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# Dew Formation and Anaerobiosis in Anaerobic Jars

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Both Authors read and approved the final manuscript.*

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## ABSTRACT

**Introduction:** This study aimed to prove whether the palladium catalyst affects the dew formation time in the anaerobic cover and whether *anaerobiosis* can be achieved without a palladium catalyst.
**Materials and Methods:** This research is a laboratory experimental study with a non-randomized control group design. In this study, replication was carried out. The amount of replication used is 10. The palladium catalyst was used in 10 experiments, whereas it was not used in ten other experiments.
**Results:** The growths of Pseudomonas aeruginosa, Clostridium tetani, and Bacteroides fragilis were observed after 48-hour incubation. The time of appearance of water condensate was observed until 24-hour incubation. The time of appearance of water condensate in the palladium-contained anaerobic jar varied between 1.37 minutes and 3.33 minutes. On the other hand, water condensate did not appear in the without-palladium anaerobic jar. No growth of Pseudomonas aeruginosa was observed in the palladium-contained anaerobic jar; on the contrary, there were ten growths in the anaerobic jar without palladium. Eight growths of Clostridium tetani were observed in the palladium-contained anaerobic jar, whereas there was no growth without palladium. No growth of Bacteroides fragilis was observed in the without-palladium anaerobic jar, whereas seven growths were observed in the anaerobic jar containing palladium. The time of appearance of water

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condensate in the palladium-contained anaerobic jar was different from that in the anaerobic jar without palladium. Significant differences ($p < 0,01$) in anaerobiosis creation between the palladium-contained anaerobic jar and the without-palladium anaerobic jar were also clearly observed.

*Keywords: Anaerobic; palladium; water condensate; pseudomonas aeruginosa; clostridium tetani.*

## 1. INTRODUCTION

Studies on the role of palladium catalysts related to anaerobiosis have not been widely carried out. There are various methods to create an anaerobic atmosphere (anaerobiosis), namely evacuation-replacement, GasPak or Oxoid disposable gas generator method, anaerobic glove box techniques, and roll tube and roll-streak tube with PRAS media [1]. The justification for conducting this experiment is based on the limited existing research on the involvement of palladium catalysts in anaerobiosis.

The use of a catalyst is essential. Srivastava, et al. [2] do not recommend the creation of uncatalyzed anaerobiosis. Palladium catalyst is the most widely used. Apart from palladium, according to Vershinin aand Kabachkov [3], platinum can also be a catalyst. Brewer's jar uses a palladium catalyst which must be preheated with an electric current. The GasPak containment uses a "cold" catalyst, consisting of alumina pellets coated with palladium, which does not require heating. This "cold" catalyst has the advantage of reducing the risk of explosion. The production of hydrogen sulfide or other volatile-metabolic bacteria products can inactivate the "cold" catalyst. If a jar is to be used, the catalyst should be replaced with a new catalyst or a catalyst that has been reactivated [1]. Min, et al., [4] states that what is meant by other volatile-metabolic products are various weak acids, such as valeric acid, isovaleric acid, butyric acid, isobutyric acid, caproic acid, isocaproic acid, acetic acid, and propionic acid.

Aside from being inactivated by hydrogen sulfide and other volatile-metabolic products, Palladium catalyst can also be inactivated by excess dew, chlorine gas, sulfur dioxide gas, carbon monoxide gas, oil, fumes of several organic solvents, and strong acids. Therefore, the use of a catalyst is only recommended once. After that, the catalyst must be reactivated every time it is used. Reactivation was carried out by heating the catalyst in a dry oven for 1.5 to 2 hours at 160°C. After that, the catalyst must be stored in a dry place. The inactivation of palladium catalysts by hydrogen sulfide is of great importance to microbiologists because some anaerobic bacteria can produce hydrogen sulfide gas, especially from liquid cultures. Examples of bacteria capable of producing hydrogen sulfide gas are Bacteroides melaninogenicus subspecies asaccharoyticus and Peptop streptococcus anaerobius [5].

The gas bag of the generating kit contains sodium borohydride and sodium bicarbonate [2]. Ten milliliters of distilled water must be poured into the gas bag of the generating kit to achieve an anaerobic atmosphere. The bag will generate H2 and CO2. O2 in the jar will soon capture H2. Then H2O formed spontaneously. The formation of H2O is indicated by condensation. The appearance of condensation can be seen by sticking dew to the inside wall of the jar. Zhu & Xu, [6] wrote that the onset of dew was less than 25 minutes. According to Legaria [7], dew is a vapor that becomes water droplets.

This research proves whether the palladium catalyst affects the dew formation time in the anaerobic cover and whether *anaerobiosis* can be achieved without a palladium catalyst.

Anaerobic conditions are also called anaerobiosis. Anaerobiosis means an atmosphere without oxygen. Quaiyum, Igarashi, Narihiro, and Kato [8] define anaerobiosis as life without molecular oxygen.

An anaerobic jar is an airtight container made of metal or translucent plastic in which an anaerobic atmosphere is created to isolate anaerobic bacteria5. There are various types of anaerobic jars, for example, Brewer, Baird-Tatlock, GasPak, McIntosh-Fildes, Oxoid, Difco, and Torbal jars. The most widely used jars in the United States are GasPak jars [9].

The Baird-Tatlock jar is equipped with a U-shaped tube manometer on the jar's side. This manometer helps check whether the catalyst has been fully activated. The Baird-Tatlock jar is made of stainless steel [9].

The Oxoid jar has a volume of 3.5 liters, and the walls are made of polycarbonate. The lid is made of sturdy metal. The clamps are also made of metal. In the center of the metal cap are 2 Schrader valves and a plus-minus pressure gauge with two valves to facilitate E/R techniques. There is also a safety valve in the lid to prevent extra gas pressure caused by using the wrong technique. The bag containing the low-temperature catalyst is attached to the bottom surface of the metal cap. It is not just E/R techniques that can be used on Oxoid jars. The Oxoid Generating Kit can also be utilized on an Oxoid jar [1].

The basic principle of various types of anaerobic jars is similar, namely removing 02 in the jar by reacting it with H2 with the help of a catalyst. The chemical reaction is 2H2 + O2 2H2O.

## 1.1 Palladium Catalyst

The reaction speed is affected by various factors. These factors are the nature of the substance, temperature, concentration, the contact surface area, and the catalyst [10]. Catalysts speed up reactions without undergoing any permanent change in the reaction. The catalyst changes the reaction mechanism. The number of reaction steps increases. The catalyst participates in one step and is reformed in one of the following steps. Suppose that without a catalyst, the reaction occurs in a single step:

$$A+BC \longrightarrow AB+C$$

then with the catalyst the number of steps increases,

$$K+BC \longrightarrow KB+C$$

$$\underline{KB+A \longrightarrow AB+K}$$
$$A + BC \longrightarrow AB + C$$

The activation energy is lower in reactions that use a catalyst, so the percentage of molecules with activation energy is important. Therefore, the reaction proceeds faster than the reaction that does not use a catalyst [11].

There are three catalysis types: heterogeneous catalysis, homogeneous catalysis, and enzyme catalysis. In heterogeneous catalysis, the reactants and catalysts are in different states. The catalyst is usually in the form of a solid, and the reactants are in the form of a gas or liquid [12]. Thus, the palladium catalyst in this study is heterogeneous.

Heterogeneous catalysts function by increasing the reaction on their surface. One or more reactant molecules are adsorbed onto the surface of the catalyst. A further consequence is increased reactivity from interaction with the catalyst surface [12].

For a reaction to occur between two molecules, the two molecules must be close enough to each other so that the molecules' outer electrons can interact. In short, the two molecules must collide. In addition to having to collide, the two molecules must have enough energy to overcome the repulsive forces between the electrons surrounding the nuclei of the two molecules. In other words, a certain amount of energy is required for a successful collision, which is called activation energy (activation energy). For a reaction to occur, the colliding molecules must have an energy equal to the activation energy. When a collision occurs, the molecules form a reactive group called the activated complex or transition state. This atomic complex is neither a reactant nor a product. These atomic complexes are volatile combinations of atoms, intermediate forms between reactants and products [6].

Reactions can occur due to effective collisions. There are two important conditions for a practical collision to occur: molecular orientation and molecular kinetic energy. The minimum kinetic energy for an effective collision is called activation energy (Ea) [8].

In the periodic system of elements, Palladium is a transition element. More precisely, Palladium belongs to group VIII. Palladium has an atomic number of 46 and an atomic weight of 106.4 (Kare, 2022).

Palladium has two valences, +2 (most common) and +4. The electron configuration of palladium is 1s2 2s2 2p6 3s2 3p6 4s2 3d10 4p6 4d10 [7]. Like other transition elements, Palladium has an incomplete d subshell [7]. This causes Palladium to become unstable and easily excited.

When Palladium is heated, the outer electrons surrounding the palladium nucleus will move to a more outer shell. This means that the electrons move to shells with higher energy levels. In other words, electrons move from the ground state/level (most stable) to the excited state/level (unstable) [6]. Furthermore, electrons will easily

move to deeper shells. A release of energy accompanies this transfer of electrons. This electron transfer, followed by energy release, is responsible for the lowered activation energy in reactions catalyzed by Palladium. As is known, before being used in an anaerobic enclosure, Palladium must be reactivated first. Reactivation means heating palladium in a drying oven for 2 hours at 160°C.

The palladium catalyst accelerates the reaction between H2 and 02 to become H20 in the anaerobic jar. Without a catalyst, a mixture of hydrogen and oxygen will not react at room temperature. The reaction will not occur if the temperature is not raised to a minimum of 400°C [11].

The palladium catalyst should be reactivated after every use. Reactivation was carried out by heating the palladium catalyst at 160°C for 1.5-2 hours in a drying oven. A catalyst that has been reactivated until the time of use arrives must be stored in a dry place [1].

At room temperature, palladium can absorb hydrogen (up to 900 times the volume of hydrogen) to form Pd2H. Until now, it is still unclear whether Pd2H is an actual compound. Not all anaerobiosis creation systems require a palladium catalyst. The sodium borohydride-sodium bicarbonate system and the evacuation-replacement method require a palladium catalyst. Those that utilize the sodium borohydride-sodium bicarbonate system include the Anaerobic System (Difco), GasGendicator (Adams Scientific), GasPak (BBL), GasPakPlus (BBL), and Anaer Generbox (bioM6rieux) [4].

Some more advanced systems with internal generators do not require a catalyst or hydrogen to produce an anaerobic atmosphere. Anaerocult A system (Merck) uses iron filings to bind oxygen. AnaeroGen system (Oxoid) utilizes ascorbic acid. AnaeroGen system (Oxoid) can absorb oxygen and produce carbon dioxide with a 9% -13% concentration. There is no hydrogen production in the AnaeroGen system (Oxoid) [13].

### 1.2 Time of Dew

Dew is vapor that becomes water droplets (Moeliono et al., 1988). Dew will slowly cover the entire inner wall of the anaerobic jar. Bhat and Khanday [1] mentions the time of appearance of dew with the term time of appearance of water condensate. Meanwhile, Pędziwiatr, Mikołajczyk, Zawadzki, Mikołajczyk, & Bedka [10] used the term moisture on the wall of the jar to describe dew that sticks to the walls of the jar. Dew is formed due to the reaction between H2 and 02 (catching 02 by H).

The formation of dew on the inner walls of the jar and the production of heat (which can be felt by touching the walls of the jar) are indicators that the palladium catalyst and gas generator kit are functioning properly [2].

## 2. METHODS

This research is a laboratory experimental study with a non-randomized control group design. In this study, replication was carried out.

The number of replications (r) was calculated by the formula [14]:

$$r \geq 2\{Z_{\alpha/2} + Z_{\beta}\}^2 \{\sigma/\delta\}^2$$

Note:

r : number of replications,
σ : standard deviation = {wte (maximum)-wte (minimum)}/4,
δ: difference in the average time of dew formation (wte) between palladium-catalyzed anaerobic jars and non-palladium-catalyzed anaerobic lids.

For research on tools commonly used α = 0.01 or 1%. This means Zα/2 is 2.58. Meanwhile, the price of β is 0.05 or 5%. This means Zβ is 1.96.

In this study the following assumption is used:

wte (maximum) = 102 and wte (minimum) = 2, then δ =. {102-2}/4 = 25, and the value of δ used is 50. If these numbers are put into the formula, we get:

$$r \geq 2\{2{,}58 + 1{,}96\}^2 \{25/50\}^2 \longrightarrow r \geq 10{,}3058$$ or 10 (rounded).

Alternatively, in other words, the number of replications required is 10.

### 2.1 Research Variable

**Variable classification**

- Independent variable: palladium catalyst
- Dependent variables: time of onset of dew, growth of Pseudomonas aeruginosa, growth of Clostridium tetani, growth of Bacteroides fragilis

- Control variable: anaerobic jar, gas generating kits

The research materials used were anaerobic jars containing gas generating kits, palladium catalysts, Pseudomonas aeruginosa grown on Simmon's Citrate Slant Agar, Clostridium tetani grown on blood agar plates, and Bacteroides fragilis grown on blood agar plates. The cover used is branded BBL. Oxoid branded gas generating kit. The palladium catalyst is also branded BBL. The jar has glass or glass walls, making dew build-up easy to monitor. In addition to the above ingredients, distilled water is also used. The research instruments used were incubators, drying ovens, test tubes, test tube covers, implant needles, loops, spirit lamps, cleaning cloths, Vaseline, stopwatches, scissors, and a 10 ml pipette.

## 2.2 Data Collection Procedures

The control group included a jar containing gas generating kit, Pseudomonas aeruginosa grown on Simmon's Citrate Slant Agar, Clostridium tetani grown on blood agar plates, Bacteroides fragilis grown on blood agar plates, and palladium catalyst.

The jar treatment group included gas generating kit, Pseudomonas aeruginosa grown on Simmon's Citrate Slant Agar, Clostridium tetani grown on blood agar plates, and Bacteroides fragilis grown on blood agar plates (without palladium catalyst).

## 2.3 The Working Methods

- Pseudomonas aeruginosa grown on Simmon's Citrate Slant Agar, Clostridium tetani grown on blood agar plates, and Bacteroides fragilis grown on blood agar plates placed in a lid,
- The gas-generating kit is opened with scissors,
- Ten milliliters of distilled water is poured into the gas generating kit,
- The gas generating kit is placed in an anaerobic vessel containing a palladium catalyst,
- Cover the anaerobic tube tightly while the stopwatch is on,
- The walls in the anaerobic containment were observed until the onset of dew so that the walls appeared to start to become opaque due to dew accumulation, and at that point, the stopwatch was turned off, and the time was recorded.
- Growth of *Pseudomonas aeruginosa, Clostridium tetani, and Bacteroides fragilis* seen after 48 hours.

## 2.4 Data Analysis Technique

The nominal scale of the palladium catalyst variable, the variable scale of dew emergence time, and the dew emergence between the control and treatment groups were compared. A statistical test was carried out with the t-test for two independent samples. With the t-test, it will be known whether dew's onset time is significantly different between the control group and the treatment group. The t-test was chosen because the sample taken was relatively small (n1 and n2 <30).

The Chi-Square Test is used to test whether the palladium catalyst with anaerobiosis is related. As is well known, both palladium catalyst and anaerobiosis variables are nominal in scale.

## 3. RESULTS

After conducting research in the Microbiology Section of the Faculty of Medicine, Wijaya Kusuma University, Surabaya, from 2 January 2022 to 22 February 2022, the results were obtained as shown in Table 1 and Table 2.

The relationship between the use of palladium catalyst and the time of dew formation (wte) is shown in Table 3.

From the figures listed in Table 3, it is clear that there is no need to carry out the t-test for two independent samples. This is due to the non-palladium-catalyzed anaerobic containment dew formation does not occur. In the palladium-catalyzed anaerobic lid, various dew formation times were obtained. The numbers obtained varied from 1.37 minutes to 3.33 minutes. The average time for dew to set in the palladium-catalyzed anaerobic hood was 2.44 minutes. The standard deviation of the time for dew to set in the palladium-catalyzed anaerobic lid is 0.64.

The relationship between the use of Palladium Catalyst and the growth of Pseudomonas aeruginosa can be seen in Table 4.

**Table 1. Observations on the time of dew formation and bacterial growth in the palladium-catalyzed anaerobic lid**

| Experiment number | emergence time | Pseudomonas aeruginosa | Clostridium tetani | Bacteroides fragilis |
|---|---|---|---|---|
| 1 | 2.50 | not growing | grow | grow |
| 2 | 1.37 | not growing | grow | grow |
| 3 | 2.45 | not growing | grow | grow |
| 4 | 2.77 | not growing | grow | not growing |
| 5 | 1.37 | not growing | not growing | grow |
| 6 | 2.70 | not growing | grow | not growing |
| 7 | 3.05 | not growing | grow | Grow |
| 8 | 2.50 | not growing | not growing | Grow |
| 9 | 2.37 | not growing | grow | not growing |
| 10 | 3.33 | not growing | grow | grow |

*Note: Bacterial growth seen after 48 hours*

**Table 2. Observations on the time of dew formation and bacterial growth in non-palladium-catalyzed anaerobic lids**

| Experiment number | emergence time | Pseudomonas aeruginosa | Clostridium tetani | Bacteroides fragilis |
|---|---|---|---|---|
| 1 | not detected | grow | not growing | not growing |
| 2 | not detected | grow | not growing | not growing |
| 3 | not detected | grow | not growing | not growing |
| 4 | not detected | grow | not growing | not growing |
| 5 | not detected | grow | not growing | not growing |
| 6 | not detected | grow | not growing | not growing |
| 7 | not detected | grow | not growing | not growing |
| 8 | not detected | grow | not growing | not growing |
| 9 | not detected | grow | not growing | not growing |
| 10 | not detected | grow | not growing | not growing |

*Notes:*
*- Bacterial growth seen after 48 hours.*
*- If up to 24 hours the dew is not visible, it means that the dew was not detected at the time of onset*

**Table 3. Observations on the time of dew onset**

| Experiment number | Dew time (minute) | |
|---|---|---|
| | Catalyzed | Not Catalyzed |
| 1 | 2.50 | not detected |
| 2 | 1.37 | not detected |
| 3 | 2.45 | not detected |
| 4 | 2.77 | not detected |
| 5 | 1.37 | not detected |
| 6 | 2.70 | not detected |
| 7 | 3.05 | not detected |
| 8 | 2.50 | not detected |
| 9 | 2.37 | not detected |
| 10 | 3.33 | not detected |

*Note:*
*- If up to 24 hours the dew is not visible, it means that the dew is not detected.*

The Chi Square test for Table 4 yields p = 0.000. This value is smaller than the α value used, which is 0.01. This means the null hypothesis is rejected, and the alternative hypothesis is accepted. So, there is a very significant relationship between the use of palladium catalysts and the growth of Pseudomonas aeruginosa.

**Table 4. Results of observing the growth of pseudomonas aeruginosa**

| | | Growth of Pseudomonas aeruginosa | | Total |
|---|---|---|---|---|
| | | **Grow** | **Not growing** | |
| Use of a Palladium Catalyst | Use | 10 | 0 | 10 |
| | not use | 0 | 10 | 10 |
| Total | | 10 | 10 | 20 |

*Chi Square Test: p = 0,000*
*Note: Growth of Pseudomonas aeruginosa seen after 48 hours.*

**Table 5. Observations on the growth of clostridium tetani**

| | | **Growth of Clostridium tetani** | | **Total** |
|---|---|---|---|---|
| | | Grow | Not growing | |
| Use of Palladium Catalyst | Use | 8 | 2 | 10 |
| | not use | 0 | 10 | 10 |
| Total | | 8 | 12 | 20 |

*Fisher's Exact Likelihood Test: p = 0,001*
*Note: Growth of Clostridium tetani seen after 48 hours.*

**Table 6. The results of observing the growth of bacteroides fragilis**

| | | **Growth of Bacteroides fragilis** | | **Total** |
|---|---|---|---|---|
| | | **Grow** | **Not growing** | |
| Use of Paladium Catalyst | Use | 7 | 3 | 10 |
| | not use | 0 | 10 | 10 |
| Total | | 7 | 13 | 20 |

*Fisher's Exact Likelihood Test: p= 0.003*
*Note: Growth of Bacteroides fragilis seen after 48 hours*

The relationship between the use of a palladium catalyst and the growth of Clostridium tetani is shown in Table 5.

Fisher's Exact Probability Test (Fisher's Exact Test) in Table 5 above yields p = 0.001. This p number is smaller than α = 0.01. This means that the null hypothesis is rejected, and the alternative hypothesis is accepted. So, there is a very significant relationship between the use of palladium catalysts and the growth of Clostridium tetani.

The relationship between the use of palladium catalyst and the growth of Bacteroides fragilis can be seen in Table 6.

Fisher's Exact Likelihood Test (Fisher's Exact Test) for Table 6 yields p = 0.003. This p-value is smaller than the α used. This means the null hypothesis is rejected, and the alternative hypothesis is accepted. So, there is a very significant relationship between the use of palladium catalysts and the growth of Bacteroides fragilis.

## 4. DISCUSSION

### 4.1 The Relationship between the Use of Palladium Catalysts and the Time of Dew Formation (wte)

Kusuma7 wrote that the dew onset time should be less than 25 minutes. Kusuma7 also stated that if the dew formation time is less than 25 minutes, it means that the gas-generating kit is functioning properly, the jar is not leaking, and the reactivation of the palladium catalyst is adequate.

Research that mentions the time of dew formation on non-palladium-catalyzed anaerobic jars has not been found. Therefore, it is very difficult to compare the time of dew formation on anaerobic jars that are not palladium-catalyzed, as shown in Table 3, with previous studies conducted by other researchers.

Without a palladium catalyst in the anaerobic containment, dew formation will not occur on the inner walls of the anaerobic containment. This is

easy to understand because the reaction between hydrogen and oxygen does not occur without the involvement of a palladium catalyst. The palladium catalyst lowers the activation energy so that the reaction occurs. As is known, for a reaction to occur, there must be a successful collision. For a successful collision to occur, there must be an effective collision. A minimum amount of kinetic energy is required for an effective collision to occur. The minimum needed kinetic energy is also known as activation energy [10].

### 4.2 Relationship between the use of Palladium Catalyst and the Growth of Pseudomonas Aeruginosa

In a palladium-catalyzed anaerobic jar, anaerobiosis can be achieved. The oxygen in the anaerobic jar is entirely bound by hydrogen so that dew is formed. Hydrogen in the anaerobic jar occurs from a series of reactions in the gas-generating kit after adding water [14].

Pseudomonas aeruginosa, based on the pressure of oxygen needed to live, belongs to the category of obligate aerobic bacteria. Pseudomonas aeruginosa requires oxygen as the final electron acceptor. Pseudomonas aeruginosa does not obtain energy via the fermentative pathway. It is easy to understand why Pseudomonas aeruginosa did not grow in palladium-catalyzed anaerobic jars.

Meanwhile, oxygen cannot be bonded by hydrogen in an anaerobic jar that is not catalyzed by palladium. Oxygen remains in the anaerobic jar. Pseudomonas aeruginosa will grow in an environment with lots of oxygen.

### 4.3 The Relationship between the use of Palladium Catalyst and the Clostridium Tetani Growth

The microbiology literature has never explained whether Clostridium tetani belongs to the category of strictly anaerobic or obligate-moderate anaerobic bacteria. What is clear is that Clostridium tetani are an obligate anaerobic bacterium. So, the presence of oxygen will kill Clostridium tetani.

In Table 5, it is clear that there was no growth of Clostridium tetani in non-palladium-catalyzed anaerobic jars. This is easy to understand because the oxygen is still intact in the non-palladium-catalyzed anaerobic containment. The presence of oxygen will inhibit the growth of Clostridium tetani [15-17]. Other studies regarding the growth of Clostridium tetani in anaerobic jars not catalyzed by palladium have not been found.

Growth of Clostridium tetani was found in 8 experiments with palladium-catalyzed anaerobic jars. This is easy to understand because Clostridium tetani is an obligate anaerobic bacterium. The absence of oxygen benefits the growth of Clostridium tetani.

Growth of Clostridium tetani was not found in 2 experiments with palladium-catalyzed anaerobic jars. Possible reasons for the absence of growth are that Clostridium tetani used is not the ATCC type, Clostridium tetani are taken from old stock, taking germs from Clostridium tetani stocks is not enough, the scratching technique is not pressing enough, and other reasons that are not yet clear. In Non-ATCC Clostridium tetani and Clostridium tetani taken from old stock, the possibility of mutations is huge so that the properties of the bacteria change.

### 4.4 The Relationship between the Use of Palladium Catalysts and the Growth of Bacteroides Fragilis

Bacteroides fragilis belongs to the category of obligate-moderate anaerobic bacteria. Bacteroides fragilis can only grow when the oxygen tension ranges from 2% to 8% (average 3%) [1].

In the non-palladium-catalyzed anaerobic housing, Bacteroides fragilis growth was not found at all. This is understandable because the oxygen pressure in the non-palladium-catalyzed anaerobic jar does not decrease. The oxygen in the non-palladium-catalyzed anaerobic jar remains intact because there is no reaction between oxygen and hydrogen. Other studies regarding the growth of Bacteroides fragilis in anaerobic jars not catalyzed by palladium have not been found.

Growth of Bacteroides fragilis was found in 7 experiments with palladium-catalyzed anaerobic jars. This is easy to understand because Bacteroides fragilis is an obligate anaerobic bacterium. The absence of oxygen actually benefits the growth of Bacteroides fragilis.

Growth of Bacteroides fragilis was not found in 3 experiments with palladium-catalyzed anaerobic jars. Possible reasons for the absence of growth

are Bacteroides fragilis planted not of the ATCC type, Bacteroides fragilis taken from old stocks, taking bacteria from Bacteroides fragilis stocks that are not abundant, less pressing technique, and other reasons that are not yet clear. In Non-ATCC Bacteroides fragilis and Baceroides fragiiiis taken from old stock, the possibility of mutation is very large, so the properties of the bacteria change.

## 5. CONCLUSION

From the description above, it can be concluded that the time of dew formation on palladium-catalyzed anaerobic jars seems to be different compared to the dew formation time on non-palladium-catalyzed anaerobic jars. The mean dew onset time on palladium-catalyzed anaerobic jars was 2.44 minutes, and the Standard Deviation of dew formation time on palladium-catalyzed anaerobic jars was 0.64. The achievement of anaerobiosis in the palladium-catalyzed anaerobic jar differed significantly compared to the achievement of anaerobiosis in the non-palladium-catalyzed anaerobiosis jar ($p < 0.01$).

This study proves that a palladium catalyst is necessary for achieving anaerobiosis in anaerobic jars. In addition, adequate reactivation must always be performed before applying the palladium catalyst.

## CONSENT AND ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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