practice, examples, and experience. There are three layers in this neural network simulation: data layers, concealed layers, and result layers. There is no specific rule that applies to hidden layers and nodes (Zahmatkesh et al., 2022a).

The five-layer hidden layer algorithm exhibited the most effective...
performance when regressed against squared values. It was a trial-and-error procedure that led to this judgment. A database was collected using the computer program MATLAB (R2021b) (Fig. 3), based on prior studies using PC-HMO and PC-Ag-NP parameters to obtain the output. When several hidden layers, nodes, and epochs are varied in an ANN analysis, an accurate prediction value can be obtained for eliminating SARS-CoV-2.

4.1.1. ANN methodology explanation

There are two layers: the data layers and the concealed layers. For input \( x_i \), the weight \( w_{ij} \) between neurons \( i \) and \( j \) of the hidden layer is multiplied by input \( x_i \). A summation of biases \( b_j \) is followed by calculating net input \( I_j \) based on the summation of biases \( b_j \) in Eq (1). By applying a logarithmic sigmoid response equation to \( x_i \), Eq (2) obtains \( y_j \) from the input layer:

\[
I_j = \sum_{i=1}^{n} x_i w_{ij} + b_j
\]

\[
f(I) = \frac{(1 - e^{-2I})}{e^{2I}}
\]

\[
y_j = f(I_j)
\]

The concealed layer transmits the \( s_j \) out a pulse to each of \( z \) neurons in a layer from which it extracts input to calculate input for the \( z \) neurons in the output layer (Eq (4)):

\[
o'z = \sum_{j=1}^{b} s_j w_{jk} + b'_k
\]

In the hidden layer, \( w'_{jk} \) represents the weights associated with interactions between \( j \) and \( k \) neurons, and \( b'k \) represents the bias.

Using Eq (5) as a final calculation, the response layers are determined using the exponential coefficient method.

\[
O_k = f'(O'_k)
\]

Two layers of feedforward and backpropagation network

\[
Z = \text{Bias} + Y_1K_1 + Y_2K_2
\]

Network training and testing

5. Result and discussion

5.1. Log reduction value calculation

SARS-CoV-2 log reduction value (LRV) has been determined by comparing each process’s total virus loads in effluent and treated wastewater based on the equations provided below. The virus concentration in secondary effluent wastewater can be calculated using secondary effluent wastewater. A flow-rate weighted average of the virus levels in the sewage before and after membrane treatment was used to

**Table 1**

| Aim     | Summary | Oligonucleotide Sequence (5’->3’ | reference |
|---------|---------|---------------------------------|-----------|
| RBD2    | Forward | GAC CCC AAA ATC AGC GAA AT      | Zahmatkesh et al. (2022a) |
| Reverse | Primer  | TCT GGT TAC TGC CAG TTG AAT     |           |
| Primer  |         | CAT TAC GTT TGG                 |           |
| Probe   |         | FAM-ACC CGG CAT BHYQ1           |           |
| ORF1ab  | Forward | FAM-ACC CGG CAT/ZEN/TAC         |           |
| Reverse | Primer  | GTT TGG TGG ACC-3IAekFQ         |           |
| Primer  |         | TTA CAA ACA TTG GCC GCA AA      |           |
| Probe   |         | GCG CCA CAT TCC GAA            |           |

\[
N_1 = Y_{11}K_1 + Y_{12}K_2 + Y_{13}K_3 + Y_{14}K_4 + Y_{10}
\]

\[
N_2 = Y_{21}K_1 + Y_{22}K_2 + Y_{23}K_3 + Y_{24}K_4 + Y_{20}
\]

\[
N_3 = Y_{31}K_1 + Y_{32}K_2 + Y_{33}K_3 + Y_{34}K_4 + Y_{30}
\]

\[
N_4 = Y_{41}K_1 + Y_{42}K_2 + Y_{43}K_3 + Y_{44}K_4 + Y_{40}
\]

\[
N_5 = Z_{51}X_1 + Z_{52}X_2 + Z_{53}X_3 + Z_{54}X_4 + Z_{50}
\]

Algorithm used in this study (Hyperbolic tangent)

\[
g(z) = \frac{\exp(2z) - 1}{\exp(2z) + 1}
\]

Fig. 2. The results of all samples tested are summarized in this report. In each block, the number represents the percentage of samples that tested positive among several tests that tested negative.
calculate the LRV in the entire WWTP. 

\[ R_{\text{Effluent Wastewater}} = -\log_{10} \left( \frac{C_{\text{Effluent Wastewater}} \cdot (Q_{\text{before treatment}} + Q_{\text{after treatment}})}{C_{i1} \cdot Q_{i1} + C_{i2} \cdot Q_{i2}} \right) \]

\[ R_{\text{Before treatment}} = -\log_{10} \left( \frac{C_{\text{before treatment}} \cdot Q_{\text{before treatment}}}{C_{i1} \cdot Q_{i1}} \right) \]

\[ R_{\text{After treatment}} = -\log_{10} \left( \frac{C_{\text{after treatment}} \cdot Q_{\text{after treatment}}}{C_{i1} \cdot Q_{i1}} \right) \]

The effluent wastewater R represents LRV after the second process at the target WWTP; the effluent water R before the second process; the effluent water R after the second process; the effluent water LRV by C before and after treatment. The final effluent of the target WWTP is characterized by its virus concentration (copies/L); the virus concentrations in the treated effluent before and after treatment; C1, C2, and the virus concentrations in wastewater before and after membrane treatment. Q before treatment, Q after treatment: flow rates of treated effluent before and after treatment (m3/d); Q1 before treatment, Q1 after treatment: flow rates of wastewater from the effluent before treatment distributed into before and after treatment (m3/d); Q2: Amount of sewage flowing from after treatment (m3/d).

5.2. SARS-CoV-2 RNA concentration in effluent hospital sewage

SARS-CoV-2 RNA amounts in middle-risk effluents ranged from 3 to 3.4 log10 copies/L to 2–2.4 log10 copies/L in low-risk effluents. A notable percentage of patients infected with SARS-CoV-2 significantly differed between moderate-risk and low-risk effluent samples (p = 0.01 < 0.05). A high SARS CoV-2 prevalence was identified between April 6 and April 12 (Fig. 4). Consequently, several mutant COVID-19 outbreaks in the study locale were not statistically strong indicators of increased SARS-CoV-2 levels in water samples. Frequent routine collection is recommended to obtain correlated data, preferably weekly. This was
likely because the measurement period was too long to capture the increasing or decreasing rate of infections.

According to the effluent sewage samples, SARS-CoV-2 RNA was significant, especially in the middle-risk fraction, whereas the amount was higher in the secondary treatment effluent fraction. It has been confirmed that SARS-CoV-2 is prevalent in 10 effluent samples in the liquid fraction, and all of them were significantly detected at 3 to 4 log10 copies/L. Past studies have also demonstrated that sewage RNA is abundant in the presence of SARS-CoV-2 RNA. According to Kitamura et al. (2021), SARS-CoV-2 prevalence was 3.0–3.6 log10 copies/L in the secondary treatment effluent fraction (Kitamura et al., 2021). Li et al. (2022) indicated that SARS-CoV-2 amounts of 1.4–6.2 log10 copies/L in the secondary treatment effluent fraction (Li et al., 2022). A secondary treatment effluent fraction of SARS-CoV-2 spiked in sewage was partitioned by Alamin et al. (2022). In the following discussion, log10 elimination rates were calculated based on SARS-CoV-2 concentrations in untreated wastewater fractions.

5.2.1. Removal of SARS-CoV-2 by MMMs
SARS-CoV-2 RNA concentrations in secondary treatment effluent (before treatment by MMMs) were between 3.4 and 3 log10 copies/L in middle-risk patients and 2.4–2 log10 copies/L in low-risk patients. There was a significant variation in the rate of determination of SARS-CoV-2 concentrations among MMMs, using both PC-HMOs and PC-Ag-NPs (p < 0.05). Nevertheless, MMMs (PC-HMO) effluent had 1.6 log10 copies/L of SARS-CoV-2, while MMMs (PC-Ag-NP) effluent had 1.3 log10 copies/L of SARS-CoV-2. According to the results, the SARS-CoV-2 concentration in PC-Ag-NP effluent was stably lower than in PC-HMO effluent. The effluent from the CAS process was reported to contain 3.29 log10 copies/L of RNA by Haramoto et al. (2020), LODs in effluent ranged from 2.2 to 3.29 log10 copies/L (Haramoto et al., 2020).

For middle-risk patients, LRVs by secondary treatment by PC-HMO ranged from 1.6 to 1.3, while low-risk patients reported LRVs between 0.96 and 1. Middle-risk patients received LRVs between 0.99 and 1.3, and low-risk patients received LRVs between 0.94 and 0.98. The SARS-CoV-2 amounts were significantly reduced among low-risk patients in May, and LRV values differed significantly between low-risk and middle-risk patients (p < 0.05). The LRVs of PC-HMO in middle- and low-risk were 1.3 and 0.96, respectively, a difference of 1.0 logs in middle-risk. There was a notable disparity in the SARS-CoV-2 RNA levels between PC-Ag-NP and PC-HMO, which demonstrates that PC-Ag-NP has a more significant effect than PC-HMO. In all processes, LRVs were almost 1.6 log or higher, indicating remarkable differences among the processes (Fig. 5). Serra-Compte et al. (2021) showed a significant decrease of 1.0–2.9 log of SARS-CoV-2, whereas the reduction measured in this study was substantially more significant (Serra-Compte et al., 2021). Serra-Compte et al. (2021) showed that SARS-CoV-2 quantification in MBR was performed on only the liquid fractions from influent wastewater. In wastewater treatment, viruses are primarily reduced by adsorption on sludge flocs and filtration of sludge where they are adsorbing.

5.2.2. ANN for eradicating SARS-CoV-2
In accordance with the validation, testing, and training of the SARS-CoV-2 removal concentration in the Fig. 6 the SARS-CoV-2 removal rates were significantly lower than the reported enteric virus removal rates for PC-HMO and PC-Ag-NP processes. Noroviruses showed LRVs ranging from 1.3 to 2.9 in activated sludge and 4.6 to 5.7 in MBR (Zahmatkesh et al., 2022a). The enteroviruses showed LRVs of 2–3 in activated sludge and 3.4–5.1 in MBR. Adenoviruses have been reported to be 2.0–3.0 in activated sludge and 3.7–5.6 in MBR. As a result of these studies, some enteric viruses were found to have lower LRVs than those produced by PC-Ag-NP and PC-HMO. Kumar et al. (2021a,b) anticipate that the SARS-CoV-2 virus will be significantly reduced in wastewater treatment over enteric viruses (Kumar et al., 2021). SARS-CoV-2 is an enveloped virus with a relatively hydrophobic bilayer membrane; therefore, it may bind more effectively to sludge flocs than non-enveloped enteric viruses. Ye et al. (2016) found that a four-fold increase in adsorption on solid particles was observed for MHV and q6 with envelopes in wastewater compared with MS2 and T3 without envelopes (Ye et al., 2016). This experiment showed a higher percentage decrease in SARS-CoV-2 in PC-HMO and PC-Ag-NP. Several factors contribute to the SARS-CoV-2 decline in these procedures, including the presence of tiny pores within PC-Ag-NP and PC-HMO membranes. Due to their density and thinness, particles adhere to the surface, which is one of the primary mechanisms underlying these processes. A remarkable difference was also observed between the reductions of SARS-CoV-2 in PC-Ag-NP and PC-HMO compared with those of encapsulated gastrointestinal viruses.

6. Comparing this study with others
It was observed that PC-HMO and PC-Ag-NP were capable of reducing 89% and 91% of SARS-CoV-2 RNA, respectively. In each case, the minimum LRV was 0.94 and 0.96, respectively. After chlorination, SARS-CoV-2 is also identified below an acceptable diagnostic threshold of 2 log10 copies/L or 2.6 log10 copies/L in the effluent. According to Kumar et al. (2021a,b), WWTP effluents containing SARS-CoV-2 RNA would be low in concentrations (Kumar et al., 2021). According to the results of this study, SARS-CoV-2 LRVs range between 1.5 and 3.9 log10 copies/L, which is comparable to or lower than norovirus and enterovirus LRVs. Through chlorination, at least 0.95 logs of SARS-CoV-2 RNA were eliminated. Enteroviruses were significantly reduced by less than 0.5 log by chlorination compared to SARS-CoV-2, which had a significantly more intensive chlorination LRV than other enteric viruses. Inactivated viruses may have retained some viral RNA, resulting in a relatively low LRV following chlorination. Chlorination will reduce SARS-CoV-2 infectivity more than viral RNA by reducing infectivity. Bivins et al. (2021) reported that SARS-CoV-2 RNA is more significant in
stabilizing the virus than its infectivity (Bivins et al., 2021). This study found a significant difference between SARS-CoV-2 RNA concentrations and infectious SARS-CoV-2 concentrations in the effluent.

6.1. Comparison of activated sludge and membranes

MMMs have been reported to reduce enteric viruses more effectively than activated sludge. Although MMMs and activated sludge significantly decreased SARS-CoV-2, the percentage decline varied widely. There are two main methods by which viruses are reduced in WWTPs: (i) adsorption on sludge particles and (ii) separation of sludge where viral particles have been adsorbing. In accordance with the previous study’s findings, high levels of MLSS within the membrane are reported to enhance adsorption. This results in the more effective removal of enteric viruses. Chaudhry et al. (2015) found membrane filtration, including cake layer filtration, to be a critical channel for removing enteric viruses from wastewater with a full-scale membrane. Chaudhry et al. (2015) found that cake filtration reduced the number of free viral particles in the liquid fraction by more than 50% (Chaudhry et al., 2015). While free viral particles are usually more prominent than the average porosity of virgin UF membranes, forming a cake layer on the surface of the membrane increases this reduction, thereby enhancing rejection. According to Wang et al. (2021), a preliminary fouling layer improved reduction by up to 0.7 log for extracellular plasmids of smaller hydraulic diameter than nominal pores (Wang et al., 2021).

For this reason, activated sludge is less effective at destroying enteric viruses than fresh sludge. Membrane filtration, however, is more efficient at destroying enteric viruses. The liquid fraction of wastewater is more effectively filtered through membranes than settled in activated sludge due to the fact that membrane filtration reduces viral particles more effectively. SARS-CoV-2 was significantly reduced in this study compared to PC-HMO, and PC-Ag-NP reported reductions in enteric viruses. PC-HMOs and PC-Ag-NPs had significantly lower levels of SARS-CoV-2 in comparison to the enteric viruses. The MMMs were significantly lower in levels of SARS-CoV-2 than enteric viruses after membrane filtration. The membrane filtration can also eliminate viral particles in liquid fractions. Compared to other enteric viruses, MMMs demonstrated a remarkable reduction in SARS-CoV-2. Compared to activated sludge, PC-HMO and PC-Ag-NP had enhanced SARS-CoV-2 reduction, which resulted in a lower LRV of SARS-CoV-2. The MMMs were reduced more steadily than in activated sludge. Because the PC-HMO and PC-Ag-NP reductions were reduced, particles adhered to the surface better.

7. Conclusion

WWTP had SARS-CoV-2 RNA levels of 3–4 log10 copies/L in secondary effluent wastewater from middle-risk and low-risk patients at the target hospital. In MMMs treatment, the recorded LRVs of SARS-CoV-2 were 1.3–1 log10 in PC-HMO for moderate risk and 0.96–1 log10 in PC-Ag-NP, while LRVs in PC-Ag-NP were 0.99–1.3 log10 for moderate risk and 0.94–0.98 log10 for low risk. SARS-CoV-2 LRV-2 was less active than typical LRVs of non-enveloped enteric viruses. There was a significant difference in optimization results between the two methodologies, with the most favorable LRV in PC-HMO being 0.96, which is more than 0.2 log higher than the most favorable LRV in PC-Ag-NP (0.94) in MMMs. This study found no significant relationships between operating parameters (DO, HRT, MLSS, SRT, etc.) and SARS-CoV-2 reduction. The findings indicate that ANNs are an excellent tool for validating, testing, and training MMMs for reducing SARS-CoV-2 in a variety of treatment procedures. Due to the fact that, in the developed ANN models, reduced SARS-CoV-2 percentages are significantly associated with observed and expected values, and ii) reduced SARS-CoV-2 efficiency percentages are estimated with R2 values ranging from 0.99 to 0.99.

Credit authors statement

Yousof Rezakhani: Collected the research articles, analyzing, testing, and prepared the first original draft, screened. Sasan Zahmatkesh: Writing-reviewing, editing Software, and analyzed the key findings, Supervision, original draft Hitesh Panchal: Software, and
analyzed, Abdoulmohammad Gholamzadeh Chofreh: Writing - review & editing, Funding, Validation Ramsha Khan: Writing - original draft, Mika Sillanpaa: Writing - review & editing, Supervisor, Validation Chongqing Wang: Supervision, Writing-review & editing, Validation Iman Ghodrati: response to reviewer & software, Melika Kariamian: response to reviewer & software, writing, editing, Mudassir Hasan: response to reviewer & funding.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgement

Throughout this study, Sustainable Process Integration Laboratory - SPIL has been supported by the EU European Structural and Investment Funds as project No. CZ.02.1.01/0.0.0/15.003/0000456, as well as the Czech Ministry of Education, Youth and Sports Operational Programme Research, Development and Education funded by the European Structural and Investment Funds, and This work was supported by the Deanship of Scientific Research at King Khalid University, Abha-KSA, for finding this research through General Research Project under grant number (R.G.P. 2/189/43).

References

Alamin, M., Tuji, S., Hata, A., Har-Ayamcura, H., Honda, R., 2022. Selection of surrogate viruses for process control in detection of SARS-CoV-2 in wastewater. Sci. Total Environ. 823, 153737.
Bivina, A., Kaya, D., Bibby, K., Simpson, S.L., Butin, S.A., Shank, O.C., Ahmed, W., 2021. Variability in RT-qPCR assay parameters indicates unreliable SARS-CoV-2 RNA quantification for wastewater surveillance. Water Res. 203, 117516.
Blackburn, B.G., Craun, G.F., Yoder, J.S., Hill, V., Calderon, R.L., Chen, N., Lee, S.H., Levy, D.A., Beach, M.J., 2004. Surveillance for waterborne-disease outbreaks – response to reviewer. Water Res. 203, 117435.
Shinta, F.R., Kamaranongrom, N., Marymato, M.A., 2019. Risk management of wastewater treatment in the wastewater treatment plant of PT. X. IPTEK J. Proc. Ser. 3, (5), 140–149.
Sun, Y., Wang, D., Tsang, D.C., Wang, L., Ck, Y.S., Feng, Y., 2019. A critical review of risks, characteristics, and treatment strategies for potentially toxic elements in wastewater from shale gas extraction. Environ. Int. 125, 452–469.
Sonawane, C., Alrabiea, A.J., Punchal, H., Chakma, A.J., Jabber, M.M., Oza, A.D., Zamatkar, S., Burdubus-Negro, D.D., Burdubus-Negro, D.P., 2022. Investigation on the Impact of Different Absorber Materials in Solar Still Using CFD simulation—Economic and Environmental Analysis. Water 14 (19), 3031.
Tietenberg, T., Lewis, L., 2018. Environmental and Natural Resource Economics. Routledge.
Wang, B., Matsura, N., Har-Ayamcura, H., Watanabe, T., Honda, R., 2021. Initial spread in sewage. Chemosphere, 135247.
Zheng, Z., Huang, S., Bian, W., Liang, D., Wang, X., Zhang, K., Ma, X., Li, J., 2019. Enhanced nitrogen removal of the simultaneous partial nitrification, anammox and nitrite oxidation processes (SPIL) has been supported by the EU European Structural and Investment Funds as project No. C2.02.1.01/0.0.0/15.003/0000456. The work reported in this paper.

powered by early warning: a perspectives of temporal variations in SARS-CoV-2-RNA in Ahmadabad, India. Sci. Total Environ. 792, 148367.
Kumar, M., Kuroda, K., Patel, A.K., Patel, N., Bhattacharya, P., Joshi, M., Joshi, C.G., 2021b. Decay of SARS-CoV-2 RNA along the wastewater treatment with upflow Anaerobic Sludge Blanket (UASB) system evaluated through two sample concentration techniques. Sci. Total Environ. 754, 142329.
Li, L., Li, H., Kuroki, H., Koda, Y., Han, L., Liu, X., Hara-Yamamura, H., Watanabe, T., Honda, R., 2021. Initial spread from wastewater from shale gas extraction. Environ. Int. 125, 469.
Naidoo, S., Olaniran, A.O., 2014. Treated wastewater effluent as a source of microbial pollution of surface water resources. Int. J. Environ. Res. Publ. Health 11 (1), 249–276.
Patriarca, R., Di Gravio, G., Costantino, F., Tronci, M., 2017. The Functional Resonance Analysis Method for a system risk based environmental auditing in a sewer plant: a semi-quantitative approach. Environ. Impact Assess. Rev. 63, 72–86.
Serra-Compte, A., González, S., Arruadas, M., Berlendis, S., Courtois, S., Loret, J.F., Schlusser, O., Yaze, A.M., Soria-Soria, E., Fittipaldi, M., 2021. Elimination of SARS-CoV-2 along wastewater and sludge treatment processes. Water Res. 202, 117435.
Shinta, F.R., Kamaranongrom, N., Marymato, M.A., 2019. Risk management of wastewater treatment in the wastewater treatment plant of PT. X. IPTEK J. Proc. Ser. (5), 140–149.
Sun, Y., Wang, D., Tsang, D.C., Wang, L., Ck, Y.S., Feng, Y., 2019. A critical review of risks, characteristics, and treatment strategies for potentially toxic elements in wastewater from shale gas extraction. Environ. Int. 125, 452–469.
Sonawane, C., Alrabiea, A.J., Punchal, H., Chakma, A.J., Jabber, M.M., Oza, A.D., Zamatkar, S., Burdubus-Negro, D.D., Burdubus-Negro, D.P., 2022. Investigation on the Impact of Different Absorber Materials in Solar Still Using CFD simulation—Economic and Environmental Analysis. Water 14 (19), 3031.
Tietenberg, T., Lewis, L., 2018. Environmental and Natural Resource Economics. Routledge.
Wang, B., Matsura, N., Har-Ayamcura, H., Watanabe, T., Honda, R., 2021. Initial behaviors and removal of extracellular plasmid gene in membrane bioreactor. J. Environ. Manag. 298, 113514.
Ye, Y., Ellenberg, R.M., Graham, K.E., Wigginton, K.R., 2016. Surviability, partitioning, and recovery of enveloped viruses in untreated municipal wastewater. Environ. Sci. Technol. 50 (10), 5077–5085.
Zamatkar, S., Amesho, K.T., Sillanpaa, M., 2022a. Critical role of Hyssop plant in the possible transmission of SARS-CoV-2 in contaminated human feces and its implications for the prevention of the virus spread in sewage. Chemosphere, 135247.
Zamatkar, S., Rezakhani, Y., Arabi, A., Hasan, M., Ahmad, Z., Wang, C., Sillanpaa, M., Al-Babani, M., Ghodrati, I., 2022d. An approach to removing COD and BOD based on polycarboxylate mixed matrix membranes that contain hydroxy manganese oxide and silver nanoparticles: a novel application of artificial neural network based simulation in MATLAB. Chemosphere 308 (2), 136304.
Zamatkar, S., Klemes, J.J., Bokhari, A., Wang, C., Sillanpaa, M., Hasan, M., Amesho, K.T., 2022b. Critical role of Hyssop plant in the possible transmission of SARS-CoV-2 in contaminated human feces and its implications for the prevention of the virus spread in sewage. Chemosphere, 135247.
Zamatkar, S., Rezakhani, Y., Arabi, A., Hasan, M., Ahmad, Z., Wang, C., Sillanpaa, M., Al-Babani, M., Ghodrati, I., 2022d. An approach to removing COD and BOD based on polycarboxylate mixed matrix membranes that contain hydroxy manganese oxide and silver nanoparticles: a novel application of artificial neural network based simulation in MATLAB. Chemosphere 308 (2), 136304.
Zamatkar, S., Klemes, J.J., Bokhari, A., Wang, C., Sillanpaa, M., Hasan, M., Amesho, K.T., 2022b. Critical role of Hyssop plant in the possible transmission of SARS-CoV-2 in contaminated human feces and its implications for the prevention of the virus spread in sewage. Chemosphere, 135247.
Zheng, Z., Huang, S., Bian, W., Liang, D., Wang, X., Zhang, K., Ma, X., Li, J., 2019. Enhanced nitrogen removal of the simultaneous partial nitrification, anammox and nitrite oxidation processes (SPIL) has been supported by the EU European Structural and Investment Funds as project No. C2.02.1.01/0.0.0/15.003/0000456. The work reported in this paper.

enhanced survery-based city zonation for effective COVID-19 pandemic preparedness for wastewater surveillance-based city zonation for effective COVID-19 pandemic preparedness.