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PURIFICATION OF DUG WELL WATER FROM *Escherichia coli* BY USING CARBON OF RICE HUSK

Hagar Karlani[1], Fahrul[1], Maria M. Meiwati[2], Herianus Manimoy[1], Loth Botahala[1*]
[1]Chemistry Study Program, Tribuana Kalabahi University, Alor Regency, Indonesia
[2]State High School 1 Kalabahi, Alor Regency, Indonesia
[*]**Email:** botahala@gmail.com

ABSTRACT

Clean water is one of the human's main needs. Dug well water in Tombang, used for various needs. However, the dug well water has been polluted by *E. Coli* bacteria which is suspected to be due to the disposal of rubbish and waste carelessly and also the distance of latrines and dug wells that are very close together. Therefore it is important to do water purification using agricultural waste that is not utilized. The analysis showed that the purification of dug well water in Tombang with rice husk charcoal had reduced *E. Coli* bacteria by 55.6% with MPN pattern 1-0-3 so that the MPN value was 11/100 mL of sample.

Keywords: Dug well water, Tombang, activated charcoal, rice husk, MPN value.

INTRODUCTION

Water can naturally form on its own from 2 diatomic molecules (H_2 and O_2). Water formation reactions can be written: $2H_2 + O_2 \rightarrow 2H_2O$ (Botahala, 2019). Water plays a very important role in human life, especially for drinking, cooking, and other needs (Karbeka, et al., 2020). In essence, nature has provided the clean water needed, but the pressure of uneven population growth and increasingly complex population activities have caused various impacts of changes in environmental order and balance. This causes water quality, quantity, and continuity to be disturbed (Fahrul, 2015). In general, most diseases suffered by humans come from the use of polluted water (Balaji R. et al., 2014 in Botahala, 2019).

Anonymous 2013, Regarding the Guidelines for the Preparation of a Water Resources Management Plan, explained that groundwater is water that is contained in the soil layer or rock below the ground surface. Found that all clean water sources on earth are formed through the hydrological cycle including groundwater. The process of groundwater formation according to (Lubis 2007 in Fahrul, 2015) is rain formed in the hydrological cycle falling to the earth's surface. This rainwater will seep into the ground with the help of the earth's gravitational force and capillaries. Under the influence of gravity, rainwater flows vertically into the ground.

Meanwhile, the capillary force flows water perpendicularly upward, downward, and horizontally (laterally). Rainwater continues to seep down until it reaches an unsaturated layer where this layer contains water and air (infiltration process). The water in this layer is used as well as water. With the percolation process, the water then drops down past the water table (water table) until it reaches the saturated layer (bedrock and fills all the soil pores). This layer only contains water. The water in the layer is used to drill wells and comes out as springs (Fahrul, 2015).

According to Marsono (2011), the quality of contaminated dug well water is caused by various factors, including household / industrial waste, garbage, and feces. Dug wells that have been used for a relatively long time are more likely to experience pollution because in addition to increasing sources of pollutants it is also easier for pollutants to seep into the well following the flow of water, while the specifications of dug wells for clean water sources, the horizontal distance of the well upstream from the groundwater flow of the source of contamination is more than 11 meters, while the distance of the communal wells (shifting) to the housing is more than 50 meters. The dug well water of several residents in Tombang, Alor district has been polluted by Escherichia coli bacteria. This is allegedly due to the careless disposal of rubbish and waste by residents, also the distance of latrines and dug wells that are very close together (Fahrul, 2015).

Escherichia coli or abbreviated as *E. coli* is a type of facultative-anaerobic bacteria that is commonly found in human intestines and also animal waste. Escherichia bacteria are straight rods, 1-4 µm in size, motile or nonmotile. These bacteria are found in the intestinal contents of humans, warm-blooded animals, and poultry. Escherichia is used as an indicator of sanitation in the coliform and fecal coliform groups (Sopandi and Wndah, 2014 in Aminah, Siti et al., 2018). This organism is easier to detect because it can survive compared to other gut pathogens and is more resistant to natural water purification processes (Aminah, Siti, et al., 2018). Most of these bacteria are harmless, but some of them can cause food poisoning and fairly serious infections in humans such as *E. Coli* type O157: H7, which causes diarrhea mixed with blood, stomach cramps, and vomiting (Halodoc, 2020). Through animal and human waste, these bacteria can produce acids and gases from lactose within 48 hours. Generally this complication is more common in children. The most serious and fatal complication of *E. coli* bacterial infection is uremic hemolytic syndrome, a condition when red blood cells become damaged and can result in kidney failure (Halodoc, 2020).

Rice husk is a potential agricultural waste for active carbon preparation (Botahala, et al, 2016; Zakir, et al., 2013). According to Botahala, et al. (2013), rice husk waste in Indonesia in

2011 amounted to 14,683,008.1 tons. Meanwhile, based on data from the Indonesian Central Bureau of Agricultural Statistics 2016, rice production in NTT from 2013 to 2015 was consecutively (in tons per year) 729,666; 825,728; 948,088 and national rice production (in Indonesia) in the same year (in tons per year) 71,279,709; 70,846,465; 75,397,841 (Botahala, 2019). When rice grains are milled, 78% of the weight is rice and 22% is unhulled (Nugraha and Antoni, 2007 in Botahala, 2013).

Thus, it can be said that the waste of rice husk in the province of NTT from 2013 to 2015 was consecutively (in tonnes per year) 160,526.52; 181,660.16; 208,579.36 while nationally (in tonnes per year) 15,681,535.98; 15,586,222.30; 16,587,525.02 and will increase every year (Botahala, 2019). Traditionally, rice husks are usually only used for household purposes, for example as conversion fuel (Wahjuni, 2011 and Mau, 2015), as rubbing ash (Botahala, 2013), and so on. The chemical composition of rice husks according to Suharno (1979) in Junaedi N., F., et al. (2010) and Karim, et al. (2012) in Botahala L. (2013) includes 9.02% water content; 3.03% Crude protein; 1.18% Fat; 35.68% Crude fiber; 17.17% Ash; 50% cellulose; 25-30% lignin; and 15-20% silica; and 33.71% basic carbohydrates. Thus, rice husk waste has the potential to be prepared into activated carbon. Activated carbon is an adsorbent in the form of amorphous and porous carbon compounds containing 85-95% carbon material. Activated carbon is commonly used to purify, decolize, detoxify, filter, or remove solutes, especially in wastewater treatment. Activated carbon is made from carbonaceous materials followed by activation with steam or carbon dioxide at high temperatures (700-1100 °C). The activation process is very important to remove carbonization products in the form of tar formed during pyrolysis, thereby opening pores (Botahala, 2019).

Pores on activated carbon according to the International Union of Pure and Applied Chemistry (IUPAC) can be grouped into 3 groups namely Micropores (D <2 nm), Mesoporous (D 2-50 nm), and Macropores (D> 50 nm) (Aznar, J. S, 2011). Of the three groups, micropores can be more effective in the entrapment process, because their surface area is much larger than mesoporous and macropores (Botahala, 2019). According to (Anzar, 2011), the carbonization process consists of 3 stages. The first stage is water removal which occurs in the temperature range of 27 °C-197 °C. The second stage is primary pyrolysis, which occurs in a temperature range of 197 °C-497 °C. at this stage the volatile matter is removed and causes a tremendous reduction in weight and also to form the basic structure. The third stage is the consolidation of the charcoal structure in the temperature range of 497 °C-847 °C. Heating up to this temperature has caused the degradation of the holocellulose and lignin components which produce gas

products (including CO_2, H_2, CO, CH_4, and benzene), liquid products (tar, high molecular weight hydrocarbons, and water) and solid products in the form of charcoal (Botahala, 2019).

During the carbonization process, the free gaps present in the carbon are either filled or at least partially blocked by the disorganized "amorphous" carbon apparently as a result of the buildup of the remaining material. The resulting carbonized product only has a very small adsorption capacity (Botahala, 2019). Such carbonized materials can then be activated by removing tar products by heating in steam or under inert gas or by extraction with a suitable solvent or by chemical reaction. After the carbonization process is continued with an activation process where this process aims to enlarge the pores, namely by breaking the hydrocarbon bonds or oxidizing the molecules that cover the surface of the charcoal so that the charcoal changes in properties, both physical and chemical, namely the surface area increases in size and affects absorption. (Botahala, 2019).

Testing the nature and quality of activated charcoal resulting from the process of activation of rice husk charcoal has been carried out (Botahala, et al., 2019). For example, activation of rice husk charcoal by activating $ZnCl_2$ solution (Zakir et al., 2013; Taba et al., 2013), and others.

Determination of water content is usually carried out in temperatures between 100 ° C - 130 ° C in order to determine the hygroscopic properties of activated charcoal (Pujiarti R. et al., 2005 in Botahala, 2019) The water content in activated charcoal is more due to the hygroscopic nature of activated charcoal itself. so that during the cooling process, water vapor from the air is absorbed into the pores. Water content is the amount of water contained in activated carbon after carbonization and activation raw materials go through the carbonization and activation stages, either chemically bound or due to external conditions such as climate, grain size, and the filtering process. The high water content will reduce the quality of activated carbon because water adsorbed on activated carbon will reduce the capacity and adsorption power of liquids and gases (Rahmawati 2006 in Botahala, 2019) but has no effect on dissolved solids (Aznar J.S., 2011).

Ash in activated carbon is the mineral content of the material contained in it which does not burn completely in the carbonization process and does not separate during the activation process. The determination of ash content aims to determine the content of activated charcoal metal oxide. Ash is an organic component that is left after the material is heated at a temperature of 600 °C-800 °C and according to Al-Qodah et al. (2009) in Botahala (2019) consists of potassium, sodium, magnesium, calcium, and other components in small amounts.

The high ash content in the sample can reduce the ability of activated charcoal to absorb gas and solution because the mineral content contained in the ash will spread into the sides of the activated charcoal so that it covers the pores of the activated carbon. The large value of the ash content is due to the charcoal process which is not tightly closed so that air contact occurs which results in the formation of charcoal being imperfect and the possibility of forming ash is getting bigger (Darmawan S., et al., 2009 in Botahala, 2019).

The quality of activated carbon based on the requirements of SNI-06-3730-1995 (Botahala, 2019 and Bapa, et al., 2019) is as shown in Table 1.

Table 1. Quality Requirements for Activated Carbon

Types of Requirements	Parameters
Part lost at heating 950 °C	Max. 25%
Water content	Max. 15%
Ash content	Max. 10%
Bonded carbon	Min. 65%
Pass 325 mesh	Min. 90
Absorption of I_2	Min. 750 mg/g
The absorption of benzena	Min. 25%

Application of rice husk has been widely carried out, as an adsorbent for methylene blue dyes (Taba et al., 2013), as silica in red bricks (Indra et al., 2013), as silica gel against adsorption of air humidity (Ummah, 2013), as an additive in the manufacture of cement (Botahala et al., 2013), as absorbents in the purification of used cooking oil (Botahala et al., 2019), and the manufacture of rice husk briquettes as an alternative fuel (Jahiding et al., 2011), and others. So that the activated rice husk charcoal can be used in the process of purifying well water from *E. Coli* bacteria.

MPN method is a technique to count the number of microorganisms. The MPN method is commonly used to test water quality or processed food ingredients and processed products. Most Probable Number (MPN) is a method for estimating the total microbial density in a sample indirectly (Aminah, Siti, et al. 2018). The MPN method uses a liquid medium placed in a test tube. The media used for the MPN method are Lactose Broth (LB), Brilliant Green Lactose Bile Broth (BGLBB), the MPN method is more sensitive and can detect coliform in very low amounts in the sample (Supardi, 1999 in Aminah, Siti, et al. 2018).

MPN is used to determine the amount of coliform in water quality tests. According to Soemarno (2002) in Sunarti, R.N (2015), there are 3 types of variations used in the MPN method, namely:

1. Variety I: 5 x 10 ml, 1 x 1 ml, 1 x 0.1 ml.
   For specimens that have been processed or the germ, the number is estimated to below.
2. Variety II: 5 x 10 ml, 5 x 1ml, 5 x 0.1 ml.
   For specimens that have not been processed or whose germ numbers are expected to be high. If necessary, planting can be continued with 5 x 0.01 ml and so on.
3. Variety III: 5 x 10 ml, 1 x 1 ml x 0.1 ml.
   There is an alternative variety for type II, if the number of tubes is limited and the media supply is also limited, the method of implementation is like type II.

In the MPN method for drinking water there are two stages of inspection (Sunarti, R.N., 2015 and Aminah, Siti et al. 2018), namely:

a. Presumptive Test
   The preliminary examination by inoculating on the Lactose Broth medium saw the presence or absence of gas formation in the Durham tube after incubation for 24 - 48 hours at 35 ° C - 37 ° C. If there is the formation of Durham gas cylinders, the drinking water test according to KepMenKes RI No.: 907 / Menkes / SK / VII / 2002. If after 48 hours no gas is formed, the result is negative and there is no need to confirm.
b. Test of Confirmation
   Examination on the confirmation test by planting on the Brilliant Green Lactose Bile Broth media saw whether or not there was a gas formation in the Durham tube after incubation for 48 hours. If gas forms in the Durham tube, the test is positive.

MATERIAL AND METHODS

Material

The materials used in this study consisted of rice husk, dug well water, Aquadest, Lactose Broth (LB), Brilliant Green Lactose Bile Broth (BGLB).

Tools

The tools used in this study consisted of Muffle Furnace, Blender, 100 mesh sifter, Hotplate Stirrer, Magnetic Stirrer, Stirring Rod, Universal Indicator Paper, Erlenmeyer, Measuring Cup, Watch Glass, Analytical Scales, Separatory Funnels, Drying Oven, Thermometer, Bottle sterile, Ose Wire, Pumpkin Measure, Cotton, Test Tubes, Lighters, Durham Tubes, Incubators, Wrapping Paper, Measuring Pipettes, Burette, Stative, Clamps, Markers, Porcelain Cups.

Working Procedure

The procedure of this research is based on research that has been done by Kalensun dkk (2012), Zakir et al. (2013), Taba et al. (2013), Fahrul (2015), Sukawaty et al. (2016), Kamaliah (2017), and Botahala et al (2019).

Rice Husk Charcoal Preparation

Rice husks that have been clean and dry, are heated in the burning furnace at a temperature of 400°C for 90 minutes up to form carbon. After that, the husk charcoal is left in the burning furnace for 24 hours. Next, the charcoal is being taken out and ground using a grinding process to form a powder and sieved with a 100 mesh sieve. The charcoal from the sieve is activated physically. Hereinafter, it is ready to be used for quality examination of activated charcoal.

Rice Husk Charcoal Active Quality Testing

Test the moisture content

A total of 1 gram of activated charcoal sample was placed in a porcelain cup of known dry weight. Then it is heated in an oven at 100°C for 3 hours, then cool in a desiccator and weighed. After that, it is followed by calculating the ash content (%) according to Botahala (2019).

$$\text{Water content} = \frac{a-b}{a} \text{ x } 100$$

Where a is the mass of the initial sample (grams) and b is the mass of the sample after drying (grams).

Ash content test

A total of 1 gram of activated rice husk charcoal was placed in a porcelain cup and then heated in a muffle furnace at 500°C for 3 hours. The weight of the sample before and after it is heated is weighed and recorded. After that, it is followed by calculating the ash content (%) according to Botahala (2019).

$$\text{Ash content} = \frac{b}{a} \text{ x } 100$$

Fly/Volatile Substances Test

A total of 1 gram of sample is placed in a porcelain cup then heated in an oven at 100°C for 10 minutes. The weight of the sample before and after it is heated is weighed and recorded. After that, it is followed by calculating the levels of flying substances according to Manurung, et al. (2018).

$$\text{Volatile content} = \frac{b-c}{b} \text{ x } 100$$

Where c is the mass after water content determination.

Bound Carbon Level Test

Bonded carbon levels are calculated from the value of volatile substances and ash content.

Bonded carbon content (%) = 100 - (volatile content + ash content)

MPN Bacteria *E. coli* examination

Examination of *E. coli* bacteria by the MPN method was carried out against dug well water samples before purification (A) and after purification (B). Purification of dug well water samples was carried out using 2 grams of activated rice husk charcoal.

Estimation Test

A total of 10 grams of Lactose Broth is mixed with 500 ml Aquades and heated using a hotplate until it boils and then sterilized using an Autoclave with a temperature of 121 ° C and a pressure of 1 atm for 15 minutes. After that, the prediction test was conducted using the MPN method using 9 tubes. Prepared 9 tubes were, for the first 3 tubes (T_1), each tube was filled with 10 ml of Lactose Broth and 10 ml of water samples. For the second 3 tubes (T_2), each tube is filled with 5 ml of Lactose Broth and 1 ml of the water sample. Whereas in the third 3 tubes (T_3) each was filled with 5 ml of Lactose Broth and 0.1 ml of water samples. After that, it was incubated at 37 ° C for 48 hours. Then the results were observed which were marked by changes in the color of the media and the formation of gas in each Durham tube.

Confirmation Test

A total of 10 grams of Brilliant Green Lactose Broth is mixed with 500 ml Aquades and heated using a hotplate until boiling then sterilized using an Autoclave with a temperature

of 121 ° C and a pressure of 1 atm for 15 minutes. Each tube of T_1, T_2, and T_3, each added 10 ml of Brilliant Green Lactose Bile Broth media. After that, all tubes were incubated at 37 ° C for 48 hours, then observed and recorded the results to be referred to in the MPN table (Most Probable Number).

RESULTS AND DISCUSSION

The quality testing of activated charcoal aims to determine the level of test parameters not exceeding the standards set in SNI 06-3730-1995 (Botahala, 2019 and Bapa et al., 2019). The activation of rice husk charcoal samples was carried out in physics with the results shown in Table 2.

Table 2. Quality of rice husk charcoal produced

No	Test Parameters (content)	Results (%)	SNI Requirements (%)
1	Water	9,33	Max. 15
2	Ash	8,67	Max. 10
3	Volatile Substances	9,33	Max. 25
4	Bound Carbon	82	Min. 65

Table 2 shows that activated rice husk charcoal has fulfilled the SNI 06-3730-1995 requirements so that it can be used to purify dug well water.

Test results on *E. Coli* bacteria before and after treatment through the estimation and confirmation test. The estimation test is intended to detect the presence of *E. Coli* bacteria contained in the dug well water samples. While the affirmation test is intended to further reinforce the presence of *E. Coli* bacteria in the water sample using bacteria growing media (Marlinda et al., 2019). Test results as shown in Table 3.

Table 3. Examination Test Results *E. Coli*.

Sample	T_1			T_2			T_3			Positive Amount		
	10	1	0,1	10	1	0,1	10	1	0,1			
(A)	+	+	+	+	+	+	+	+	+	3/3	3/3	3/3
(B)	+	+	+	-	-	-	+	+	+	3/3	0/3	3/3

Examination test results in Table 3 show that sample (A) showed the presence of *E. coli* bacteria in dug well water, both T_1, T_2, and T_3 with MPN 3-3-3 pattern. Sample (B) in Table 3 is a test sample that has been purified using activated rice husk charcoal. The results show that the test results on the T_2 variable show negative values with the MPN pattern 3-0-3 (33.3%

negative). These results are still temporary so a confirmation test is needed. The confirmation test results are listed in Table 4.

Table 4. The Confirmation Test Results *E. Coli*.

Sample	T_1			T_2			T_3			Positive Amount			MPN (per 100 mL sample)
	10	1	0,1	10	1	0,1	10	1	0,1				
(A)	+	+	+	+	+	+	+	+	+	3/3	3/3	3/3	1100
(B)	-	-	+	-	-	-	+	+	+	1/3	0/3	3/3	11

The confirmation test results in Table 4 show that the 3 sample test variables (A) show the presence of *E. Coli* bacteria significantly in dug wells, with MPN pattern 3-3-3 so that the MPN value indicates 1100/100 mL. The presence of *E. Coli* bacteria in dug well water is thought to be caused by human activities in the environment. For example, the distance of the toilet is very close to the dug well so that activities such as bathing, washing, etc. produce uncontrolled wastewater, which can be absorbed into the dug well.

The sample (B) in Table 4 is the confirmation test sample after purification using activated rice husk charcoal. As many as 55.6% of *E. Coli* bacteria were removed from water samples using activated rice husk charcoal with MPN pattern 1-0-3 so that the MPN value was 11/100 mL.

CONCLUSION

After the confirmation test, 100% dug well water samples containing *E. Coli* bacteria were marked with a 3-3-3 MPN pattern so that the MPN value was 1100/100 m / L. However, using activated rice husk charcoal can reduce the amount of *E. Coli* bacteria in the dug well water samples by up to 55.6% by forming a 1-0-3 MPN pattern so that the MPN value becomes 11/100 m / L.

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