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Reduction of Microbial Content (*Escherichia coli*) in Well Water Using Various Processes: Microfiltration Membranes, Aeration and Bentonite Adsorption

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Abstract

Water is a basic need for living things. This research aims to know the reduction of microbe content (*Escherichia coli*) in well water by using microfiltration membrane, adsorption using bentonite and aeration. The capability of those three methods in reducing *E. coli* was examined on the variety of time contact: 30, 60, 90, 120, 180 and 300 minutes. The result of the research shows that using those methods has shown that the optimum percentage of *E. coli* reduction by using microfiltration membrane with 23 Most Probable Number (MPN)/100 mL of *E. coli* initial concentration in well water and became 0 MPN/100 mL by 100 % of *E. coli* reduction. Adsorption using bentonite resulted in a 78% reduction in *E. coli* and reduction by using aeration, the reduction of *E. coli* by 21%. This study shows that microfiltration has the best ability compared to other methods.



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1. Introduction

Water is used for drinking, washing, irrigation, power generation and so on. In principle, water that is used must meet specific requirements, both physically, chemically and biology. Water is also a medium for the growth of dangerous pathogenic microorganisms for health. Many microorganisms are defined as causing disease through beverage intermediaries such as *Salmonella typhi*, *Shigella*, *Escherichia coli*, *Vibrio cholera* and *Campylobacter jejuni*. Based on the Regulation of the

Minister of Health of the Republic of Indonesia Number 492/MENKES/PER/IV/ 2010, the number of most probable number (MPN) *E. coli* in water is 0/100 mL of the sample [1]. Coliform bacteria can be divided into two groups: fecal coliform, for example, *E. coli* and non-fecal coliforms such as *Enterobacter aerogenes*. *E. coli* is a bacterium that comes from an animal or human feces, while *Enterobacter aerogenes* are usually found in animals or plants that have died. So, there are *E. coli* in drinking water shows that drinking water was once contaminated with feces humans and may contain intestinal pathogens [2]. There

is a statement that clear water is not necessarily clean. This is connected with circumstances that water, since coming out of springs or wells, actually contains microbes, particularly bacteria or microalgae. In physiqués of water that are contaminated or polluted, such as rivers, ponds, and lakes, various microbial groups including *E. coli* bacteria are present. These microbes not only contribute to the discoloration of the water, but also produce toxins that can lead to disease, promote the growth of harmful algae, and accelerate the corrosion and degradation of the surrounding environment [3].

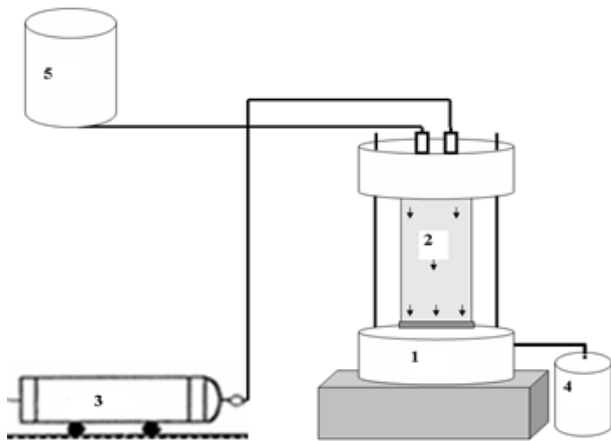
In this study, a method has been used simply for the separation or reduction of microbes from well water that can produce water which is free from *E. coli* content by using membrane technology [4]. Separation of bacteria in water can also be done by using other chemical compounds, for example by adding bentonite in water. Based on chemical properties, bentonite has cation and bentonite exchange capacity has the ability to absorb colors. Addition Oxygen allegedly can also be used to reduce microbial content in water, with increased oxygen content in water, substances and carbon dioxide content of water evaporate and the dissolved minerals will oxidize to form a precipitate that can be eliminated by sedimentation [5]. By using microfiltration membranes, bentonite and additions oxygen (aeration) is expected to reduce the content of microbes (*E. coli*) from well water thus improving water quality. The purpose of this study is to reduce the content (*E. coli*) of well water by using a microfiltration membrane and knowing the difference between filtering membrane by addition or absorption by bentonite and addition of oxygen (aeration). This research is expected to provide information to the public about how to reduce or eliminate the content of microbes or bacteria (*E. coli*) present in the water well. And can be applied in daily life, so that the quality of the well water is one source of the need for clean water free from *E. coli* bacteria.

A membrane can be defined as a thin layer which selectively separates the two or more component substances than mixtures. Separation with this membrane is an alternative good enough for now technically and economically. The development of this membrane technology experienced a very rapid increase because the advantages they have are very suitable used for the process of separation and purification. This technique is also very economical in energy use. The membrane process is a relatively new method of separation, now. This membrane process is used in a wide range of applications. From the economic side will continues continued development of first-generation membrane processes such as microfiltration,

ultrafiltration, hyper filtration or reverse osmosis and electro dialysis. The development of this process also continued in second-generation membrane processes such as gas separation, evaporation, membrane distillation and liquid membrane [6]. This membrane has a pore size between 0.05 to 10 μm and a thickness between 10 to 150 μm . Microfiltration is used in a variety of applications in the industry, especially for the separation of particles $>0.1 \mu\text{m}$ in size from the solution. One of its main applications in the industry is sterilization and clarification in the pharmaceutical industry. Microfiltration can also be used for separating particles during the process of making ultrapure water in the semiconductor industry. Microfiltration can be defined as the separation of a single particle from an article other size of certain fluids (liquid or gas). Membrane separation technology is based at the molecular size. The semipermeable membrane in the system Pressure is given in part membranes and water and low molecular weight components in raw water flowing through pores as permeate, components with large molecular weight and suspended solid flow and become part of the concentrate [7].

Bentonite is the term for clay containing montmorillonite in the world trade and including dioctahedral groups. The naming of the type of clay depends on inventors or researchers, for example, geologists, mineralogists, industrial minerals and others. Bentonite can be divided into 2 groups based on the content of aluminium silicate hydrous, which is activated clay and fuller's earth. Activated clay is clay that has less pale power, but power the pale could be increased through certain processing. Meanwhile, fuller's earth is used in fulling or cleaning wool material from fat [8].

Aeration is a treatment process where water is made in close contact with air with the aim of increasing the oxygen content in the water. With increasing, the oxygen content of volatile substances such as hydrogen sulphide and methane affects the taste and smell can be removed. The carbon dioxide content of water will be reduced. Soluble minerals such as iron and manganese will oxidize to form deposits that can be eliminated by sedimentation and filtration [9]. The most probable number (MPN) method using the approach has been proven very good for estimating microbial populations, especially if microbes are present in very large quantities little in food or water samples [10]. Sampling, shipping and examination of water samples must be carried out by aseptic and can represent the water being examined. The use of tools, media and reagents and the implementation of the test must be in accordance with the type the bacteria to be determined [11].



Information:
 1. Microfiltration Membrane
 2. Sample place
 3. Air pump (pressure)
 4. Permeate
 5. Bait

Figure 1. Schematic of microfiltration process.

2. Materials and Methods

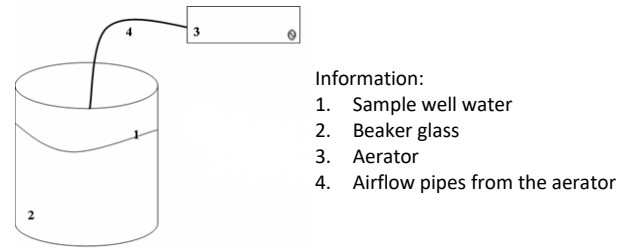
Tools used: test tubes, Durham tubes, beaker glass, ovens (heaters), pipettes drop, volume pipettes, autoclaves, microfiltration membranes, vacuum pumps, petri dishes, tweezers, magnetic stirrer, hot plate and aerator. Materials needed include well water, aqua dest, Lactose Broth (LB) media, Green Bile Lactose Broth (BGLB) media and natural bentonite. Membrane specifications: fiber volume of 150 mL, height 9 cm and diameter 4.4 cm

2.1. Testing of samples with membranes

The steps of the microfiltration process are as follows: Water has flowed through the inlet to the bait membrane surface. Filtering is done based on time variations (30, 60, 90, 120, 180 and 300 minutes) and then the filtrate which is a product of microfiltration process is collected and analysis to determine the amount of *E. coli* in the permeate (Figure 1) [12]. The membrane module consists of one tube made of fiber with two: the top lid is installed with two pipes, inserting pressure and bait or sample and the bottom lid as a membrane mounting area. This microfiltration membrane is made of polyethylene polymers with a pore size of 0.45 μm . The operation of this tool is equipped with a coagulation system and disinfection to help increase filtering out contaminants. Flow will be processed maximum with a normal pressure (1 bar) [13].

2.2. Flux determination

Flux value is the amount of permeate volume that passes through a unit surface area of the membrane at a certain



Information:
 1. Sample well water
 2. Beaker glass
 3. Aerator
 4. Airflow pipes from the aerator

Figure 2. Schematic addition of oxygen (aeration) with an aerator.

time with the presence of a driving force in the form of pressure [7]. The formula used in the calculation of flux is presented in Equation 1.

$$J = \frac{V}{(A \times t)} \quad (1)$$

Membrane area (A) is the area of the membrane that is passed through by well water. Permeate is taken every 30 minutes and is carried out for six hours.

2.3. Absorption using natural bentonite

A total of 4 grams of bentonite with 100 mesh particle size, put into the beaker 200 mL glass, added 100 mL of the sample and stirred with a magnetic stirrer (Figure 2). Stirring time variation (30, 60, 90, 120, 180 and 300 minutes) and then filtered with the paper filter it. The filtrate was taken and counted the amount of *E. coli* [8].

2.4. Aeration

A total of 100 mL of well water samples were put into the beaker glass, mounted on the device aerator as in the picture. Then flowed air from the aerator at a speed note, the sample is stirred using a magnetic stirrer. The treatment is done with variations time (30, 60, 90, 120, 180 and 300 minutes). Then the sample is allowed to stand for 1 hour and counted the amount of *E. coli* [9].

2.5. Determination of the Amount of *E. coli* in the sample

Prepare 9 Lactose Broth (LB) media tubes or more equipped with Durham tube: 3 tubes containing 10 mL each, 3 tubes containing 5 mL each, 3 tubes containing 5 mL each. Sterilized by autoclave up to 121°C [14]. Original sample pipettes (without dilution): 10 mL of each sample is put into 3 tubes, 1 mL of each sample is put into 3 tubes, 0.1 mL of sample is inserted each into 3 tubes. Incubate at 37°C for 24–48 hours. Observe the number of positive tubes (the Durham tube rises to the top). Take 1-2 use aseptic liquid BGLB media and put it in a positive tube, then incubate at 44°C for 24 hours. Then observe the number of positive BGLB tubes (Durham tubes going up). The observations are then referred to in the MPN

Table 1. Decreasing the amount of *E. coli* with respect to time in well water samples using microfiltration membrane.

No.	Time of Treatment (Minute)	Analysis results (MPN*/100 mL)	Rejection Percent (%)
1	0	23	Control
2	30	0	100
3	60	0	100
4	90	0	100
5	120	3	79
6	180	0	100
7	300	3.6	74

*MPN = Most Probable Number

Table 2. Results of calculating flux values.

No.	Time		Flux Value (mL/m ² seconds)
	Minute	Second	
1	30	1800	0.099
2	60	3600	0.085
3	90	5400	0.075
4	120	7200	0.067
5	180	10800	0.055
6	300	18000	0.0048

table to determine the number of *E. coli* bacteria in 100 mL or gram sample [15]. Each treatment is carried out on well water samples containing *E. coli* distinguished by varying the time of treatment, ranging from 30, 60, 90, 120, 180 and 300 minutes. Time 0 is the time that well water samples containing *E. coli* have not carried out any treatment or used as a control (blank), the treatment starts at 30, 60, 90 and 120 minutes with an interval of 30 minutes, the next time is 180 and 300 minutes [12].

3. Results and Discussions

In this study, a decrease in *E. coli* in well water was carried out using microfiltration membranes, adsorption and aeration. The presence of *E. coli* shows that drinking water was once contaminated with human feces and may be able to contain intestinal pathogens. Therefore, the drinking water standard requires *E. coli* to be 0/100 mL, based on the Regulation of the Minister of Health of the Republic of Indonesia Number 492/MENKES/PER/IV/2010 [1].

3.1. Microfiltration membrane

The microfiltration membrane used here has a pore size of 0.45 µm and a thickness of 10 µm. Microfiltration is usually used in a variety of applications in the industry, especially for separation of particles >0.4 µm. In this study, the microfiltration membrane used to filter *E. coli* in well-sized well water samples 0.5-5 µm [16]. The results show that microfiltration membrane can reduce *E. coli* concentration in well water samples (Table 1).

Based on the analysis conducted on the permeate, it was found that there was no presence of *E. coli* in the well water over a period of time, indicating good microfiltration capability (Table 1). After that time the amount of *E. coli* seen again at 3.6 MPN/100 mL from the initial *E. coli* concentration of 23 MPN/100 mL. This matter caused by the microfiltration membrane is no longer effective to hold the content of *E. coli* from the well water sample, due to fouling on the surface and pores membrane. This means that the microfiltration membrane can indeed be used to decrease bacteria (*E. coli*) in water.

3.2. Determination of flux on the microfiltration membrane.

Flux is a physical quantity that is used as a variable in determining the ability of the membrane that is used, the greater the flux value of a membrane can be stated that the membrane has an optimal ability [17]. Flux can be defined as the amount of permeate volume (separation) that passes through the membrane unity of membrane surface area unity of time, following flux profile with time like Table 2 [18].

According to [19], the permeate flux values are seen based on variations a predetermined time which can be seen in the graph above, which is getting longer the time given permeate flux value decreases, this is due to the longer time the microfiltration membrane operation, more and more fouling occurs in the membrane microfiltration by *E. coli* bacteria. This fouling will increasingly increase, until cover the pores of the membrane, which makes the membrane work harder and resulting in a decrease in the number of permeates produced, according to Figure 3.

3.3. Adsorption using Bentonite

According to [20], bentonite can absorb harmful compounds, such as elements metal element is dangerous because bentonite has the ability to absorb metal from the air, water and soil because of its unusual structure, which is "porous" which is able to absorb material substances very large amounts of pollutants. So, in this study we used bentonite to absorb or bind *E. coli* bacteria that are in clean water or drinking water. The results of research conducted to reduce the concentration of *E. coli* in well water by using natural bentonite can be seen in Table 3.

Stirring has been done by adding natural bentonite to water samples containing *E. coli*. It is expected that the amount of *E. coli* present in the sample can be absorbed by bentonite which has adsorption properties [8]. The

Table 3. Reduction in the amount of *E. coli* with stirring time from well water samples with use natural bentonite as an absorbent.

No.	Time of Treatment (Minute)	Analysis results (MPN*/100 mL)	Rejection Percent (%)
1.	0	14	Control
2.	30	12	14
3.	60	11	21
4.	90	9.1	35
5.	120	3.1	78
6.	180	3.6	74
7.	300	6.2	56

*MPN = Most Probable Number

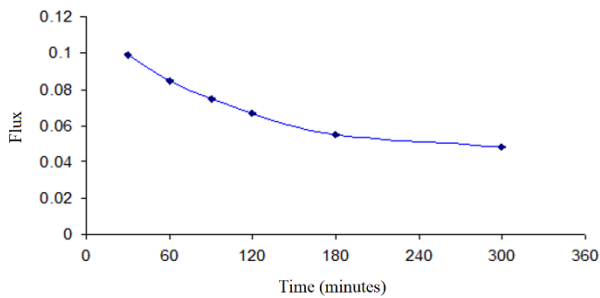


Figure 3. Flux profile with time.

number of bentonites for each treatment is fixed, as much as 4 grams with the sample volume used is 100mL. The sample used was taken from the well water of the Meesalee village community, Indrapuri sub-district which is consumed daily, where the amount of *E. coli* is contained in the well water amounting to 14 MPN / 100 mL. Based on Table 3, it appears that the longer the contact time or stirring, then the amount of *E. coli* contained is also less or percent absorption increased. The optimum absorption condition is obtained at 120 minutes contact time with percent its absorption is 78% of the initial *E. coli* amount of 14 MPN/100 mL. After reaching the maximum then the absorption percent drops, this is likely due to the bentonite pores already covered or saturated, both by *E. coli* itself and other soluble chemicals.

3.4. Aeration

Dissolved oxygen is the amount of oxygen contained in water and is measured in ppm units [21]. Biological Oxygen Demand (BOD) is the amount of oxygen needed by bacteria to decompose (oxidize) almost all dissolved organic substances and some substances organically suspended in water. Chemical Oxygen Demand (COD) is a measure of water pollution by organic substances which naturally can be oxidized through the process microbiological, and resulting in reduced oxygen dissolved in water. In this study, the addition of oxygen or aeration was carried out so that the content *E. coli*

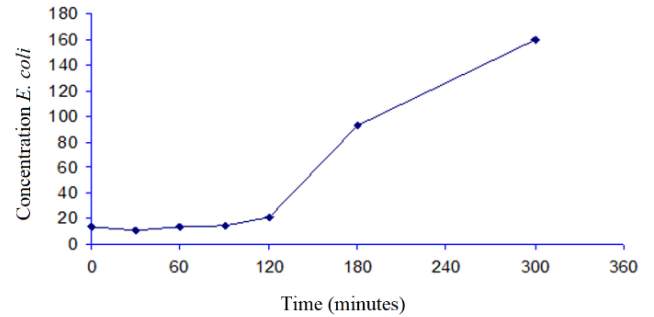


Figure 4. Effect of time on the amount of *E. coli* with oxygen addition (Aeration).

Table 4. Decreases in the amount of *E. coli* with respect to aeration time in well water samples.

No.	Time of Treatment (Minute)	Analysis results (MPN*/100 mL)	Rejection Percent (%)
1.	0	14	Control
2.	30	11	21
3.	60	14	0
4.	90	15	7
5.	120	21	50
6.	180	93	564
7.	300	160	1043

*MPN = Most Probable Number

contained in the sample, which is expected community well water consumed can be derived even free from *E. coli* bacteria content [21].

Based on research studies that have been carried out that the addition of oxygen or aeration can reduce metal content in water. However, the effect of microbial reduction has never been observed like *E. coli* bacteria. The results of research that has been done to reduce *E. coli* concentration in well water with the addition of oxygen or aeration, can be seen in Figure 4.

Based on Figure 4 and Table 4 observations, it turns out that the aeration mechanism cannot be used for the purpose of reducing or removing *E. coli* from well water. From the amount of *E. coli* initial of 14 MPN/100 mL, at 30 minutes the addition of air the concentration of *E. coli* fell by 21%. Then at 60 minutes to 300 minutes, the concentration of *E. coli* continues to rise up to 1043%. This is likely caused by *E. coli* bacteria that will quickly multiply themselves in the presence addition of air or also called aerobic bacteria.

5. Conclusions

The study found that the microfiltration membrane used to reduce *E. coli* levels in the water was very effective. Bentonite is also used to absorb harmful compounds such as metallic elements. Bentonite is very suitable, efficient and readily available for absorbing *E. coli*.

However, the addition of oxygen (aeration) to the process should not be used as air can help bacteria to multiply. Further research is needed to determine the amount of pressure applied, which bentonite is activated first, or other variations and optimizations.

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