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ARTIKEL PENELITIAN

Unleashing the Power of Palladium Catalyst: Unveiling the Role in Dew Formation and Anaerobiosis within Anaerobic Jars

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Abstract: Anaerobiosis is essential for studying and cultivating specific microorganisms, and anaerobic jars are commonly used to create anaerobic conditions. **Purpose**: To determine the essentiality of a palladium catalyst in creating anaerobiosis. The growth of specific microorganisms and the appearance of water condensate in anaerobic jars with and without the catalyst are observed. **Methods**: A non-randomized control group design is employed with 10 replications. Ten experiments use a palladium catalyst, while another 10 do not. After a 48-hour incubation, the growth of Pseudomonas aeruginosa, Clostridium tetani, and Bacteroides fragilis is observed. The appearance of water condensate is monitored within a 24-hour incubation period. **Results**: Water condensate appears within 1.37 to 3.33 minutes in the palladium-contained anaerobic jar, whereas it does not appear without the catalyst. No growth of Pseudomonas aeruginosa is observed with the palladium catalyst, but ten growths occur without it. Eight growths of Clostridium tetani are observed with the catalyst, whereas none occur without it. Bacteroides fragilis does not grow without palladium, but seven growths are observed with the catalyst. Significant differences ($p < 0.01$) in anaerobiosis creation are observed between the two types of anaerobic jars. **Conclusion**: The presence of a palladium catalyst is crucial for creating anaerobic conditions in anaerobic jars. Without the catalyst, specific microorganisms fail to grow, and water condensate does not appear, indicating ineffective anaerobiosis. These findings stress the necessity of the palladium catalyst for successful anaerobic experiments, highlighting its significance in promoting reliable microbial growth under anaerobic conditions.

Keywords: anaerobic, c*lostridium tetani*, palladium, *pseudomonas aeruginosa*, water condensate.

INTRODUCTION

Studies on the role of palladium catalysts related to anaerobiosis have not been widely carried out. Anaerobiosis, also known as anaerobic conditions, as are defined as life without molecular oxygen. An anaerobic jar is an airtight container made of metal or translucent plastic in which an anaerobic atmosphere is

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created to isolate anaerobic bacteria. There are various methods to create an anaerobic atmosphere, namely evacuation-replacement, GasPak or Oxoid disposable gas generator method, anaerobic glove box techniques, and roll tube and roll-streak tube with PRAS media. Among the various types of anaerobic jars (e.g., Brewer, Baird-Tatlock, GasPak, McIntosh-Fildes, Oxoid, Difco, and Torbal masks), the GasPak mask is the most widely used in the United States.[2,5,7,11]

The principle of the jar above is similar, namely removing oxygen from the jar by reacting it with hydrogen with the help of a palladium catalyst.[4] The use of a catalyst is essential. Srivastava, et al.,[14] do not recommend the creation of uncatalyzed anaerobiosis. Palladium and platinum can also be a catalyst.[16] 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.[2,9]

Palladium catalyst can be inactivated by hydrogen sulfide, volatile-metabolic products, 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. 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.[13]

Catalysts speed up reactions without undergoing any permanent change in the reaction, changes the reaction mechanism.[3]

The reaction speed is affected by various factors such as the nature of the substance, temperature, concentration, the contact surface area, and the catalyst.[3] The catalyst speeds up the reaction without undergoing a permanent change in the reaction, changes the mechanism of the reaction, increases the number of reaction steps, and participates in one

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step and is reformed in one of the following steps.[10]

In the periodic system of elements, Palladium is a transition element belongs to group VIII. Like other transition elements, Palladium has an incomplete d subshell. This causes Palladium to become unstable and easily excited. [6,8]

When Palladium is heated, the outer electrons surrounding the palladium nucleus will move to a more outer shell. Furthermore, electrons will easily move to deeper shells. A release of energy accompanies this transfer of electrons.[17]

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). 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).[1,9]

Dew is vapor that becomes water droplets. Dew will slowly cover the entire inner wall of the anaerobic jar. The time of appearance of dew with the term time of appearance of water condensate. Dew is formed due to the reaction between H_2 and O_2 (catching O_2 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.[2,14]

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[15]:

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

Note:

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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:

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

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

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.

Data Collection Procedures

The control group included a jar containing gas generating kit, Pseudomonas aeruginosa grown on

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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).

The working method:

- 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.

Data analysis technique

A statistical test was carried out with the t-test for two independent samples. The Chi-Square Test is used to test whether the palladium catalyst with anaerobiosis is related.

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.

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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

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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

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

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.	1a37	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.

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 and the standard deviation is 0.64.

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

Table 4. Results of observing the growth of Pseudomonas aeruginosa.

Use of a Palladium Catalyst	Growth of Pseudomonas aeruginosa		Total
	Grow	Not growing	
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.

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The chi square test for table 4 yields p = 0.000. So, there is a very significant relationship between the use of palladium catalysts and the growth of Pseudomonas aeruginosa.

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

Table 5. Observations on the growth of Clostridium tetani

Use of a Palladium Catalyst	Growth of Clostridium tetani		Total
	Grow	Not growing	
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.

There is a very significant relationship between the use of palladium catalysts and the growth of Clostridium tetani (p= 0,001).

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

Tabel 6. The results of observing the growth of Bacteroides fragilis

Use of a Palladium Catalyst	Growth of Bacteroides fragilis		Total
	Grow	Not growing	
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.

There is a very significant relationship between the use of palladium catalysts and the growth of Bacteroides fragilis (p = 0.003).

DISCUSSION

The relationship between the use of palladium catalysts and the time of dew formation (wte)

Kusuma wrote that the dew onset time should be less than 25 minutes. Kusuma 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. [7]

Research that mentions the time of dew formation on non-palladium-catalyzed anaerobic jars has not been

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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.[3]

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.[15]

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.

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

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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.[6] 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.

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%).[2]

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

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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 Bacteroides fragilis taken from old stock, the possibility of mutation is very large, so the properties of the bacteria change

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.

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