The effect of ozonation on dissolved oxygen and microbiological content in refill drinking water

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Abstract. One indicator of safe drinking water is its microbiological content of 0 colonies/100 ml. Ozonation is one method for killing microbes contained in drinking water. Disinfection in refillable drinking water that uses ozone needs to be studied, considering that the drinking water put into used gallons is washed again and can be drunk directly by consumers. It should be suspected that the drinking water used from drinking refill water contains bacteria, a dangerous virus. So the purpose of this study is to find out the microbiological content in refill drinking water with variable storage time for refill drinking water. The research method used is an observational research method. The parameters observed were temperature, pH, total microorganisms and dissolved oxygen content where the dissolved oxygen content indirectly indicates the reaction process of ozonation and bacterial activity contained in refill drinking water. The results showed that at pH showed a decrease from day to day, things this is caused by chemical reactions and microbiological activities. TPC shows that the smallest TPC value is 1 colony/100 ml on the second day, this is due to the ozone reaction in the water here H₂O₂ and (OH *) are very strong oxidizers which work optimally on the second day and then increase to 13 colonies/100 ml on day 5. The result of DO examination was an increase in DO on the second day to 8.2 mg/l which afterward finally decreased again the following day, this was due to an ozone reaction and bacterial activity in the water, therefore it was recommended that storage of drinking water after ozonation be stored for 2 days, and need renewal in the cleaning and disinfection of gallons to be used.

Keywords: Refill drinking water, disinfection, microorganisms, ozonation, dissolved oxygen (DO)

1. Introduction
In accordance with the Republic of Indonesia Law Number 18 of 2012 on Food, beverages are foods originating from both treated and untreated water which must be prevented from possible biological, chemical and other objects including additives, raw materials and materials, which is used in the process of preparing, processing and/or making food or drinks [1].

Water is the main requirement of humans for their survival, water sources can be obtained from groundwater and surface water. But not all water sources can be used by humans to meet drinking water needs. Drinking water is water that goes through the process both with and without treatment that meets health requirements and can be drunk directly. Drinking water is safe for health if it meets
the requirements of physics, microbiology, chemistry and radioactive which are contained in mandatory parameters and additional parameters. This requirement is regulated in the Regulation of the Minister of Health of the Republic of Indonesia No.492/MENKES/PER/IV/2010 [2].

In Indonesia, especially in urban, drinking water is managed by the local government, namely the PAM (Drinking Water Company). The water produced by PAM has fulfilled the requirements of drinking water but because in the distribution using very long piping and leakage is still found, the government urges the community to boil the water before it is used as drinking water. There are also people who use groundwater with wells or bore wells that do not necessarily meet the requirements of drinking water so that it requires processing. Due to the limited-service of drinking water from PAM and the much-contaminated groundwater and limited, especially during the dry season, many companies began to sell drinking water using packaging. Although this bottled water is relatively more expensive than PAM water, bottled water is relatively more practical, hygienic and easily available everywhere.

Recently we can easily find refill drinking water depots in most parts of the urban city hat offer services to fill customers gallons from various brands with treated drinking water. The price offered is relatively cheap compared to buying bottled drinking water produced by large-scale industries.

Refill drinking water is treated water that comes from springs that have passed the stages of cleaning the water content from all germs and bacteria contained in it without having to be cooked (traditional way), so that the water can be drunk directly. Efforts to fulfill and maintain healthy and hygienic drinking water conditions that are free from the dangers of biological contamination are disinfection methods. The disinfection method for current refill drinking water is using the method of ozonation, ultraviolet light and a combination of the method of ozonation with ultraviolet light. This study focused on disinfection using ozone.

Ozone was first discovered by CF Schönbein in 1840. Naming was taken from the Greek ozein (ὄζειν) which means "to smell" or smell. Ozone is a compound that can kill bacteria and has strong oxidation data. Since the last few decades, several countries in Europe have used ozone to treat drinking water, as well as America and even Japan [3]. Ozone is bactericidal, virucide, algicide, fungicide, and converts complex organic compounds into simpler compounds [4]. While the physical properties - ozone chemistry include [5]:

| Description                     | Value of                                      |
|---------------------------------|-----------------------------------------------|
| Chemical Formula                | O₃                                            |
| Molecular weight, M             | 47.998 g/mol                                   |
| Appearance                      | Blue colored gas                              |
| Melting Point, ° K              | 80.7                                          |
| Boiling point, ° K              | 161.3                                         |
| Volume, ml/mol                  | 147.1                                         |
| Surface tension at 90 ° K, dyne/cm | 58.4                                      |
| Solubility in water             | 0.105 g/100 ml (0°C)                          |

Ozone (O₃) at one time is considered the ideal water disinfectant due to very high oxidizing power and therefore biocidal activity is very fast at very low concentration. It also destroys odor, taste, color, and leaves no toxic residue after treatment. It breaks down pesticides and causes the cleavage of large molecular organic compounds [6]. Through the process of oxidation, ozone will damage the outer wall of microorganism cells (lysis cells) while killing it. Also through the oxidation process by free radicals such as hydrogen peroxide (H₂O₂) and hydroxyl radical (• OH) which are formed when ozone breaks down in water [7].
Figure 1. Chemical bonding of ozone

\[
\begin{align*}
O_3 + H_2O & \rightarrow H_2O_2 + O_2 \\
O_3 + H_2O & \rightarrow 2OH^* + O_2
\end{align*}
\]

The workings of OH * itself are to reduce or attach to bacterial cell walls over time a hole in the bacterial cell wall will occur and eventually, the bacteria will damage and dead [4].

Figure 2. The effects of ozone on bacteria [8].

The process of disinfection using ozone can kill germs but also produce oxygen which can add a sense of freshness to those who drink it [9]. Disinfection procedure in refill drinking water treat water with ozone and then left for 1 day before being put into gallons of consumers. While the procedure for disinfection of industrial-scale bottled drinking water, water that has been processed and ozonized is directly entered into the gallon and then quarantined 2-3 days before being distributed to consumers.

Refill drinking water that is disinfected using ozone needs to be studied considering that refillable drinking water is put into the gallon by the consumer. It should be suspected that this disinfection process is not perfect so that the drinking water still contains a lot of bacteria, dangerous viruses, also OH * radicals which are strong oxidizers. So the purpose of this study is to determine the effect of drinking water storage time on the ozonation process with bacterial content parameters, and dissolved oxygen content.

2. Materials, tools, and methods

2.1. Materials

- Refill drinking water was obtained from 5 refill drinking water depots using ozone disinfectant in the East Bandung area.
- Agar for bacterial growth media.
- Solid media used are Plate Count Agar (PCA)
2.2. Tools
Tools used: gallons for refill drinking water samples, beakers, measuring cups, analysis balance, micron wire, micron pipettes, Erlenmeyer flasks, autoclave, Petri dishes, incubators, pH meter, DO meter and colony counter.

2.3. Methods
This research is a type of observational research. This research was chosen because researchers only took measurements and observations without intervening.

The research begins by selecting a drinking water refill depot that is one franchise so that it has similarities in the process, equipment and source of raw water.

Furthermore, it is sampling, samples consist of raw water that has not been disinfected and drinking water that has been disinfected. Samples were put into 5 gallons of packaging, these were done at 5 different refill drinking water depots.

All these samples were taken on the first day where bottle I = will be analyzed on the first day, bottle II = will be analyzed on the second day, bottle III = will be analyzed on the third day, bottle IV = will be analyzed on the fourth day and bottle V = will be analyzed on the fifth day. Each treatment was carried out for 5 different depots. The temperature is measured using a thermometer with a probe on a sample dipped it will be visible figures in Celsius degree temperature, pH was measured using a pH meter is by dipping electrode into the sample, pH value will show on the monitor. DO (Dissolved Oxygen) is measured using DO meter, i.e. by dipping the electrode into the sample and its value will be seen on the monitor. Microorganisms analysis, i.e. by using quantitative methods, known as Total Plate Count (TPC).

The Total Plate Count (TPC) test method is carried out in several stages:

a. Sample homogenization, as a preliminary stage in testing which is useful for freeing bacterial cells that may be protected by sample particles and to obtain the best possible bacterial distribution.

b. The dilution stage, using a diluent solution that functions to reanimate bacterial cells that may lose their vitality because the conditions in the sample are less favorable.

c. The stage of mixing with the solid media used.

d. Incubation and observation stages. Incubation is carried out at the appropriate temperature and time and conditions are made in such a way as to suit the microbial properties.

e. Interpretation of results in the form of numbers in colonies (cfu) per ml/g or colony/100 ml.
3. Results and discussion

3.1. pH analysis of refill drinking water
The results of the examination of pH values in refill drinking water samples can be seen in the table below:

| Day | Sample  |
|-----|---------|
|     | I  | II  | III | IV  | V   |
| 1   | 8.71| 8.95| 8.92| 8.89| 8.78|
| 2   | 8.64| 8.77| 8.86| 8.72| 8.65|
| 3   | 8.53| 8.63| 8.76| 8.56| 8.56|
| 4   | 8.38| 8.39| 8.54| 8.41| 8.41|
| 5   | 8.32| 8.28| 8.46| 8.32| 8.33|

Figure 4. Stages of the research process.
Figure 5. Results of pH inspection.

From table 2 and figure 5 it can be seen that pH decreases day by day, this is because of both the chemical and biological processes.

3.2. Refillable DO (Dissolved Oxygen) analysis

The results of the examination of DO levels in refill drinking water samples can be seen in the table below:

Table 3. Results of DO analysis.

| Day | Sample  | I  | II | III | IV  | V  |
|-----|---------|----|----|-----|-----|----|
| 1   |         | 7.73| 8.16| 8.17| 8.17| 8.06|
| 2   |         | 7.74| 8.18| 8.19| 8.15| 8.04|
| 3   |         | 7.74| 8.2 | 8.18| 8.14| 8.02|
| 4   |         | 7.72| 8.2 | 8.17| 8.14| 7.99|
| 5   |         | 7.71| 8.19| 8.15| 8.1 | 7.87|

Figure 6. Results of examination of DO levels.
From table 3 and figure 6 there is an increase on the second day, while on the third day only sample II has increased, then has decreased until the fifth day. The increase in DO levels was caused by the reaction of ozone in water which was still not perfect on the first day so that the reaction still continued until the second day and the third day until finally it only experienced a peak and decreased again. A reaction of ozone in water:

\[
\begin{align*}
O_3 + H_2O & \rightarrow H_2O_2 + O_2 \\
O_3 + H_2O & \rightarrow 2OH + O_2
\end{align*}
\]

The reaction shows that the decomposition of ozone in water to produce oxygen.

3.3. Turbidity (turbidity) test of refill drinking water

Turbidity test results in samples of refill drinking water can be seen as follows:

**Table 4. Turbidity test results.**

| Day | Sample | I | II | III | IV | V |
|-----|--------|---|----|-----|----|---|
| 1   | 0      | 0 | 0  | 0   | 0  | 0 |
| 2   | 0      | 0 | 0  | 0   | 0  | 0 |
| 3   | 0      | 0 | 0  | 0   | 0  | 0 |
| 4   | 0      | 0 | 0  | 0   | 0  | 0 |
| 5   | 0      | 0 | 0  | 0   | 0  | 0 |

**Figure 7. The** results of the examination turbidity.

From table 4 and figure 7 shows that the analyzed samples have turbidity zero.

3.4. Temperature test of refill drinking water

The results of temperature test on refill drinking water samples are as follows:
Table 5. Temperature test results.

| Day | Sample (°C) | I   | II  | III | IV  | V   |
|-----|-------------|-----|-----|-----|-----|-----|
| 1   | 27.70       | 27.70| 27.70| 27.80| 27.80|
| 2   | 28.00       | 28.00| 28.00| 28.00| 28.00|
| 3   | 27.20       | 27.20| 27.20| 27.20| 27.20|
| 4   | 26.60       | 26.60| 26.60| 26.60| 26.60|
| 5   | 27.10       | 27.10| 27.10| 27.10| 27.10|

Figure 8. Temperature test (°C).

From table 5 and figure 8 the temperature looks up and down depending on room temperature when measuring temperature.

3.5. Microbiological test
The results of the microbiological test with the TPC method on refill drinking water samples were a decrease in the number of colonies on the second and third days of the sample.

Table 6. TPC results.

| Day | Sample | I   | II  | III | IV  | V   |
|-----|--------|-----|-----|-----|-----|-----|
| 1   | 3      | 4   | 3   | 2   | 3   |
| 2   | 1      | 1   | 1   | 1   | 1   |
| 3   | 1      | 2   | 1   | 1   | 2   |
| 4   | 3      | 3   | 2   | 2   | 2   |
| 5   | 3      | 4   | 3   | 2   | 3   |
From table 6 and figure 9 it can be seen that the lowest bacterial content of all samples is on the second day, this occurs because of the reaction of ozone in water:

\[
O_3 + H_2O \rightarrow H_2O_2 + O_2 \]

\[
O_3 + H_2O \rightarrow 2OH + O_2
\]

It appears that ozone depletions in water produce H$_2$O$_2$ and OH which are strong oxidizers as killers of bacteria contained in water. From the results of the experiment, it was seen that the lowest bacterial content of all samples was on the second day, this shows that the optimum ozonation process can be seen in the following day, almost all samples showed an increase in the number of microbes.

4. Conclusions and suggestions

The study can be concluded as follows:

a. The pH examination results showed a decrease in pH from day to day, this was due to chemical reactions and microbiological activities

b. The result of the DO examination was an increase in DO on the second day which finally declined again the next day

c. The TPC test results revealed that the TPC value was the smallest on the second day, this was due to the ozone reaction in the water where H$_2$O$_2$ and (OH) were very powerful new oxidizing agent works optimally on the second day

Based on the test results, it is highly recommended:

a. Storage water after ozonation should be kept for 3 days in order to optimize the disinfection process using ozone

b. It is necessary to update the cleaning and disinfection of used gallons

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