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Safe, Effective, and Cost-Efficient Air Cleaning for Populated Rooms and Entire Buildings Based on the Disinfecting Power of Vaporized Hypochlorous Acid

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Abstract**—**Pathogen-carrying aerosol particles are recognized as important infection carriers like those in the current Corona pandemic. This infection route is often underestimated yet represents the infection route that has been least systematically countered to date. Particularly, the transmission indoors is of the highest concern but current indoor safety measures (e.g.: distancing, masks, filters) provide only limited protection. Inhalation of hypochlorous acid (HOCl) containing aerosols may become an alternate route to attack the incubating microbes in-situ and so potentially lead to a reduction of symptoms of already infected individuals. We investigated a facility-wide air-disinfection concept utilizing the potential of vaporized HOCl to become a disinfecting agent for populated indoor atmospheres. Aerosolized bacterial microbes were used as surrogates for a viral contamination, particularly the enveloped coronavirus. For the room air purification tests we aerosolized bacterial suspensions into lab chambers preloaded with vaporized HOCl solutions. Concentration of 'free active chlorine' in the test chamber atmosphere was determined with a special gas sensor system (Draeger AG, Lübeck, Germany) controlling the amount of vaporized HOCl via an aerosolis® device (oji Europe GmbH, Nauen, Germany). We could confirm the disinfecting power of HOCl in suspensions and determined the high efficacy of vaporized HOCl to disinfect atmospheres of populated indoor places at safe and non-irritant levels.

Keywords**—**Hypochlorous acid, HOCl, indoor air cleaning, infection control, microbial air burden, protective atmosphere.

I. Introduction

HYPOCHLOROUS acid (HOCl) is a potent broad spectrum fast-acting antimicrobial agent with a favorable safety profile. It also is key actor of the body's innate immune response system. It has the highest redox potential of all physiological intracellular occurring defense mechanisms (e.g.: H_2O_2). It was entered into the 'List N' of United States Environmental Protection Agency (EPA) for use in disinfection against the coronavirus pandemic [1], [2].

All practical pathways of administering HOCl have been investigated and demonstrated a safe and effective way to booster the innate immune response [3]-[15]. The methods span nasal and pharyngeal inhalation, topical applications (e.g.: wound care), and gastro-intestinal and even systemic intra-venous (i.v.) delivery.

Increasing evidence is emerging of the beneficial effects of inhaling micro aerosolized HOCl as a routine intervention in the prevention and treatment of respiratory virus infections, including SARS CoV-2 [3]-[6]. The treatments reduce nasal and pharyngeal viral load and can minimize the progression and/or spread of the disease.

Nasal-spray treatments for respiratory tract viruses have been explored in several pre-clinical and other trials [3], [7]-[12]. In these nasal formulations, HOCl has shown bactericidal, fungicidal, or virucidal effects [7], [10], [12]-[19]. Several of these antiseptics have demonstrated the ability to cut the viral load of SARS-CoV-2 by 99.9% - 99.99% in 15–30 s *in vitro*. Several such products are already commercially available for prevention or early treatment of COVID-19 and have shown promising results [8], [20]. In this way HOCl has proven to serve as a potential solution for upper respiratory tract hygiene assisting intra-cellular defense mechanisms by its extra cellular attack on adsorbed pathogens (not yet inserted their RNA into intracellular space).

It is important to note that the use of disinfectants has seen quite an increase to combat the current pandemic. However, many of commonplace disinfecting substances and application procedures have not been designed nor tested for intensive applications around humans [3], [21]-[24]. Toxicological evidence of serious adverse side-effects of disinfectants has become a more intensely studied field of research. In particular, the unintended exposure to certain disinfectants – like inhalation of aerosols created by general cleaning procedures - is an area of major concern [23], [24].

Extensive research has been done previously at exposure to most frequently used disinfectant compounds, that is, quaternary ammonium salts (QAS), sodium hypochlorite, hydrogen peroxide, ozone, glutaraldehyde, and alcohols of various types. These commonly used disinfectants were identified as potential cause for a series of pulmonary and ocular conditions for health workers and individuals when used regularly (e.g.: COPD, asthma, eye irritation) [25]-[29].

In particular, the harmful and toxic effect of sodium hypochlorite (NaOCl) is often confused with the safe utilization of HOCl, when abiding to the well documented legal

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concentration levels.

HOCl, however, when used as a sole component within approved limits shows no negative side effects on living cells in topical, inhaling and even systemic applications. Safety of any HOCl application is of course the most important concern. In animal studies with massive HOCl vapor exposure (way beyond necessary limits to be effective as a virucide agent) no detectable blood parameter change, nor any significant change of lung function was observed [8]. Also, in human studies no observable changes in the endoscopic scores were detected after 8 weeks of regular exposure with HOCl via nasal irrigation [7].

II. Methods and Materials

A. Suspension Tests

The biocidal effect of HOCl was quantified by a series of standard suspension deactivation tests, performed according to the methods of: CEN Technical committee 216: EN 1276, 13624, and 14476.

B. Room Air Purification

For room air purification tests we aerosolized bacterial suspensions (with a protein load of 0.1 or 0.3%) into lab chambers preloaded with vaporized HOCl solutions. Tests were carried out in two controlled measuring chambers (1 m^3 and 34 m^3).

Total amount of ‘free active chlorine’ was determined by volume of vaporized HOCl, performed with an aerosolis® device (oji Europe GmbH, Nauen, Germany). Concentration of HOCl follow-on products were continuously measured by hand-held gas sensor devices specific for Cl_2, ClO_2 and HCl (Draeger AG, Lübeck, Germany).

We used our own developed two step experimental procedure to determine the efficacy of vaporized biocide in the gas phase, because no standard method is available yet:

- Bacterial decay measurements (‘BLANCs’)
- HOCl biocidal effectiveness measurements

Aerosolizing a bacterial suspension into a test chamber results in a concentration profile determined by three factors: (1) number of injected bacteria and (2) self-decay rate of aerosolized bacteria, and (3) HOCl biocidal effect. Taking BLANC measurements as baseline and separate HOCl laden measurements as cumulated bacterial self-decay/HOCl effect tests, allows to net out the biocidal HOCl effect.

C. Materials

Used test organisms for suspension tests: *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) [4]. We used vaccinia virus (strain Elstree) ATCC VR-1549 as a test virus together with Vero-B4-A 33 (DSM) indicator cells [4].

Used test organisms for room air purification are *Staphylococcus aureus* and *Staphylococcus warnerii*, *Pseudomonas aeruginosa*, and *Escherichia coli* K12 (strains all as above).

TABLE I
Investigated Microbes in Aerosolization Experiments

Bacterium	Type	Envelope
Pseudomonas aeruginosa	gram -	liquid membrane
Staphylococcus aureus	gram +	murein capsid
Staphylococcus warnerii	gram +	murein capsid
Escherichia coli	gram -	liquid membrane

A commercial preparation of HOCl was used as biocidal agent: Biodyozon Clean Air (Biodyozon GmbH, Dreieich, Germany) with a 1,000 ppm HOCl stock solution, diluted with distilled water to 500 ppm.

III. Results

A. Suspension Tests

In standard suspension experiments we tested all relevant organisms with varying concentrations of our HOCl solution at low soil conditions (0.03% protein). Vaccinia virus as a model for enveloped viruses appeared to be even more sensitive [30]. An overview of the obtained RF values is given in Table II.

TABLE II
Biocidal Effect of HOCl on Various Microbes [Suspension Tests]

conc. [ppm]	*Pseudom. aeruginosa*	*Staph. aureus*	*E. hirae*	*E. coli*	*C. albicans*	*Staph. warnerii*	*Vaccinia virus*
	Standard EN 1276				EN 13624	Additional organisms	EN 14476
	Time: all 30 sec						
800	> 5,33	> 5,22	> 5,16	> 5,11		nt	> 4,75
600	> 5,33	> 5,22	> 5,16	> 5,22		nt	nt
400	> 5,33	> 5,22	> 5,16	> 5,11	> 4,11	nt	nt
300	> 5,46	> 5,32	> 5,54	> 5,05		nt	> 4,75
200	> 5,46	> 5,32	> 5,54	> 5,05	> 4,11	> 5,50	> 4,50
100	2,35	2,85	5,02	> 5,05		> 5,05	> 4,50
50	2,74	< 0,95	1,52	4,53	< 0,74	1,66	> 4,00
10	< 1,09	< 0,95	< 1,17	< 0,68		< 0,68	< 0,50

Under the selected conditions (room temperature, low organic load and incubation time 30 sec), sufficient efficacy was found for all organisms (4 bacteria according to EN 1276, *C. albicans* according to EN 13624 as well as vaccinia virus according to EN 14476) at concentrations from 200 ppm HOCl [4]. At a concentration of 50 ppm, however, efficacy against bacteria and yeasts was no longer sufficient. Against vaccinia virus, 50 ppm was still just sufficient, but 10 ppm no longer.

B. Room Air Purification Tests

Fig. 1 shows the biocidal effect of an HOCl laden atmosphere. The net effect is determined by comparing the decay measurement (red line) to a BLANC run (grey line). The dotted lines show the exponential fits starting with the time when the bacterial injection phase ended (t > 300 s) [4].

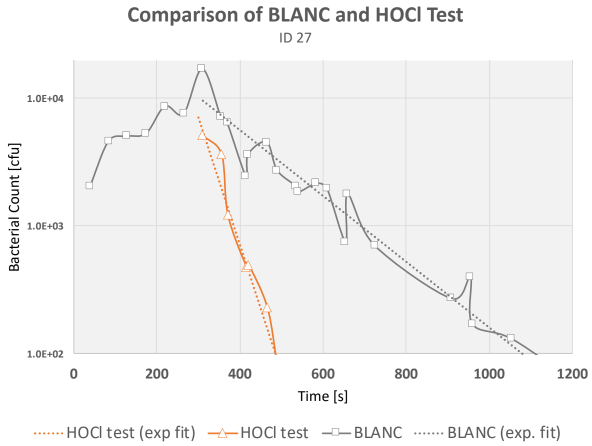

Fig. 1 BLANC and corresponding HOCl measurement

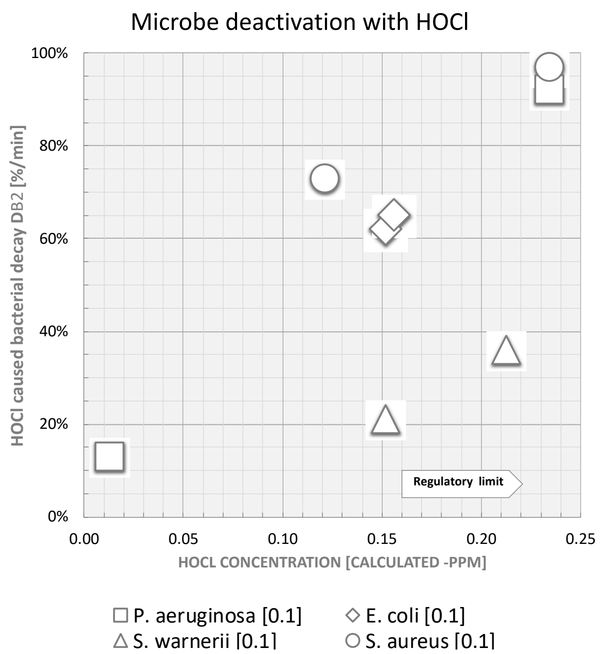

Fig. 2 Disinfection rates DB2 for various microbes at different HOCl concentrations

The determination of the net bacterial inactivation by HOCl (D_{B2}) is obtained under the premise that the bacterial self-deactivation (d_2), as determined through the BLANC tests (grey line), and the HOCl caused effect are independent processes and behave multiplicatively to yield D_{comb}, which is measured in the HOCl tests (orange line). This provides for D_{B2}:

$$D_{B2} = 100 - \frac{100 - D_{Comb}}{100 - d_2}$$

Fig. 2 shows the results for the disinfection rates D_{B2} for studied bacteria at different HOCl in-air concentrations.

The deactivation rate is proportional to the HOCl concentration (within species). The highest values are observed for Gram-negative microbes. The EU limit for safe long-term exposure to a free-chlorine laden atmosphere is depicted with the vertical dashed line at 0.21 ppm [4].

IV. Discussion

The results of the suspension test support the suitability of the biocide preparation used here. Bacteria and vaccinia virus show high susceptibilities to HOCl (vaccinia is even more sensitive). These results suggest (according to CEN TC 216) high sensitivity of all enveloped viruses (including SARS-CoV-2) to HOCl.

To measure the virucidal efficacy of an HOCl laden atmosphere is problematic since it would be requesting quantitative recovery of infectious virus particles from the air. Molecular biological detection of viral RNA via PCR methods would include inactivated virus particles as well. To overcome this principal hurdle, we used in the tests representative bacteria as surrogate organisms for pathogens. The vaccinia virus is such an accepted surrogate virus for all enveloped viruses (e.g.: SARS-CoV-2) [4].

Our results demonstrate that HOCl can be used as an effective air cleaning agent. Aerosolized HOCl solutions vaporize within seconds resulting in an HOCl laden atmosphere which can slowly transition into a series of chlorine carrying products (e.g.: Cl_2O, ClO_2, Cl_2, Cl·), all of which are summed up as 'free active chlorine' according to EU regulations.

In such an 'active' atmosphere, virus laden aerosol particles and any other airborne microbes are deactivated [4], [31]. The required concentrations of free-chlorine to gain a substantial bacterial deactivation are well below legal limits, safe, and non-irritant [4], [25], [32], [33].

The results indicate that enveloped viruses - given their chemical and structural similarity with Gram-negative bacteria - can be progressively deactivated with increasing HOCl concentration. Aerosolized infectious organisms are attacked by biocidal molecules either by droplet merge (aerosol/aerosol) or from the gas phase [34]-[37], which suggest the transferability of our suspension and in-air test results: If the studied bacteria are deactivated, so will be aerosolized enveloped viruses.

V. Conclusion

The importance of our results is twofold:

- Infection prevention: HOCl activated air may offer a low-cost, efficient way to secure a pathogen reduced/free facility atmosphere.
- Disease progression: HOCl enriched air has the potential to contain or even invert disease progress.

Facility management may play an important role in future infection control. Even in general terms, because the potential of HOCl to serve as a safe, efficient, and cost-effective indoor disinfectant points way beyond COVID related applications. Our results suggest that use of HOCl based room air decontamination counters the need for high air exchange rates for infection control and in doing so would offer significant energy savings.

Further developments will comprise controlling the amount of vaporized HOCl via an aerosolis® device (oji Europe GmbH, Nauen, Germany) combined with a special gas sensor system (Draeger AG, Lübeck, Germany) and control unit.

Today, any microbial insertion (through viral spreaders) will only be partly contained with incumbent safety measures. We confirmed our hypothesis of the high disinfecting power of HOCl-laden atmospheres. The method can be used in populated indoor environments because it is safe at the investigated concentration levels according to many peer-reviewed studies [25], [38], [39]. Our early results suggest that HOCl based air-cleaning for populated rooms should be considered as a potential alternative and should be further evaluated.

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