EFFECTIVENESS OF CHLORINE DESINFECTANTS TO ESCHERICHIA COLI REMOVAL IN PURUS PADANG WATER WELL

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ABSTRACT

One of the requirements of drinking water is the bacteriological content, there is no Escherichia coli bacterium. Based on researched, the content of E.coli in water well from densely populated areas and poor environmental sanitation were evaluated. The content of E.coli can eliminated by disinfection process using chlorine. Optimum dose and contact time are specified on the artificial solution experimental. The water wells in Purus Padang area had a high content of E.coli that is >1,6.10^5 cell/100 ml because of environmental pollution. In optimization experiments the optimum chlorine dose is 50 mg/l with a contact time of 30 minutes to eliminated a number of E.coli bacteria from >1,6.10^5 sel/100 ml to 0 sel/100 ml. The death rates of E.coli value for each dose of chlorine and contact times ranged from 0.1 to 0.7/min. The growth rate of E.coli in chlorine dose 10-40 mg/l and contact times 10-40 minutes is 0.03 to 0.09/min. Metals iron and manganese are in the $Fe(NO_3)_2$ and $Mn(NO_3)_2$ could decrease the effectiveness of disinfection reached 0.9% and 0.6% at the optimum conditions of artificial solution. The experiments on water well samples in Purus area obtained the efficiency of E.coli removal reached 99.9% at optimum conditions with 0.4 mg/l of chlorine residual. Based on the experimental results, that chlorine as a disinfectant effectived to eliminated the content of E.coli bacteria in the water wells.

Keywords: Water wells, Disinfection, Escherichia coli, Chlorine.

INTRODUCTION

The ground water is one of drinking water used by the community which is obtained by dug wells. Approximately 45% of people in Indonesia used water wells as a clean water means, and about 75% using dug wells (Chandra, 2007). On of the requirements of drinking water should qualified bacteriology conditions. Water that does not meet the demand of bacteriolical requirements to be caused of waterborne disease. The parameters used in the bacteriological requirements of the water is Escherichia coli bacterium (Suriawiria, 2005).

E.coli bacteria content in the water can be removed by disinfection (Sutrisno, 2002). Chlorine is a disinfectant that is often used in disinfection because it is effective, economically, stable and can be stored for longer (Sururi, 2008). Disinfectant performance can be reduced by the presence of compounds such as iron and manganese in water (Sururi, 2008). Temperature and pH also influenced the effectiveness of disinfectants (Waluyo, 2009). Quality of water wells influenced by the environmental conditions. In the densely populated areas and poor environmental sanitation is expected to reduce the quality of well water.

Purus Padang in the district of West Padang with high population density so that needs relatively high of water. A total of 2.334 households in the district use the wells as sources of drinking water (Bapedalda, 2010). From the condition of settlement in the region as well as the Purus drains and sewers that mix is expected to contaminate water well. Research Syadikin (2003) states that the number of groundwater bacterial cells in Purus region was 3.145 cells/ml. Through experiments using sunlight, the amount of bacteria in groundwater can be reduced to 1.110 cells/ml (64.71%). Disinfection by sunlight are flawed because it depends on the intensity of solar radiation and weather.

Based on the research above, it is necessary to research on other water well disinfection alternative with chlorination to the high content of E.coli. This research will be known to the effectiveness of chlorine in the water wells of E. coli designated in Purus region with variations of chlorine dose and contact time. It also examined the effect of iron and manganese are often found in groundwater on the performance of disinfectants.

METODOLOGY OF RESEARCH

a. Material

The tools that used in this research are test tube and durham to the MPN test, erlenmeyer, incubator (QL Model 12-140E), Oven (Memmert), Analytical Balance (And 12,329,241), and Shaker (Wiseshake SHO-2D).

Escherichia coli bacteria are used for artificially solution experiments that obtained from the Laboratory of Mathematics and Natural Sciences Andalas, pure water (sterile distilled water), the water wells sample in Purus and chlorine as a disinfectant. Nutrient Agar (NA) and Nutrient Broth (NB are the media for E.coli bred. MPN test for the calculation of E.coli bacteria using Lactose Broth (LB) and Brilliant Green Lactose Bile Broth (BGLB) media. In addition to experimental ion effects of disinfection used metals iron and manganese compounds in the form of $Fe(NO_3)_2$ dan $Mn(NO_3)_2$.

b. Bacteria Breeding

Taken as many as 1-2 ose of bacterial colonies and streaking in NA media as slant breeding. After that incubation at room temperature (30oC) for 24 hours. Further breeding is done in liquid media by entering 1 sterile bacterial loop into sterile Erlenmeyer containing 200 ml of NB. Whisk in the shaker and incubated at room temperature for 24 hours.

c. Characterization of Water Well Purus region

Water well samples are chosen with the highest content of E.coli bacteria. Physical and chemical parameters measured of water well samples that smells, color, TDS, turbidity, temperature, TSS, iron, manganese, COD, BOD, pH, cadmium, chloride, hardness, zinc, sulfate, copper, ammonia, nitrite, and nitrate. Compared with the drinking water quality standard.

d. Optimization Experiments on Artificial Solutions

Optimization experiments conducted to obtain chlorine dose and optimum contact time in the allowance E.coli bacteria. A number of E. coli bacteria used in this experiment according to the number of E. coli in water well samples measured.

- Chlorine Dose

A variation of chlorine dose are obtained by measuring the value of DPC. DPC is calculated based on the relationship between residual chlorine with initial chlorine to the following equation (Ditjen. PPM & PLP, 1998):

$$\text{DPC} = \text{klor segera - sisa klor} \qquad \text{.............(1)}$$

Chlorine dose range affixed taken two below and above the DPC calculation has been done is C_1, C_2, C_3 (DPC), C_4, and C_5.

- Contact time

Variation of contact time based on research by Surusri (2008) to the disinfection time are 10, 20, 30, 40, 50 minutes.

In this experiment, the E.coli bred put into erlenmeyer (250 ml) containing 100 ml of sterile water and chlorine dosage that has been prescribed. Closed the erlenmeyer with sterile cotton and shake with a speed of 80 rpm, creating aerobic conditions. This condition simulates an open water well. Each dose of the chlorine shake with contact time for 10, 20, 30, 40 and 50 minutes. Count the number of E. coli after treatment for each dose and contact time and removal efficiency with the following equation:

$$\square = \frac{\text{E.coli initial -E.coli residue}}{\text{E.coli initial}} \text{ X } 100\% \qquad \text{...........(2)}$$

Ct concept is a basic theory of disinfection. CT values can be calculated with the following equation:

$$CT = konstan \qquad \text{...........(3)}$$

where:

C = concentration of disinfectant (mg / l)
T = time of contact

e. Chlorine Residuals

Residual chlorine measured at the optimum dose and contact time. It is to check the chlorine residual levels are within acceptable limits is 0.2-0.5 mg / l (Waluyo, 2009). Chlorine residual measurements using the following equation:

$$\text{Residual chlorine} = \frac{1000}{\text{ml sample}} \text{x ml Thio Sulfat x N Thio Sulfat x 35,45} \qquad \text{.......(4)}$$

f. Effect of Disruptors Ion

Disruptors Ion in this research is the metal iron and manganese compounds in the form of $Fe(NO_3)_2$ and $Mn(NO_3)_2$ is added to a artificial solution under optimum conditions. Variation range of ferrous metals are 0.15. 0.3 and 0.45 mg / l, and manganese variations are 0.2, 0.4 and 0.6 mg / l based on the content of the sample. The Results of the experiments influenced of iron and manganese can be compared with the results of experiments on artificial solution without confounding compounds by looking the contents of E.coli.

g. Inactivation of E.coli bacterium

inactivation of E.coli after khlor present can calculate with the following equation (Metcalf, 2007):

$$\ln \frac{Nt}{No} = -k.t \qquad \text{................(5)}$$

$$\ln \left(\frac{Nt}{No}\right) = -k'Ct \qquad \text{.................(6)}$$

$$C^n k' = k \qquad \text{..................(7)}$$

$$\log \text{inaktivasi} = \log \left(\frac{N0}{Nt}\right) \qquad \text{..................(8)}$$

Where:

No	= a number of microorganisms at 0
Nt	= a number of microorganisms at t
C	= concentration of disinfectant (mg/l)
k	= death rate (1/min)
k '	= constant of specific destruction (l/mg.min)
t	= time (minutes)
n	= solubility constant

h. The growth rate of E. coli Bacteria

The E.coli growth estimated occur after the disinfectant exterminate decreased. The growth rate can calculate with the following equation (Monod *equation*):

$$\ln \left(\frac{Xt}{Xo}\right) = \mu.t \qquad \text{...................(9)}$$

Dimana:

X_o	= a number of microorganisms at 0
X_t	= a number of microorganisms at t
t	= time (minutes)
μ	= spesific growth rate

i. Water well samples desinfection in optimum condition

The effectiviness of chlorine in optimum conditions for the water well samples knowed from this research. Put the water well samples in erlenmeyer 250 ml with *optimum chlorine dose to 100 ml and shak for* optimum contact time. Measured the amount of E. Coli, removal efficiency and residual chlorine.

j. Analysis of Measurement

In this research the parameter measurement are:

- A number of E.coli in water well sample and E.coli bred calculated by the MPN methode;
- Residue of chlorine in optimum condition was measured by iodometric titration;
- Physical and chemical parameters of water well sample by *standar method.*

RESULTS AND DISCUSSION

a. Characteristics of water well sample

The physical, chemical and biologycal parameters of sample can viewed in Table 1.

Tabel 1. Concetration of physical, chemical and biologycal parameters

No	Parameters	Unit	Permenkes No.492/Menkes/ Per/IV/2010	Sample
	Physical			
1.	Smells		Tidak berbau	-
2.	Color	TCU	15	2,375
3.	TDS	mg/l	500	896
4.	Turbidity	NTU	5	5,7
5.	Temperature	°C	28± 3°C	29
6.	TSS	mg/l		252
	Chemical			
1.	Iron	mg/l	0,3	1,103
2.	Manganese	mg/l	0,4	0,6
3.	COD	mg/l		192
4.	BOD	mg/l		7,1
5.	pH	mg/l	6,5 – 8,5	7,5
6.	Cadmium	mg/l	0,003	0,267
7.	Chloride	mg/l	250	618,8
8.	Hardness	mg/l	500	23
9.	Zinc	mg/l	3	0,967
10.	Sulfate	mg/l	250	111,6
11.	Copper	mg/l	2	0,485
12.	Ammonia	mg/l	1,5	1,1
13.	Nitrite	mg/l	3	0,849
14.	Nitrate	mg/l	50	1,098
	Biologycal			
15	E.coli	cell/100ml	0	$>1,6.10^5$

In the table it can be seen TDS content of water well samples is 896 mg/l that passed the standard of quality. High content of TDS are caused by the presence of organic pollution. Purus water well turbidity is 5.7 NTU. Basically the turbidity caused by water containing of mud, organic materials and small particles suspended. Suspended solids and turbidity were positively correlated (Effendi, 2003). Chemical parameters such as BOD and COD has a high value becaused the high content of organic matter contained in water well. High levels of organic pollution caused by domestic and industrial waste.

The content of chloridein water well is 618.8 mg/l caused of seawater intrusion. Sulfate, nitrate, nitrite and ammonia parameters are still below the standards, *while the metal content of iron, manganese and* cadmium passed the quality standard are 1.103 mg/l, 0.6 mg/l and 0.267 mg/l. In general, ground water contains a lot of iron and manganese will increase with human activity and industry (Widowati, 2008)

A number of Escherichia coli bacteria in Purus water *well samples is > 1.6 x105 cell/100 ml that passed the* standard for drinking water. Marwati studied (2008) resulted in an average content of water well for E. Coli is 1100 cell/100 ml for Puskesmas I, South Denpasar. This indicates that the Purus water well has been contaminated. Suriawiria (2005) stated that the presence of microbial pathogens will increase if the high of organic matter content in water.

High content of E. coli is also influenced by the location and condition of the well, where the distance well with a discharge of toilet is 2 meters and 6 meters from the septic tank. Based on the results of the statistical analysis by Hasnawi (2012) note that the distance of wells with proven pollutant have an influence on the content of the Escherichia coli bacteria. The minimum distance of well and pollution sources such as latrines, cattle sheds, bins, and so on is 15 meters (Chandra, 2007). Construction of water wells and the way decisions can affect the bacterial content in water (Depkes RI, 1985).

b. Reproduce of Escherichia coli to Artificially Solution experiments

E.coli bacteria cultured in media NA and NB media. Based on microscopic observations, the physiological of E.coli bacteria bred that has the rod-shaped (bacillus) and belong to the class of gram-negative bacteria because it produces a red color when gram staining. Observations E.coli bacteria using a microscope can be seen in Figure 2.

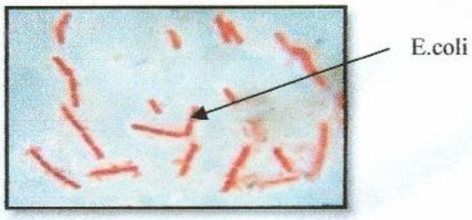


Figure 2. Escherichia Coli Bacteria reproduce on NA media with 1000x magnification

Gram-negative bacterial cell wall composed of peptidoglycan and consists of 10-20% polysaccharides, proteins and fats. Gram-negative bacteria do not bind crystal violet in gram staining because most of the cell wall consists of a lipoprotein and lipopolysaccharide (Block, 2000). Lipid soluble by alcohol, so complex crystal violet dye in the cell *walls of bacteria can not be sustained (Pelczar, 2006).*

c. Chlorine Dose on Artificial Solution

DPC calculation is obtained by reduction of residual chlorine with chlorine immediately using equation (1). Based on the calculation of the value obtained DPC is 30 mg/l, then set the range of variation of chlorine dose are 10, 20, 30 (DPC), 40, and 50 mg/l. Susanto researched (1998), the dose of chlorine is 19mg/l can reduced the amount of coli bacteria in peat water coli from 250 cell/100ml to 0 cell/100. A high doses of chlorine for Purus water wells due to the great content of E. coli bacteria is $>1.6x10^5$ sel/100ml. Large number of microorganisms, especially pathogenic microbes will require higher the disinfectant dosage (Waluyo, 2009).

d. Optimization Dose of chlorine and disinfection contact time at Artificial Solutions Experiment

In the optimization experiments the initial number of bacteria contained in the artifisial solution according to the number of E. coli in water well sample is > 1.6 $x10^5$ cell/100ml. The results of E. coli after the dosing of chlorine 10, 20, 30, 40 and 50 mg/l for each contact time 10, 20, 30, 40, 50 minutes can be seen in Figure 3. Removal efficiency of E. coli in artificial solution experimental can be calculated based on the relationship between the initial amount of total E.coli with E.coli measured after treatment by using equation (2) and can be seen in Table 2.

Based on the MPN test results can be seen the same tendency during 10 minute contact time for each additional dose of chlorine, which drastically decrease the number of E.coli. Chlorine dose 10, 20, 30, 40 and 50 mg/l can reduce the content of E.coli from > 1.6 x 105 cell/100ml to 370, 180, 180, 180, 360 cell/100ml with removal efficiency reached 99.8%. Decrease in the number of bacteria caused by inactivation ability of chlorine. Mechanism of disinfectant in killing the bacteria that is by inhibiting cell wall synthesis, damage the membrane plasma, inhibiting protein synthesis and nucleic acid and stop metabolism (Sumbali, 2009). This cause the bacteria die or disappear.

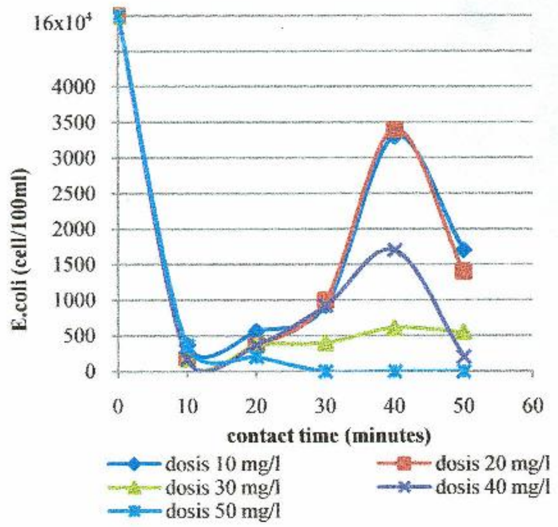


Figure 3. Graph of the number of E.coli to Contact Time for Chlorine Dosing Addition of 10-50 mg / l in the artificial solution experiments

Table 2. Removal efficiency of E. coli in chlorine dose 10-50 mg/l and contact time 10-50 minutes

Chlorine dose (mg/l)	Removal efficiency (%) in contact time (t)				
	10 minute	20 minute	30 minute	40 minute	50 minute
10	99,8	99,7	99,4	97,9	98,9
20	99,9	99,8	99,4	97,9	99,1
30	99,9	99,8	99,8	99,6	99,7
40	99,9	99,8	99,4	98,9	99,8
50	99,8	99,9	100	100	100

On the addition of chlorine dose 10-40 mg/l, after 10 minutes consist increase the number of cells at 20 minutes contact time to 560, 370, 370, 370 sel/100 ml and continued increase until 40 minutes contact time. This becaused the power of chlorine decreases and bacteria begin to utilize nutrition from NB media in medium for growth. An increasing a number of E.coli is also characterized by the value of the removal effeciency.

After 10 minutes of contact time can be said that E.coli follow the phases of growth. When the chlorine decreased, the bacteria would adapt to their environment and begin to grow slightly in accordance with the phase lag in the sigmoid growth curve of bacteria. In the lag phase of microorganisms perform metabolic activities such as transport of nutrients. At the 30 minute contact time for the chlorine dose of 10-40 mg/l, the bacteria are in the exponential phase (log phase). During the log phase, the population has doubled at constant speed dependent intrinsic properties of bacteria and environmental conditions.

40 minutes of contact time in chlorine dose of 10-40 mg/l, the bacteria are in the static phase where bacteria growing number equals the number of dead bacteria and continued to decline until the 50 minute contact time to be 1700, 1400, 550, 200 cells/100ml. This is because the amount of nutrients needed by the bacteria to grow less and less, the cell autolysis and decreased cellular energy so many dead bacteria.

The differences can be seen in the chlorine dose 50 mg/l, which decreased the amount of E. coli bacteria occurs from the first minute and so on until the constant point at 30-50 minutes of contact time. Based on the results of MPN test there are not bubbles gas in the Durham tube, so it can be said that the number of E. coli is 0 sel/100 ml with a value of 100% removal efficiency. This indicates that the chlorine effective to destroy at greatest contact time.

Based on the number of E. coli above the results, optimum conditions of disinfection with chlorine in a artificial solution experiment is 50 mg/l of chlorine dosage and 30 minutes of contact time, it can be set aside E.coli tol 0 sel/100 ml with 100% of removal efficiency.

Sururi studied (2010) using ozone to remove the number of E. coli produces 100% efficiency on a 2 minute of contact time. When compared with the above studies, disinfection with chlorine has a longer contact time than ozone disinfectant. But more advantageous in terms of cost.

e. C.T concept

Effectiveness of disinfection can be described as Ct. C is the disinfectant concentration of disinfectant and t is the time for inactivation of the population in certain conditions (pH and temperature) and calculated by equation (3). Can be seen in Table 3 Ct value for each treatment.

Table 3. Ct value in optimation treatment

Chlorine dose (mg/l)	C.t (mg.min/l)				
	10 minute	20 minute	30 minute	40 minute	50 minute
10	100	200	300	400	500
20	200	400	600	800	1000
30	300	600	900	1200	1500
40	400	800	1200	1600	2000
50	500	1000	1500	2000	2500

f. Chlorine residue on Artificially Optimum Conditions

Chlorine residual measurements performed after achievement of optimum conditions in this artificial solution experiment on the addition 50 mg/l of chlorine dose for 30 minutes of contact time. Chlorine residuals were measured by using equation (3). Based on the calculation results obtained residual chlorine value is 3.5 mg/l for 50 mg/l of the initial chlorine dose. The high residual chlorine in artificial solution experiment was caused by a high dose of chlorine. In the chlorine dose of 30 mg/l (DPC), optimum conditions have not been achieved due to the persistence of the content of E.coli, whereas for 50 mg/l of chlorine dose, E. coli can set aside up to 100% at 30 minutes of contact time. Residual chlorine is beyond quality standard that is 0.2-0.5 mg/l. High residual chlorine is not good for health as it can cause negative effects such as irritation and toxic.

g. Effect of Iron and Manganese on Disinfection in Artificially Optimum Conditions

Variation of iron content used in this experiment are 0.15, 0.35 and 0.45 mg/l in the form of the compound $Fe(NO_3)_2$ and manganese in the form of compounds $Mn(NO_3)_2$ are 0.2, 0, 3 and 0.6 mg/l at the optimum conditions. Effect of iron and manganese for disinfection can be seen in Table 4. Iron metals influence on disinfection because is still presence of E.coli. At manganese content 0.2 mg/l, which still contained 180 cells/100 ml the number of E. coli . As for the addition of higher levels of manganese is 0.6 mg/l in the solution, the number of E. coli was reduced to 930 cells/100 ml. Based on these results it can be concluded that the iron and manganese compounds may reduce the disinfection performanced because of the persistence of E. coli that live in optimum condition.

Table 4. Effect of Iron and Manganese on Disinfection

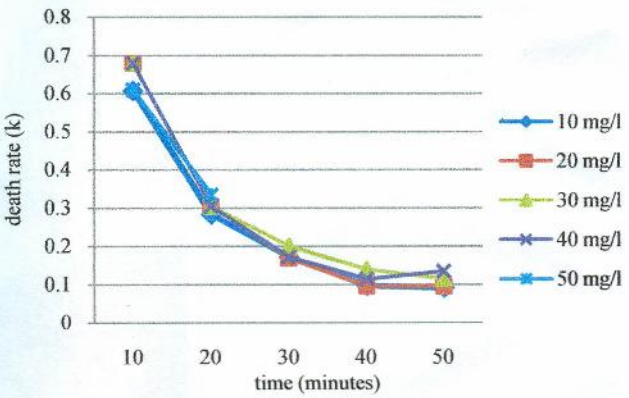
Metal	Concentration (mg/l)	A number of E.coli ($x10^2$sel/100ml)	Removal effeciency (%)
Iron	0,15	6,1	99,6
	0,30	9,2	99,4
	0,45	14	99,1
Manganese	0,2	1,8	99,9
	0,4	4	99,75
	0,6	9,3	99,4

E. coli removal efficiency decrease is caused by chlorine reacts first with substances and reducing agents such as Fe^{2+} and Mn^{2+}. The greater concentration of Fe^{2+} and Mn^{2+} in the water that longer the contact time and disinfectant dose required in the process of disinfection (Sururi, 2008). Iron metals provide a greater influence than the metal content of manganese becaused a number of E. coli in water with iron had higher removal efficiency.

h. Inaktivation of *Escherichia Coli*

- **Optimization experiment**

E.coli growth rate calculated using equation (5). In Figure 4 can be seen in the tendency of the death rate of E.coli in optimization experiments.



Gambar 4. Death rate (k) in contact times for 10-50 mg/l of chlorine dose

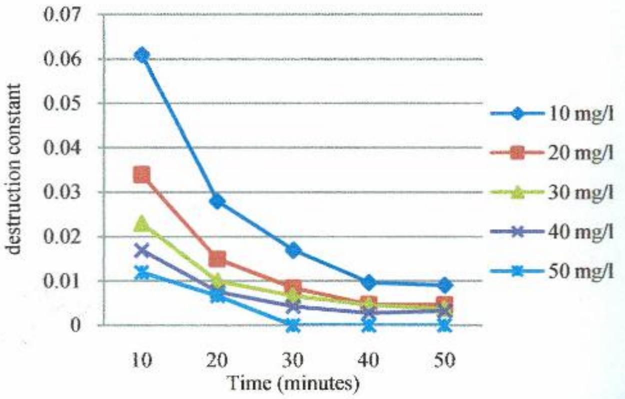
The value of death rate (k) in the 10 minutes of high value for each dose are 0.670, 0.679, 0.679, 0.679 and 0.611 l/min, is characterized by reduction in the number of E. coli larger than other contact time. In Figure 4 can be seen the same trend for each dose of chlorine, where the value of the growth rate has decreased from the first until the end of contact time. However, there was no significant difference between the death rate graphs for each dose because the value of the death rate is only influenced by the contact time.

Value of E.coli death rate (k) in nature with natural processes by Menon (2003) is 0.13 to 0.57x10⁻

^{3}/minutes. Escherichia coli 0157: H7 in the Kunun-zaki, Nigeria according to Thomas (2012) had 2.5 x10^{-3}/minutes of a death rate at 4°C and 3.8 x10^{-3}/menit at 27°C of temperature. When compared with the study, the value of the death rate of E. coli in this study is much higher. It is influenced by the presence of chlorine disinfectant compounds used in this experiment compared with the above two studies that utilize natural processes in the inactivation of E. coli.

Specific destruction constant (k ') *is the ratio between* ln (Nt/No) with concentration of chlorine and disinfection contact time can be calculated using equation (6). While the dissolution coefficient (n) is the relationship between the death rate (k) with Specific destruction constant (k ') in accordance with equation (7). The value of k' to views in Figure 5 and n values in Table 5.

Specific destruction constant value is directly proportional with the death rate that has decreased value until the last of contact time. In Figure 5 can be seen the difference in specific destruction constant chart in addition influenced by contact time, chlorine dose is also an impact on the value of k'.



Gambar 5. Specific destruction constant value (k') in contact times for 10-50 mg/l of chlorine dose

Tabel 5. Dissolution coefficient (n) in 10-50 mg/l of chlorine dose and 10-50 minutes of contact times

Chlorine dose (mg/l)	Dissolution coefficient value (n)				
	10 minutes	20 minutes	30 minutes	40 minute	50 minute
10	1,00004	1,00038	1,00012	0,99986	1,00052
20	1	1,00058	0,99966	0,99901	1,00079
30	1	1,00051	1,00042	0,9995	1.0014
40	1	1,00047	1,00008	1,00092	1,00062
50	1,00013	0,99982	-	-	-

Based on the calculation of Specific destruction *constant* (k') *are 0,061, 0,034, 0,023, 0,017 and 0,012* L/mg.min to 10-50 mg/l of chlorine dose and 10 minutes of contact time. Bichi's researched (2012) about kinetics of water disinfection using *Moringa Oleifera* seed extract obtained Specific destruction constant of E.coli reached 3,76 L/mg.min, so it is more effective than using chlorine disinfection.

N values in table 5 is close to 1. If the value of n <1 the disinfection process is more influenced by the contact time compared with the concentration of disinfectant. While the value of n> 1 the dose of disinfectant is the dominant factor to controlling the disinfection process, however, the value of n is generally close to 1 (Metcalf, 2007). This indicates that the chlorine dose and contact time are equally affect the disinfection process in this study.

Inactivation of E. coli bacteria levels are measured on a logarithmic scale by reducing the initial value of log *bacteria (No.) with the log of bacteria at time t (Nt).* Based on calculations using equation (7), for the 10 mg/l of chlorine dose and 10 minutes of contact time that No is 1,6.10^{5} cell/100 ml and Nt is 370 cells/100 ml. Comparison between the log No values and Nt is the 2.6-log that mean removal efficiency reached 99.8%. Value of log E. coli inactivation in the addition of chlorine dose is shown in Table 6 below.

Table 6. log inactivation in 10-50 mg/l of chlorine dose and 10-50 minutes of contact times

Chlorine dose (mg/l)	log inactivation in time (t)				
	10 minute	20 minute	30 minute	40 minute	50 minute
10	2,6-log	3-log	3-log	3-log	2,6-log
20	2,5-log	2,6-log	2,6-log	2,6-log	3-log
30	2,2-log	2,2-log	2,6-log	2,2-log	-
40	1,7-log	1,7-log	2,4-log	2-log	-
50	2-log	2,1-log	2,5-log	3-log	-

Inactivation of E. coli using an antimicrobial with 27% of citric acid by Gurtler (2011) obtained the value of log inactivation of E. Coli are 2,60, 4,32 and 6,95 log at 45, 50 and 55°C. In this experiment, chlorine disinfectant can set aside of E.coli up to 3 log its meaning that citric acid is superior compared with chlorine.

- **Ion disruptors effect experiment**

Death rate (k), specific destruction constant (k') and dissolution coefficient (n) values in ion disruptors effect experiment can be seen in Table 7 and the death rate graph for 50 mg/l of chlorine dose and 30 minutes of contact time can be seen in Figure 6.

Table 7. k, k' and n values in Inactivation

Ion disruptors	Dosage (mg/l)	Death rate/k (1/minutes)	Specific destruction constant/k' (l/mg.min)	Dissolution coefficient /n
Iron	0,15	0,186	0,0037	0,999
	0,30	0,172	0,0034	0,999
	0,45	0,158	0,0032	0,999
Manganese	0,2	0,226	0,0045	1
	0,4	0,2	0,004	0,998
	0,6	0,17	0,0034	1,018

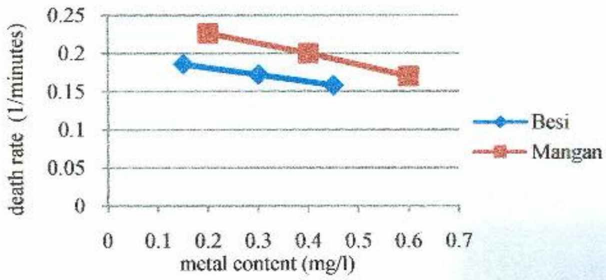


Figure 6. k value at iron content (0,15, 0,3, 0,45 mg/l) and manganese (0,2, 0,4, 0,6 mg/l) on artificial solution

Based on the table above, the value of the death rate (k) was highest in the lowest content of the metal both iron and manganese. So the smaller content of iron and manganese in the water the less influence on disinfection. Moreover, it can be seen that the metal iron is more influential on disinfection performance, evidenced by the lower of death rate value than manganese.

i. The growth rate of E. coli in the Optimization Experimental after Phase Lag

Specific growth rate of bacteria in this experiment are in the 10-40 minute of contact time for the 10, 20, 30, and 40 mg/l of chlorine dose. Calculation of the growth rate using equation (9). The ln (Xt/X0) value plotted against the time and matched with Figure 7 in order to get the slope of the curve. From the slope of the line equation obtained specific growth rate value of E. coli (μ).

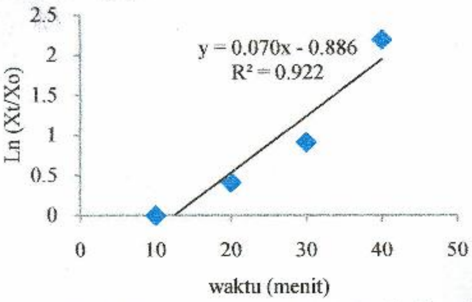


Figure 7. Linear curve $\ln(X_t/X_0)$ in 10-40 minutes of contact times and 10 mg/l of chlorine dose

Calculation results for E. coli specific growth rate using 10-40 mg/l of chlorine dose and 10-40 minute of contact time can be seen in Table 8.

Table 8. Specific growth rate value in 10-40 minutes of contact time at artificial solution

Chlorine dose (mg/l)	Growth rate (μ) (minutes^{-1})	r^2
10	0,07	0,922
20	0,098	0,987
30	0,037	0,907
40	0,076	0,995

At the table can be seen there is no significant difference between the growth rate for 10-40 mg/l of chlorine dose. Compared with the specific growth rate of isolates T5 lactic acid bacteria on MRS media based on Yuliana study (2008) that is 0.0598/h in the absence of toxic compounds. Differences growth rate of various bacteria caused by the of μ is the specific value and is determined by the type of microorganism and growth conditions (Supariasih, 2008). Besides food ingredients contained in the media where her life also affect the rate of growth of the bacteria.

j. Experiment of disinfection water well sample in optimum condition

The content of E.coli bacteria in water well samples was reduced from 160.000 cells/100ml to 180 cells/100ml after giving 50 mg/l of optimum chlorine for 30 minutes of contact time, with a removal efficiency reached 99.9%. Compared with experimental removal efficiency at optimum conditions an artificial solution that reaches 100%, the decreased of removal efficiency due to confounding iron and manganese contained in the water wells. Removal efficiency of E.coli by chlorine will determine that chlorine disinfection method feasible applied in society.

Chlorine residue in optimum dosage and contact time on water well samples was calculated using equation (3) is 0.4 mg/l. Chlorine Rresidual is still in quality standard of drinking water. Chlorine residual is lower than the rest of the optimum conditions in artificial solution caused by the influence of physical and chemical characteristics of water wells such as iron and manganese, which can be disrupt of disinfection. Observations of bacteria contained in the Purus water well samples microscopically can be seen in Figure 8.

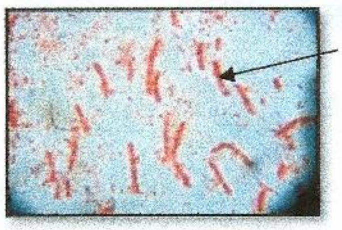


Figure 8. *Escherchia Coli* bacteria in water well sample with 1000x magnification

The picture shows the content of E.coli bacteria in water well samples, which E. coli is a gram negative bacteria and shaped bacillus. Gram-negative bacteria will bind of red when gram staining. There are other bacteria contained in the well water samples are gram-positive bacteria which characterized by colored purple and cocus.

Wells where the sampling has 1 meter of diameter at 5 metres of a depth, so the volume of water in the wells is 15,7 m^3. By the use of chlorine for disinfection to public water wells depending on the needs of water used per day.

When compared with Syadikin study (2003) on the elimination of bacteria by solar radiation in the same region got a bacterial removal efficiency reached 64.71% from 3145 cells/ml, then the elimination of bacteria using chlorine in this study have a higher removal efficiency is 99,9%. This is because chlorine has a greater effectiveness and will immediately react if put into water.

k. Evaluation of Chlorine Disinfectants Performance in Purus Water Wells Purus

Physical parameters such as smells, color, and temperature of the well water is still at the drinking water quality standard according to Permenkes No.492/Menkes/Per/IV / 2010. Some other physical parameters such as TDS content and turbidity over the limit of quality standards due to the domestic and industrial waste pollution of nearby water well sampling location. Chemical parameters such as BOD and COD has a high value due to the high content of organic matter contained in the well water. Parameter values of sulfate, nitrate, nitrite and ammonia are still well below the standards. Metal content of iron, manganese and cadmium passed the quality standard are 1,103 mg/l, 0,6 mg/l and 0,267 mg/l. Groundwater naturally contains a lot of iron and manganese because of ground water is in contact with a variety of material contained within the earth.

Bacteriological parameters of E.coli bacteria content obtained a number of E. coli in Purus water well is > $1{,}6.10^5$ sel/100ml, indicating the well water has been contaminated. Pollution can be caused by domestic sewage, industrial and livestock manure. Purus region is a densely populated area so that the resulting high domestic waste that would affect the water well bacteriological conditions. Conditions or well that are less maintained and spacing with septic tanks and other sources of pollution that does not meet the requirements could result in higher organic matter is the source of nutrients for E. coli growth.

Artifisial experiment using distilled water and breeding of E.coli to get the optimum dose and contact time. On artificial solution, given 10-50 mg/l of chlorine dose which is obtained from the calculation of the DPC for 10-50 minutes of contact time with the initial number of E. Coli is > $1{,}6.10^5$ sel/100 ml. Optimum conditions occur in 50 mg/l of chlorine dose and 30 minutes of contact time with 100% of removal efficiency according drinking water quality standard. High doses of chlorine due to the amount of E. coli content in the water wells. The residual chlorine at the optimum condition that is 3.5 mg/l.

Specific growth rate of bacteria after 10 minutes to 40 minutes of contact time on 10-40 mg/l of chlorine dose are 0.07, 0.098, 0.037 and 0.076/min. The growth rate value had no significant difference caused there are NB content in the medium for bacterial growth nutrients. Death rate of bacteria on 10-50 minutes of contact time and 10-50 mg/l of chlorine dose has a different value. The death rate was highest in the 10-minute of contact time for chlorine as a disinfectant to kill bacteria. Dissolution constants values for each dose and contact time close to 1 that means the chlorine dose and contact time are equally affecting disinfection.

Metals iron and manganese can reduce the performance of disinfectant. Before killing the bacteria, chlorine will oxidize iron and manganese metal in advance so that the chlorine exterminate reduced, according to the following reaction:

$$2Fe^{2+} + Cl_2 + 6H_2O \longrightarrow 2Fe(OH)_3 + 2Cl + 6H^+$$

$$Mn^{2+} + Cl_2 + 2H_2O \longrightarrow MnO_2 + 2Cl^- + 4H^+$$

Based on the results, the iron gives the greater influence than manganese in the disinfection process. It is characterized by the measurement results of a larger number of E. coli bacteria after disinfection processes in medium containing iron compounds. Formation of Fe^{3+} and Mn^{4+} is affected by pH. The reaction of Fe^{3+} formation can occur rapidly than the slow formation of Mn^{4+} when pH below 9,5.

Chlorine disinfection research at the optimum conditions for Purus water well sample can eliminate E. coli content of the water wells up to 99.9% with 0.4 mg/l of residual chlorine which still in limit of quality standards. Different removal efficiency is caused by water well contains several compounds of high iron and manganese and the amount of TDS and turbidity that passes the quality standard.

CONCLUSION

From the results obtained in this study of chlorine disinfectant effectiveness to Escherichia coli bacterium in Purus water wells can conclude the following:

1. E.coli bacteria content in Purus water well is > $1{,}6.10^5$ cells/100 ml which passed the quality standard (Permenkes No.492/Menkes/Per/IV/ 2010);
2. Optimum chlorine dose is 50 mg/l for 30 minutes of contact time with a removal efficiency reached 100% in the initial amount of E.coli> 1,6.105 cells/100 ml to 0 cell/100 ml;
3. E. coli death rate value for each dose of chlorine and contact time ranged from 0,1 to 0,7/min;
4. Concentration of Chlorine (C) and contact time (t) equally affect the disinfection process in the artificial solution experiment;
5. The growth rate of E. coli after a 10 minutes of contact and 10-40 mg/l of the chlorine dose is 0.03 to 0.09/min cause decreased performance of disinfection and the content of the substrate in the medium;
6. Effect of Iron with 0,15, 0,3 and 0,45 mg/l concentrations reduce the effectiveness of disinfectants in optimum condition reached 0.38%, 0.575% and 0.875%. while 0,2, 0,4 and 0,6 mg/l of manganese concentration reached 0.112%, 0.25% and 0.581%;
7. E.coli bacteria removal efficiency of water water samples in optimum condition that is 99.9% by the end of the number of E.coli is 180 cells/100 ml

and 0.4 mg/l of residual chlorine due to the confounding matter content in the well water.

ADVICE

Based on the research that has been done, some things that can be done for further research are as follows:

1. Continuing research with other disturb compounds factors to determine how much influence these compounds to chlorine disinfection;
2. Doing research on the frequency of affixing proper chlorine in water well;
3. Conduct preliminary research on E. coli with other disinfection alternatives.

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