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Reducing Chemical Oxygen Demand, Total Coliform, and Escheria coli levels at the UPTD Health Laboratory in Kudus district using ZSM-5/TiO_2 with TiO_2 Mass Variations

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KEYWORDS
COD, Coliform, E. coli, ZSM-5-TiO_2 Powder.

ABSTRACT
The Kudus health laboratory is a laboratory that carries out health examination services, due to several activities in the laboratory (the use of chemicals in the Hematology laboratory, Clinical Chemistry, and Health and Environmental Chemistry and handling of samples produces liquid waste). Liquid waste is hazardous material containing bacteria, viruses and chemical compounds. One of the parameters of Chemical Oxygen Demand (COD) number contamination with COD threshold value according to the Regional Regulation of Central Java Province No. 5 of 2012, namely 80mg /L. The aim of this research is to reduce COD levels, the number of Coliforms and E. coli in wastewater at the Kudus Regency Health Laboratory UPTD with mass ratio of dengan ZSM-5:TiO2 20:1, 20:3, 20:5 and 20:7 and characterization using X-Ray Diffraction and Scanning Electrone Microscope. This research is an experimental research supported by literature study with variations in the ratio of Zeolite ZSM-5-TiO2 20: 1, 20: 3, 20: 5, and 20: 7 and the duration of immersion for 30 minutes. The results of this study showed that the percentage reduction in COD levels after soaking ZSM-5-TiO2 zeolite powder with variations in the ratio of 20: 1, 20: 3, 20: 5, and 20: 7 with 30 minutes of soaking time respectively increased from 25.51; 29.82; 42.58; and 55.34%. The best percentage reduction in the number of Coliforms and E. coli was obtained in the ZSM-5/TiO2 powder 20:1, of Coliforms decreased by 99.57% and the number of E. coli decreased by 100%. Conclussion: The best percentage reduction in COD was obtained in the ZSM-5/TiO2 powder 20:7 and number of Coliforms and E. coli were obtained in the ZSM-5/TiO2 powder 20:7.

1. INTRODUCTION

Waste water is the remainder of a business and/or activity that produces liquid either from industry or a health laboratory which, if not handled properly, will cause pollution. Every business that produces waste such as factories, clinical laboratories, health centers and hospitals is required to have a Waste Water Treatment Plant (IPAL) with the aim of treating the waste water first before being discharged into the environment (Sulistiyawati, 2019). The Health Laboratory Regional Technical Implementation Unit (UPTD) in Kudus district is a unit that carries out health services, especially in the laboratory sector, including clinical, hematology, chemistry and microbiology laboratories which every day produce solid and liquid waste including Chemical Oxygen Demand (COD) levels, total Coliforms, and E. coli.

Chemical Oxygen Demand levels is the amount of oxygen needed to oxidize organic substances in one liter of liquid sample. Based on the regulations of the Ministry of Environment and Forestry (2016), the permitted threshold for COD levels is 100 mg/L, total Coliform is 3000 CFU/100 mL, E. coli (0 CFU/100 mL. Reducing waste levels before being discharged into the environment requires adsorbents. Adsorbents can come from natural materials such as Moringa seed powder and natural zeolites, while synthetic adsorbents such as Zeolite Socony Mobile-5 (ZSM-5). ZSM-5 was synthesized from a mixture of silica (SiO4-), alumina (AlO43-) and organic base at a temperature of 90^{0}C for 4 days (Mukaromah et al., 2016; Mukaromah & Ariyadi, 2017). ZSM-5 has unique thermal stability, selectivity, functions as an adsorbent because it has pores, as an ion exchanger and a network structure. Apart from ZSM-5, TiO_2 powder can also be used as a pollutant reducer, because it acts as a photocatalyst which accelerates reactions with the help of light, and has a semiconductor structure which is characterized by the presence of a filled valence band and an empty conduction band. The work of TiO2 can be increased by impregnating it on supporting media such as Zeolit, Bentonit, or ZSM-5 (Alfiani et al., 2018; Hamed et al., 2022; Maria Liliriani & Alfiani, 2019; Prima, 2012).

Prima's research results (2012) show that TiO_2/Zeolite with a mass ratio of ZSM-5:TiO_2 20:1 can degrade Congo red dye 81.66%, whereas when using zeolite, TiO_2 and without a TiO_2 catalyst respectively 80.69; 78.87 and 57.63%. The results of research by Faridah et al (2018) using TiO_2/ZSM-5 1.25% w/v to degrade Cr(VI) ions with variations in solution pH resulted that at a solution pH of 2, the Cr(VI) solution could be degraded by 37.55%; whereas when using zeolite and TiO2 alone it is 24.72% and 24.15% respectively. The research results of Khasanah et al. (2019) that the use of TiO2/ZSM-5 membrane with gauze 304-200 and gauze 304-400 with the initial number of E. coli bacteria in a volume of 5ml was 12 x 107; 7ml 63.2 x 107 and 9ml 66 x 107. The highest percentage reduction in the number of E. coli bacteria was 90.25% from the average value of four repetitions using 304-400 gauze buffer with a suspension volume of 7 mL (Khasanah et al., 2019). The research results of Zilfa, Suyani and Nuansa (2015), the percentage of carbaryl degradation during 60 minutes using ozonolysis without using a catalyst was 42.14%, and 56.62% with the addition of 15 mg TiO_2/Zeolite. TiO_2/Zeolite is effectively used as a good catalyst in the carbaryl degradation process by ozonolysis (Zilfa & Nuansa, 2015).

Research on the use of ZSM-5/TiO_2 to reduce Cr (VI) levels, and Methylene Blue levels and ZSM-5/TiO_2 membranes to reduce levels of Chemical Oxygen Demand COD, the number of Coliforms and E. coli in waste water at the Kudus Regency Health Laboratory UPTD with comparison The masses of ZSM-5:TiO_2 20:1, 20:3, 20:5 and 20:7 have never been reported, so this research is important to do.

The aim of this research is to reduce COD levels, the number of Coliforms and E. coli in wastewater at the Kudus Regency Health Laboratory UPTD with mass ratio of dengan ZSM-5:TiO_2 20:1, 20:3, 20:5 and 20:7 and characterization using X-Ray Diffraction and Scanning Electrone Microscope.

2. METHOD

This type of research is experimental research, with a randomized research design with initial, final tests and a control group. The research was carried out in the UPTD laboratory, Kudus Regency Health Laboratory and UNIMUS Chemistry Laboratory. The independent variable in this study was ZSM-5/TiO_2 powder with a mass ratio of ZSM-5:TiO_2 of 20:1, 20:3, 20:5 and 20:7 and the dependent variables were COD content, number of Coliforms and E. Coli. The research object was waste water samples from the UPTD IPAL outlet of the Kudus Regency Health Laboratory which were taken every day for 5 consecutive days.

Tools and Materials

Tools used in the research include: autoclove, petri dish, 1 set of filter membrane tools, measuring cup, Erlenmenyer, measuring pipette, oven, sample bottle, incubator. Materials include: waste water, ZSM-5/TiO_2 powder, Chromocult Coliform Agar (CCA) media. $K_2Cr_2O_7$, Ferrous ammonium Sulfate (FAS), concentrated H_2SO_4, ferroin indicator.

Research Procedures

1. Preparation of ZSM-5/TiO_2 with a mass ratio of ZSM-5 to TiO_2 of 20:1; 20:3; 20:5 20:7
   ZSM-5 at low temperature (90°C) according to the procedure of Mukaromah et. al (2016). Next, impregnate TiO_2 into ZSM-5 (with a mass ratio of ZSM-5 to TiO_2 of 20:1), namely 20 g of ZSM-5 synthesized at low temperatures and 1 gram of TiO_2 according to the procedure of (Nurprihandayani et al., 2018). This procedure was repeated for a mass ratio of ZSM-5:TiO_2 respectively 20:3; 20:5; and 20:7.
2. Reagents for COD Analysis
   a. Preparation of 0.1N Potassium Chromate ($K_2Cr_2O_7$) Standard Solution
      $K_2Cr_2O_7$ standard solution 0.1 N was prepared by dissolving 4.9031 grams of $K_2Cr_2O_7$ powder with distilled water until the solution volume is 1000 mL.
   b. Preparation of 0.1 N Ferrous Ammonium Sulfate (FAS) Standard Solution
      Ferro Ammonium Sulfate solution 0.1 N was made by dissolving 39.2130 grams of $Fe(NH_4)_2(SO_4)_2.6H_2O$ in 500 mL of distilled water, adding 20 mL of concentrated H_2SO_4, cooling and bringing the solution volume to 1000 mL.
      FAS Normality= ((Volume x Normality) $K_2Cr_2O_7$)/(FAS Volume)
3. Preparation of Chromocult Coliform Agar (CCA) Media
   Chromocult Coliform Agar (CCA) media was weighed at 26.5 grams and dissolved in sterile distilled water until the solution volume was 1000 mL. Next, it is heated over a water bath for 30 minutes until completely dissolved, then cooled to 45□C, and poured aseptically into a Petri dish, waited until it cools and solidifies.
4. Waste Water Sampling at Outlets
   The sterile sample bottle is put into the control tank (IPAL Outlet) until it is full, then removed and labeled and identified. Every day 2 bottles of samples are taken, one bottle to immediately check COD levels, initial Coliform and E. coli counts and one bottle for treatment of the sample.
5. Procedures for Reducing COD Levels and the Number of Coliforms and E. coli in wastewater at the UPTD Health Laboratory of Kudus Regency
   a. Tool Sterilization
      All tools used, including glass materials, were sterilized in an autoclove at 121°C with a pressure of 1 atm for 30 minutes, then dried in an oven at 60°C for 30 minutes.
   b. Erlenmeyer washing for determining COD levels using the bichromatometry

method

The erlenmeyer was filled with 50.0 mL of distilled water, 5 ml of 2 N H_2SO_4 and 0.01 N $KMnO_4$ was added so that it turned pink and heated until it boiled, then the liquid contained in the erlenmeyer was discarded.

c. Treatment of COD samples with the addition of ZSM-5/TiO_2 powder with mass variations of ZSM-5:TiO_2 20:1; 20:3; 20:5; and 20:7

Four Erlenmeyers that have been washed are prepared, each filled with 50.0 mL of waste water, added with 0.5 g of ZSM-5/TiO_2 20:1 powder and put into a BSC level IIA with dimensions of 170x60x66 cm. Next, it was stirred at a speed of 320 rpm and exposed to a 40 watt UV lamp for 60 minutes (the distance between the sample and the UV light was 40 cm). After that, it was filtered using Whatman 42 filter paper, and the filtrate was checked for COD levels, the number of coliforms and E. coli. The procedure was repeated 3 times and repeated with variations in the mass of ZSM-5:TiO_2 20:3; 20:5; and 20:7.

6. Analysis Procedures:

a. COD level

COD level examination uses the bichromatometry method (Khasanah, U., 2019). The initial sample or sample after treatment was pipetted 20.0 mL into an Erlenmeyer, added + 0.4 g $HgSO_4$; 10.00 mL $K_2Cr_2O_7$ 0.1000 N and 30 mL concentrated H_2SO_4 through the walls of the Erlenmeyer, installed a condenser (air cooler), and refluxed by heating at a temperature of 80°C for 2 hours. After cooling, ferroin indicator was added and the remaining 0.1 $K_2Cr_2O_7$ was titrated with 0.1 N Ferro Ammonium Sulphate (FAS) solution from green-blue to brown-red. Blanks were carried out according to the sample treatment procedure by replacing the sample with 20.0 mL of distilled water.

$$\text{COD concentration} = \frac{1000}{\text{Vol sample}} \times (\text{Vblank-Vsample}) \times \text{N FAS}\} \times \text{BE } O_2 = \ldots\text{mg/L}$$

b. Detection of Coliform and E. coli Colonies

A sample of 20.0 mL the initial sample and after treatment was filtered with 0.45 µm filter membrane aseptically (Merck), then the filter membrane was placed on Chromocult Coliform Agar (CCA) media, incubated for 24 hours at 37□C. If the colony is purple it means there is E. coli, and if it is red it means there is Coliform. The red colonies are followed by an oxidase test using Bactidentoxidase reagent by inoculating the colony onto a Bactidentoxidase stick and leaving it for 20 – 60 seconds. After that it is read by looking at the color, if it is blue to purple blue (oxidase +) then the colony is non-coliform but if this does not happen Colony color changes (oxidase -), then the colony is Coliform. Next, the number of Coliforms and E. coli is calculated in CFU/100ml units.

$$\textit{Reduction in COD levels or Number of Coliform and E.coli}\ (\%) = \frac{(\textit{Initial level} - \textit{Final Level})}{\textit{Initial Level}} \, x\, 100$$

3. RESULT AND DISCUSSION

Determination of initial COD levels and after adding ZSM-5- TiO2 is shown in Table 1.

Table 1. Initial COD levels and after immersion with variation mass ratios of ZSM-5-TiO2 for 60 minutes

Variations in Mass Comparison	COD Level (mg/L)

ZSM-5/TiO_2	
0 : 0	62.7±7.6
20:1	46.7±7.1
20:3	44.0±8.0
20:5	36.0±5.3
20:7	28.0±.8.0

From Table 1, it was found that the initial COD level in Kudus Health Laboratory waste was 62.7 mg/L, with a ZSM-5-TiO2 ratio of 20:1, 20:3, 20:5, and 20:7 for 60 minutes. COD has decreased. The percentage reduction in COD levels after adding ZSM-5-TiO2 with variations in the mass ratio of ZSM-5:TiO2 is presented in Figure 1.

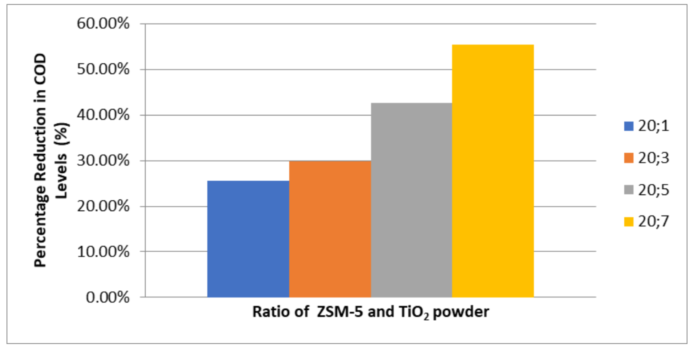

Figure 1. Percentage graph of COD reduction levels

The highest percentage reduction in COD levels of 55,34% was obtained by adding 500 mg of ZSM-5/TiO2 20:7 (Figure 1), where the amount of TiO2 added was (7000 mg/27000 mg) x 500 mg = 129.63 mg/50 mL sample. This study is in line with Sari's research (2019), the reduction in COD levels of 150 ppm in synthetic waste CI Reactive Red 2 using 6 g/L TiO2 (120 mg/50 mL sample) photocatalyst during 12 hours of irradiation was 59%.

Normality test using the Shapiro-Wilk method.

Tests of Normality

		Kolmogorov-sminov[a]			Shapiro-wilk		
	Concentration variations	Statistic	df	Sig.	Statistic	df	Sig.
The results of measuring COD levels in water using variations in the amount of TiO2	20:1	.219	3	-	.987	3	.780
	20:3	.314	3	-	.893	3	.363
	20:5	.175	3	-	1.000	3	1.000
	20:7	.175	3	-	1.000	3	1.000

a. Lilliefors Significance Correction

Based on the results of the normality test using the Shapiro-Wilk method, p-values were obtained for concentration variations of 20:1; 20:3; 20:5; and 20:7 of 0.780, 0.363; 1.000 and 1.000. Because the p-value is more than 0.05, it can be concluded that the data is normally distributed.

Table 2. Tests of homogeneity of variances The results of measuring COD levels in water using variations in the amount of TiO2

Levene statistic	df1	df2	Sig.
.171	3	8	.913

Based on the results of the homogeneity test using the Levene method in the table above, a p-value of 0.913 was obtained. Because the p-value is more than 0.05, it can be concluded that the data is homogeneous or the same.

Table 3. ANOVA

	Sum of square	df	Mean squares	F	Sig.
Between groups	640.000	3	213.333	2.025	.189
Within groups	842.667	8	105.333		
Total	1482.667	11			

Based on the hypothesis testing table using the One Way Anova test above, a significant p-value of 0.189 was obtained. Because the significance value of the p-value is more than 0.05, it can be concluded that there is no effect of concentration variations of 20:1, 20:3, 20:5, and 20:7 on COD levels.

Number of Coliforms and E. coli initially and after adding ZSM-5/TiO_2 powder

The number of Coliforms and E. coli initially and after adding ZSM-5/TiO_2 powder with varying amounts of TiO_2 is presented in Table 4 and Figure 2.

Table 4. Initial number of Coliforms and E. coli (CFU units/100 mL) with the addition of ZSM-5/TiO_2 powder, varying the ratio of ZSM-5: TiO_2

Sample (day -)	**Initial**		**Variations in the Mass Ratio ZSM-5/TiO_2**							
			20:1		**20:3**		**20:5**		**20:7**	
	Coliform	*E. coli*	*Coliform*	*E. coli*	*Coliform*	*E. coli*	*Coliform*	*E. coli*	*Coliform*	*E. coli*
1	43000	500	190	0	490	0	2700	30	41	0
	55000	600	170	0	500	0	2600	20	35	0
2	101000	2100	0	0	150	20	50	0	240	50
	140000	2700	0	0	160	10	40	0	230	60
3	33000	400	10	0	4300	40	900	0	6200	30
	38000	330	10	0	4700	40	900	0	6300	30
4	30000	400	500	0	100	0	900	0	6400	30
	32000	500	600	0	300	0	800	0	6900	20
5	30000	340	0	0	900	0	1700	30	120	0
	22000	280	0	0	800	0	1300	10	130	0

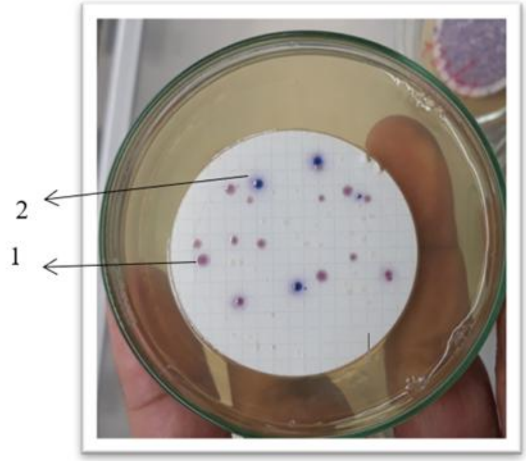

Figure 2. Colonies on CCA media (1. Coliform colonies, 2. E. coli colonies)

The percentage (%) reduction in the number of Coliforms after treatment is shown in Figure 3 and E. coli ini Figure 4.

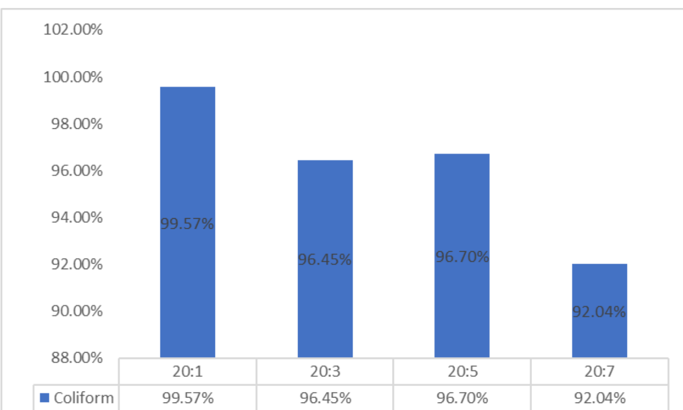

Figure 3. The percentage (%) reduction in the number of Coliforms after treatment

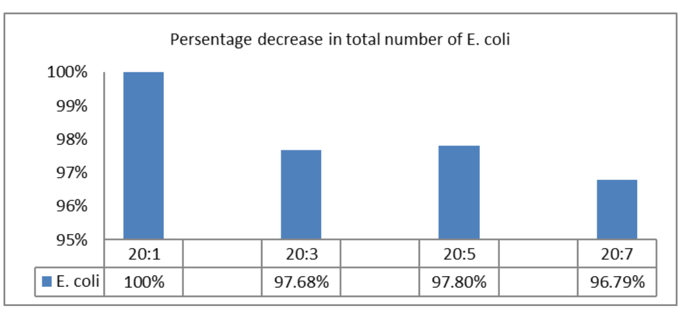

Figure 4. Percentage decrease in total number of E. coli after treatment

Figure 3 and 4 show the percentage decrease in total number of Coliforms and E. coli from samples from the UPTD IPAL outlet of the District Health Laboratory. Kudus with the addition of variations in the ZSM-5/TiO2 powder ratio of 20:1; 20:3; 20:5 and 20:7 have decreased. The highest percentage reduction in the number of coliform and E. coli using ZSM-5/TiO2 20:1 powder resulted in 99.57% and 100% respectively, where these results were higher than the UV light control, ZSM-5, and variations in the amount of TiO2 alone (Figure 5 and Figure 6).

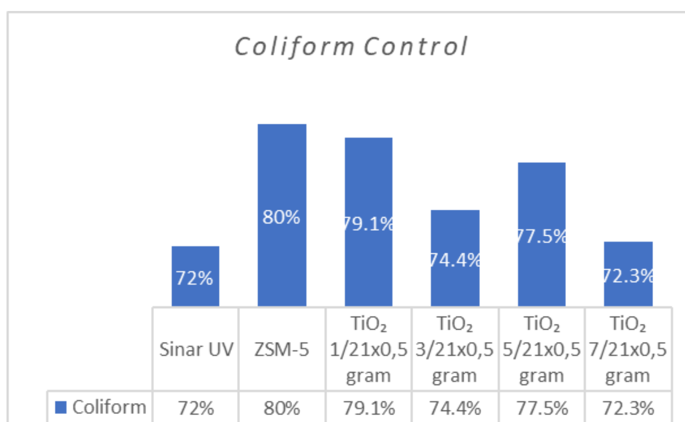

Figure 5. Graph of Percentage Reduction in the Number of Coliforms

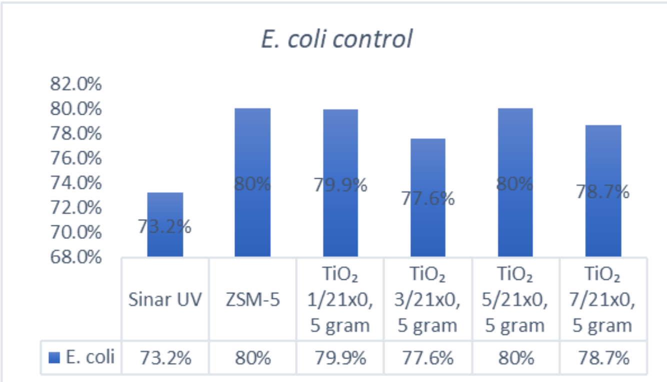

Figure 6 Percentage graph of reduction in the number of E. coli

Figures 5 and 6 show that the reduction in the number of coliforms and E. coli using UV light control, ZSM-5, and TiO2 alone, the results were lower than using ZSM-5/TiO2. From the research results, the data were tested for normality using the Shapiro Wilk test, it was obtained that the percentage reduction in the number of Coliforms with variations in the mass ratio of ZSM-5/TiO2 was 20:1; 20:3; 20:5; and 20:7 respectively obtained a sig value of 0.000; 0.001; 0.279; and 0.001. Percentage reduction in the number of E. coli with varying mass ratios of 20:3; 20:5; and 20:7 respectively obtained a sig value of 0.000; 0.003; and 0.037, while for ZSM-5/TiO2 20:1 because the data is constant so it is considered abnormal data, the Shapiro-Wilk test is used. The data results were not normal, so it was continued with the Kruskal Wallis Nonparametric Tests. The results of the Kruskal Wallis statistical test decreased the number of Coliforms with a value of $p < 0.05$, so there was an influence of variations in the ratio of ZSM-5/TiO2 powder. The results of the Kruskal Wallis statistical test decreased the number of E. coli with a p value of > 0.05, so there was no effect on variations in the ratio of ZSM-5/TiO2 powder.

TiO2 photocatalyst which has been impregnated on ZSM-5 support material (ZSM-5/TiO2) with varying amounts of TiO2 is characterized using SEM presented in Figure 7 and XRD presented in Figure 8.

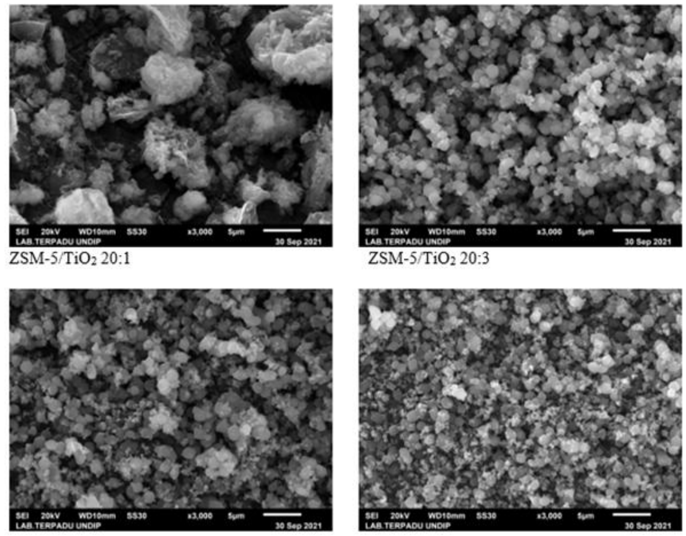

Figure 7. Morphology SEM with 1500x OF ZSM-5-TiO2, with 20 g of ZSM-5 and TiO2 mass A 1g; B 3g; C 5g; D 7g

Figure 7 shows that the greater the amount of TiO2 impregnated on ZSM-5, the larger the particle size, the greater the surface area of the catalyst.

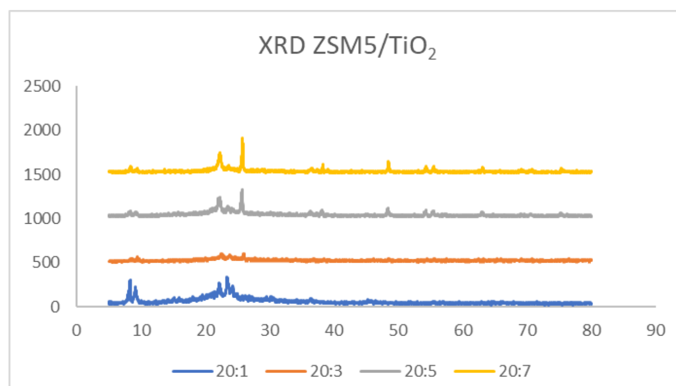

Figure 8. X-ray diffraction pattern of ZSM-/TiO2 POWDER (The integrated Laboratory of Diponegoro University Semarang)

Discussion

This research is about "Reducing Chemical Oxygen Demand, Total Coliform, and Escheria coli levels at the UPTD Health Laboratory in

Kudus district using ZSM-5/TiO2 with TiO2 Mass Variations". The anatase type of TiO2 used has an Eg value of 3.2 eV, and has the potential as an antibacterial because it has OH radicals that can inactivate bacteria. Reactive radical species (ROS) consisting of (OH•- and O2 •-) produced from the photogeneration process on the surface of titania are strong oxidants to degrade organic compounds from bacterial cell walls and membranes (Hogg; 2005). TiO2 photocatalyst after absorbing photons, can produce hvb+ and ecb- species. The hvb + species when reacting with >TiIVOH can produce •OH radicals. Meanwhile, the hvb+ species and ecb species can recombine (recombination) while releasing heat. Photodegradation reactions or reactions that destroy organic compounds are basically oxidation reactions induced by UV/Visible light. This reaction can take place if there is a light source, substrate, oxygen and photocatalyst in a system. The photon energy provider can come from sunlight or UV lamps. When TiO2 is exposed to light or photon energy of hv equal to or greater than the bandgap energy (Eg) of the photocatalyst semiconductor, TiO2 will absorb light, producing •OH radicals on the catalyst surface [•OH(s)]. The greater the amount of catalyst used, the greater the surface of TiO2 that absorbs ultraviolet radiation, so that the amount of •OH radicals produced also increases. Another limitation of TiO2 is that it has low absorption capacity and is difficult to reuse, so further modification is needed by impregnating kaolin. TiO2 impregnated on kaolin can increase the surface area of TiO2 and its catalytic activity (Lu et al., 2017).

With the increasing amount of •OH radicals, the effectiveness of COD photodegradation increases. The effectiveness of the catalyzed photodegradation process can be influenced by the catalyst, reactants and process conditions. The role of the catalyst can be represented by the amount of photocatalyst. The amount of photocatalyst will determine the amount of photocatalyst surface that can provide •OH radicals. Thus, a large amount of •OH radicals can increase the effectiveness of the COD photodegradation reaction and reduce the number of Coliform and E. coli bacteria.

The use of TiO2 if not balanced by the ability to adsorb target compounds can reduce the ability of the degradation process, so that the degradation process does not run well or is less than optimal (Aji et al., 2016). Therefore, supporting materials are needed to maximize the work of photocatalytic by using high adsorption materials. Several types of materials that have high adsorption capacity are activated carbon, zeolite, ZSM-5 and silica gel (Anggraini, 2010; Damchan et al., 2008). TiO2 can be loaded on a supporting material that has a fairly high adsorption capacity. Several types of porous materials that can be used as adsorbents include silica gel, activated carbon, zeolite, and bentonite. Silica in ZSM-5 inserted into the TiO2 crystal framework can increase the acidity and hydrophilicity of the surface. The acidity of TiO2 causes an increase in hydrophilic capacity and is able to adsorb more OH radicals. Thus, TiO2 has the potential as an antibacterial agent, with the addition of SiO2 to ZSM-5 being able to increase the hardness, transparency and tensile strength properties (Clarissa et al., 2021).

ZSM-5/TiO2 can reduce COD levels in wastewater because ZSM-5 contains aluminosilicate, built from (SiO4)4- and (AlO4)5- and has a tetrahedral bond with oxygen atoms forming a pentasil chain and becomes one of the media used as an ion exchanger that has the ability to absorb organic substances in Kudus health laboratory liquid waste, while TiO2 with the presence of electron jumps from the valence band to the conduction band in semiconductor metals when exposed to a photon energy. This electron jump causes holes (electron holes) to appear that can interact with solvents (water) to form OH- radicals. OH radicals are active and can continue to decompose target organic compounds into H2O and CO2. By reducing organic substances in wastewater, the amount of oxygen needed to decompose organic substances is reduced, so it can be concluded that the effective and highest comparison variation is a comparison of 20:7 which can reduce COD levels by 55.34%.

The addition of ZSM-5/TiO2 powder with various ratios to the wastewater outlet of the Kudus District Health Laboratory UPTD has been proven to be able to reduce the number of Coliforms, as evidenced by the decrease in the number of coliforms for the ratio of the number of ZSM-5/TiO2 powders of 20:1; 20:3; 20:5; 20:7; respectively, the decrease in the number of coliforms was 99.57%; 96.45%; 96.70%; and 92.04%. This is in accordance with the research of (Aji et al., 2016), that the largest decrease in BOD and COD values was obtained by using TiO2-Bentonite, respectively 18.40 ppm and 10.05 ppm. The ability of the TiO2-Bentonite composite is greater than that of the TiO2 and bentonite catalysts alone.

4. CONCLUSION

The best percentage of COD reduction in Kudus health laboratory liquid waste samples was 55.34% using ZSM-5/TiO2 20:7 with an initial COD level of 62.7 mg/L, while the best percentage of Coliform and E. coli reduction using ZSM-5/TiO2 20:1 was 99.57% and 100% respectively.

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