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Inactivation of Avian Influenza Virus of Subtype H5N1 and H9N1 in The Vaccine Industrial Wastewater Treatment with an Advanced Oxidation Process Base on Ozone

E Enjarlis[1*], Karna Wijaya[1], Eva Fathul Karomah[2], Shabri Huda[1]

[1]*Department of Chemical Engineering, Institut Teknologi Indonesia, Jl. Raya Puspiptek Serpong, South Tangerang, Banten, 15314 Indonesia*
[2]*Department of Chemical Engineering, Universitas Indonesia, Kampus Baru UI Depok, Depok, West Java, 16424 Indonesia*

Abstract. This study aims to compare the effectiveness of oxidation and adsorption technology in the inactivation of Avian Influenza (AI) virus subtypes H5N1 as well as H9N1 and remove the content of COD, BOD, and TSS in industrial wastewater of Avian Influenza (AI) vaccines production. The experiment variables are the number and type of oxidizers (O_3 and H_2O_2), the number of adsorbents granular activated carbon (GAC), and the type of Advanced Oxidation Process (AOP) based on O_3 (O_3/H_2O_2 and O_3/GAC). The measured parameters include virus inactivation test (CT Value) and the number of AI virus subtypes H5N1 and H9N2 as well as TSS, COD, and BOD of wastewater from the vaccine industry. The results showed that the AOP with O_3/H_2O_2 at a dose of 0.00013 g O_3/ml of wastewater is the most effective technology in the inactivation of AI virus of subtypes H5N1 and H9N1 and the decrease in the content of COD, BOD, and TSS. Furthermore, approximately 2% of H_2O_2 and ozonation for 20 minutes with CT Value equal to No CT, virus quantity was 0.0 thousand units/0.1 mL and pH 7.16, while percentage removal of TSS, COD, and BOD were 58% (86.42 mg/L), 49% (575 mg/L), 52% (304.42 mg/L. For the COD and BOD values to meet the quality standards, it is recommended to apply the O_3/H_2O_2 technology in series with an additional processing time of approximately 10 minutes, or as alternative processing can be continued at the Wastewater Treatment Plant (WWTP) which is already owned by the Vaccine Industry.

Keywords: Advanced Oxidation Process; Inactivation of Avian Influenza Virus (H5N1 and H9N1); Ozonation; Wastewater of vaccine industry

1. Introduction

The COVID-19 virus epidemic had a significant impact on human life and the economy of countries around the world (Nur *et al.*, 2022). This showed that outbreaks of other deadly viruses, such as Avian Influenza (AI) are expected to be transmitted to animals and humans. In Indonesia, the AI virus vaccine industry in West Java, in the production process, generates wastewater from live egg allantois, tank washing, and sterilization. The wastewater is processed by the disinfection method at a steam temperature of 85^0C for 45 minutes. However, the products obtained usually contain AI virus strains H5N1 and H9N1

*Corresponding author's email: en.jarlis@iti.ac.id, Tel.: +62-813-81234418;
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activity with COD, BOD, and TSS values that exceed quality standards. AI virus is a single-stranded RNA virus of the family Orthomyxoviridae, which is deadly zoonotic and contagious to humans (Everest *et al.,* 2021; Mostafa *et al.,* 2018). Therefore, to prevent environmental pollution and the spread of avian influenza Virus subtypes H5N1 and H9N1 from liquid waste from the Avian Influenza virus vaccine industry, the waste must be treated with the right technology. The 2 categories of these viruses are Low Pathogenic Avian Influenza (LPAI), including H9N1, which has 9 subtypes and High Pathogenic Avian Influenza (HPAI), which includes H5N1 with 5 subtypes. Furthermore, the viruses are divided into subtypes based on 2 surface proteins, namely hemagglutinin (HA) and neuraminidase (NA) (CDCP, 2022; Everest *et al.,* 2021; Huang *et al.,* 2021; Koutsakos, Kedzierska, and Subbarao, 2019). The HA is a type of glycogen and a class 1 fusion protein that has a multifunctional activity for attaching viruses to cells, while NA is an enzyme that releases viruses for complete infection (Kosik and Yewdell, 2019; Naguib *et al.,* 2019).

The oxidation process using ozone and Advance Oxidation Procces (AOP) base on Granular Activated Carbon (GAC) and Ozone are more environmentally friendly, efficient and effectively in treating industrial wastewater containing AI viruses. This method is also superior to the use of other chemicals such as alcohol, formalin, surfactants, sodium dichloro in the inactivation of microorganisms on the surface of solid objects. Ozone of oxidizers are strong and unstable, have a broad spectrum of antimicrobials and are reactive to proteins and lipids (Tizaoui, 2020; Megahed, Aldridge, and Lowe, 2018). In water, ozone is decomposed into free radicals such as $HO^{\bullet}$, $HO_2^{\bullet}$, $O_3^{\bullet-}$ and O_2 (Fu *et al.,* 2019). The inactivation of the AI virus by this compound is influenced by dose, time, temperature, and relative humidity (Dubuis *et al.,* 2021; Kong *et al.,* 2021). Hydrogen peroxide (H_2O_2) is also a strong oxidizer, with an oxidation potential of 1.77 Volts, which reacts and damages the structure of microorganisms by disrupting their metabolic stability. The same study also showed that H_2O_2 was an extremely effective inactivating for both RNA and DNA viruses in less than 2 h after exposure to a 3% aqueous solution of H_2O_2 (Elveborg, Monteil, and Mirazimi, 2022). The advantages of activated carbon include its high absorption capacity, with a surface area of 500 to 1500 m2, and its good absorb inorganic and organic pollutants such as phenol and heavy metal ions in the water and wastewater (Hoang *et al.,* 2022; Desmiarti *et al.,* 2019), as well as during ozonation (O_3/GAC) has been reported to improve oxidation performance through acts as an initiator and increased transformation of O_3 to OH radicals (Rekhate and Srivastava, 2020).

Advanced Oxidation Process (AOP) is higher oxidation with $^{\bullet}OH$ as an oxidating potential of 2.8 volts and is non-selective, the most reactive free radical formed in vivo (Martemucci *et al.,* 2022). The $^{\bullet}OH$ formation systems are homogeneous such as O_3/H_2O_2 (Fan, Sokorai, and Gurtler, 2020) and heterogeneous, namely O_3/GAC (Chen *et al.,* 2021; Wang *et al.,* 2020). In O_3/H_2O_2 systems, $^{\bullet}OH$ and oxidants ($O_2^{\bullet-}$ and O_2) are produced through a reaction between H_2O_2 and water to generate HO_2^-, which further reacts with O_3 (Kim *et al.,* 2021). Meanwhile, the mechanism of O_3/GAC in wastewater treatment is such that organic micropollutants whose low reactivity to ozone can be removed by either (i)$^{\bullet}OH$, especially micro-hydrophilic pollutants and/or (ii) adsorption on the surface of activated carbon for hydrophobic micropollutants (Lisovskayaa *et al.,* 2021; Wang *et al.,* 2018; Vega and Valdes, 2018). Ozone has a low solubility at room temperature, and the solubility of ozone can be increased by using an aerator pump so that it can reduce the size of the ozone bubble by up to 90% and increase the effective ozone solubility to 0.47 ppm (Verinda *et al.,* 2022).

Inactivation of AI Viruses subtypes H5N1 and H9N1 has been carried out in drinking water treatment (Lenes *et al.,* 2010). However, research on AI virus inactivation in

wastewater containing COD, TSS, and BOD exceeding the standard has not been carried out using oxidation processes (O_3 and H_2O_2) and Advanced oxidation (O_3/H_2O_2 and O_3/GAC). The purpose of this study was to compare the processes of oxidation (O_3 and H_2O_2), adsorption (GAC), and Advanced oxidation of the vaccine Industrial wastewater treatment: inactivation of AI Viruses subtypes H5N1 and H9N1 and their impact on decreasing COD, BOD, and TSS values.

2. Methods

2.1. Equipment and Materials

Oxidation reactor from glass material size ID 4.0 cm and H 60 cm, equipped with incoming and outgoing ozone gas ports, Plate Magnetic Stirrer 500 – 1500 rpm (Thermo Scientific, USA), Biosafety Cabinet model BSC-1500IIB2-X (MEDFUTURE, China), Analytical Balance Sheet model ABJ320-4NM, max 220 grams (KERN, Germany), Realtime PCR Quantstudio5 (Thermo Scientific , USA), Ozone Generator with capacity 3 grams O_3/h (Quanju, China), Egg Candler 2,000 mAh (Magicfly, China), Egg Incubator model T-JZ1056 (Tengao, China), Laminar Air Flow (LAF) for PCR model 321 PCR Workstation (Captair Bio, Malaysia), and pH Meter work on ATC (Mettler Toledo, Indonesia, Sulfuric acid (Merck, Indonesia), Hydrogen Peroxide (H_2O_2) (Sindopex Perotama Indonesia), GAC (Granular Activated Carbon) (Bumi Agung Chemistry, Indonesia), Viral Nucleic Acid Extraction Kit (Geneaid, Taiwan), Quantitect, RT-PCR Kit (Qiagen, USA), Primer & Probe (Macrogen, Korea Selatan), Egg Specific Pathogen Free (SPF) (SPF Egg Plant, Indonesia), Iodine (I_2) (Merck, Indonesia), Potassium Iodide (KI) (Merck, Indonesia), Sulfuric Acid (H_2SO_4) (Meck, Indonesia), Sodium Thiosulfate (Merck, Indonesia) and Kanji Solution $(C_6H_{10}O_5)n$ (Merck, Indonesia).

2.2. Preparation and Characteristics of Liquid Waste of AI Virus Vaccine Industry

Liquid waste of H5N1 and H9N1 virus vaccine industry from PT. Vaksindo Satwa Nusantara (Ungas vaccine industry) Gunung Putri, Bogor Regency. The collecting tank at the sampling point was used to collect 500 mL in a tightly closed glass bottle and stored at 4^{o}C. The characteristics of liquid waste from the vaccine industry before treatment are stated in Table 1.

Table 1 Characteristics of Liquid Waste Industry Vaccine of Virus AI Subtype H5N1 and H9N1

Parameter	Before Inactivation	*Quality Standards	Unit
Temperature	26	-	^oC
pH	6,52	6,0 – 9,0	-
Specific gravity	970,2	-	kg/m^3
TSS	149	100	mg/L
COD	1,172,85	300	mg/L
BOD	585,42	100	mg/L
Quantity of Viruses H5N1	138,786,192	-	*Quantity* (Unit/0.1mL)
Quantity of Viruses H9N1	22,091,644,00	-	*Quantity* (Unit/0.1mL)

* PERMENLH RI Number 5 of 2014 concerning Wastewater Quality Standards (PharmaceuticalIndustry Appendix XXXIX)

2.2. Qualitative and Quantitative Analysis of AI Viruses in liquid waste (OIE, 2014)

2.3.1. Inoculation of Wastewater Samples on Eggs

A total of 0.1 mL of wastewater was inoculated onto 11-day-old SPF (Specific Pathogen Free) eggs, which were then incubated at a temperature of 37°C during 5 days. The eggs

were observed (candled) using an egg candler tool to determine the growth of embryos at dead or live eggs. RNA extraction was performed after incubation.

2.3.2. Ribonucleic Acid (RNA) Extraction

Allantois sample was obtained from inoculation on eggs, and RNA (Ribonucleic Acid) was extracted using a Viral Nucleic Acid Extraction Kit from Genaid with the appropriate procedure on the www.Geneaid.com website.

2.3.3. Mixing RNA (RT-PCR)

RT-PCR (Quantstudio5) was used to detect AI viruses of subtypes H5N1 and H9N1 qualitatively and quantitatively. The RNA in samples was amplified with Quantitect Probe RT-PCR Kit reagents with procedures according to using specific primers (www.macrogen.com) for each strain of the virus. Meanwhile, the cut-offs for each of the H5N1 and H9N1 methods are presented in Table 2. CT is a measure of disinfectant concentration (C) multiplied by the time (T) required to achieve a given inactivation level of a microorganism.

2.3.4. Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), dan TSS Analysis

Measurement of COD, BOD and TSS of wastewater Industry vaccine AI virus subtype AIH5 dan AIH9 before and after treatment from the Indonesian National Standard (SNI) (BSN, 2019), for COD with SNI6989.2: 2019, BOD with SNI 06-6989.14-2004 and TSS with SNI 06-6989.3- 2004.

Table 2 Cut Off Each Method (H5N1 and H9N1)

Target	H5N1	H9N1
Positive	CT < 30	CT < 30
Indeterminate	30 < CT < 35	30 < CT < 35
Negative	CT > 35	CT > 35

2.4. Wastewater Treatment of Vaccine Industry and AI Virus Inactivation

A total of 200 mL wastewater from the AI subtypes H5N1 and H9N1 vaccine industry was placed in the oxidation reactor, O_3 gas flowed up to 144 mg/L. min for 10, 20, 30, 40, and 50 minutes, and the remaining ozone was analyzed using the iodometry approach (Chasanah *et al.,* 2019). In the adsorption experiments with GAC (5.0 and 7.5 and 10 % w/v), approximately 200 mL of wastewater was stirred with a magnetic stirrer in the reactor for 20 Minutes. Subsequently, treatment with H_2O_2 oxidizers was carried out at 2.0, 4.0, and 6.0 %v/v, while ozone-based AOP (O_3/H_2O_2 and O_3/GAC) was conducted at different doses, H_2O_2, and ineffective GAC.

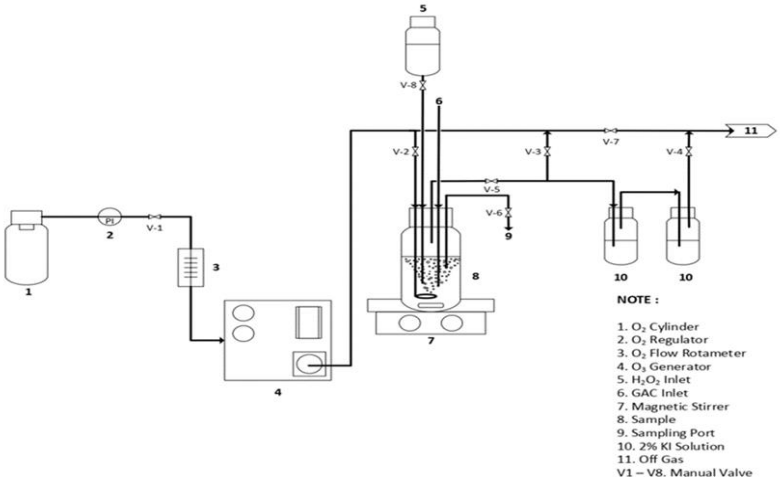

Figure 1 Process flow diagram Wastewater Treatment of Vaccine Industry and AI Virus Inactivation with Ozonation and AOP base on Ozone (O_3/H_2O_2 and O_3/GAC)

3. Results and Discussion

3.1. Inactivate of AI Virus in wastewater of vaccine Industry with H_2O_2.

Table 3 shows the inactivation data of H5N1 and H9N1 viruses by H_2O_2 at 2.0, 4.0, and 6.0%. The optimum inactivation was obtained at H_2O_2 of 6% with each No CT and quantity value of approximately 0.0 units/0.1 mL, and the egg is still alive. This indicates that the virus is inactive (dead) and is incapable of damaging the egg. At a concentration of 4%, the egg was still alive, showing that the virus was inactive, where the H5N1 and H9N1 quantity values had approximately 0.0 Units/0.1 mL and 17.60 units/0.1 mL with No CT and a value of 35.51, respectively the eggs also die in H_2O_2 of 2% which indicated that the virus is still active and can infect eggs in both subtypes, with quantity values of 4,261.81 units/0.1 mL and 10,404.53 units/0.1mL with CT values of 35.31 and 27.41, respectively. Previous reports stated that the effectiveness of H_2O_2 in the inactivation of the AI virus occurs at concentrations of H_2O_2 > 5 % micro aerosol (Neighbor *et al.,* 1994), and the inactivated virus by H_2O_2 still has the ability to induce an immune response in the same level as live viruses (Dembinski *et al.,* 2014). When compared to the maximum removal of Paracetamol in Pharmacy wastewater, it reaches 80% with the use of O_3: H_2O_2 (1: 0.25 or 25% H_2O_2). Therefore, to minimize costs, the use of H_2O_2 needs to be combined with ozone gas. Ozone is a selective oxidant, but the addition of H_2O_2 is generated in situ.

Table 3 Data of AI Virus of subtypes H5NI and H9N1 in wastewater of vaccine industry before and after inactivation with H_2O_2

Oxidizer	Concentration (%)	Egg		H9N1		H5N1	
		Result	Dead (Day to)	CT Value	Quantity (Unit/0.1 mL)	CT Value	Quantity (Unit/0.1 mL)
Before treatment	-	Die	1	14.79	220,091,644	20.05	138,786,192
After	2.0	Die	2	27.41	4,261,81	35.31	10,404.53
treatment	4.0	Live	-	35.51	17.60	No CT	0.0
by H_2O_2	6.0	Live	-	No CT	0.0	No CT	0.0

Hydrogen peroxide is a strong, broad-spectrum inactivating agent that can decompose into water, oxygen, and highly reactive hydroxyl free radicals (•OH). These radicals can cleave or crosslink a wide range of biomolecules, including proteins, nucleic acids, and lipids (Lisovskaya *et al.,* 2021). The H_2O_2 reaction in AI virus inactivation in the wastewater of the vaccine industry is as follows: H_2O_2 acts as an oxidant by producing hydroxyl free radicals (•OH), which attack the essential cell components, including lipids, proteins, and DNA as well as RNA (Ofoedu *et al.,* 2021; Elveborg, Monteil, and Mirazimi, 2022).

3.1. Inactivate of AI Virus in wastewater of vaccine Industry with H_2O_2.

Table 3 shows the inactivation data of H5N1 and H9N1 viruses by H_2O_2 at 2.0, 4.0, and 6.0%. The optimum inactivation was obtained at H_2O_2 of 6% with each No CT and quantity value of approximately 0.0 units/0.1 mL, and the egg is still alive. This indicates that the virus is inactive (dead) and is incapable of damaging the egg.

3.2. Inactivation of AI Virus in the wastewater of Vaccine Industry with O_3

The inactivation of AI viruses of subtypes H5N1 and H9N1 by ozone in the wastewater of the vaccine Industry in Table 4 shows a very significant effect. At 50 minutes of ozonation (0.0325 mg O_3/mL) for H5N1 viruses, the decrease in CT and the quantity of viruses reaches 100%, namely No CT and a quantity of 0.0 units/mL or complete inactivation. This is very important to restrict any possibility of DNA/RNA mutations (Hossain, 2022). Meanwhile, AIH9 has a CT value of approximately 34.27, a virus quantity of 407.4 units/mL, and more

glycogen than H5N1. Since the viruses are in organic wastewater, H9N1 inactivation needs greater ozone (Kong *et al.,* 2021).

Table 4 AI virus of subtypes H5N1 and H9N1 in Wastewater of Vaccine Industry Before and After Inactivation with O_3

Oxidizer	Time (Minute)	Egg		H9N1		H5N1	
		Result	Dead (Day to)	CT Value	Quantity (Unit/mL)	CT Value	Quantity (Unit/mL)
Before Treatment	-	dead	1	14.79	220,916,440	20.05	138,786,192
After treatment by O3	10	dead	2	23.65	544,294,4	27.53	10,816,205.0
	20	dead	2	23.89	464,639,5	27.93	8,308,768,1
	30	dead	2	24.82	246,359,7	29.27	3,490,141,9
	40	dead	3	30.85	4,132.5	36.97	23,571,5
	50	dead	3	34.27	407.4	No CT	0.0

AI virus is a single-stranded RNA virus that can be decomposed by ozone (Martemucci *et al.,* 2022; Blanco *et al.,* 2021; Mostafa *et al.,* 2018; Megahed, Aldridge, and Lowe, 2018) and radical OH from ozone decomposition.

3.3. Inactivation of AI Virus in wastewater of Vaccine Industry with GAC

The inactivation of AI viruses of subtypes H5N1 and H9N1 by GAC in Table 5 seems less effective, where at 10% GAC, a quantity of 1,577.9 units/0.1 mL and 6,618.28 units/0.1 mL was detected in the liquid waste. This indicated that it takes a GAC > 10% to achieve a CT value and quantity of viruses of approximately 0. The removal of viruses with GAC through the adsorption process depends on the dose of activated carbon or adsorption capacity, the contact time between activated carbon and adsorbate/virus (Zhang *et al.,* 2021; Dotto and McKay, 2020; Wang *et al.,* 2020; Matsushita *et al.,* 2013).

Table 5 Data of AI virus of subtypes H5N1 and H9N1 in wastewater of industry vaccine before and after Inactivation with GAC

Adsorbent	Amount (%)	Egg		H9N1		H5N1	
		Result	Dead (day to)	CT Value	Quantity (Unit/0.1 mL)	CT Value	Quantity (Unit/0.1 mL)
Before treatment	-	dead	1	14.79	220,091,644	20.05	138,786,192
After treatment byGAC	5.0	dead	2	21.34	161,120.20	31.49	123,230.86
	7.5	dead	2	23.74	51,391.30	35.25	10,780.20
	10	dead	2	26.76	6,618.28	38.22	1,577.97

3.4. Inactivate of AI Virus in wastewater of vaccine Industry with O_3/H_2O_2 and O_3/GAC

Figure 2 shows the number of AI viruses of the subtypes H5N1 and H9N1 in the vaccine industry wastewater after inactivation with O_3/H_2O_2 at H_2O_2 (2% and 4%) and O_3/GAC at GAC (5.0% and 7.5%) as well as various doses of ozone. The inactivation of AI virus of subtype H5N1 with O_3/H_2O_2 at concentrations of 2% and 4% of H_2O_2 in the wastewater was carried out until the quantity of the virus became 0.0 units /mL and No CT, respectively, at 20 minutes (0.00013 gr O_3/mL) and 15 minutes (0.0001 gr O_3/mL) of ozonation, while with O_3/GAC at GAC 5.0% and 7.5% occurred at 25 minutes of stirring. Furthermore, inactivation of H9N1 with the use of O_3/H_2O_2 at 2% and 4% was achieved at the 20th minute of ozonation (0.00013 gr of O_3/mL of waste), and O_3/GAC both for GAC 5.0% and 7.5% was the 25th minute. The difference in ozonation time or dose in the inactivation of H5N1 and H9N1 viruses with O_3/H_2O_2 at 4% H_2O_2 is because H9N1 has a HA of 9 proteins, which is greater than H5N1 with HA 5 proteins and NA one protein (CDCP, 2022; Koutsakos, Kedzierska, and Subbarao, 2019). Therefore, it takes a long time or a greater ozone dose for

the formation of OH radicals (Wang *et al.,* 2018), especially since the virus is in the wastewater that has a fairly high COD value (Kong *et al.,* 2021).

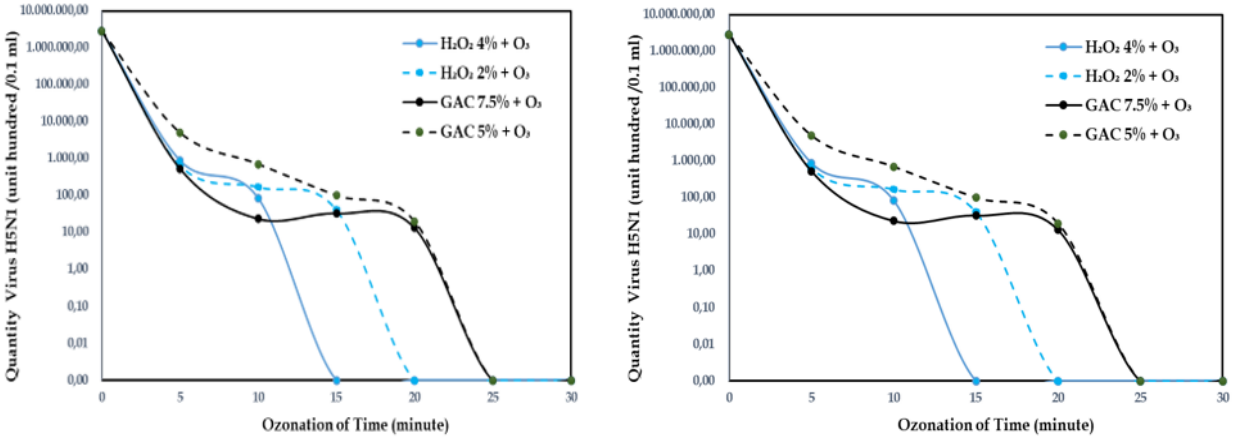

Figure 2 The quantity of AI viruses of the subtypes H5N1 and H9N1 in wastewater of the vaccine industry after inactivation with O_3/H_2O_2 and O_3/GAC

The formation of OH-radical in wastewater with O_3/GAC was faster, and after 6 h of operation, O_3 initially led to an increase in Brunauer-Emmett-Teller (BET) surface area on the GAC (Vatankhah *et al.,* 2019). There are 3 phases in the reaction at O_3/GAC, namely gaseous (ozone gas), liquid (wastewater of vaccine Viruses), and solid (GAC) as adsorbent and a catalyst depending on the site (Wang *et al.,* 2020). Figure 3 shows that (1) Ozone gas (O_3g) dissolves in liquid waste (O_3l) and degrades AI viruses, (2) dissolved ozone (O_3l) by OH ions is decomposed into OH-radicals and also degrades AI viruses in liquid waste (3) dissolved ozone (O_3l) and AI viruses are adsorbed on the surface of the GAC and also degrades the viruses, and (4) part of the ozone on the surface of the GAC is decomposed to form free radicals (OH and Oxygen) (Beltrán, Rey, and Gimeno, 2021; Wang *et al.,* 2020) which also degrade the AI viruses.

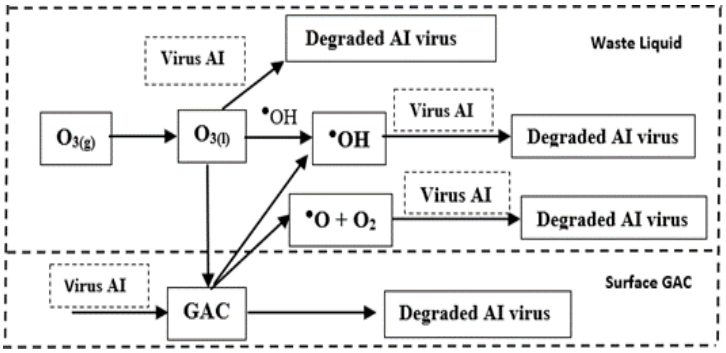

Figure 3 Mechanism of AI Virus Degradation and Inactivation with O_3/GAC in wastewater of Industry of vaccine

3.5. Effect of O_3/H_2O_2 and O_3/GAC on the removal of COD, BOD, and TSS in liquid waste from the vaccine industry

Figure 4 shows that the inactivation of H5N1 and H9N1 with O_3/H_2O_2 has a significant impact on the elimination of TSS, COD, and BOD compared to O_3/GAC. Based on the results, TSS was removed by 58% to 61% and has met quality standards, COD by 49 to 50%, and BOD by 51 to 52 %. When compared to COD removal of leachate, it decreased by 27% - 45% through ozonation (Moersidik, Annasari, and Nugroho, 2021). In textile industrial wastewater, the COD removal was 79.31% with 48 -72 hours through a combination of MBBR and Ozonation technology (Suryawan *et al.,* 2021), as well as in PLTU wastewater,

the removal of COD is 83.33% through O_3/H_2O_2 with H_2O_2 of 1 mL/L and O_3 of 0.3 m^3/hour (Jasim *et al.*, 2021).

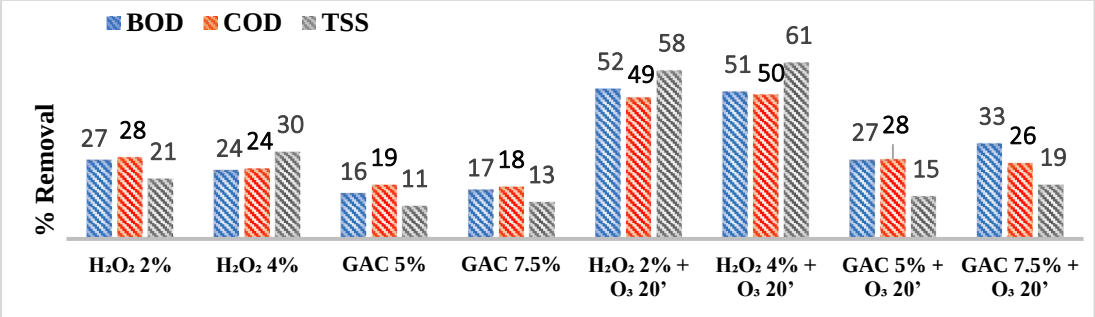

Figure 4 The effect inactivation of AI Virus of Subtype H5N1 and H9N1 with O_3/H_2O_2 and O_3/GAC on Removal TSS, COD and BOD in wastewater of Vaccines industrial of AI viruses

Table 6 Summary of studies removal virus on the surface of solid, Water and Wastewater

Methods	Material	Removal Efficiency Contaminant	Experimental medium	Reference
Oxidation	H_2O_2 (6%; 20 min contact)	H5N1 and H9N1(100%)	Vacine industry Wastewater	(This study)
	H_2O_2 aerosol (> 5%; 20 min contact)	Inactive H5N1	Drinking Water	(Neighbor *et al.*, 1994)
	H_2O_2 (3%; 2h contact)	RNA and DNA viruses	Wastewater	(Elveborg, Monteil, and Mirazimi, 2022)
	H_2O_2 (2.1%; 10 min exposure could)	Norovirus and Bacteriophage by 4 log10 units.	on stainless steel surfaces	(Li *et al.*, 2011)
	O_3 (0.0325 mg/mL; 50 min exposure)	H5N1 and H9N1(100%)	Wastewater	(This study)
	O_3 (20 ppm and10 min exposure)	SARS-CoV-2 (99.8% and 99.9%)	on porous and nonporous surfaces	(Uppal *et al.*, 2021)
	O_3 (30 to 50 mg/m^3 and 20-30 min exposure)	Inactive SARS-CoV-2	In large rooms	(Blanco, *et al.*, 2021)
Adsorption	GAC (10%) with 20 min contact time	H5N1 (99.97%) and H9N1(99.99 %)	Vacine industry Wastewater	(This study)
	GAC/NP6 (5 mg mL^{-1} and 11.01 min contact)	Bacteriophage T4 99.99% (4.0 log)	Virus model in Water	(Shimabuku *et al.*, 2018)
	GAC (5 mg mL^{-1} and 3096.72 min contact)	Bacteriophage T499.99% (4.0 log)		
AOP	H_2O_2 (2%) and O_3 (20 min)	H5N1 and H9N1(100%); TSS (58%); COD (52%); BOD (49%)	Vacine industry Wastewater	(This study)
	H_2O_2 (4%) and O_3 (20 min)	H5N1 and H9N1(100%); TSS (61%); COD (51%); BOD (50%)		
	H_2O_2 (1 mL/L) and O_3 (0.3 m^3/h)	for oil (89.79 %); COD 83.33 % and TOC (70%)	Oily Wastewater	(Jasim *et al.*, 2021)
	GAC (5%) and O_3 (25 min)	TSS (28%); COD (27%); BOD (15%); H5N1 and H9N1(100%)	Vacine industry Wastewater	(This study)
	GAC (7.5%) and O_3 (25 min)	TSS (23%); COD (33%); BOD (19%); H5N1 and H9N1(100%)		
	GAC (50 gr); O_3 (155 mg/h); 1h contact	COD (46.26 %); TSS (12.38%)	Tofu Industry Wastewater	(Karamah, Adripratiwi, and Anindita, 2018)

In O_3/H_2O_2, ozone decomposition in OH radicals becomes faster with the presence of H_2O_2 (Cuerda-Correa *et al.,* 2019; Wang *et al.,* 2018), and it's a significant impact on the removal of TSS, COD, and BOD However, COD and BOD still did not meet the quality standards of 300 mg/L and BOD 100 mg/L, respectively. The use of O3/GAC for the inactivation of viruses H5N1 and H9N1 was less effective, as the percent removal of TSS, COD, and BOD was only 14.77% and 18.79%, 27.60%, and 26.32%, and 27.44% and 33.01%, respectively. Compared to the Tofu Industrial wastewater, the COD reduction reached 46.26% and TSS 12.38% through a combination of ozonation (155 mg O_3/hour) and GAC (50 gr) (Karamah, Adripratiwi, and Anindita, 2018). Visual observations also show that the results of wastewater treatment of the AI virus with O_3/H_2O_2 are clearer than the use of O_3/GAC, H_2O_2, and GAC alone.

4. Conclusions

Advanced Oxidation Process (AOP) based on Ozone and Hydrogen Peroxide (O_3/H_2O_2) is proven to be effective and economical in inactivating AI viruses (H5N1 and H9N1) in AI vaccine industry wastewater. The use of ozone as much as 0.00013 g O_3/ml for 20 minutes and as much as 2% H_2O_2 can cause the AI virus to die with a CT of 0.0 unit/0.1 mL. Researchers suggest that stakeholders from the Ministry of Environment have a policy so that liquid and solid waste from the vaccine industry is treated using O_3/H_2O_2 technology at least at the final processing stage before being discharged into the environment. In addition, the use of AOP technology (O_3/H_2O_2) in vaccine wastewater treatment can simultaneously reduce TSS, COD, and BOD values; 58% (86.42 mg/L), 49% (575 mg/L) and 52 % (304.42 mg/L). If the COD and BOD values of vaccine industrial wastewater are large enough, then in processing the amount of ozone can be increased by extending the processing time or as alternative processing can be continued at the Wastewater Treatment Plant (WWTP) which is already owned by the Vaccine Industry, so that the impact on humans can be prevented, considering that the AI virus shows symptoms of resistance to all types of drugs on the market. Whereas in drinking water treatment, it is recommended that at the disinfection process stage it is enough to use O_3/GAC or Ozonation only if a pandemic situation occurs. There are two problems encountered in this study, namely optimizing the contact of ozone gas, H_2O_2 with the sample, so that the removal of COD and BOD is maximized. In addition, researchers must ensure that they have received the vaccine because the AI virus is zoonotic. The future research, it is hoped that the AOP method will be tested against other virus variants found in the wastewater, for example hospital wastewater.

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