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Antimicrobial Activity in the Gasphase with Hypochloric Acid

Abstract:

Background: The study investigated if the disinfecting potential of Hypochlorous acid (HOCl) in suspensions are transferrable to in-air cleaning applications and to what extent aerosolized HOCl solutions can deactivate indoor microbial contaminations in-air at or below legal limits.

Material and Method: For the liquid disinfection we used a standard suspension disinfection test protocol. For the in-air tests we conducted several experiments where aerosolized bacterial suspensions were injected into lab chambers preloaded with different HOCl gas concentrations.

Results: In suspension experiments we found sufficient efficacies for all studied organisms at minimum concentrations of 200 ppm HOCl. The in-air measurement set-up allows to follow microbe deactivation by HOCl interaction. The deactivation rate increases with the HOCl concentration, and the values are highest for Gram-negative bacteria.

Conclusion: We confirmed our hypothesis of the high disinfecting power of HOCl in-air at safe levels for populated indoor places. The investigated bacteria provide a model system for infectious particles, including enveloped viruses (to which Coronavirus belongs). These early results suggest that HOCl should be further evaluated as an air-cleaning method which may complement established concepts.

Keywords: HOCl, hypochlorus acid, air disinfection, indoor air cleaning, coronavirus, aerosolization

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1 Introduction

Virus-carrying aerosol particles are recognized as infection carriers in the current Corona pandemic, but their high-risk potential is often underestimated and represents the infection route that has been least systematically countered to date.

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Clinical and conventional hygiene masks do not provide sufficient protection due to insufficient filtration efficiency for aerosol particles and even FFP2 masks often do not achieve their theoretical protection level because of insufficient edge closure [1]. As a result, aerosols currently represent an insufficiently contained mode of disease transmission at public indoor spaces (e.g.: offices, schools, gastronomy).

The study investigated if the disinfecting potential of HOCl in suspensions are transferrable to in-air cleaning applications and to what extent aerosolized HOCl solutions can deactivate indoor microbial contaminations in-air at or below legal limits.

2 Methods

For the liquid disinfection we used a standard suspension deactivation process. To create an atmosphere with free floating HOCl solo or in laden water particles one must aerosolize the HOCl solution with particle sizes below 10 µm. This allows the particles staying afloat long enough to vaporize within seconds.

While being afloat HOCl disintegrates into active components like Cl_2O, ClO_2, Cl_2, and finally is reduced to HCl. Such 'active' with HOCl loaded atmosphere has the potential to interact with virus laden aerosol particles and effectively deactivate (oxidize) any air-born microbes [2]. For the in-air tests we conducted several experiments where aerosolized bacterial suspensions were injected into lab chambers preloaded with different HOCl gas concentrations.

The biocidal effect of HOCl was quantified for two domains:

- Biocidal effect in suspensions
- Room air purification

2.1 Suspension tests

Suspension tests have been performed according to the methods of the CEN Technical committee 216 (Chemical disin-fectants and antiseptics): EN 1276 (Bactericidal efficacy), EN 13624 (Levurocidal efficacy) and EN 14476

(Virucidal efficacy).

The following test organism were used: Enterococcus hirae DSM 3320 (corresponding to ATCC 10541), Pseudomonas aeruginosa DSM 939 (ATCC 15442), Staphylococcus aureus DSM 799 (ATCC 6538), Escherichia coli K12 DSM 11250 (=NCTC 10538) and Candida albicans DSM 1386 (=ATCC 10231).

Vaccinia virus (strain Elstree) ATCC VR-1549 was used as test virus in combination withVero-B4-A 33 (DSM) indicator cells.

2.2 Room air purification

We performed two types of atmospheric tests:

- Bacterial decay ('BLANC' measurements)
- HOCl biocidal effectiveness

The BLANC measurements provided the baseline for the calculation of the HOCl biocidal effect for atmospheric tests. With DIN EN 17272 (Airborne disinfection), a method for the efficacy of nebulized biocide for surface disinfection has also been published since 2020[3]. For the efficacy of nebulized biocide in the gas phase or in aerosols itself, however, no methods are yet available. Therefore, in a newly developed experimental procedure, bacteria were aerosolized, and the disinfecting effect of the vaporized biocide (HOCl) was investigated.

The test organisms used were Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli K12, gram-positive and Gram-negative bacteria (from DIN EN 1276, or closely related organisms).

A commercial preparation of Hypochloric acid was used as biocidal agent: Biodyozon (Biodyozon GmbH, Dreieich, Germany) with a 1000 ppm HOCl stock solution. For the tests with aerosolized HOCl a 500-ppm solution was obtained by dilution the stock solution with distilled water.

The tests were carried out in two sizes of measuring chambers (1m^3 and 34 m^3), which allowed continuous monitoring of temperature and barometric pressure. The 1m^3 chamber consists of a cubic stainless-steel housing. The larger chamber was a 3.4m x 4m x 2.5m specially prepared office container. Both chambers have pass-through channels or doors for microbial inflow and windows for sampling (gas filter sampling of room air with corresponding recirculation of the filtered/analyzed air sample). HOCl aero-solubilization is performed in the room itself using the aerosolis® device from oji-Europe.

3 Results

3.1 Suspension Experiments

In suspension experiments we found sufficient efficacy against all studied organisms (4 bacteria according to EN 1276, C. albicans according to EN 13624 as well as vaccinia virus according to EN 14476) at minimum concentrations of 200 ppm HOCl. Vaccinia virus as a model for enveloped viruses appeared to be even more sensitive.

Table 1: Biocidal effect of HOCl on various microbes

	P. aeruginosa	S. aureaus	E. hirae	E. coli	C. albicans	Vaccinia virus
conc [ppm]	Standard EN 1276				EN 13624	EN 144476
800	>5.33	>5.22	>5.16	>5.11		4.75
400	>5.33	>5.22	>5.16	>5.11	>4.11	nt
300	>5.46	>5.32	>5.54	>5.05		4.75
200	>5.46	>5.32	>5.54	>5.05	>4.11	4.50
50	2.74	<0.95	1.52	4.53	<0.74	4.00
10	<1.09	<0.95	<1.17	<0.68		0.50

[suspension Measurement]

3.2 Biocidal effect of vaporized HOCl

The in-air measurement set-up allows to follow microbe deactivation by HOCl interaction. The deactivation rate increases with the HOCl concentration, and the values are highest for Gram-negative bacteria.

The following graph shows the result of a typical HOCl induced biocidal measurement together with the corresponding BLANC test runs. The dotted lines represent the exponential fits of the two curves for the period post the bacterial injection phase.

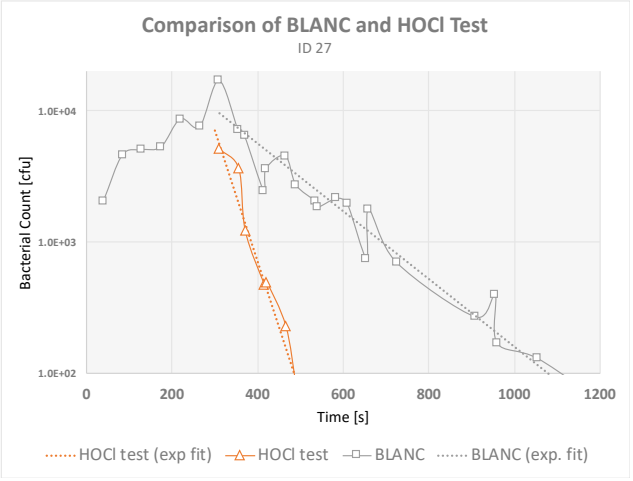

Figure 1: Typical result of a BLANC and a corresponding HOCl measurement

The following table and graph show the results for the decay of various representative bacterial organism types induced by HOCl at different in-air concentrations. Comparing corresponding BLANC and HOCl test runs and computation of the corresponding HOCl deactivation rate (in %/min).

Table 2: HOCl induced deactivation of 4 different bacteria populations

ID	Microbe	Charge	Envelope	HOCl conc (ppm)	HOCl conc (mg/m3)	HOCl induced microbe deact [%/min]
1	Pseudomonas aeruginosa	gram -	lipid membran	0.01	0.03	13%
3	Pseudomonas aeruginosa	gram -	lipid membran	0.23	0.54	98%
27	Escherichia coli	gram -	lipid membran	0.15	0.35	62%
28	Escherichia coli	gram -	lipid membran	0.15	0.35	72%
46	Staphylococcus aureus	gram +	Murein capsid	0.13	0.30	73%
47	Staphylococcus aureus	gram +	Murein capsid	0.26	0.59	97%

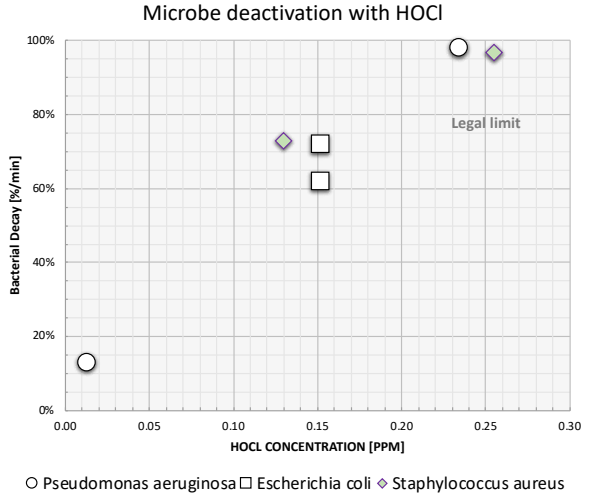

Figure 2: Microbe deactivation in-air with HOCl

Within species the deactivation rate increases with the HOCl concentration. The absolute values are highest for the Gram-negative bacteria. The vertical dashed line marks the EU legal limit of 0.21 ppm of in-air free chlorine (e.g.: HOCl) in populated rooms.

4 Discussion

The results of the measurements with HOCl are very consistent. The HOCl deactivation levels could be determined in over 90% of the measurements. The remainder of the experiments is still under evaluation.

The results of our measurements support the hypothesis that HOCl can be used as an effective air cleaning component. The concentrations which result in a substantial bacterial deactivation are dominantly well below legal limits and safe [4-7].

For the investigated Gram-negative species (Pseudomonas aeruginosa, E. coli) we can stipulate an almost linear relationship between HOCl concentration and bacterial deactivation rate. This effect is very important considering the chemical and principal structural similarity of the envelope of Gram-negative and coronavirus lipid envelopes. It supports the assumption that enveloped viruses can be progressively deactivated with increasing HOCl concentration. When we transfer the results to a simple table of bacterial decay and HOCl exposure time, it becomes apparent how effective HOCl would reduce a bacterial or presumably viral load in a contaminated indoor atmosphere.

The table below shows the deactivated percent of the bacterial load as a function of the bacterial decay rate (due to HOCl biocidal effect) along the HOCl exposure time. For the measured Gram-negative bacteria at a concentration of 0.11 ppm (half the legal limit), the bacterial deactivation rate of 50% would result in a so-called LOG 3 reduction within only 10 minutes.

Table 3: Microorganism reduction along exposure time in an HOCl atmosphere [min]

	HOCl exposure time [min]					
	10	20	30	40	50	60
Bacterial decay 10%	65.132%	87.842%	95.761%	98.522%	99.485%	99.820%
[%/min] 20%	89.263%	98.847%	99.876%	99.987%	99.999%	100.000%
30%	97.175%	99.920%	99.998%	100.000%	100.000%	100.000%
40%	99.395%	99.996%	100.000%	100.000%	100.000%	100.000%
50%	99.902%	100.000%	100.000%	100.000%	100.000%	100.000%
60%	99.990%	100.000%	100.000%	100.000%	100.000%	100.000%
70%	99.999%	100.000%	100.000%	100.000%	100.000%	100.000%
80%	100.000%	100.000%	100.000%	100.000%	100.000%	100.000%
90%	100.000%	100.000%	100.000%	100.000%	100.000%	100.000%

In real-life situations one must expect a continuous virus injection through a present spreader. Therefore, the so-called LOG reduction - as used in filter classification protocols - can hardly be applied to this situation.

In real life any microbial insertion (through spreader) will be only partly counteracted with a bacterial consumption process (e.g.: ventilation, filtering, air cleaning with HOCl, self-decay through oxidization, or biological consumption through present people). All these effects have their efficiency and limits in reducing the indoor air microbial load.

TRANSFERABILITY OF THE RESULTS TO VIRUSES

An analogous study like the investigations carried out here with bacteria, with viruses is not possible.
So far, the recovery of aerosolized infectious virus particles in

a reliable quantitative manner has only been shown once[6].
Although the presence of viruses in the ambient air can be determined by means of molecular biological methods (PCR), a quantitative statement on the infectivity of the virus particles has not yet been possible [8-9].
Since the efficacy of the biocide does not directly attack the integrity of the viral RNA, but probably first attacks the spike proteins on the surface of the virus, molecular biological detection is of no help here.

Therefore, representative bacteria were used as surrogate organisms for infectious particles in the tests. The suspension test results demonstrate that the sensitivity to HOCl being approximately in the same concentration range for all organisms, with vaccinia virus at the lower limit, i.e., even slightly more sensitive overall (see above).

Vaccinia virus is considered a surrogate virus for all enveloped viruses in the tests of CEN TC 216, i.e., with sufficient efficacy against vaccinia virus, efficacy against all enveloped viruses (which includes SARS-CoV-2) can be assumed. The effect of the biocide in the nebulized germ-containing aerosol droplets is not completely known.

Nevertheless, it can be assumed that the organisms in the droplets are present in a micro-suspension, into which the biocide molecule then enters either itself as aerosol droplet or from the gas phase [10-16]. In this respect, the results from the macroscopic suspension tests can be transferred. If the bacteria used are clearly deactivated, it can therefore also be assumed that nebulized enveloped viruses are deactivated.

5 Conclusion

We validated the disinfecting effects of HOCl in suspensions and additionally, confirmed our hypothesis of the high in-air disinfecting power of HOCl at safe levels for populated indoor places. The investigated microbes (Gram-positive, Gram-negative bacteria and Vaccinia virus) can be understood as a general model system for infectious particles, including enveloped viruses (to which Coronavirus belongs). The method is harmless, and no tissue irritation is to be expected at the investigated concentration levels, which many peer-reviewed studies support [17-22].

These early results suggest that HOCl based in-air cleaning should be further evaluated. The method offers real life applications due to the broad operating window of HOCl between effective and safe concentration levels and may present a valuable addition to existing infection control procedures like ventilation, high class air filter systems and personal hygiene protection.

Author Statement

Research funding: oji Europe GmbH; Conflict of interest: Authors state that they are affiliated with oji Europe. Dr. Boecker and Mr. Zhang are consultants to the company, Dr. Breves is head of the microbiology lab where the measurements were conducted, Prof. Bulitta is member of the scientific advisory panel. Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

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