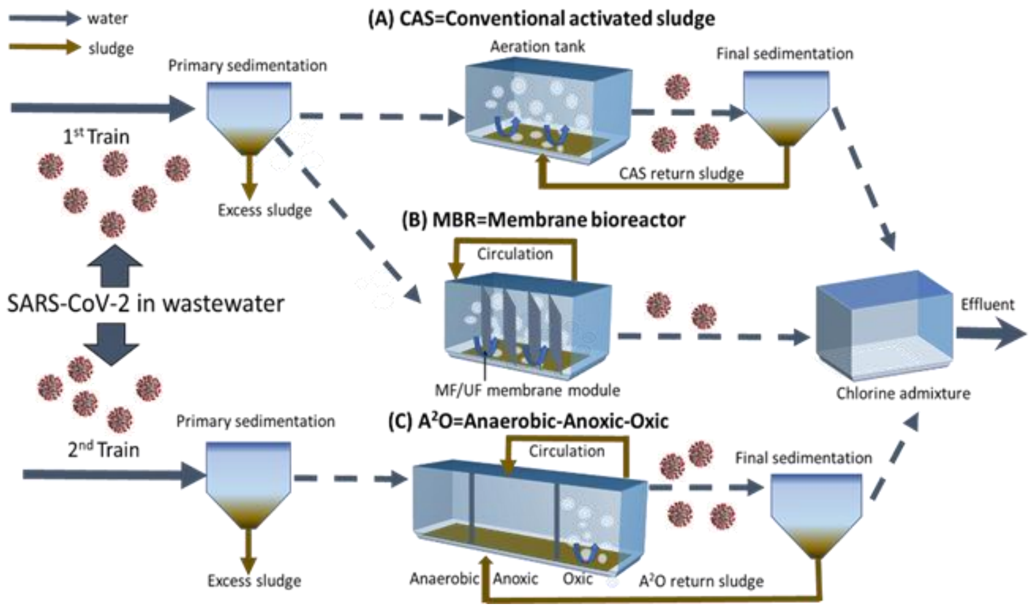
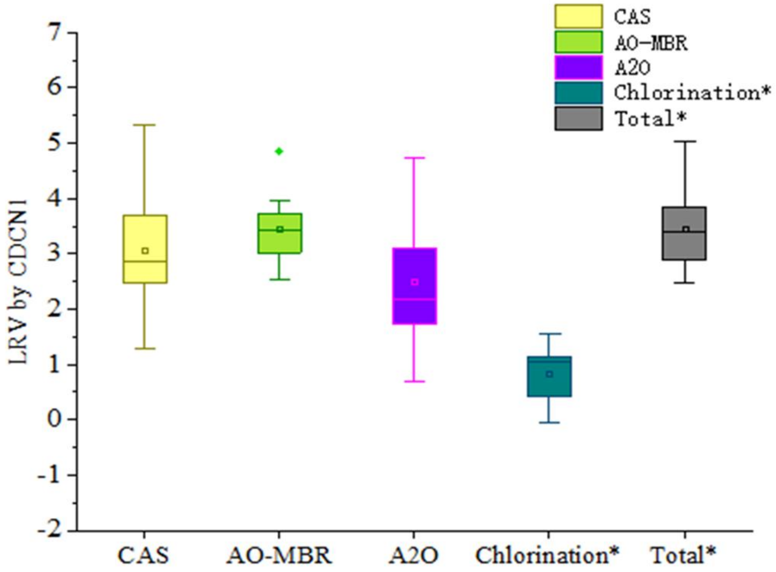
Removal Performance of SARS-CoV-2 in Wastewater by Membrane Bioreactor, Anaerobic-Anoxic-Oxic and Conventional Activated Sludge Processes





Rongxuan Wang [a], Md. Alamin [b], Shohei Tsuji [b], Hiroe Hara-Yamamura [b], Akihiko Hata [c], Bo Zhao [d], Masaru Ihara [d, e], Hiroaki Tanaka [d], Ryo Honda [a, d] *

Background: potential risk of SARS-CoV-2 in wastewater

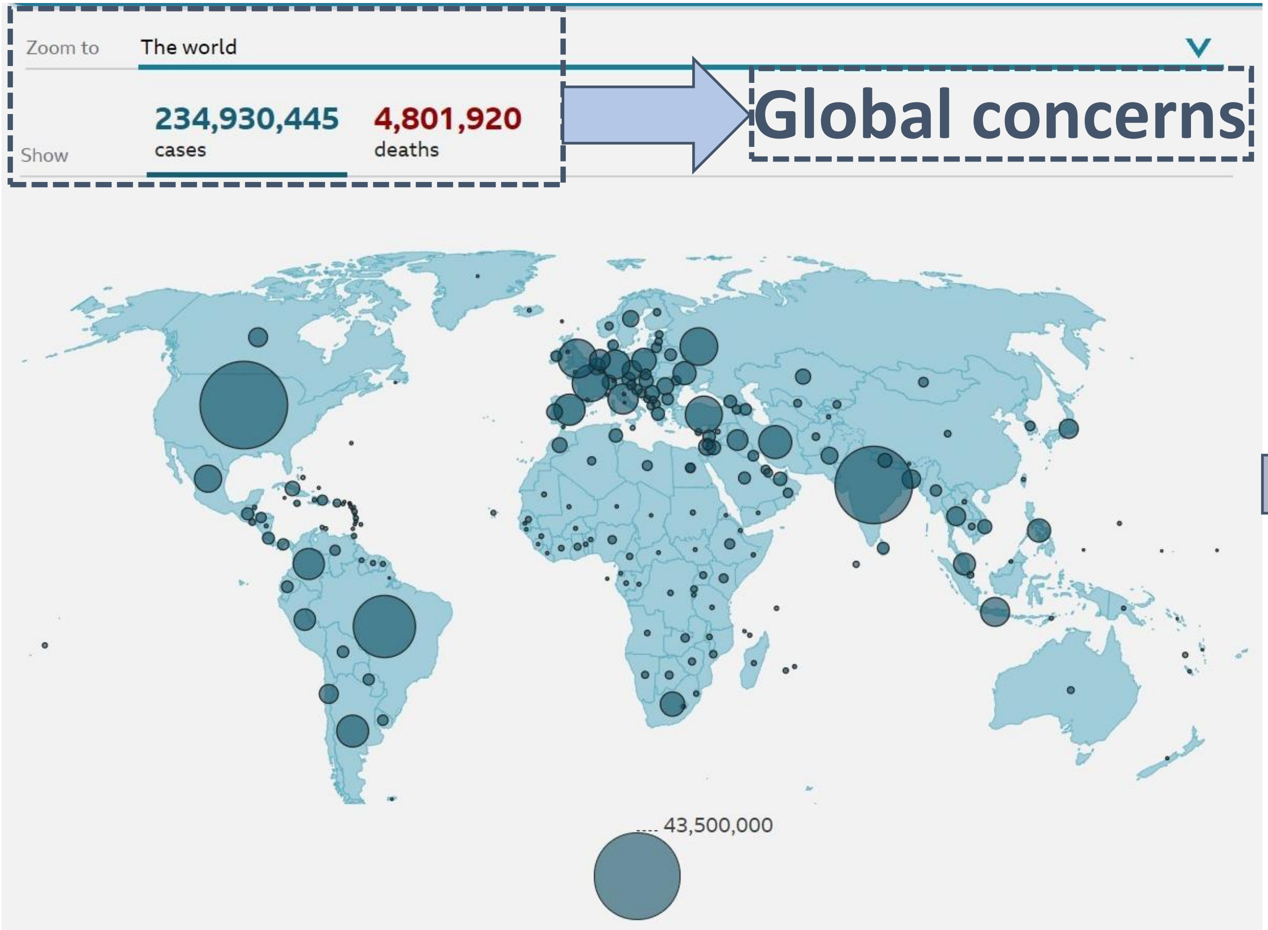
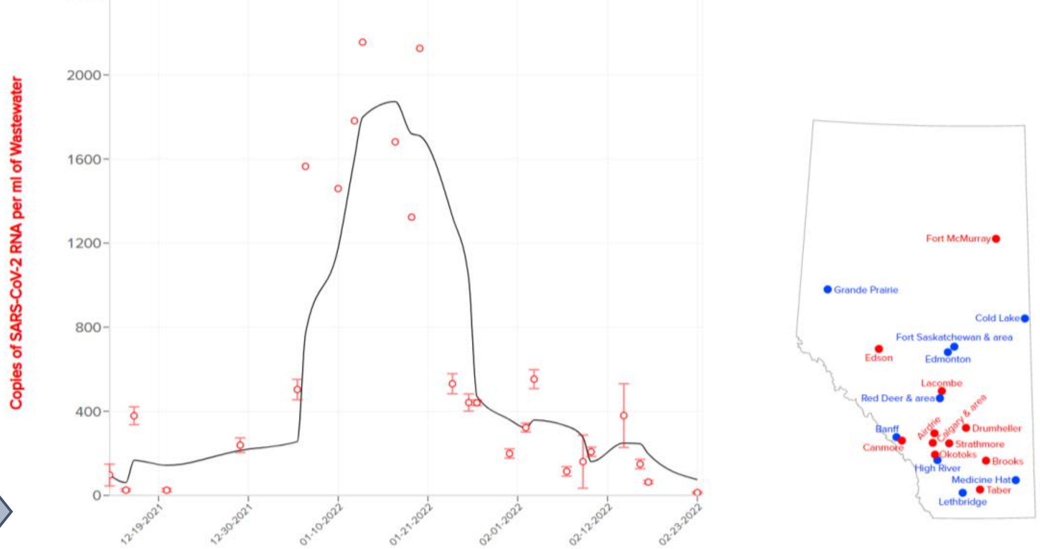


Figure 1. Accumulation of COVID-19 confirmed cases and death in the world. Circles show number of confirmed coronavirus cases per country.

Source derived from : Johns Hopkins University, national public health agencies
Figures last updated 4 October 2021, 10:13 BST



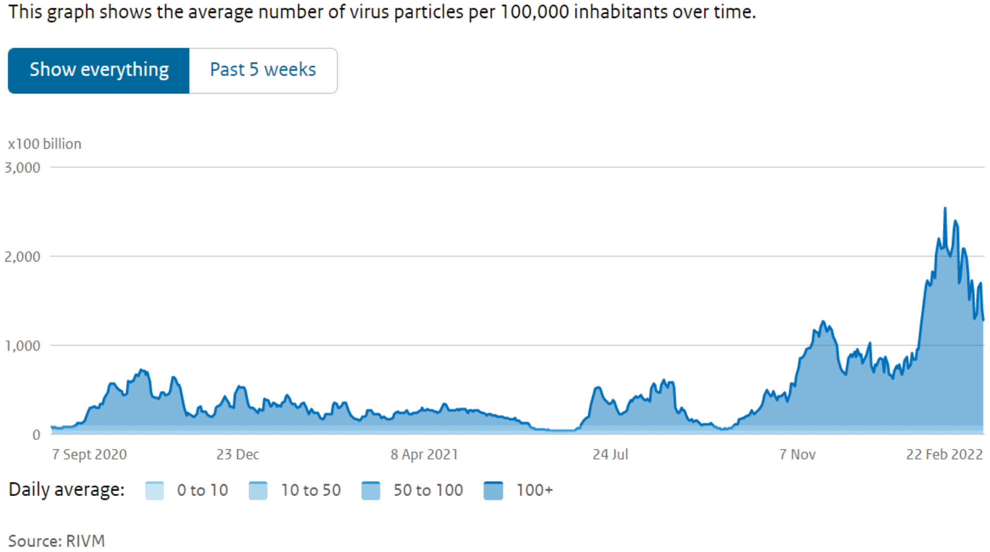


Figure 2. surveillance of SARS-CoV-2 RNA in wastewater

Source derived from : https://covid-tracker.chi-csm.ca/
https://coronadashboard.government.nl/landelijk/rioolwater

Background: detection of SARS-CoV-2 in wastewater

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First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan

Eiji Haramoto [a,*], Bikash Malla [a], Ocean Thakali [b], Masaaki Kitajima [c]

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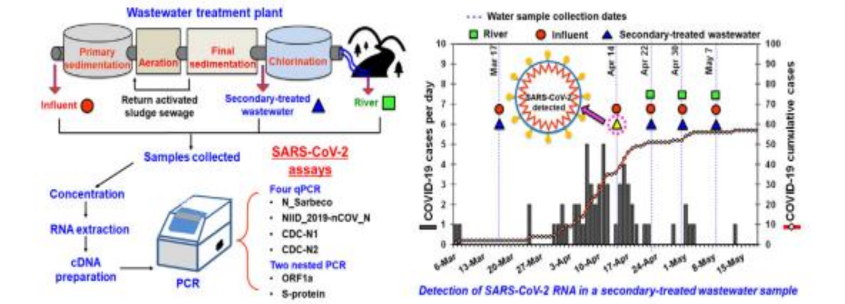
HIGHLIGHTS

- First environmental surveillance for SARS-CoV-2 RNA in Japan was carried out.
- SARS-CoV-2 RNA was detected in a secondary-treated wastewater (2.4×10^3 copies/L).
- None of influent and river water samples tested positive for SARS-CoV-2 RNA.
- SARS-CoV-2 RNA was detected when the reported cases in the community were high.
- Applicability of EMV method for detection of SARS-CoV-2 in water is demonstrated.

GRAPHICAL ABSTRACT



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Detection of SARS-CoV-2 in wastewater in Japan during a COVID-19 outbreak

Akihiko Hata [a], Hiroe Hara-Yamamura [b], Yuno Meuchi [a], Shota Imai [a], Ryo Honda [b,c,*]

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[b] Faculty of Geosciences and Civil Engineering, Kanazawa University, Japan
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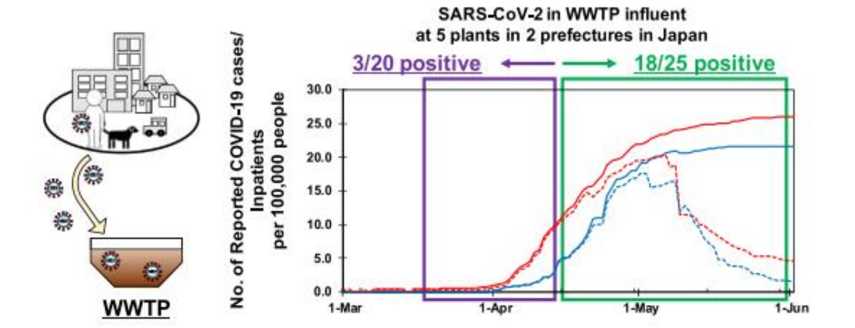
HIGHLIGHTS

- Presence of SARS-CoV-2 RNA in wastewater was studied in two prefectures in Japan.
- At the start of the study, no cases of COVID-19 had been reported in the study area.
- SARS-CoV-2 detection frequency increased along with the number of reported cases.
- SARS-CoV-2 was detected even when the number of cases was <1.0 per 100,000 people.
- The detection frequency remained high even after increase in the cases stopped.

GRAPHICAL ABSTRACT



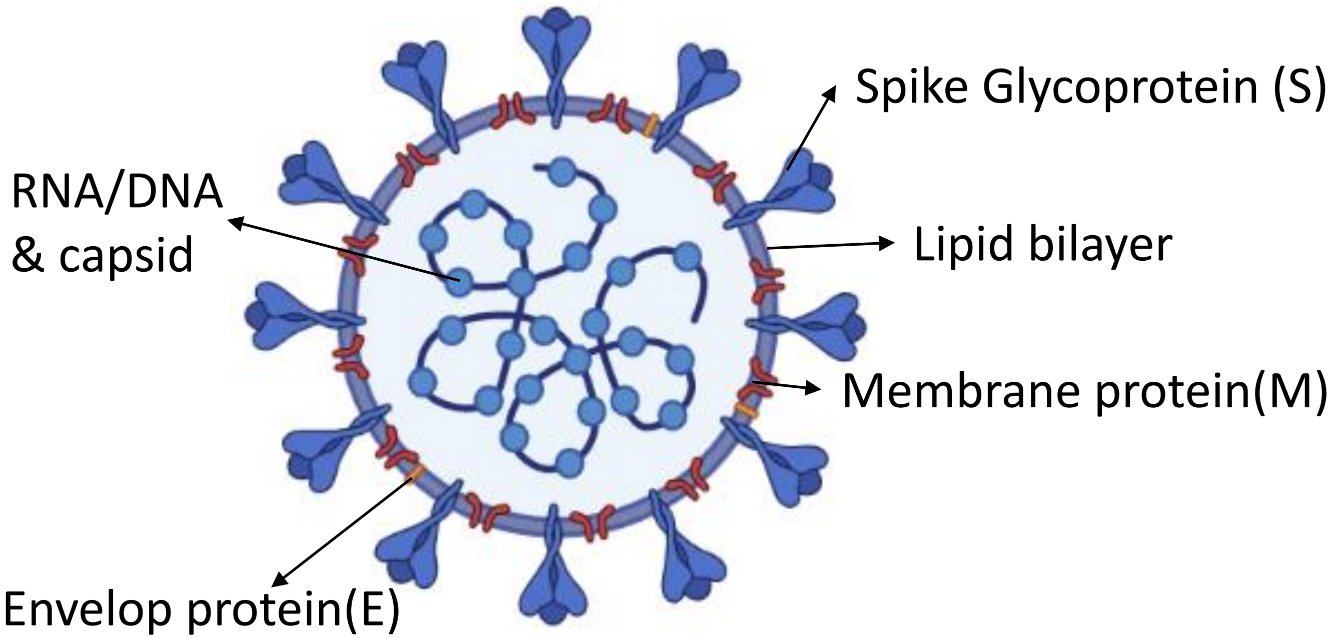
- ***SARS-CoV-2 RNA*** was **DETECTED** in ***wastewater*** in Japan
- ***SARS-CoV-2 RNA*** was quantified ***2400 copies/L*** in an effluent of ***secondary-treated wastewater***.

* Presence of ***SARS-CoV-2 RNA*** was founded in ***wastewater*** In two Prefectures in Japan.

- ***Wastewater-based epidemiology (WBE)*** act as an early warning of ***COVID-19*** outbreaks in Japan

Background: nonenveloped virus and enveloped virus

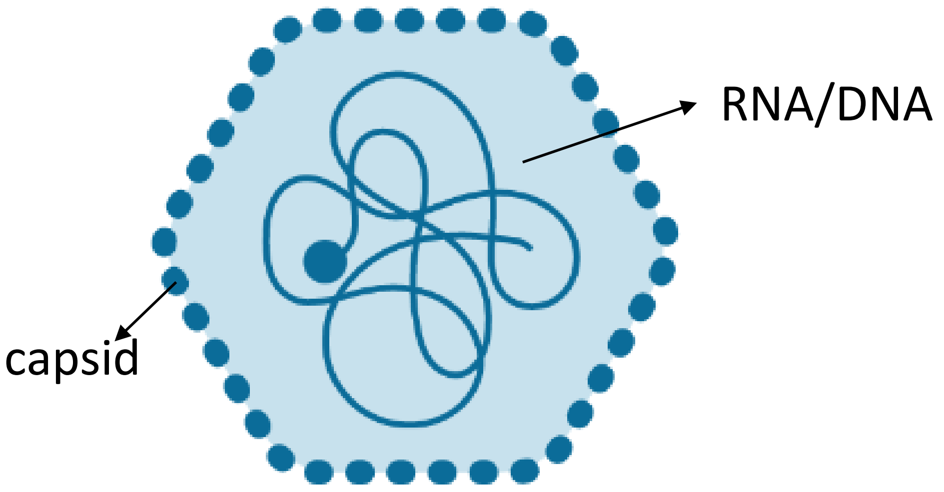
Enveloped virus



Enveloped virus: **Coronavirus**, phi6, influenza virus, etc.

- **differences between enveloped and nonenveloped virus (no enveloped protein carry)**

Non-enveloped virus



Non-enveloped virus: Norovirus, hepatitis E virus (HEV), hepatitis A virus (HAV), etc.

Created in biorender. com

Background: removal of nonenveloped virus in wastewater

Kumar et al., (2021) npj. Clean Water. https://doi.org/10.1038/s41545-021-00098-2

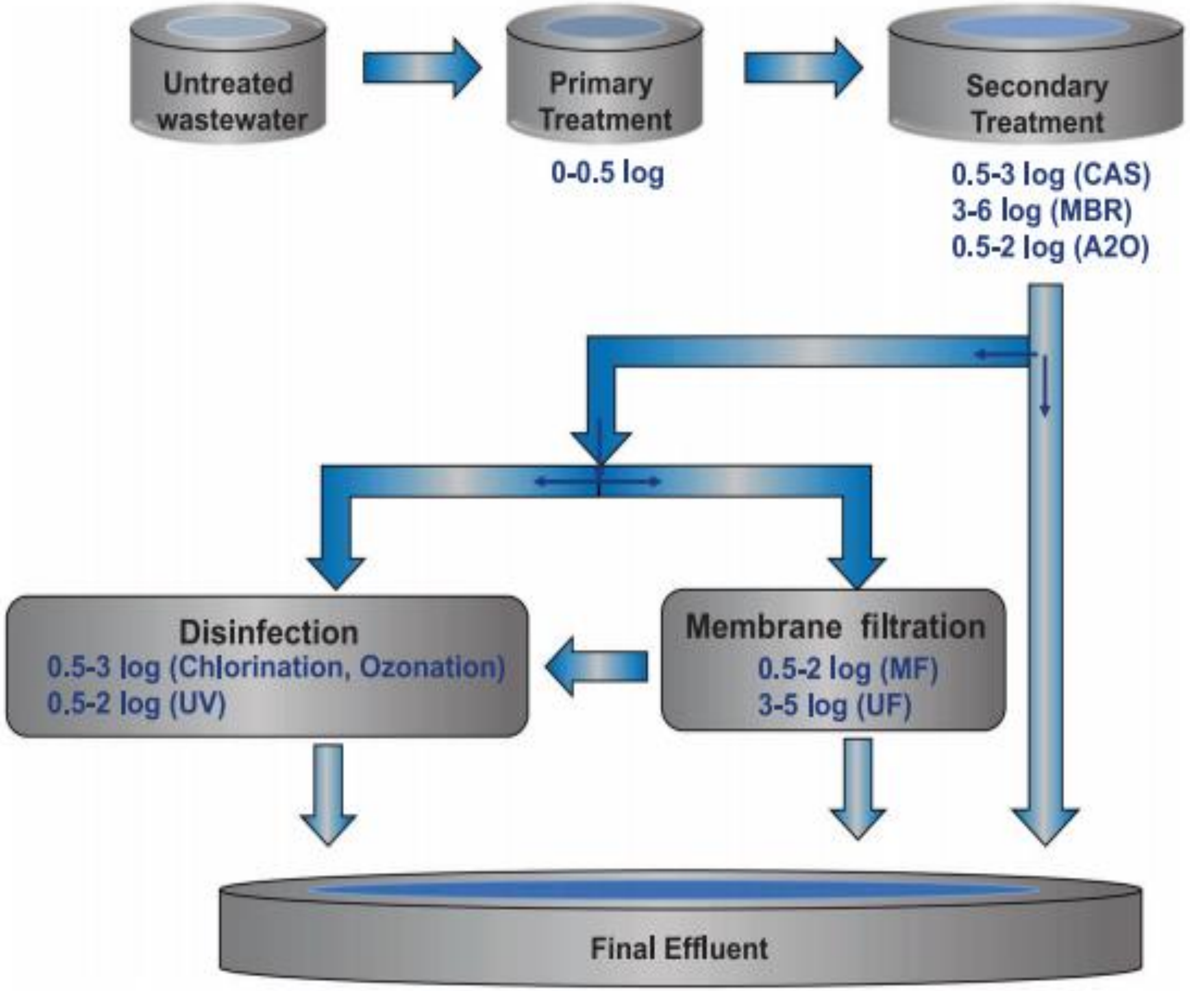


Fig. 2 Typical log removal values (LRVs) of viruses in the wastewater treatment process. CAS conventional activated sludge process, MBR membrane bioreactor process, A2O anaerobic–anoxic–oxic process, UV ultraviolet disinfection, MF microfiltration, UF ultrafiltration.

- **The typical concentration of influent**

MBR: 10^5 copies/L-10^9 copies/L
CAS : 10^2 copies/L-10^{10} copies/L
A2O : 10^1 copies/L-10^3 copies/L
After chlorination:10^2 copies/L-10^7 copies/L

- **The concentration of effluent**

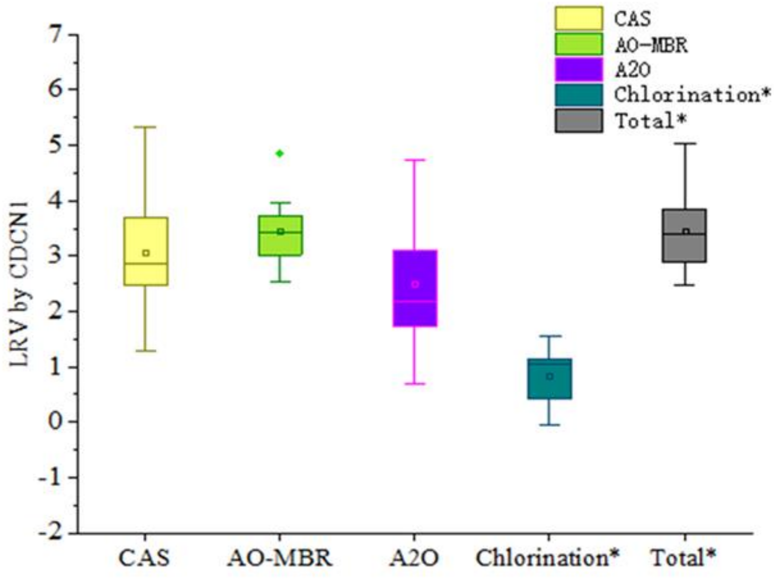
MBR: 10^2 copies/L-10^4 copies/L
CAS: 10^{-1} copies/L-10^9 copies/L
A2O : 10^2 copies/L
After chlorination:10^0 copies/L-10^5 copies/L

SARS-CoV-2 is unknown

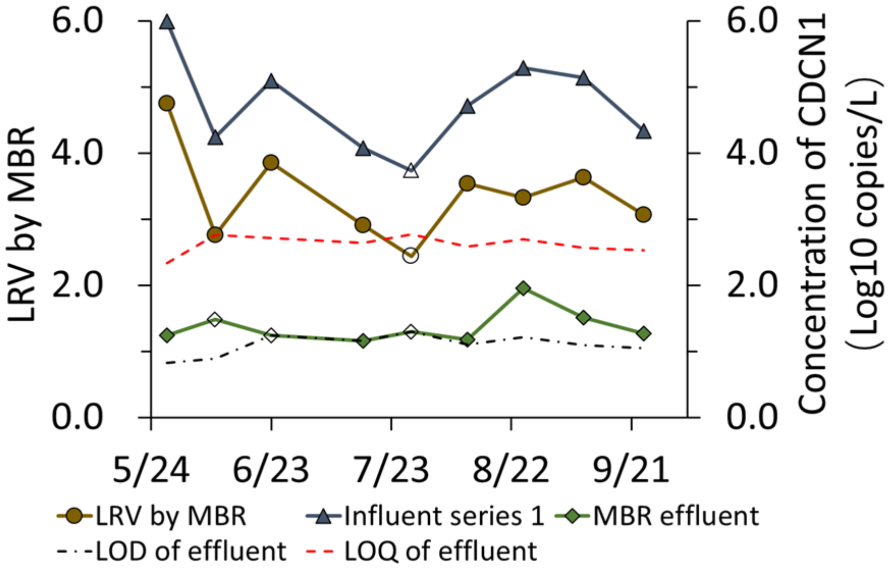
Objective: investigate removal of SARS-CoV-2 in real WWTP

- To clarify ***removal performance*** of ***SARS-CoV-2*** in real WWTPs.
- To compare removal performance of three secondary treatment processes ***(MBR, CAS, A2O)*** and ***chlorination*** in SARS-CoV-2 reduction.
- To evaluate applicability of ***PMMoV*** as a process control for ***SARS-CoV-2*** in wastewater.

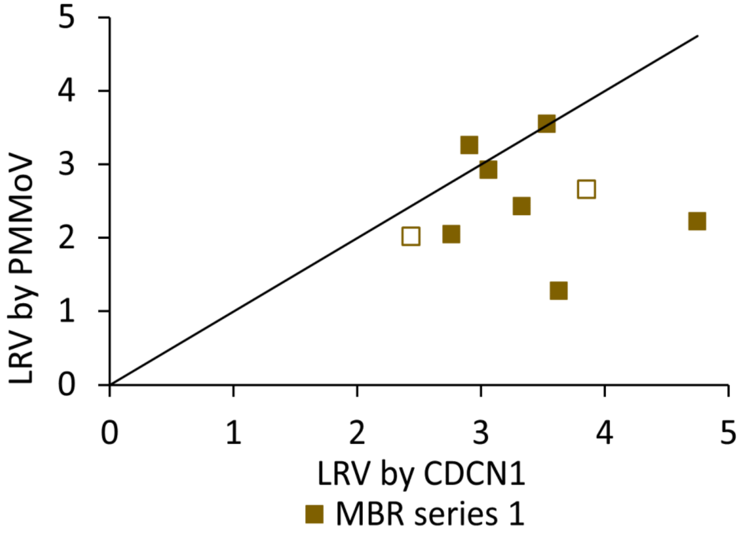
(*i*)

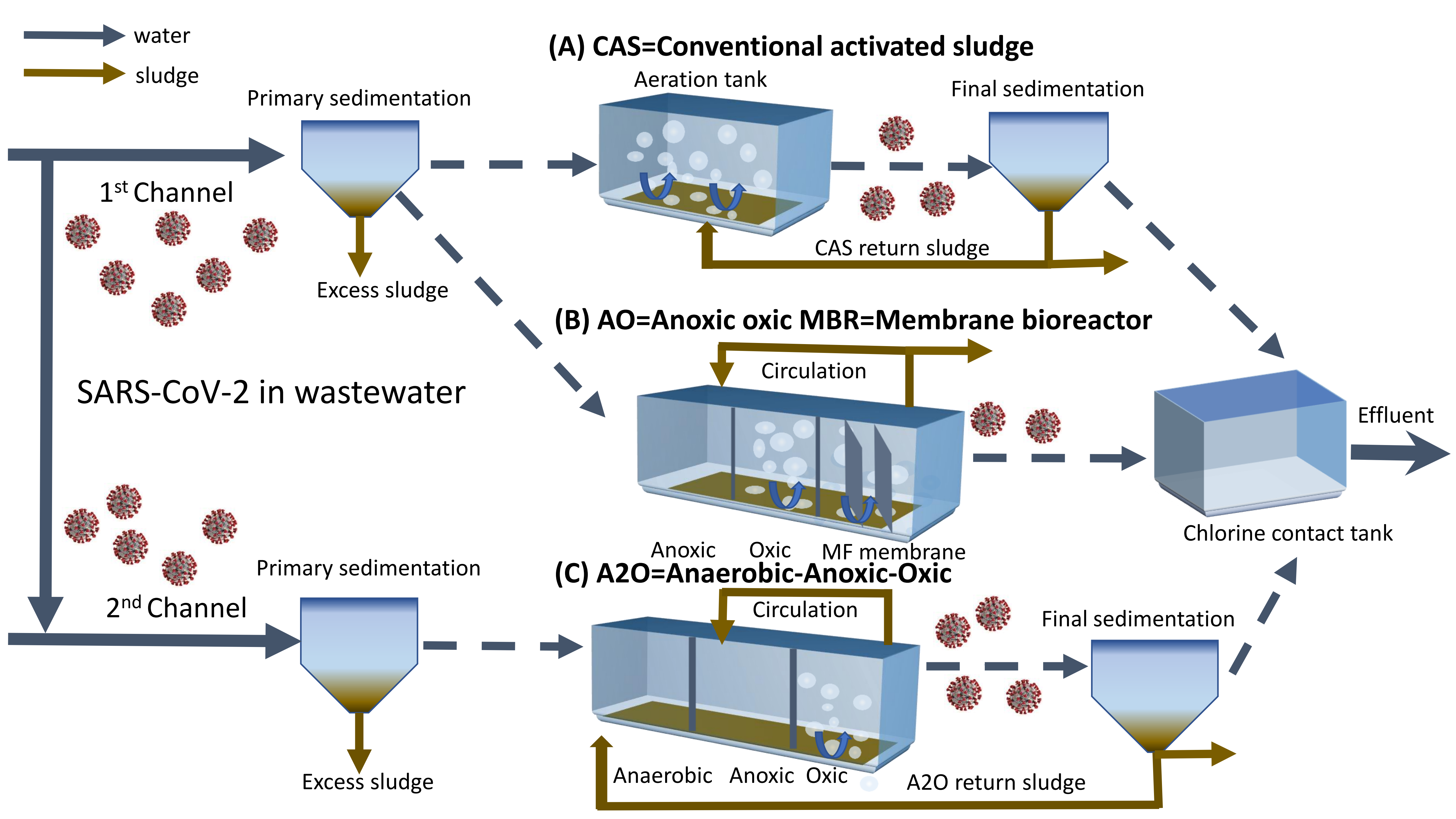


(*ii*)



(*iii*)




water
sludge
Primary sedimentation
(A) CAS=Conventional activated sludge
Aeration tank
Final sedimentation
1st Channel
Excess sludge
CAS return sludge
(B) AO=Anoxic oxic MBR=Membrane bioreactor
Circulation
SARS-CoV-2 in wastewater
Effluent
Chlorine contact tank
Anoxic
Oxic
MF membrane
Primary sedimentation
(C) A2O=Anaerobic-Anoxic-Oxic
2nd Channel
Circulation
Final sedimentation
Excess sludge
Anaerobic
Anoxic
Oxic
A2O return sludge

Method: sampling information and population coverage

Table 1. flow rate in conventional activated sludge, membrane bioreactor and anaerobic anoxic oxic process

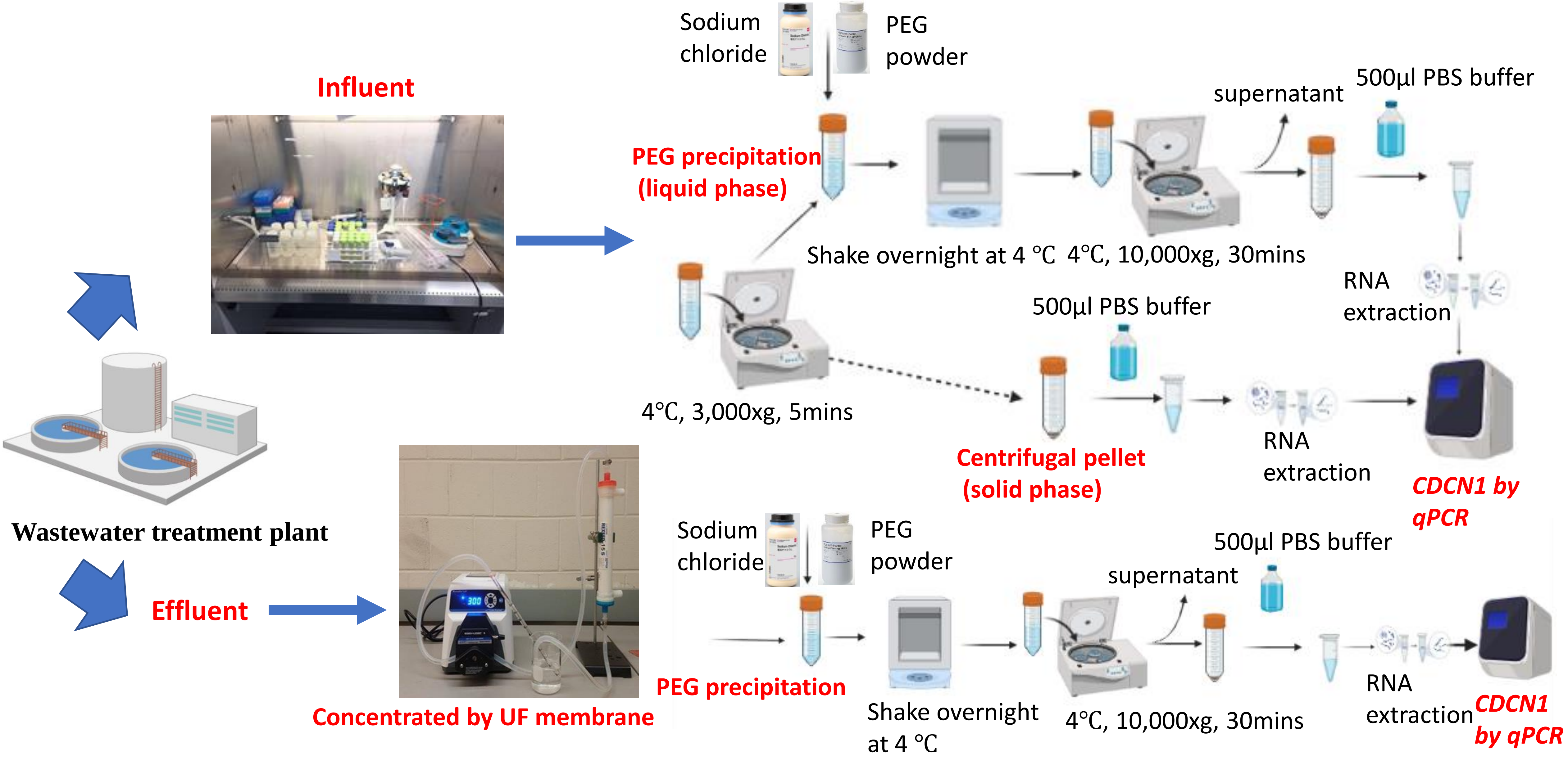
Flow rate		Until July	Unit	in August and later	Unit
1st train (CAS+MBR) 24,000 m3 or 34,000 m3	1st train	24,000	m3/d	34,000	m3/d
2nd train (A2O) 38,000 m3/day	MBR	14,000	m3/d	14,000	m3/d
flow ratio of CAS:MBR = 12000:10000	CAS	10,000	m3/d	20,000	m3/d
flow ratio of 1st (MBR+CAS):2nd (A2O)= 240:380 until July	2nd train	38,000	m3/d	38,000	m3/d
flow ratio of 1st (MBR+CAS):2nd (A2O)=340:380 since August					

Table 2. Data on population coverage of WWTPs in the target city

	Catchment area	Population coverage	Designed Population capacity (m3/y)	Coverage population (city)
Inf series	ha	persons	persons	person
WWTPs	4,281	270,104	276,735	840,000

Date: from May 28 to September 24, 2020
Sample volume: ***250*** mL of influent wastewater
10 L of secondary treatment effluents from CAS and MBR
9 samples of ***influent*** in each train, ***9 samples*** in each process and ***9 samples*** of ***final effluent*** of chlorination process

Method: detection of SARS-CoV-2 in the influent and effluent



Results: SARS-CoV-2 in Influent and COVID-19 confirmed cases

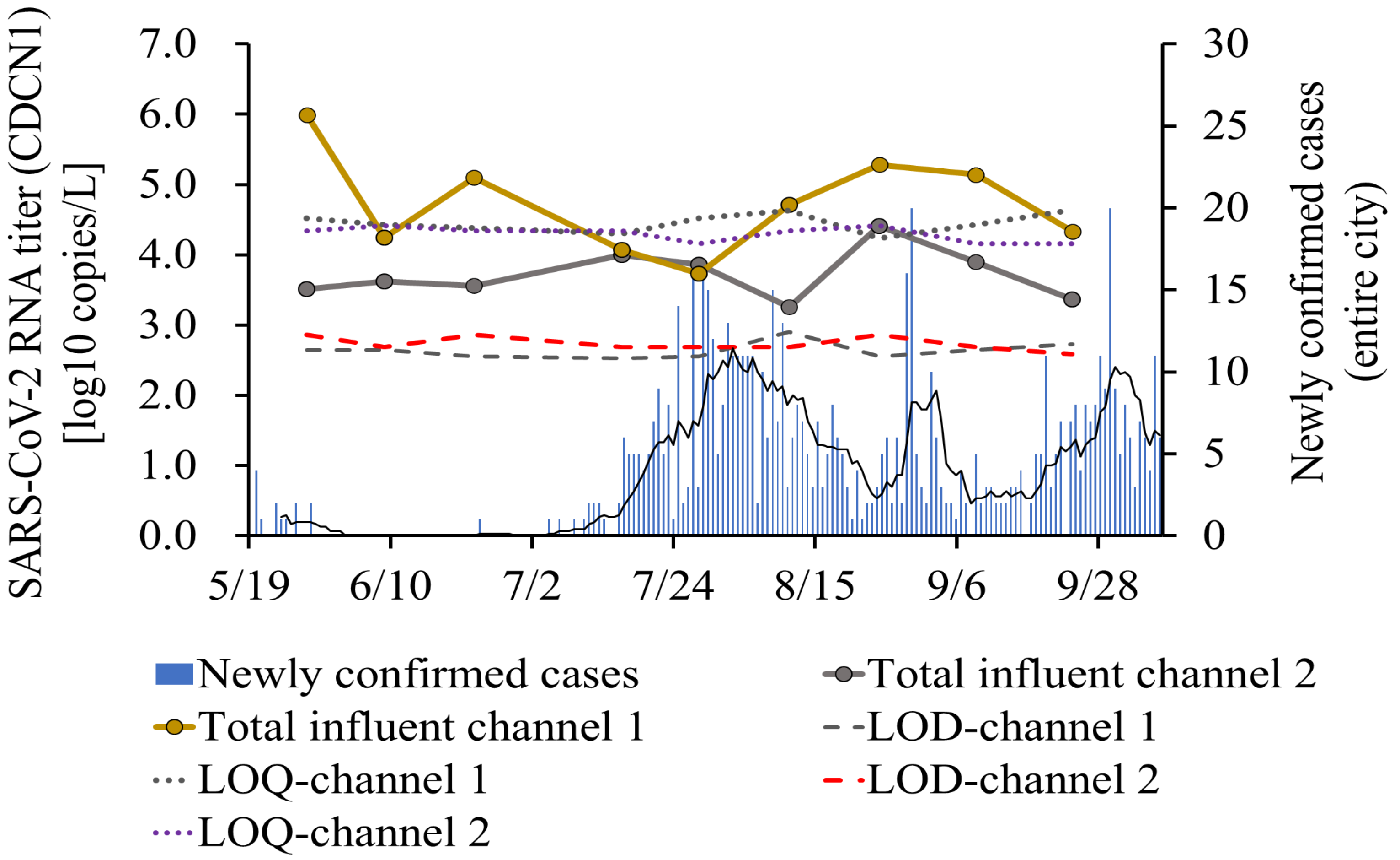


Figure 1. Comparison of SARS-CoV-2 RNA concentration in influent (log10 copies/L) and newly confirmed cases.

the Total concentration of SARS-CoV-2 RNA was *3.3-6.0 log copies/L* in *influent*

Results: LRV of CDCN1 after secondary treatment

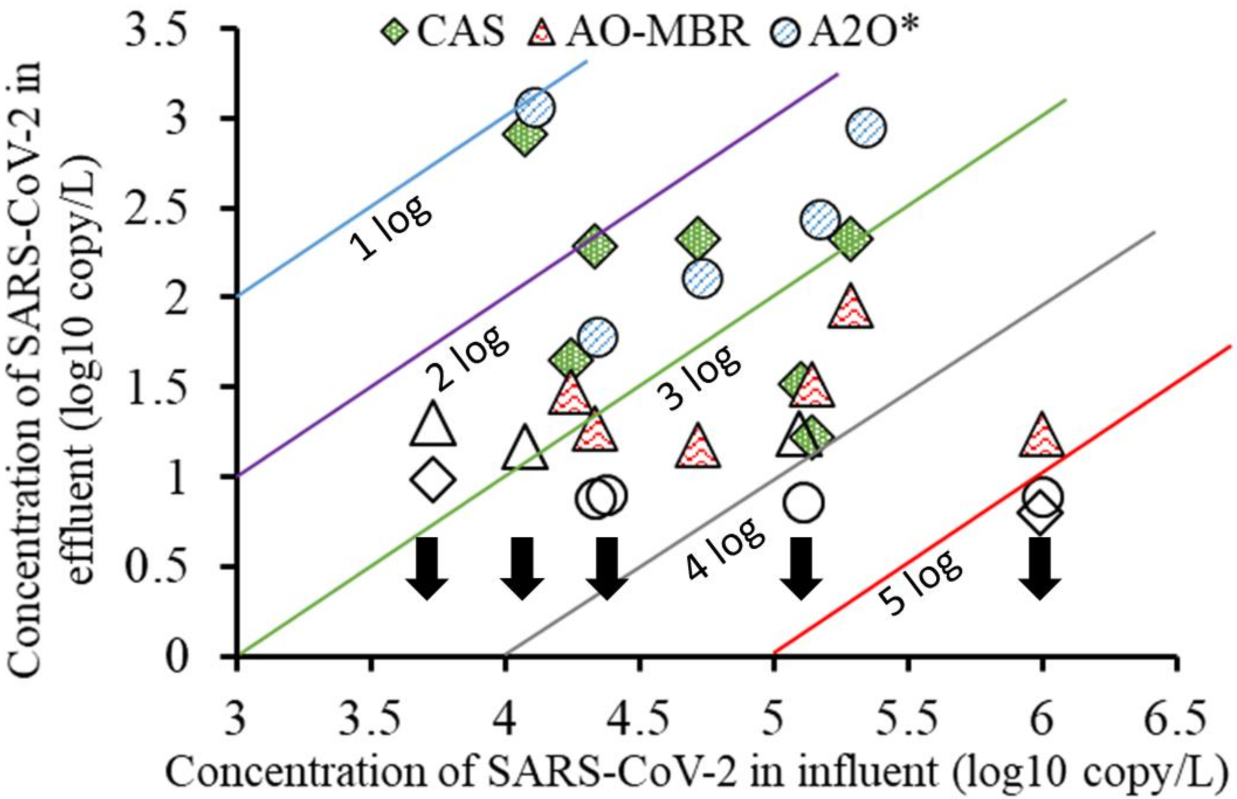


Figure 2. Concentration of SARS-CoV-2 in influent was in related to concentration of SARS-CoV-2 in effluent (log10 copy/L). Blank stand in figure indicate that SARS-CoV-2 was positive in influent but negative in the corresponding effluent. **__Notice__: MBR have never shown the reduction value < 2log.*

- The ***reduction of SARS-CoV-2*** was mostly in range of ***2-4 log*** in the three processes.
- ***SARS-CoV-2 RNA*** concentration in ***CAS effluent*** was ***N.D-2.91 log10*** copies/L.
- ***SARS-CoV-2 RNA*** concentration in ***MBR effluent*** was ***N.D-1.96 log10*** copies/L.
- ***SARS-CoV-2 RNA*** concentration in ***A2O effluent*** was ***0.89-3.07 log10*** copies/L.

Results: LRV of CDCN1 by each process and disinfection

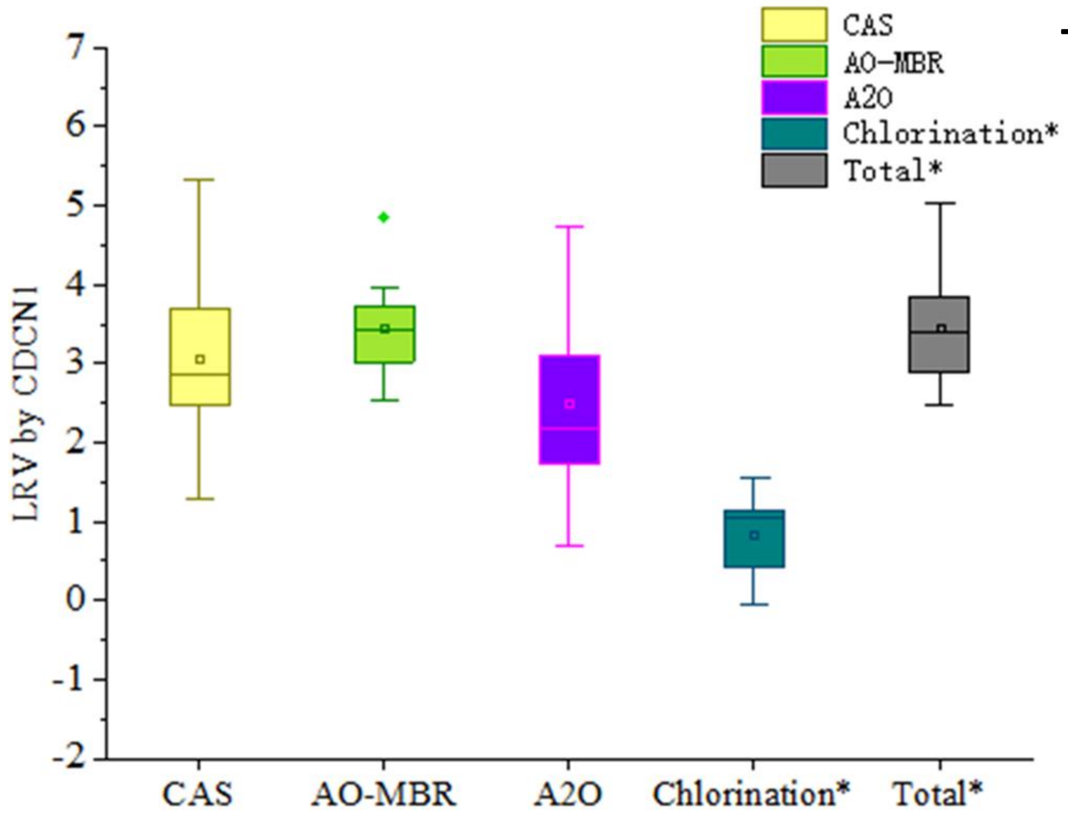


Table 1. comparison of LRV of CDCN1 by each process and disinfection.

Treatment process	average
CAS	3.1 ± 1.1
AO-MBR	3.5 ± 0.65
A2O	2.5 ± 1.2
Chlorination*	>0.85 ± 0.54
Total*	>3.5 ± 0.72

Figure 3. boxplot profile indicated distribution of LRV by CDCN1 in (a) CAS, (b) AO-MBR process and (c) A2O process. *chlorination represent minimum LRV, the real total LRV is higher than this min total LRV. **<u>*Notice*</u>**<u>*: MBR have never shown the reduction value < 2 logs.*</u>

- ***<u>LRV of CDCN1 by MBR process</u>*** (3.5 ± 0.65 log) was more stable than ***<u>CAS process</u>*** (3.1 ± 1.1 log).
- ***<u>LRV of CDCN1 by A2O process</u>*** (2.5 ± 1.2 log) was not significantly different from ***<u>CAS process</u>*** (3.1 ± 1.1 log)

Results: comparison with other studies

Table 2. comparison of removal of SARS-CoV-2 in various wastewater treatment processes.

Country	Treatment processes In WWTPs	Concentration in influent (log10 copies/L)	Concentration in effluent (log10 copies/L)	Log removal value (LRV) (Log10 copies/L)	References
Japan	CAS	3.73-5.99	0.80-2.91	3.1±1.1	**My study**
	MBR	3.73-5.99	1.16-1.96	3.5±0.65	
	A2O	3.26-4.41	0.86-3.07	2.5±1.2	
	chlorination	1.15-2.86	<0.83-1.30	>0.97±0.50	
Spain, France	Activated sludge	3.29±0.67	2.26±0.47	1.03±0.59	Serra-Compte et al., 2021
	Activated sludge plus nutrient removal	3.65±0.68	2.28±0.70	1.37±0.72	
	MBR	3.89±0.89	2.13±0.35	1.96±0.93	
India	CAS	3.17	2.40	0.77	Kumar et al., 2021
	chlorination	3.17	2.46	0.71	
	UASB	3.54	<LOQ (2.23)	>1.3	Kumar et al., 2021
Paris	WWTPs	4-7	ND-5	2	Wurtzer et al., 2020a
Spain	Secondary treatment (activated sludge/A2O/extended aeration), disinfection, NaClO/UV	<3.53	<3.40	>0.1	Randazzo et al., 2020b

Wastewater treatment plant=WWTPs, Membrane bioreactor=MBR, Conventional activated sludge= CAS, Anaerobic-anoxic –oxic=A2O, Upflow Anaerobic Sludge Blanket=UASB, Limit of quantification=LOQ

Results: potential of PMMoV as a performance indicator

Purpose of **performance indicator virus** in wastewater

- To check the removal performance of the target virus in wastewater independent of outbreak situation in the sewershed.

Three requirements for performance indicator virus

1. To be abundant in wastewater
2. To have high concentration to be detected after treatment.
3. LRV is consistently lower than the target virus.

Results: potential of PMMoV as a performance indicator

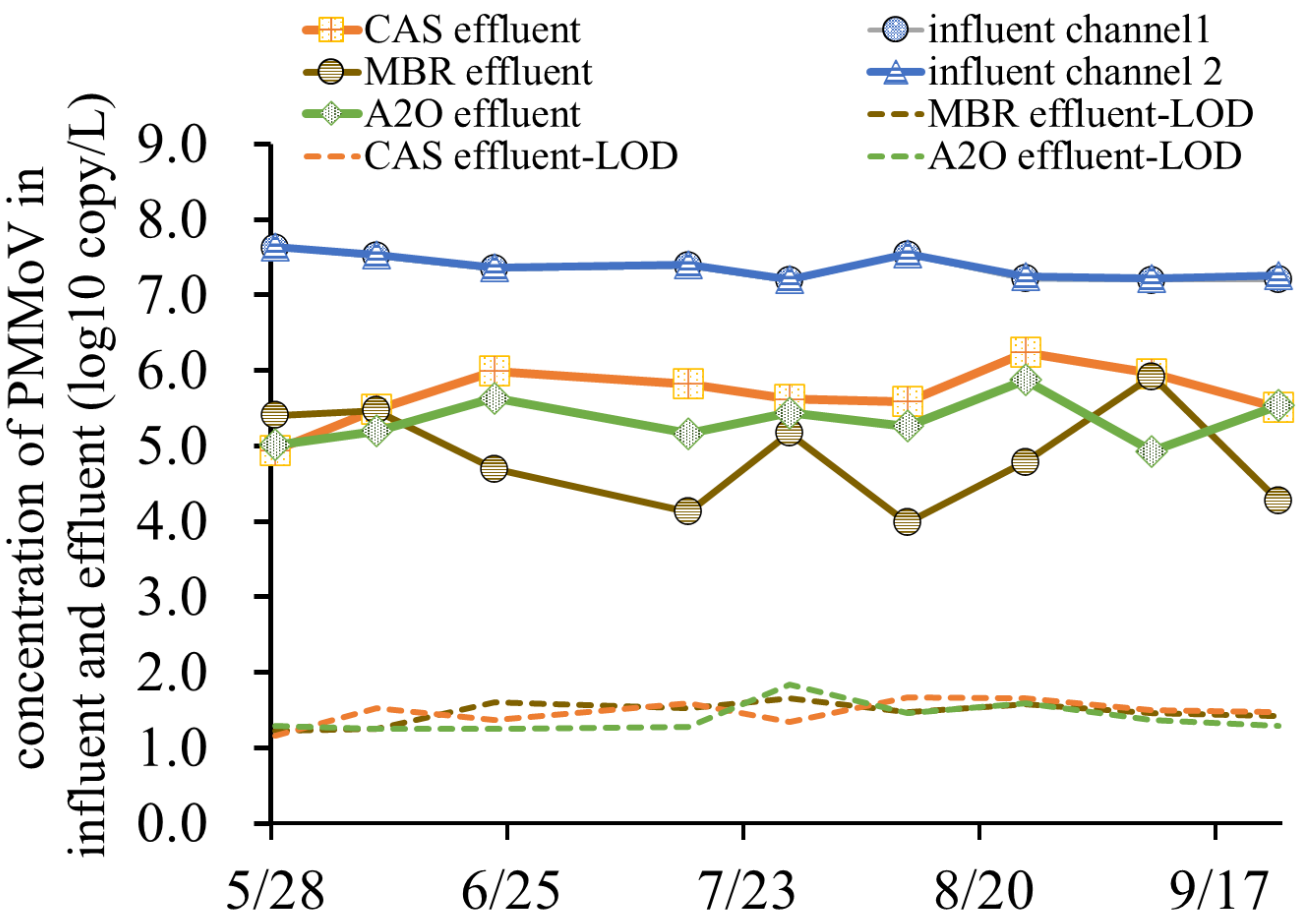
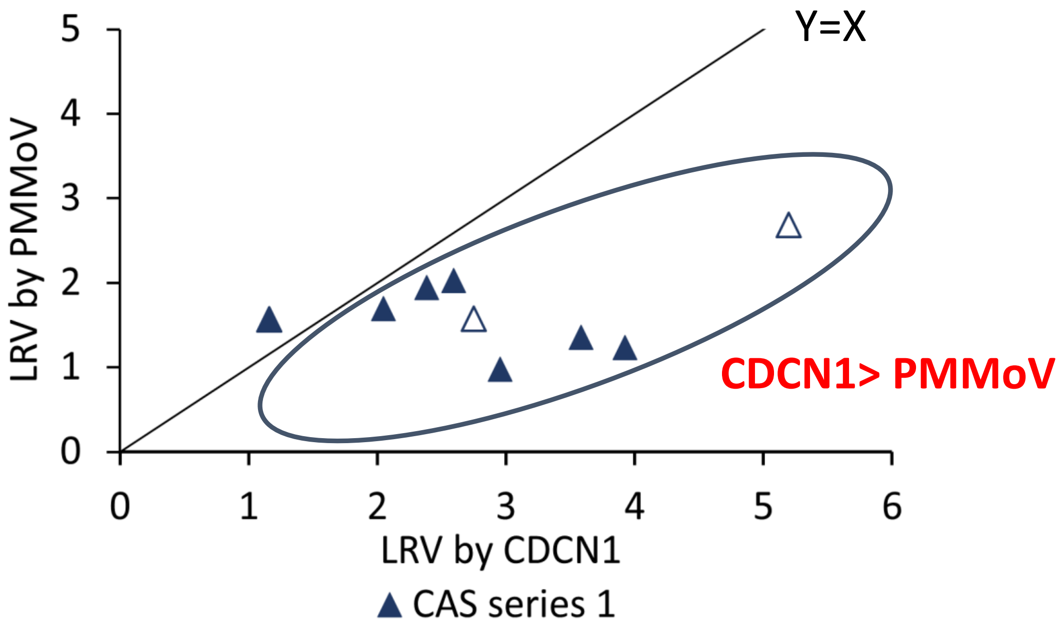


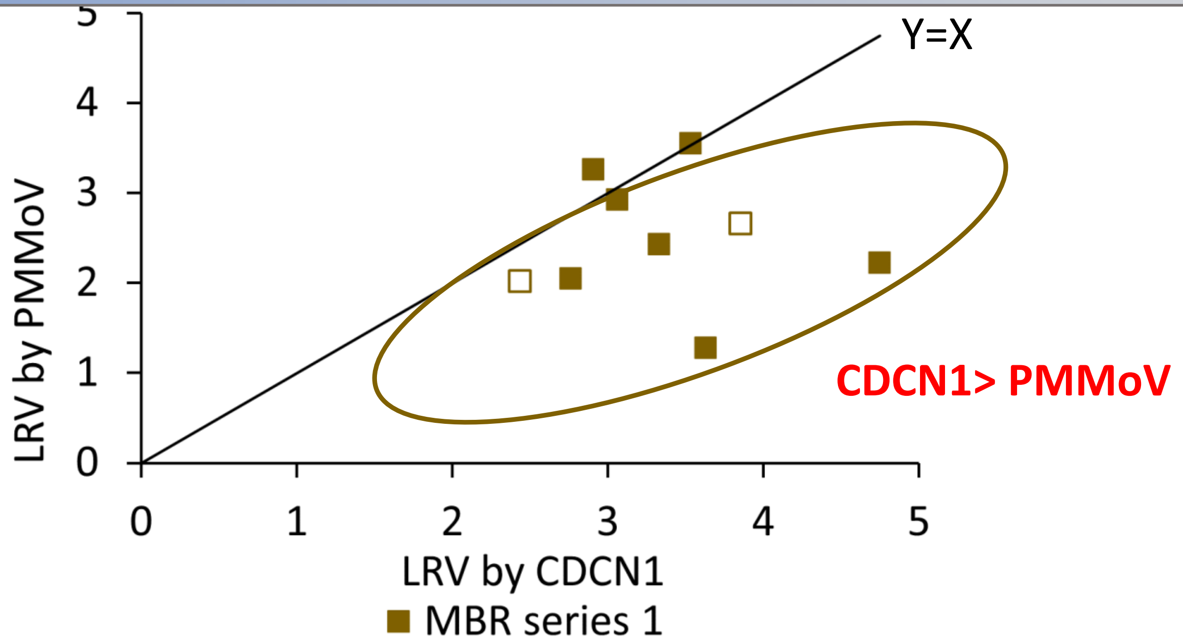
Figure 4. Time series change with influent and effluent of PMMoV concentration (log10 copy/L).

✓1. PMMoV is always abundant in wastewater.

✓2. PMMoV is present at high concentration to be detected after treatment.

Results: potential of PMMoV as a performance indicator





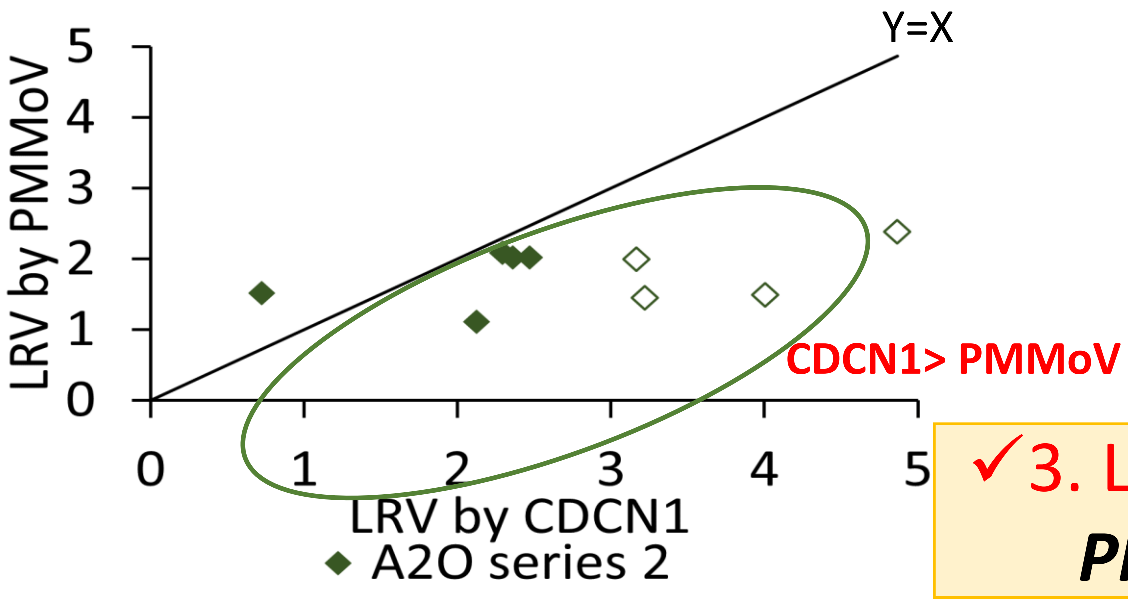


Table 3. comparison Log removal value of CDCN1 and PMMoV

process	LRV by CDCN1	LRV by PMMoV	*P* value
MBR	3.5±0.65	*2.6±0.66*	0.031*
CAS	*3.1±1.10*	*1.8±0.48*	0.008**
A2O	*2.5±1.2*	*1.5±0.48*	0.019*

Where, *= P<0.05: significant difference, * *= P<0.01: highly significant difference

✓3. LRV of PMMoV is lower than the SARS-CoV-2

PMMoV is a good performance indicator.

Conclusions

- ✓ The ***total LRV*** after disinfection was ***3.5 log or higher***, which was higher than typical LRV of nonenveloped enteric virus.
- ✓ The ***removal of SARS-CoV-2 in secondary treatment*** by ***MBR*** (3.5 ± 0.65 log) was ***more stable*** than ***CAS*** process (3.1 ± 1.1log)
- ✓ The ***removal of SARS-CoV-2 in secondary treatment*** by ***A2O*** process (2.5 ± 1.2 log) was not significantly different from ***CAS*** process (3.1 ± 1.1log).
- ✓ ***PMMoV*** ***is a good indicator*** virus to evaluate removal of SARS-CoV-2 in WWTP.

Acknowledgements

- JST CREST (Grant No. JPMJCR20H1)
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Supplementary: LRV by CAS, MBR and A2O

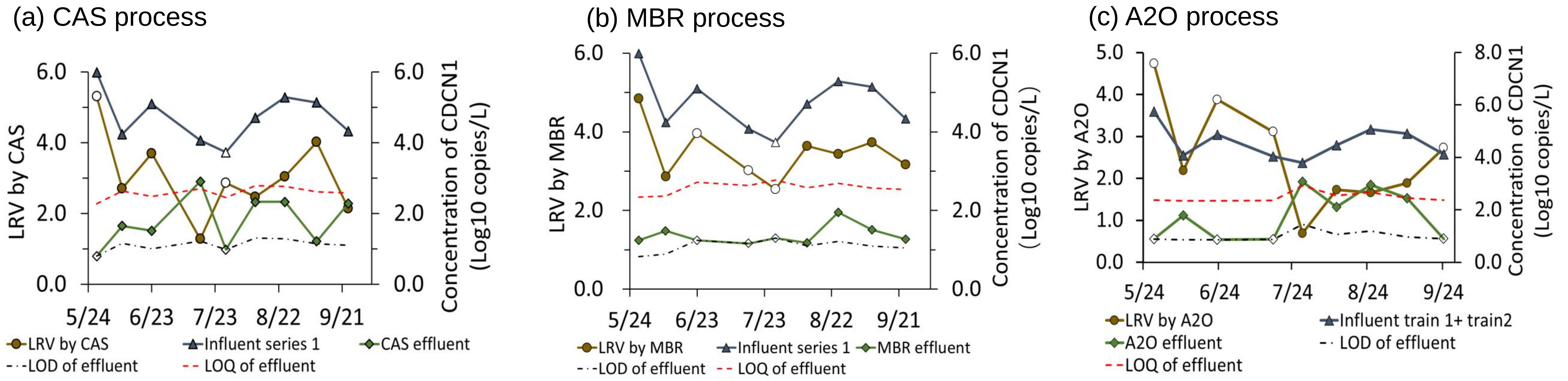


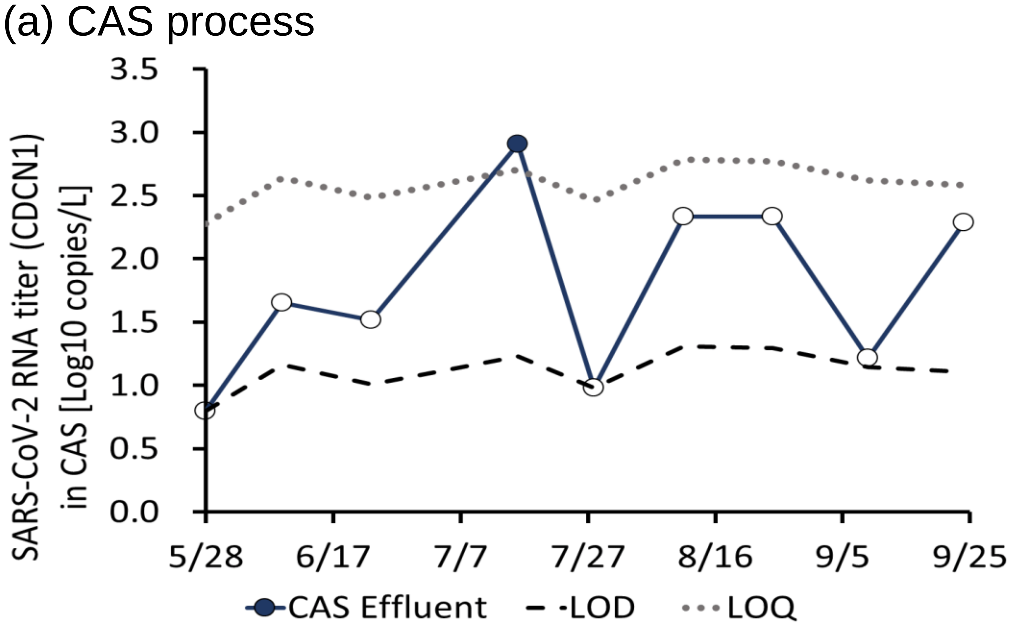
Figure 1. CDCN1 concentrations in effluent and log removal values (LRV) in (a) CAS and (b) MBR process. The blank mark means below the LOD (undetected). series 1 influent concentration in CAS and MBR process, series 2 influent concentration in A2O process.

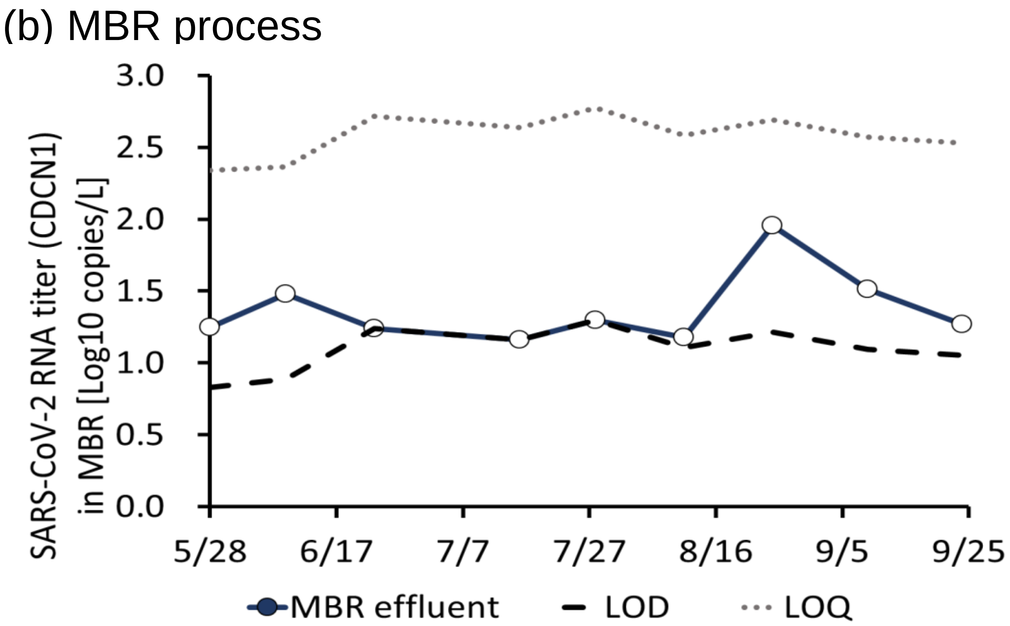
SARS-CoV-2 RNA concentration **in CAS effluent** was N.D-2.91 log10 copies/L.
LRV in CAS process reached **3.1 ± 1.1** log.

SARS-CoV-2 RNA concentration **in MBR effluent** was N.D-1.96 log10 copies/L.
LRV in MBR reached **3.5 ± 0.65** log.

SARS-CoV-2 RNA concentration **in A2O effluent** was 0.89-3.07 log10 copies/L.
LRV in A2O reached **2.5 ± 1.2** log.

Supplementary: effluent concentration of CAS, MBR, A2O





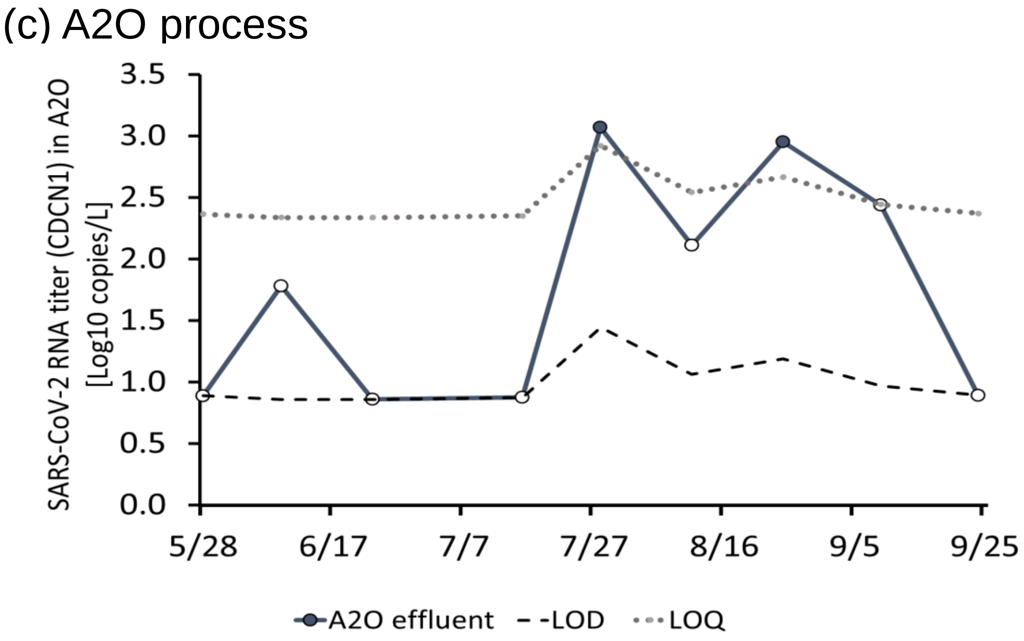


Figure 2. Comparison of SARS-CoV-2 RNA concentration in effluent. Blank plots stand for below the lower limit of quantification (LOQ), indicating that the true value is possibly lower than the plotted value.

Table 1. LOD and LOQ of effluent in each process

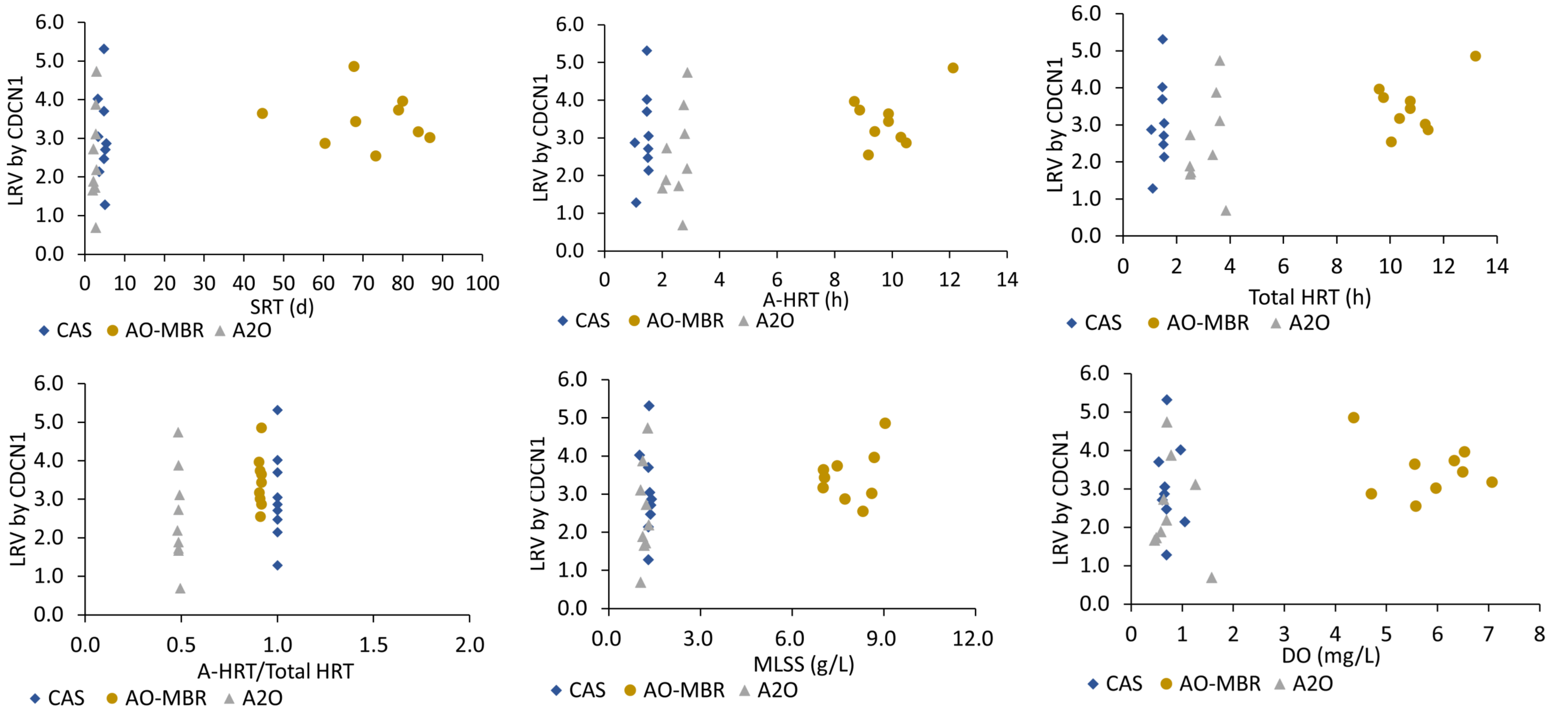
	Average LOD (log10 copy/L)	Average LOQ (log10 copy/L)
CAS effluent	*1.11±0.15*	*2.59±0.15*
MBR effluent	*1.10±0.15*	*2.58±0.14*
A2O effluent	*1.01±0.19*	*2.48±0.19*

Supplementary: removal of enteric virus in wastewater

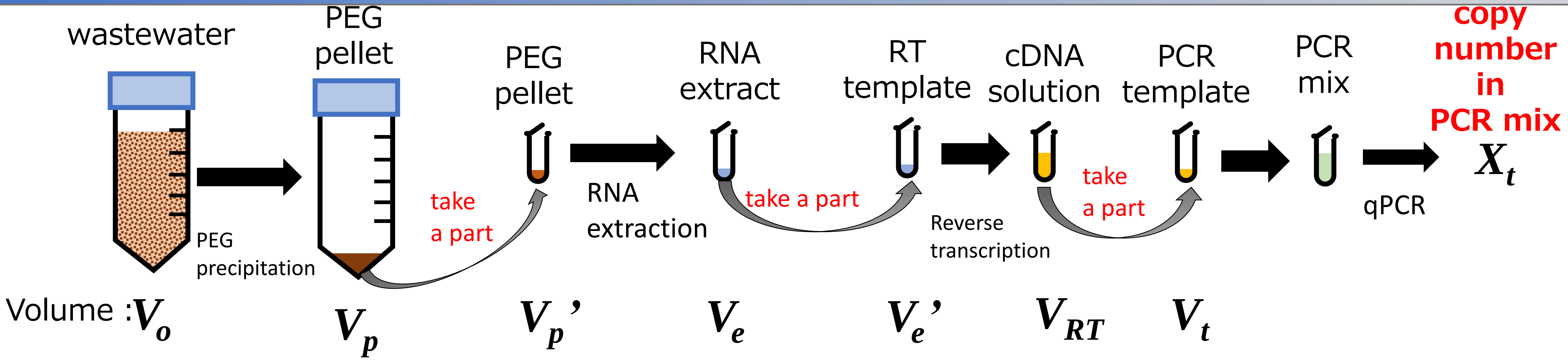
Table 2. Summary of virus concentration and log removal values in various (A) wastewater treatment, and (B) disinfection processes.

Virus	Process of WWTPs	Concentration in influent (copies/L)	Concentration in final effluent (copies/L)	LRV (log reduction)	References
(A) Wastewater Treatment Systems					
GI-Norovirus	CAS	4×10^4–8.2×10^9	1.4×10^2–2.5×10^4	0.50–2.87	99–101
	MBR	10^6–10^9	1×10^3–1×10^4	2.40–4.30	102–105
	A2O	1×10^1–1×10^3	1×10^2	1–2	106–108
	Trickling filter	1.5×10^1–1×10^5	1×10^{-1}–1.5×10^3	1.5–3.5	100,108
	WSP	1×10^1–1×10^9	1.4×10^2–2.5×10^7	0.5–2	103,108
GII-Norovirus	CAS	4×10^2–8.2×10^9	1.4×10^{-1}–2.5×10^3	1.5–3	99–101
	MBR	10^5–10^8	1×10^2–1×10^3	1.1–5.3	102–105
	A2O	1×10^1–1×10^3	1×10^2	1–2	106–108
	Trickling filter	1.5×10^1–1×10^5	1×10^{-1}–1.87×10^4	2.5–3.5	100,108
	WSP	1.5×10^2–1×10^7	1×10^{-1}–1×10^6	0.5–1.5	103,108
GIV-Norovirus-	CAS	1×10^4–1.5×10^5	1.4×10^{-1}–2.5×10^1	4–5	100
	Trickling filter	1.5×10^3–1×10^5	1×10^{-1}–1.87×10^3	2–4	100,108
Murine Norovirus	CAS	–	–	2–3	106
	MBR	–	–	1–3	106
	A2O	–	–	0–1	106
PMMoV	CAS	1×10^6–1×10^{10}	1×10^5–2.5×10^9	2–3	100,109
	MBR	1×10^5–1×10^6	1×10^3–1×10^4	0.70–2	110
	Trickling filter	1×10^5–1×10^6	1×10^5–2.5×10^5	0.5–1	100
Adenovirus	CAS	1×10^2–1×10^6	1×10^0–2.5×10^1	2–3	105,111
	MBR	1×10^3–1×10^6	1×10^1–1×10^3	3.7–5.6	104,111,112
	A2O	1×10^5–1×10^6	1×10^4–1×10^5	0.4–1.6	104
	Trickling filter	1×10^5–1×10^6	1×10^4–1×10^5	0.5–2	100
	WSP	1×10^1–1×10^2	1×10^0–1×10^1	0.7–1	108
Enterovirus	CAS	1×10^4–1×10^6	1×10^0–2.5×10^1	0.5–2.5	100,112
	MBR	1×10^3–1×10^5	1×10^2–1×10^3	1.52–3.89	99,105,106,112,113
	A2O	1×10^2–1×10^5	1×10^3–$1 \times 10^{4.5}$	0.5–1	106
	Trickling filter	1×10^5–1×10^6	1×10^2–1×10^3	2.5–3	100

Supplementary: parameters VS LRV by CDCN1 in CAS, MBR, A2O



PEG precipitation with centrifuge・2-step RT-qPCR in influent



copy number in PEG pellet : $X_p = X_e \cdot \frac{V_p}{V'_p}$

copy number in extract : $X_e = X_{RT} \cdot \frac{V_e}{V'_e}$

copy number in cDNA : $X_{RT} = \frac{X_t}{V_t} \cdot V_{RT}$

Total virus concentration in wastewater

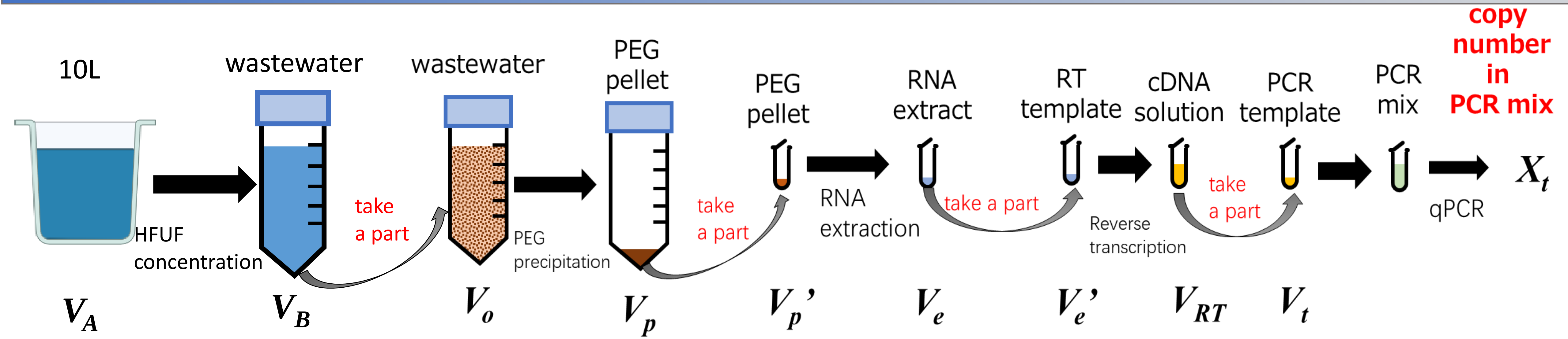
$$C_0 = \frac{X_p}{V_0} = \frac{X_t}{V_t} \cdot \frac{V_p}{V'_p} \cdot \frac{V_e}{V_e'} \cdot \frac{V_{RT}}{V_0}$$

Concentration factor by PEG precipitation: $\frac{V_0}{V_p}$

Concentration factor by RNA extraction: $\frac{V_p'}{V_e}$

Dilution factor by RT: $\frac{V_e'}{V_{RT}}$

PEG precipitation without centrifuge 2-step RT-qPCR in effluent



copy number in HFUF : $X_B = X_p \cdot \frac{V_B}{V_0}$

copy number in PEG pellet : $X_p = X_e \cdot \frac{V_p}{V'_p}$

copy number in extract : $X_e = X_{RT} \cdot \frac{V_e}{V'_e}$

copy number in cDNA : $X_{RT} = \frac{X_t}{V_t} \cdot V_{RT}$

Total virus concentration in effluent:

$$C_0 = \frac{X_p}{V_0} = \frac{X_t}{V_t} \cdot \frac{V_p}{V'_p} \cdot \frac{V_e}{V_e'} \cdot \frac{V_{RT}}{V_A} \cdot \frac{V_B}{V_0}$$

Concentration factor by UFMF: $\frac{V_A}{V_B}$

Concentration factor by PEG precipitation: $\frac{V_0}{V_p}$

Concentration factor by RNA extraction: $\frac{V_p'}{V_e}$

Dilution factor by RT: $\frac{V_e'}{V_{RT}}$