# Yuni Astuti - The Effectiveness of Sludge in the Leaf Fermentation Process

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### The Effectiveness of Sludge in the Leaf Fermentation Process

Yuni Astuti1\*, Syamsul Arifin Syarifudin1, Maesyaroh1, Ranti An Nisa1

<sup>1</sup>Biology Education, University of Muhammadiyah Prof. DR. HAMKA, Jl. Tanah Merdeka, Kp. Rambutan, Jakarta Timur, Indonesia 13830

\*yuni.astuti@uhamka.ac.id

Abstract. Wet leaves that an electromposed with mud can cause the leaf tissue to peel and become transparent. This scientific study aims to determine the effect of various mud media from rivers, ponds and gutters on the flaking of soursop (Annona muricata L.) leaf tissue. This study uses 3 variations of mud taken from river ponds and ditches designed using the RAL design (Complete Randomized Design). Each mud medium in a container was filled with fresh soursop leaf swith the leaf criteria of the fourth node of each tree branch. The Kruskal Wallis hypothesis test with a significance level of 0.05 was used in making decisions, which was then continued with the LSD test to determine the difference in effect between treatments. In the calculation of the peeling percentage of leaf tissue, it was found that the river and pond mud soaking media had a percentage of 100% and only 6.9% of the gutter, which means that river and pond mud had good potential as a medium for leaf fermentation. In the immersion photo, it was found that the river mud had more complete stripping of tissue. The exfoliating quality of leaf tissue in this study was influenced by the type of sludge medium and its pH.

#### 1. Introduction

Leaf litter is a daily-generated litter. The problem of leaf litter cannot be solved without proper management. Priscilia et.al. (2016) found that 97.32% of road park waste is leaf litter. Leaf litter can come from dry leaves that fall and wet leaves that result from pruning plants. According to Yulipriyanto (2009), leaf litter in wet conditions is usually generated when tree pruning is being carried out. Wet leaf litter that falls into the water will decompose and experience sedimentation at the bottom of the waters, which has the risk of increasing the volume of water and triggering floods. According to Rosyidie (2013) and Kodoatie & Syarief (2006), factors causing floods include land use change, garbage disposal, erosion and sedimentation (Zhang, 2018).

Sedimentation is the deposition of particles originating from decomposed organic materials which settle on the bottom of the waters, which then accumulate and become mud. Mud that has accumulated on the bottom of the water would increase the volume of water and potentially causing floods. Usually the Jakarta Public Works office will perform mud dredging to prevent the accumulation of mud on the riverbed. However, this is still insufficient because there is no further utilization. According to Rachman & Moch (2015), currently this river sludge treatment has not been optimal. The management of the sludge is only in the form of dredging by the Public Works office, which are being dumped at disposal sites

The use of sludge as a medium for making transparent leaves is economically and environmentally friendly (Saima et al., 2013). As explained by Suryandari & Tutik (2017), the processing system has a very low budget, whereby only by soaking the leaves in water or mud within a certain period of time it

can produce transparent leaves that are aesthetic. The types of leaves that can be used to produce transparent leaves are teak leaves, sandpaper and soursop leaves.

A research on tissue peeling has also been conducted by Mir et.al. (2013) who explained that the fermentation of bodhi leaves using 2% yeast could provide the best exfoliating results, which do not leave any leaf tissue remnants on the leaf bones. Meanwhile, a study by Whittenberger & Naghski (1948) discovered that *Clostridium roseum* bacteria culture plays an active role in digesting the walls of mesophyll parenchyma cells, which allows a good separation between epidermal tissue and leaf venation. Both of these studies focused on the concentration levels of the types of microorganisms used.

The existing research demonstrates that the process of peeling leaf tissue using mud media has rarely been studied. This is despite the fact that mud media can also potentially be used for leaf decomposition as interpretation and interpretation as interpretation a

This study aims to determine the effect of various types of sludge on the flaking of soursop (Annona muricata L.) leaf tissue. Leaves that have become transparent can be used as leaf bone crafts that have high economic and aesthetic value. Transparent leaves can also be used as learning media in schools regarding leaf anatomy.

## Methodology

The method used in this research is a true experiment. Various kinds of sludge originating from rivers, ponds and ditches were made as variables and the frequency of peeling soursop (*Annona muricata L.*) leaf tissue was placed as the dependent variable. The mud media was taken from various places in the Ciliwung river area; one pond in the Cinere area and one of the waterways in the Jagakarsa area. This research used special tools and materials such as; *vortex*, micro pipette, petri dish, *drygalski*, incubator, sterile distilled water, *De Man* solid jelly medium, *Rogosa* and *Sphare* jelly (MRS jelly).

#### Research Scope

The operational objective of this study was to calculate the percentage of the frequency of encountering tissue peeling on soursop (Annona muricata L.) leaves. The research was conducted at the UHAMKA FKIP Greenhouse. The study was conducted from February to August 2019.

The sample used was fresh soursop leaves which were taken using purposive sampling technique, with the criteria of leaves that were on the 4th node of each plant branch to equalize the age of the leaves. Sample repetitions were calculated using Federer's formula and 10 replications were obtained for each immersion medium.

The research design was CRD (Completely Randomized Design), to make all samples experienced the same conditions.

#### Research Procedures

The procedure of this research includes t soaking the soursop leaves and calculating the number of microorganisms present in each medium using the TPC test.

- 1. The soaking of soursop leaves
  - The process of soaking leaves began with selecting leaves from one soursop tree ( $Annona\ muricata\ L$ .) by taking leaves at the fourth node. The mud along with the water was put into a jar with a hole in the lid and a hose connected to the mineral water bottle was attached. Leaves were submerged and a wire mesh was added to prevent the leaves from floating to the surface. We waited for about 2 weeks soaking time to see the results. The temperature and pH were measured every day on each immersion medium at the same time. After 2 weeks, the leaves were removed and cleaned. Leaves that have been cleaned were calculated for the percentage of their peeling using the  $10\ X\ 10\ cm^2$  square that has been made.
- Calculating the number of microorganisms present in each mud immersion medium using the TPC test

In the TPC (Total Plate Counting) test, the procedure was carried out by laboratory staff in the InaCC (Indonesian Culture Collection) laboratory. The sample tested was in the form of a mud bath. The study aimed to see the ratio level of the number of bacteria that developed during the study. The procedure was carried out using the agar dispersion technique, with the following steps: A total of 1 mL of sample was inserted into a test 2 be containing 9 mL of sterile distilled water, and homogenized using the vortex ( $10^{-1}$  dilution). Then, 1 mL of the  $10^{-1}$  dilution was put into a test tube containing 9 mL of sterile distilled water ( $10^{-2}$  dilution). It was vortexed until homogeneous and a serial dilution was made to  $10^{-7}$ . After that,  $200 \,\mu$ L of suspension from  $10^{-1}$   $10^{-7}$  dilution was taken using a micropipette and put in a petri dish containing De Man, Rogosa and Sharpe agar (MRS agar) solid media. Each dilution was made in 2 replications. The suspension on surface of the MRS media was spread evenly using drygalski. The petri dishes were then incubated at room temperature for 24-48 hours. The bacterial colonies that grew on the surface of the media were counted, and the abundance of the number of bacteria was obtained by multiplying the number of bacterial colonies that grew by 10. It was then multiplied again by the dilution factor.

#### Data Collection and Data Analysis

The data of this research are the frequency of encounters of peeling leaf tissue, which is adopted from the formula for calculating the frequency of encounters with lichens by Arifwibowo (2017) and Soerianegara and Indrawan (1998). The formula that has been modified is as follows:

$$FPPJD = \frac{\text{the length of peeling leaf}}{\text{the length of leaf}} \times 100\%$$

Information:

FPPJD: the frequency of the exposure of leaf tissue exfoliation

Data were collected using squares with a scale of  $10 \times 10 \text{ cm}^2$ , in 1 square consisting of 100 samples and each sample has a length of  $1 \times 1 \text{ cm}^2$ . To simplify the percentage calculation, coding was used for each sample affected by the leaf. Code number 1 indicated "peeling was found" while for code number 0 means that "there was no peeling part" in the snippet.

The data obtained were then analyzed using the SPSS version 25. The prerequisite test results showed that the data were abnormal and not homogeneous so that the hypothesis test was continued with the Kruskal Wallis test which aimed to determine the effect of the treatment given on the peeling of the leaf tissue that occured. A follow-up test in the form of the LSD (Least Significance Different) test was carried out to determine whether there were differences in the effect between each mud immersion medium.

#### 3. Result and Discussion

The data in this research were in the form of: 1) data on the frequency of encounters with peeling leaf tissue; 2) data on physical properties of river, pond and gutter soaking media, and data on physical properties measured in the form of pH and water temperature and. 3) data on the number of microorganisms in river mud media, ponds and gutters tested using the TPC (Total Plate Counting) technique.

#### Data on the Frequency of the Exposure of Leaf Tissue Exfoliation

Data on the frequency of the exposure of leaf tissue exfoliation is the average percentage of each medium that was calculated. This can be seen in Figure 1.

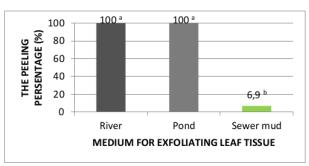


Figure 1. The frequency of the exposure of leaf tissue exfoliation

Figure 1 shows that river and pond mud immersion had a peeling percentage of 100% while sewer mud immersion had an exfoliation percentage of 6.9%. These findings indicated that river and pond mud immersion had a great potential as a medium for exfoliating leaf tissual than soaking sewage mud.

In the Kruskal Wallis test data, it was obtained the Asymp value. Sig.  $0.\overline{000}$  <0.05, which means that  $H_1$  was accepted and  $H_0$  was rejected. This indicated that there was a significant difference in the effect between various kinds of mud immersion media in the process of exfoliating the soursop leaf tissue.

The results of the LSD test showed that the river and pond did not have a significant difference in effect. However, the results of the ditch on the river or ditch on the pond were different, both of which had a significant difference in effect.

In the photographic evidence, it was found that the river mud had very clean flaking tissue, as shown in Figure 2.



Figure 2. Immersion Result in River, Pond and Sewer Mud

#### Data on Physical Properties of River, Pond and Gutter Soaking Media

The physical data of the mud immersion obtained were temperature and the degree of acidity (pH) of the mud immersion during the study. The average temperature in each medium included soaking rivers (30.4oC), ponds (30.5oC) and gutters (30.4oC). The pH value of river mud immersion was 7.2, pond 6.9 and ditch 5.5. The data showed that rivers and ponds had a neutral pH, while sewers had a more acidic pH.

#### Data on Physical Properties of River, Pond and Gutter Soaking Media

Data on the number of microorganisms was used to determine the number of microorganisms present in each sludge immersion medium in units (CFU / ml). The counted microorganisms were bactia, where each bacteria was counted using the TPC technique. Data on the number of bacteria per mL, can be seen in Table 1.

Table 1. Data on the number of bacteria in each immersion medium in units (CFU / mL).

Sample X	Numbers of Bacteria (CFU/mL) 5,8 x 10 <sup>3</sup>	
River Immersion		
Sewer Soaking Water	$6.0 \times 10^4$	
Pond Water	Cannot be counted (Full)	

Source: INaCC LIPI Laboratory, Cibinong Biological Research Center

The number of bacteria in each immersion medium has differences as shown in Table 4. The river mud had a very small number of bacteria compared to soaking ditches and ponds. However, the exfoliation that the river soaked leaves had was very good.

The Kruskal Wallis test results showed that there was an effect of various immersion media on the exfoliation of leaf tissue. In Figure 1, it was found that by calculation, the percentage of leaf peeling in river mud and ponds had the same percentage. However, Figure 2 shows that leaves from the river mud immersion were peeled off completely and did not leave any peeling residues, while the leaves from the soaked pond mud did not have perfect exfoliation. This showed that river mud had the best quality as an immersion medium for exfoliating leaf tissue.

The clean exfoliation of soursop leaf tissue soaked in mud media was caused by several factors, including: the thickness of the leaf tissue, the types of microorganisms that played a role, and the length of soaking time. The main factor in exfoliating leaf tissue was the type of microorganism that acts as a decomposer. This factor is crucial because decomposer microorganisms can help the process of peeling leaf tissue. As written by Waluyo (2013), leaves that fall and directly enter the water will settle at the top sludge, which enable decomposing microorganisms to use leaves as a source of decomposition.

Microorganisms can break down soursop leaf tissues because in the soursop leaves contain compounds that can be broken down, such as cellulose compounds, carbohydrates and fats. As described by Devianti & Indah (2017), several studies report that several chemical properties such as the initial content of Lignin, cellulose and carbohydrates significantly influence the level of leaf litter decomposition. According to Suradinata (1998), the main compounds in the cell wall are cellulose, hemicellulose, pectin and lignin, which are present in cells with varying amounts based on the nature of the cell concerned. These compounds can be processed by microorganisms as a source of energy for their survival.

Suitable environmental conditions can also support the growth of decomposing microorganisms, which are characterized by the presence of certain temperature and pH. In Suwatanti & Widiyaningrum (2017) and Indriani (2007), it is explained that the optimal temperature in the decomposition process is 30-50oC. On average, the three immersion media had a temperature of not more than 31oC, meaning that each medium had undergone a decomposition process. Nonetheless, the results of the soaked gutter leaves did not have good peeling, meaning that temperature was not the main factor causing the increase in decomposing microorganisms.

The difference in pH for each sludge medium is also an important factor, as written in Widarti et.al. (2015) which states that the optimum pH for the decomposition process ranges from 6.5 to 7.5. In the average pH results of each medium, the pH of the rivers and ponds were more neutral, namely 7.2 and 6.9, making the process of decomposing leaf tissue to occur perfectly. However, in Figure 2, it can be seen that the results of river peeling were cleaner than the pond. This is because the more neutral the pH is, the more optimal the work of microorganisms is in breaking down the soursop leaves. Widarti et.al. (2015) also explained that the pH of the ripe decomposition is usually close to neutral. The more neutral the pH is, the more optimal the peeling of the leaf tissue is. In contrast, however, the average pH of the

sewer tends to be more acidic. This is possible because when pH is too acidic it will inhibit the performance of decomposing microorganisms.

The temperature and pH of the river mud immersion were at optimal limits, making the leaf decomposition to be perfect. This was probable due to optimal conditions in the river mud immersion, which made more and more microorganisms. One of the microorganisms that play an important role in the process of decomposing organic materials in soursop leaves is decomposer bacteria. As stated by Mufaidah et.al, bacteria as decomposers play an active role in providing nutrients in the bottom of the waters such as organic sedimentary materials that settle on the bottom of the waters.

The sludge that has been used as a soaking medium for soursop leaves was also subjected to a TPC test as shown in Table 1. The amount of bacteria content associated with the percentage of leaf peeling in Figure 1 explains that the bacterial content in river mud immersion was less than the bacterial content in sewer sludge. This was possible because the counted bacteria can come from bacteria that do not act as decomposition but rather disease bacteria or pathogens, as also discovered in Aditama et.al. (2013) who explained that sewer mud is identical to dirtiness, namely the smell is rotten and stinging with germs.

Pathogenic bacteria played a role in spreading disease and they did not act as decomposers. This was also supported by the pH of the sewer media which tended to be more acidic, making the decomposer bacteria to not work optimally. The TPC test on the pond sludge immersion media showed that the pond bacteria content was so large that it was countless. In the results of calculating the percentage of peeling leaves, the pond mud media showed that it has the potential to be a good immersion medium. Nevertheless, Figure 2 showed that the results of soursop leaves soaked using pond mud were not as good as soursop leaves in river mud immersion. This was possibly because several bacteria that acted as decomposers and pathogens had the same number.

The pH of the pond sludge immersion tended to be close to neutral, which is the optimal factor for the work of decomposer bacteria, but this pH may also trigger the growth of pathogenic bacteria that have optimal growth in the pH range.

In the TPC test results, the river immersion media had a smaller number of bacteria compared to the pond and sewer sludge soaking media, while the pH of the river soaking tended to be more normal than the two soaks, this made the performance of the short bacteria work more optimally. The amount of bacteria contained in the river mud bath was less, but in Figure 2 showed that the river soaking had good potential in exfoliating leaf tissue. This is possible because most of the bacteria act as decomposers.

The exfoliation of leaf tissue was not only based on the number of bacteria that played a role, but also the presence of other microorganisms. Therefore, a system is needed to involve various types of microorganisms, namely the simba system. According to Waluyo (2013), the simba system is a symbiotic system of life between two or more bodies during biological or microbiological processes (Kant, 2018). The simba system applied turned out to be beneficial in terms of time, yield, efficiency and the stability of the use of raw materials during the process compared to a single system that only used one type of culture in the process.

Each microorganism has a different role to play in influencing the peeling of leaf tissue. Fungi have their own role in penetrating the cuticles and epidermis of plants. According to Nurwahyuni et al. (2015) and Struck (2006), mold hyphae can enter plant tissue without damaging the epidermal tissue because hyphae only penetrate the cuticle layer by releasing the enzyme cutinase, which is a biocatalyst, in the process of cuticle degradation (Grübel et al, 2014). Afterwards, the mycelium grew between the cuticles and the cell walls of the epidermis. Meanwhile, according to Waluyo (2013), aquatic fungi can digest protein, sugar, starch and fat, in addition to digesting pectin, hemicellulose, lignin and chitin.

Meanwhile, the leaf cuticle that has been penetrated by fungal hyphae can facilitate the entry of bacteria that play a role in the process of destroying the inside of the leaf such as palisade tissue and sponges. The composition of the leaf mesophyll is also mostly composed of carbohydrates and cellulose, making it easier for bacteria to digest and process parts of the leaf mesophyll. This showed that fungi and bacteria had an important role in decomposition.

The bone of soursop leaves would be left behind and very difficult to destroy by microorganisms. According to Waluyo (2013), lignin is a component of wood and in anaerobic conditions lignin is very resistant to microbial attack.

#### 4. Conclusion

The type of sludge characteristics and its pH are important factors in determining the quality of the exfoliating results of leaf tissue.

#### 5. Acknowledgement

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