Ecotoxicity evaluation of pure peracetic acid (PAA) after eliminating hydrogen peroxide from commercial PAA

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Abstract In recent years, disinfection of inflowing Combined sewer overflows (CSO) water in the CSO discharge structures has been studied using Peracetic acid (PAA) to minimize the impact from the discharge of untreated CSO to the surface waters.

Degradation of hydrogen peroxide was slower than PAA when it was used to disinfect CSO. All previous toxicity studies was based on commercial PAA mixture and variance on toxicity value was observed due to different PAA: hydrogen peroxide ratio. In this **s**tudy, hydrogen peroxide was eliminated from the PAA mixture using potassium permanganate to avoid the strict environmental risk assessments of hydrogen peroxide to obtain the permit from the authorities. Ecotoxicity data of PAA without hydrogen peroxide was obtained by conducting the battery of ecotoxicity test: the bioassays using *Vibrio fischeri*, *Daphnia magna* and *Pseudokirchneriella subcapitata*.

Effect concentration (EC_{50}) of PAA without hydrogen peroxide was 0.84 mg/L for *Vibrio fischeri* and 2.46 mg/L for *Pseudokirchneriella subcapitata*, respectively whereas lethal concentration (LC_{50}) was 0.74 mg/L for *Daphnia magna*. The toxicity results showed that pure PAA was less toxic to the most commonly used aquatic species for toxicity tests compared to the commercial PAA.

Keywords: Peracetic acid, Hydrogen peroxide, Disinfection, Combined sewer overflows, Ecotoxicity

1. Introduction

Combined sewer overflows (CSO) is a variable mixture of wastewater and rain water. Quality of receiving surface waters get deteriorates when untreated CSO is discharged into it. In recent years, the effect of CSOs on water bodies used for recreational purpose has caught significant attention in Europe. European Union have defined the bathing water standards for recreational purposes in the directive 2006/7/EC and to qualify for a good quality of bathing water, the number of indicator bacteria should not exceed 500 MPN *E. coli* and 200 MPN *Enterococcus* per 100 mL water intended for recreational purposes (Directive 2006/7/EC, 2006).

Good bathing water quality can be maintained by disinfection of the inflowing CSO to the receiving waters which will reduce the number of indicator bacteria. There are various well known disinfectants used in the water industries and recently, peracetic acid (PAA) and performic acid (PFA) has been used to disinfect CSO (Chhetri *et al*., 2016b, 2015, 2014). PAA is a well-known disinfectant which was introduced to wastewater treatment approximately 30 years ago(Antonelli *et al*., 2013, 2006; Baldry, 1983; Falsanisi *et al*., 2006; Kitis, 2004; Luukkonen *et al*., 2015). Commercial PAA is a quaternary equilibrium mixture of PAA, acetic acid and hydrogen peroxide:

$$CH_3COOH + H_2O_2 \rightleftharpoons CH_3COOOH + H_2O \quad \text{Eq. 1}$$

$$CH_3CO - OOH + 2e^- \rightarrow CH_3CO - O^- + HO^- \quad \text{Eq. 2}$$

The residues after PAA use are acetic acid, hydrogen peroxide and water. The degradation of hydrogen peroxide is slower than PAA (Chhetri *et al*., 2014; Wagner *et al*., 2002) and it has a stringent discharge limit to the surface water. Furthermore, ecotoxic evaluation of residual PAA and its degradation product, hydrogen peroxide, is important to minimize its impact to the aquatic ecosystems when PAA is used for full scale disinfection.

The aim of this study was to study the ecotoxic effect of PAA when hydrogen peroxide was eliminated from the commercial PAA. The ecotoxic effect of PAA without hydrogen peroxide was studied using the bioassays using *Vibrio fischeri* (*V. fischeri*), *Daphnia magna* (*D. magna*) and *Pseudokirchneriella subcapitata* (*P. subcapitata*) which was later used for aquatic environmental risk assessment.

2. Materials and methods

2.1. Chemicals and chemical analysis

ABTS (2,2”-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt), potassium permanganate and PAA solution containing 30–40% (w/w) of technical grade were purchased from Sigma–Aldrich (Brøndby, Denmark).

PAA concentration was analyzed using the colorimetric method described by Chhetri *et al*. (2014) based on selective oxidation of ABTS by PAA without interference from hydrogen peroxide. Hydrogen peroxide was analyzed using the titanium oxide-oxalate colorimetric assay (Antoniou and Andersen, 2015). Hydrogen peroxide from commercial PAA solution was removed by titration with potassium permanganate ($KMnO_4$). Endpoint of titration was determined by slight appearance of pink color of permanganate where hydrogen peroxide was eliminated from the commercial PAA.

2.2. Bioassays

The toxicity towards the photobacterium *V. fischeri* was measured with a commercial assay kit marketed as BioTox™ (AboatoxOy, Finland) according to ISO 11348-3, 2007. Prior to the assay the pH of all samples was adjusted to 7.0 ± 0.2 with NaOH or H_2SO_4 solutions. NaCl was added to obtain a final chloride concentration of 2% w/v in the samples. The toxicity towards the freshwater microalgae *P. subcapitata* was determined using modified ISO 8692, 2012. Laboratory culture of *Pseudokirchneriella subcapitata* was obtained from the Norwegian Institute for Water Research, Oslo, Norway (NIVA). The ISO algal medium was used to prepare a range of concentrations of the PAA, which was then inoculated with exponentially growing algae to a density of 10^4 cells/mL. The immobilization test with the crustacean *D. magna* were performed using method and testing conditions prescribed by ISO 6341, 2012. The tests with *D. magna* neonates less than 24 h old were incubated at 20±2°C in the dark mixed with samples. The *D. magna* neonates were considered immobilized after 48 h of incubation with the test sample, if they remained settled at the bottom of the test container and did not start swimming within 15 s of observation.

3. Results and discussion

3.1. Toxicity values

Effect concentration (EC_{10} and EC_{50}) of PAA without hydrogen peroxide, commercial PAA and hydrogen peroxide obtained from *V. fischeri* and *P. subcapitata* toxicity and lethal concentration (LC_{10} and LC_{50}) obtained from *D. magna* is reported in table 1. Among three aquatic species, PAA without hydrogen peroxide was most toxic to *D. magna* followed by *V. fischeri*. Toxicity (EC_{50}) of PAA towards *V. fischeri* was 0.84 mg/L whilst toxicity from commercial PAA towards *V. fischeri* was 0.47 mg/L (Chhetri *et al*., 2017). Moreover, toxicity value of PAA (2.46 mg/L) towards *P. subcapitata* was double than commercial PAA mixture (1.38 mg/L) (Chhetri *et al*., 2016a). Antonelli *et al*., (2009) have different EC_{50} values of commercial PAA for *V. fischeri* (0.13 mg/L) and *P. subcapitata* (8.89 mg/L) compared to our study. Both *V. fischeri* and *P. subcapitata* are microorganisms and micro algae, respectively, which were less sensitive to PAA when hydrogen peroxide was eliminated from the commercial PAA mixture. However, toxicity (LC_{50}) of PAA towards *D. magna* was almost similar to commercial PAA mixture (Chhetri *et al*., 2016a). *D. magna* is crustacean which has a different cell structure and morphology than *V. fischeri* and *P. subcapitata* and this alter the toxic mechanism of PAA towards these aquatic species. Liu *et al*., (2015) found different toxicity (LC_{50}) for *D. magna* ranging from 0.18-0.77 mg/L when PAA formulation with diverse PAA: H_2O_2 ratios was used (Table 1). Furthermore, PAA mixture with less hydrogen peroxide concentration showed low toxicity towards *D. magna* whilst toxicity values towards *D. magna* increased when more hydrogen peroxide was present in PAA formulation. Hydrogen peroxide exhibit toxicity towards the aquatic species but the toxic inhibition occur at higher values; EC_{50} of 5.67 mg/L for *V. fischeri*, 2.90 mg/L for *P. subcapitata* and LC_{50} of 3.46 mg/L for *D.* magna which were less than commercial PAA mixture. The results obtained in this study showed that when hydrogen peroxide was eliminated from the commercial PAA, it also reduce the toxicity towards the aquatic species. Toxicity value (EC_{50}) of commercial PAA on *V. fischeri* and *P. subcapitata* was nearly half than PAA alone and this might be due to the synergic effect of hydrogen peroxide presented in the commercial PAA. Flores *et al*., (2014) have proposed two stages of an attacking scheme of PAA to the microorganisms when used for disinfection. In first step, PAA eliminated some specific components (e.g. catalase enzyme) from the cell of the microorganisms, which would otherwise inhibit the parallel action of hydrogen peroxide. However, when hydrogen peroxide was eliminated from commercial PAA, synergic effect of hydrogen peroxide was also eliminated which was observed experimentally by comparing the toxicity values of PAA and commercial PAA.

Table 1. Effect concentration (EC_{10} and EC_{50}) of PAA, commercial PAA and hydrogen peroxide on *V. fischeri* at 30 min, algal growth rate inhibition at 72 h and lethal concentration (LC_{10} and LC_{50}) of PAA, commercial PAA and hydrogen peroxide on *D. magna* at 48 h contact time from eco-toxicity tests based on nominal concentration. 95% confidential interval in parenthesis.

Test		PAA (mg/L)	Commercial PAA* (mg/L)	Hydrogen peroxide (mg/L)
Vibrio fischeri	EC_{10}	0.47 (0.38-0.58)	0.27 (0.16-0.27); 0.13**	1.06 (0.52-2.16)
	EC_{50}	0.84 (0.78-0.91)	0.42 (0.41-0.44)	5.67 (4.20-7.65)
Pseudokirchneriella subcapitata	EC_{10}	2.14	0.23 (0.10-0.53)	1.78 (1.63-1.94)
	EC_{50}	2.46	1.38 (0.96-1.99); 8.89**	2.90 (2.87-2.92)
Daphnia magna	LC_{10}	0.45 (0.20-0.59)	0.53 (0.28-0.66)	2.59 (1.79-3.01)
	LC_{50}	0.74 (0.55-0.91)	0.78 (0.59-0.95); 0.18-1.1***	3.46 (2.97-3.96)

* Chhetri *et al.*, (2017, 2016b)
**Antonelli *et al.*, (2009)
***ECETOC (2001) & Liu *et al.*, (2015)

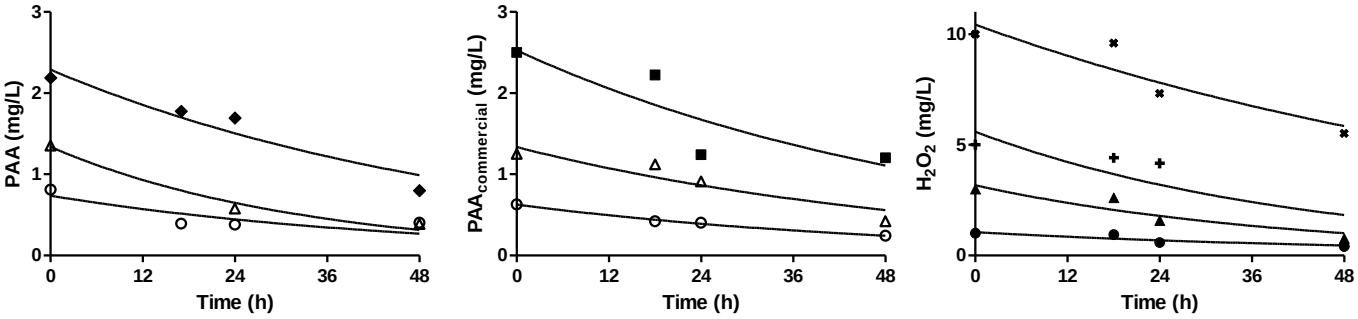

Figure 1. Concentration profiles of PAA without hydrogen peroxide, commercial PAA and hydrogen peroxide in Daphnia test medium

3.2. Concentration profiles in test media

Concentration profiles of PAA without hydrogen peroxide, commercial PAA and hydrogen peroxide in Daphnia test medium was obtained by measuring concentration over time (Figure 1). Low concentration of PAA was not possible to obtain due to limit of quantification of colorimetric assay. First order degradation kinetics model described in equation 3 was used for curve fitting in figure 1.

$$C_t = C_0 \cdot e^{-kt} \quad \text{Eq. 3}$$

In Equation (3) C_t is the residual disinfectant concentration at time t, C_0 is the applied disinfectant dose, k is the rate constant, and t is time.

A slow degradation of PAA, commercial PAA and hydrogen peroxide was observed in Daphnia test medium. PAA degrades slower in samples which was observed in our previous studies when concentration profiles were observed in the algal test medium and in combined sewer overflows (Chhetri *et al*., 2016a, 2016b, 2014). According to CLP regulation, toxicity values (EC/LC_{50}) less than 1 mg/L gives a classification as ''Acute toxic'' i.e. very toxic to the aquatic organisms (EU Commission, 2011). PAA without hydrogen peroxide will be considered as very toxic towards *V. fischeri* and *D. magna* according to CLP regulation however, that will not be considered towards *P. subcapitata*. Overall, it is evident that PAA was less toxic to the micro algae and microorganisms when hydrogen peroxide was eliminated from the mixture of PAA compare to the commercial PAA.

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