



Comparative Disinfection and Residual Effect of Peracetic Acid with Other Disinfectants in Aquaculture

Suvana S^{1,2*}, Muralidhar M², Lalitha N², Avunje S³, Baldev E², Kumararaja P², Saraswathy R², Vinay TN³, Gopinath D², Rajesh R² and Nagavel A²

¹ICAR- Central Research Institute for Dryland Agriculture, Hyderabad, India

²ICAR- Central Institute of Brackishwater Aquaculture, Chennai, India

³ICAR- Central Institute of Freshwater Aquaculture, Bangalore, India

*Corresponding Author: Suvana S, ICAR- Central Research Institute for Dryland Agriculture, Hyderabad, India.

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Suvana S., et al.

Abstract

Peracetic acid (PAA) is a promising disinfectant for use in aquaculture due to its strong oxidizing properties and relatively harmless decomposition products compared to other commonly used disinfectants such as bleaching powder and sodium hypochlorite. This study evaluated the residual effect and efficiency of PAA under controlled conditions at low (5 ppt) and high (25 ppt) salinities. The degradation of PAA was rapid, with an exponential decrease in residual content observed over 48 hours. Residual PAA was higher in high saline water compared to low saline water. PAA at 10 mg l⁻¹ significantly reduced heterotrophic bacterial counts and inhibited the growth of luminescent *Vibrio* sp. However, the efficiency of PAA decreased in high turbidity samples due to the presence of organic matter. A comparative study with bleaching powder and sodium hypochlorite at 10 mg l⁻¹ chlorine showed that PAA residues diminished within 2 days, while residual chlorine from the other disinfectants persisted for 4 days. The disinfection efficiency of PAA was comparable to that of bleaching powder and sodium hypochlorite. The study concludes that PAA can be used as an alternative disinfectant in aquaculture reservoirs, as its residual effect is lower and less toxic than other commonly used disinfectants, and it effectively inhibits the growth of *Vibrio* sp.

Keywords: PAA; Residual Effect; Salinity; Bacterial Count; Turbidity

Introduction

Aquaculture systems are prone to bacterial, viral, fungal, and parasitic infections. Disinfection in aquaculture is crucial for maintaining a healthy aquatic environment, preventing disease outbreaks, and ensuring the sustainability of fish and shrimp farming. Peracetic acid (PAA) is a relatively new compound suggested for disinfection in aquaculture. Peracetic acid is an antimicrobial disinfectant registered by the US Environmental Protection Agency for use in agriculture, food processing and medical facilities [1]. In Europe, PAA is also approved for use in veterinary medicine and is one of the very few compounds approved for use in aquaculture as a disinfectant. Peracetic acid has been recognised as a sustainable disinfectant in aquaculture.

PAA is a strong oxidant and disinfectant. Its oxidation potential is larger than that of chlorine or chlorine dioxide [2]. The potential of PAA for direct oxidation and destruction of cell wall of microbial pathogens qualifies it as a potential disinfectant in waste water treatment [3]. The PAA has a pH of less than 2 with specific gravity varying from 1.10 to 1.11, depending on temperature. The presence of acetic acid gives it a pungent odour. The PAA product is a clear, colourless liquid [4]. Commercially, this product is available in concentration from 2-15% (wt/wt). Solutions exceeding 15% exhibit explosive nature, instability and reactivity [5]. The activity of PAA is very high even in very low concentrations and works across a wide range of microbes. The germicidal property is found to be bactericidal at 0.001%, fungicidal at 0.003% and sporicidal at 0.3%. The disinfection efficiency can be ranked as bacteria > virus > bacterial spores > protozoan cysts [6].

Acetic acid, hydrogen peroxide, oxygen and water are the decomposition products of PAA [7]. PAA is consumed in aqueous systems mainly by three reactions: spontaneous decomposition, hydrolysis and transition-metal catalysed reaction. The spontaneous decomposition of PAA to acetic acid and oxygen mainly occurs in the pH range of 5.5 to 8.2 [7]. PAA is more potent antimicrobial agent than hydrogen peroxide, even-though hydrogen peroxide is also a disinfecting agent. The disinfectant activity is based on the release of active oxygen. Compared to other disinfectants like, bleaching powder and sodium hypochlorite, the residual effect of PAA is less harmful. No to little toxic or mutagenic byproducts are produced after the reaction of PAA with organic material present in treated wastewater [8]. The oxidation of natural organic matter in water by PAA produces carboxylic acids. The PAA treated water does not produce any halogen containing disinfection by products [8].

This is one major advantage compared to other disinfectants like gaseous chlorine, sodium hypochlorite, or ozone. The chlorine-based disinfectants produce toxic and halogenated by-products (chlorinated) after their reaction with organic matter.

As a better management practice in brackish water shrimp aquaculture, source waters are to be disinfected in reservoir ponds. Bleaching powder and sodium hypochlorite are commonly used for this purpose and only dechlorinated water has to be used in culture ponds. Many farms would like to use PAA in aquaculture as it is being used in other waste water treatment units as disinfectant and also due to its faster degradation. The purpose of the study was to evaluate the residual effect and efficiency of peracetic acid as a disinfectant under controlled conditions compared to other disinfectants like bleaching powder and sodium hypochlorite under low and high saline conditions.

Materials and Methods

Three sets of batch experiments were conducted to evaluate the efficiency and residual effect of peracetic acid as a disinfectant and also to compare the effect of PAA with other disinfectants being used in aquaculture. The experimental details are as follows:

Experiment I- Determination of optimum dose of PAA for disinfection

A study was conducted with a commercial grade peracetic acid of 15% concentration at 0, 2, 5 and 10 mg l⁻¹ concentration. The experimental set up was maintained at 5 and 25 ppt salinity in triplicates. The residual PAA was determined using iodometric titration [9]. The residual PAA was determined at an interval of 30 min, 1h, 2h, 4h, 6 h, 12h, 24h and 48h after application of PAA. The sampling was continued till the residual PAA was reduced to zero.

The disinfection efficiency of commercial grade PAA of 15% concentration was determined by studying its effect on heterotrophic bacterial count and luminiscent *Vibrio* sp. The effect on bacterial count was studied using PAA at 2, 5 and 10 mg l⁻¹ conc at 5 and 25 ppt salinity. A microbial inhibition study [10] was conducted to determine the effect of PAA on luminescent *Vibrio* sp at concentration ranging from 2 to 100 ppm.

Experiment II- PAA as a disinfectant in turbid samples

The efficiency of peracetic acid in turbid samples in both low (5ppt) and high (25 ppt) saline waters, ranging from 20 to 320 NTU was determined. The turbidity was created using both organic (using feed) and inorganic loads (using bentonite clay) [11]. The residual effect and efficiency (by heterotrophic bacterial count) of PAA was determined at regular intervals.

Experiment III- Comparative study of PAA with other disinfectants

A comparative study was conducted between PAA and the other commonly used disinfectants being used in aquaculture like bleaching powder and sodium hypochlorite. The residual effect and efficiency of all the three disinfectants were studied at 10 mg l⁻¹ concentration.

Statistical analysis

The statistical analysis to determine the effect of salinity on the residual products was done using T-test (SPSS).

Results and Discussion

Residual effect and efficiency of PAA

The Figure 1 shows the degradation pattern of PAA at different concentrations (0, 2, 5, 10 ppm) under low (5 ppt) and high salinity (25 ppt). An exponential decrease in the residual content was observed in both the salinities during the study period of 48 h. The consumption of PAA in water was rapid.

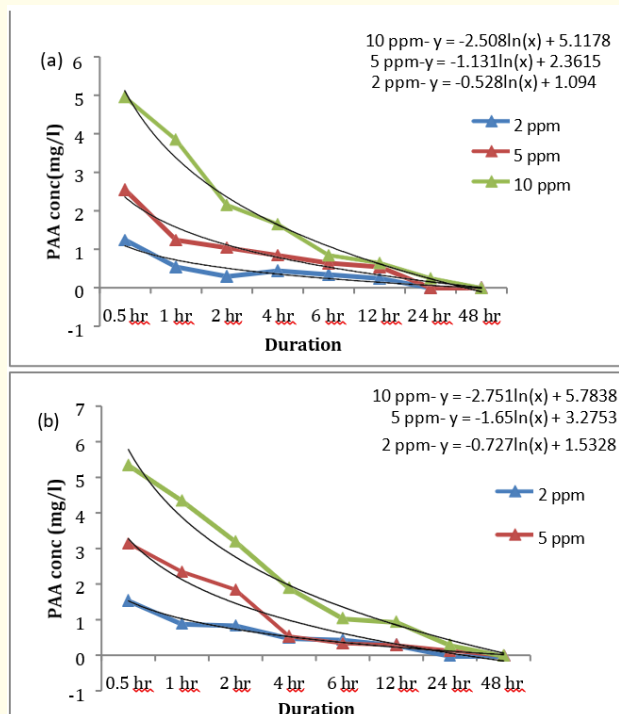


Figure 1: (a) Residual effect of PAA in 5 ppt water
(b) 25 ppt water.

The residual PAA reduced to zero after 24 h of application at 2 and 5 mg l⁻¹ doses and after 48 h at 10 mg l⁻¹ in both low and high saline waters. The degradation was observed to be comparatively faster in high saline water than that in low saline. The residual content was comparatively more in high saline water compared to the low saline.

The study on the efficiency of PAA on the heterotrophic bacterial count showed that the count had reduced from 7.6 x 10⁶ cfu/ml in control to 11.4x 0⁴cfu/ml in low saline and 6 x 10³ cfu/ml

in high saline water after 24 h of application at 10 mg l⁻¹ concentration (Figure 3). The PAA at 2 and 5 mg l⁻¹ was insufficient for disinfection as no significant reduction in counts was observed. The efficiency of PAA on luminescent *Vibrio* sp conducted by microbial inhibition study at concentrations ranging from 2 to 100 mg l⁻¹, showed that at concentrations of 8 mg l⁻¹ and above the growth of *Vibrio* sp was inhibited as evident from the inhibition zone (Figure 2). A slight inhibition zone was developed at 6 mg l⁻¹ also, though the concentration of 8 mg l⁻¹ and above was effective in inhibiting the growth of *Vibrio*.

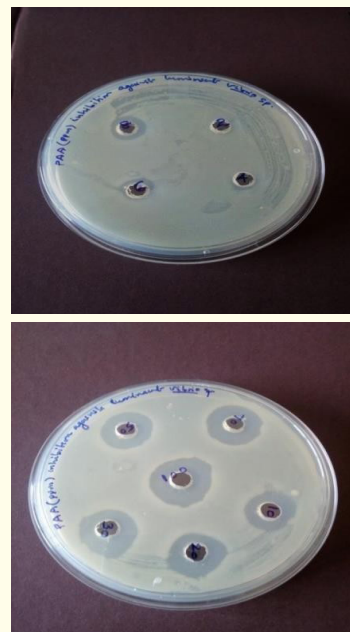


Figure 2: Microbial inhibition study of PAA on *Vibrio* sp.

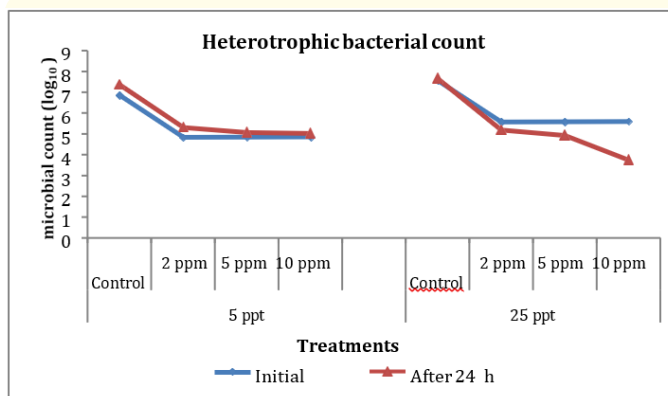


Figure 3: Effect of PAA on the heterotrophic bacterial count

The consumption of PAA in samples of high turbidity (organic load) was very rapid. The analysis of the residual effect of PAA (at 10 ppm) on turbid samples (ranging from 20 NTU to 300 NTU) indicated that there were no residues after 30 minutes in samples from 40 NTU and above (Figure 4). In low turbid samples, the residual effect was zero after one hour. The organic load in the water accelerated the decomposition of PAA. The heterotrophical bacterial count showed that there was no significant decrease in the counts in any of the treatments indicating lower efficiency in the turbid samples.

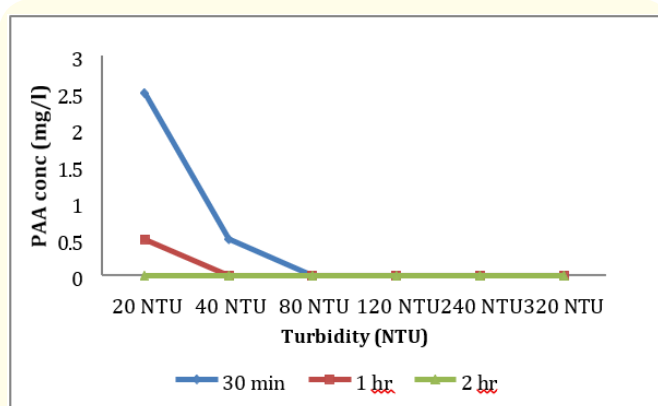


Figure 4: Residual effect of PAA in turbid samples.

Comparative study of PAA with other disinfectants

The residual effect of bleaching powder at the rate of 10 mg l⁻¹ Cl, in 5 and 25 ppt water showed that under higher salinity the total residual chlorine content was 2.4mg Cl₂/L compared to 0.8 mg Cl₂/L in low saline water after 4 h of application and it decreased to 0 after 72 h of application (Figure 5). The free chlorine content after the addition of bleaching powder reduced from 0.5 mg Cl₂/L after 4 h of application to zero by 24 h in low saline and from 2.1 to 0.2 mg Cl₂/L after 48 h of application in high saline water. The residual chlorine content after application of sodium hypochlorite (SHC) in 25 ppt water was 1.6 mg Cl₂/L compared to 0.7 mg Cl₂/L in low saline water after 4 h of application and reduced to zero after 72 h of the application. The free chlorine content in samples treated with SHC decreased from 0.5 to 0.1 mg Cl₂/L in low saline water after 48 h of application and from 1.4 to 0.3 mg Cl₂/L within the same duration. The residual chlorine content was significantly

higher under high saline water compared to low saline (P < 0.05) and was significantly lower (P < 0.05) in sodium hypochlorite than bleaching powder.

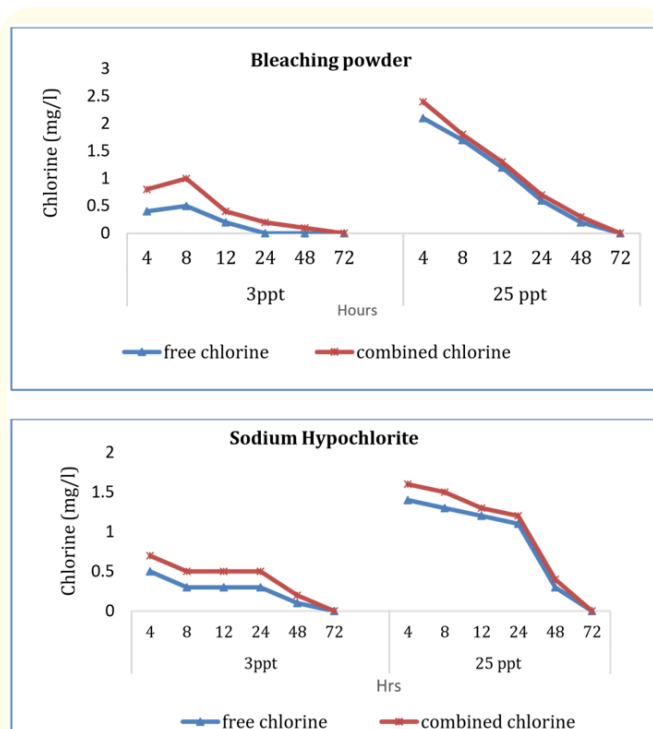


Figure 5: Residual effect of (a) bleaching powder and (b) sodium hypochlorite.

The residual effect of peracetic acid at 10 mg l⁻¹ concentration was reduced to zero after 48 h, and the residues- acetic acid, hydrogen peroxide, and water are comparatively less toxic.

The disinfection efficiency of bleaching powder and sodium hypochlorite at 10 mg l⁻¹ chlorine in 5 and 25 ppt salinity showed that the heterotrophic bacterial count reduced from 8.3 x 10⁴ cfu/ml in low saline and 38 x 10⁴ cfu/ml in control samples to nil and remained same after 24, 48 and 72 h of treatment (Table 1).

Our results showed a rapid exponential decrease in PAA content after addition in water under different salinities. The residual content was comparatively higher in the high saline water than that in low saline water. This supports the study conducted by [12] which

Table 1: Heterotrophic bacterial count after the addition of bleaching powder and sodium Hypochlorite at two different salinities.

Disinfectant	Salinity (ppt)	CFU/ml (72 hrs)
Control	5	8.3 x 10 ⁴
	25	38 x 10 ⁴
Bleaching powder	5	Nil
	25	Nil
Sodium hypochlorite	5	Nil
	25	Nil

displayed a significant decrease in the half-life of PAA in 1 and 3 % salinity. [13] observed a 93.3 to 99.9 % decline in PAA added after 3.5 h of application, which was similar to the present study results of 85-90 % decline in PAA within 2 to 4 hours of addition. The ionic composition of the water may play an important role in the degradation of PAA under different salinity [12]. The degradation of PAA observed in high saline was comparatively slower than the low saline water contrary to the findings by [12]. PAA degrades completely within several hours after application, depending on the concentration [14] and results in harm-less residues [4]. It is the peroxide of acetic acid and is commercially available in an equilibrium mixture with acetic acid, hydrogen peroxide and water. The mixture is represented by the following equilibrium [13]:



In the present study, the heterotrophic bacterial count was found to reduce and inhibit the growth of luminescent *Vibrio* sp. with the application of PAA at 10 mg l⁻¹ concentration. [15] reported that PAA at low concentrations (0.99 and 1.06 mg/l) was not sufficient to remove contamination by *Salmonella* in the effluents. [16] reported a reduction (log reductions ranging from 1 to 5) in fecal coliforms and *E.coli*, which was influenced by the PAA doses, residual content and the contact time. PAA at concentration 10 mg l⁻¹ was effective in total inhibition of *Aphanomyces* spore germination in both liquid and petri dish culture tests [17]. [18,19] cited the bactericidal and sporicidal activity of PAA in combination with H₂O₂. [20] observed that the synergistic sporicidal effect of PAA with H₂O₂ was largely due to PAA and not due to H₂O₂. The sulfhydryl and sulfur bonds in proteins, enzymes and other metabolites are oxidised and the double bonds are reacted, impeding molecular function [5]. It acts as a protein denaturant [4,5]. It is

speculated that the chemiosmotic function of lipoprotein cytoplasmic membrane is disrupted and transport process through rupture or dislocation of cell wall is caused by PAA [21]. PAA activity decreases at higher pH. The undissociated acid is the biocidal form of PAA [22]. The PAA has a pKa of 8.2, i.e. at pH above 9, the dissociated form is the predominant species and its disinfection efficiency is lower [23].

The high turbidity created with the high organic matter load accelerated the degradation of PAA. Pedersen, *et al.* [13,14] reported the faster decomposition of PAA in water with high organic load. Similar results were observed by [12]. The biofilm on the water surface (created under the high turbid conditions) diminished the activity of PAA as these consume the PAA and thereby reduces their disinfection efficiency. [7] observed an increase in disinfection efficiency of PAA with a decrease in total suspended solids and biological oxygen demand.

Comparison of the residual content of PAA with bleaching powder and sodium hypochlorite at the rate of 10 mg l⁻¹ showed that application of bleaching powder and sodium hypochlorite (SHC) required 4 days for the residual chlorine content to diminish compared to a period of 2 days for PAA. The bleaching powder on dissolution in water, breaks into hypochlorous acid and hypochlorite ions. Hypochlorous acid is more toxic than hypochlorite ion. Laboratory trials has showed that application of commercially available bleaching powder at the rate of 5 mg l⁻¹ and 20 mg l⁻¹ chlorine was effective in killing *Vibrio* sp in filtered and unfiltered sea water. The residual chlorine content in the case of SHC was comparatively less, as being in the liquid form, the reaction was faster than bleaching powder. [24,25] in their studies had demonstrated the biocidal efficiency of chlorine against virus and bacterial pathogens.

Conclusion

PAA is a strong oxidising agent and it breaks down to hydrogen peroxide and acetic acid in water, which further breaks into water and oxygen. These are rather harmless compared to the by-products formed by the dissolution of bleaching powder and sodium hypochlorite. It takes utmost 4 days for the residual chlorine to reduce to zero when applied at 10 mg l⁻¹ in the case of bleaching powder and sodium hypochlorite, which is the effective dosage, whereas in the case of PAA the residues were nil within 2 days. The disinfection efficiency of PAA is also on par with that of other two chemical disinfectants. As the residual effect of PAA is lower and less toxic than bleaching powder and sodium hypochlorite, it can be used as an alternative disinfectant in reservoirs being used in aquaculture farms. Compared to other disinfectants under study, the by-products of PAA are comparatively harmless and inhibits the growth of *Vibrio* sp. The use and the dose of PAA suitable during the culture period is an area where further studies need to be conducted.

Conflict of Interest

The authors have no conflict of interest to declare.

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