Application of fungi *Aspergillus* sp. from leaf litter *Rhizophora mucronata* Lamk. to accelerate decomposition on various salinity levels in Belawan

Yunasfi¹*, N Hakiki¹ and I E Susetya²

¹ Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Medan, Indonesia
² Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia

*E-mail: yunasfi@usu.ac.id

Abstract. The decomposed leaves of *R. mucronata* are a source of organic material or nutrients for plants and various types of biota associated in the Belawan Mangrove Area. One of the microorganisms that play a role in the decomposition process is *Aspergillus* sp. The purpose of this study was to determine the acceleration of the rate of decomposition and measure the carbohydrate and protein content of *R. mucronata* leaf litter that had been decomposed by *Aspergillus* sp at various levels of salinity in Belawan. This research was conducted in June 2019 - March 2020. The average litter decomposition rate was obtained using the Olson Formula (1963): \( \ln \left( \frac{X_t}{X_0} \right) = -kt \), analysis of carbohydrate and protein content using SNI methods 01-2891-1992. The fastest decomposition rate of *R. mucronata* leaves is at station 1 with salinity 0-10 ppt which is 10.02 / year and the lowest is at station 2 with salinity 11-20 ppt which is 7.51 / year. The highest average carbohydrate content at station 1 is 7.02 and the lowest content of station 2 is 5.18%. The highest average protein content at station 3 ppt is 5.62% and the lowest is station 2 which is 4.28%.

1. Introduction
The current condition of some mangrove forests in Indonesia is not good. Mangrove forest damage is caused by various factors, such as reclamation, conversion into settlements, ponds and so on. One of the most pronounced impacts due to the absence of mangroves was when the Tsunami occurred in Nangroe Aceh Darussalam and North Sumatra Provinces. Areas that are overgrown by mangroves are relatively more protected than other areas that are not overgrown with mangroves [1].

Mangrove ecosystems are a type of forest that grows in tidal areas that are flooded at high tide and free from inundation at low tide whose plant communities are tolerant of salt. Mangrove weathering materials come from mangrove tree organs, namely leaves, flowers, branches, twigs and a number of other tree parts that fall to the forest floor called litter [2].

One of the fertility factors in mangrove ecosystems is leaf litter that falls and undergoes a decomposition process. The results of decomposition contribute to organic material that plays a role in the growth and development of plants, fish, shrimp, crabs and other microorganisms in mangrove forests. Litter decomposed by microorganisms will produce organic material that is absorbed by plants and some will be dissolved and carried water recede into the surrounding waters. One of the microorganisms that play a role in the decomposition process is fungi [3].
Fungi are one of a group of microorganisms that play a very important role in the process of decomposition of plant material litter. In addition to fungi, other groups of organisms such as bacteria, worms, crabs and others, as well as environmental factors also influence the process of litter decomposition. Utilization of various types of fungi which is estimated to play a role in the decomposition process of mangrove leaf litter is one of the efforts that can be used to exploit the biological potential found in mangrove ecosystems [4]

This study applied isolates of *Aspergillus* sp. obtained from the decomposition of *R. mucronata* leaf litter in the previous year. Therefore, the present study aimed (1) to determine the acceleration of the decomposition rate of *R. mucronata* leaf litter by fungi *Aspergillus* sp. at various levels of salinity in Belawan, (2) measure the content of carbohydrates and protein in leaf litter *R. mucronata* which has been decomposed as fungi *Aspergillus* sp. at various levels of salinity in Belawan.

2. Materials and Method

2.1. Time and location
This research was conducted in December 2019 - March 2020. The collection of *R. mucronata* leaf litter was carried out in Belawan. Determination of the dry weight of *R. mucronata* leaf litter was carried out at the Forest Cultivation Laboratory, Faculty of Forestry, University of North Sumatra. Determination of carbohydrate and protein content is carried out at the Medan Industrial Research and Standardization Center. The locations of stations 1 - 3 can be seen in Figure 1.

![Figure 1. Location of the research station. (a) salinity 0 - 10 ppt, (b) salinity of 11 - 20 ppt, (c) salinity of 21 - 30 ppt.](image)

2.2. Tools
The tools used in this study are litter bags made of nylon with 1 mm mesh size, aluminum foil, permanent markers, hand refractometer, Petri dishes, ovens, analytical scales, sewing needles, sewing thread, razor blades, ropes, gloves, cameras, cameras digital, Kjeldahl flasks, distillers, electric heaters / burners, Erlenmeyer flasks, upright coolers, measuring flasks, funnels, goiter pipettes, stop watch, measuring cups, burettes and drip pipettes.
The material used in this study was *R. mucronata* leaf litter, fungi isolates type *Aspergillus* sp. obtained from the previous year's experiment, 70% alcohol, name label, sample envelope, sterile water, selen mixture, Conway indicator, boric acid solution 4% H$_3$BO$_3$, HCl 0.1 N, 40% NaOH, 3% HCl, 30% NaOH, Litmus paper, PP indicator, Luff-Schoorl solution, 20% KI, 25% H$_2$SO$_4$, 0.1 N Na$_2$S$_2$O$_7$ and 0.5% starch solution.

2.3. Determination of location
The study was conducted in the Belawan mangrove area. Determination of the station point is done by measuring the level of salinity using a refractometer. Station 1 with salinity 0 – 10 ppt, station 2 with salinity 11 – 20 ppt, station 3 with salinity 21 – 30 ppt. The technique of collecting data by using purposive sampling (taking data through consideration) is to determine 3 points of observation station based on differences in salinity.

2.4. Research procedure

2.4.1. *Rhizophora mucronata* leaf litter collection in the field
As much as 50 g of *R. mucronata* leaf litter is put into a 40 x 30 cm litter bag and made of nylon with 1 x 1 mm mesh. The number of bags containing litter prepared was 54 bags (18 bags x 3 levels of salinity).

2.4.2. Fungal application on leaf litter
The fungi isolate that will be used is *Aspergillus* sp. This isolate was first rejuvenated by planting it on PDA media until it had sufficiently grown. Each type of fungi that has grown on this PDA media is then taken by cutting to 5 x 5 x 2 mm. These pieces of agar that have been overgrown with fungi are then put into test tubes filled with 2.5 ml of sterile water to be used as suspensions. As much as 2.5 ml of the suspension are spread evenly on the leaf litter found in the litter bag.

2.4.3. *Rhizophora mucronata* leaf litter placement in the field
Litter bags containing *R. mucronata* leaf litter are placed in a field that has various levels of salinity, according to treatment. Previously the level of salinity has been measured using hand refractometer. Determination of the station point is done by measuring the salinity level using a refractometer. Station 1 with salinity 0 – 10 ppt, station 2 with salinity 11 – 20 ppt, station 3 with salinity 21 – 30 ppt. Determination of the coordinates of the station is done using a GPS (Global Positioning System). A total of 54 litter bags were divided into 18 for each salinity, each containing 50 g of leaf litter *R. mucronata* is placed randomly by binding the four corners of the litter bag at the root or base of the nearest tree trunk. The old data collection period of decomposition is done after the litter is placed in the field with various levels of salinity, during the following time: days – 15, days – 30, days – 45, days – 60, days – 75, days – 90. For each observation, 3 sample bags of litter bags were taken for up to 75 days, and each time 3 observations were made.

2.4.4. Analysis of *R. mucronata* leaf litter decomposition rate
The litter left in the bag which comes from each level of salinity taken every 15 days is cleaned of mud and then air dried. Litter that has dried in the wind is put into the oven at 75 °C for 3 days or until the weight is constant. The dry weight value obtained is used to determine the rate of decomposition based on the weight of litter in the bag before and after decomposition. The litter decomposition rate is obtained using the formula [5]:

\[
R = \frac{W_0 - W_t}{T}
\]

Where:
\[
R = \text{decomposition rate (g/day)}
\]
\[
T = \text{observation time (days)}
\]
Wo = initial litter dry weight (g)
Wt = dry weight of litter sample after the t-observation time (g)

Estimation of the average value of litter decomposition rates is carried out according to the following equation [5]:

\[ X_t = X_0 \cdot e^{-kt} \]
\[ \ln \left( \frac{X_t}{X_0} \right) = -kt \]  

Where:
Xt = litter dry weight after the observation time (g)
X0 = initial litter weight (g)
e = natural logarithm number (2.72)
k = value of litter decomposition rate
t = observation time (days)

2.5. Analysis of carbohydrate and protein content found in R. mucronata leaf litter experiencing decomposition at various salinity levels

2.5.1. Carbohydrate content analysis
Carbohydrate content in R. mucronata leaf litter which decomposed can be determined by using the method of SNI 01-2891-1992. Hydrolysis of carbohydrates into monosaccharides which can reduce Cu\(^{2+}\) to Cu\(^{1+}\). The excess of Cu\(^{2+}\) can be controlled by Yodometry. The content of carbohydrate in the decomposition of R. mucronata leaves can be determined by first calculating the ash content in the following way:

Weigh carefully about 5 g of the sample into a 500 ml erlenmeyer, then add 200 ml of 3% HCl solution, simmer for 3 hours with an upright cooler. Cool and neutralize with 30% NaOH solution (with litmus and phenolphthalein) and add a little CH\(_3\)COOH 3% so that the atmosphere of the solution is slightly acidic, then transfer the contents into a 500 ml measurement and squeeze until the line mark and then strain. Pipette 10 ml filter into a 500 ml erlenmeyer, add 25 ml Luff Schoorl solution (with a pipette) and some boiling stones and 15 ml distilled water. Heat the mixture with a steady flame, try so that the solution can boil within 3 minutes (use a stop watch), continue to simmer for exactly 10 minutes (counted when starting to boil) then quickly cool in a tub of ice. After chilling add 15 ml of 20% KI solution and 25 ml of 25% H\(_2\)SO\(_4\) slowly, then pull the turret immediately with a 0.1 N tio solution (use a 0.5% starch solution pointer) also do the blank. Calculation: (Blank titers) x N tio x 10, equivalent to reduced irrigation. Then look in the list of Luff-Schoorl how many mg of sugar is contained for ml of tio used.

\[ \text{Glucose content} = \frac{W_1 \times f_p}{W} \times 100\% \]

\[ \text{Carbohydrate content} = 0.9 \times \text{glucose content} \]  

Where:
W1 = sample weight
W = glucose contained for ml used (mg)
fp = dilution factor

2.5.2. Protein content analysis
For protein analysis, it is carried out using the Semjikro Kjeldahl method SNI 01-2891-1992. The Kjeldahl method is a simple method for the determination of total nitrogen in amino acids, proteins and nitrogen-containing compounds. Protein analysis of the Kjeldahl method can basically be divided into three stages: (1) the destruction stage, (2) the distillation stage and (3) the titration stage.
This method is suitable for semimicrobial use, because it only requires a small number of samples and reagents and a short analysis time.

The detailed analysis method is described as follows: weigh 2 g of the sample, put it in a 100 ml Kjeldahl flask, then add 1 g of the selenium mixture and 25 ml concentrated H_2SO_4, then heat it on an electric heater or an incendiary flame until it boils and the solution becomes clear greenish (about 1 - 2 hours). Allow to cool, then dilute and put into 100 ml measuring cup, right up to the line mark.

Pipette 25 ml of solution and put it into a distiller, add 5 ml of 40% NaOH and a few drops of PP indicator. Flute for about 9 minutes, as a container to use 25 ml of 4% boric acid which has been mixed with the Conway indicator. Rinse the cooling end with distilled water. Then titrate with 0.1N HCl solution. Work on blanking.

Determination of protein content can be done by the formula:

\[
\text{Protein content} = \frac{(V_1 - V_2) \times N \times 0.14 \times f_k \times f_p}{W}
\]  

Where:
- \(W\) = sample weight
- \(V_1\) = HCl volume of 0.1 N used in the sample spin
- \(V_2\) = volume of HCl used in blanking
- \(N\) = normality of HCl
- \(F_k\) = protein from food in general 6.25, milk and processed products 6.38, peanut oil 5.46
- \(F_p\) = dilution factor

3. Results and Discussion

3.1. Decomposition rate

Rhizophora mucronata leaf litter which underwent a decomposition process for 90 days was marked by a reduction in dry weight and different physical changes at each observation station. The changes in physical shape from intact to small pieces that can be seen in Figure 2. Based on the data of weight loss or residual litter of R. mucronata leaves in Figure 2 can be known the average rate of leaf litter decomposition R. mucronata periodically (g/day) can be seen in Table 1.

| Station | Time (Days) | Average |
|---------|-------------|---------|
| 0       | 15          | 0.47    |
| 1       | 0           | 0.47    |
| 2       | 0           | 0.47    |
| 3       | 0           | 0.49    |
| Average | 0           | 0.49    |

From the table above it can be seen that the rate of leaf litter decomposition. The highest R. mucronata is at the beginning of the composition of the 15th day and then continues to decline periodically until the end of the observation time of the 90th day. The average decomposition rate at the beginning of the observation is known 2.45 g/day and at the end of the observation of 0.49 g/day. The leaf litter of R. mucronata undergoing a decomposition process was observed once every 15 days until reaching the 90th day. Depreciation of R. mucronata leaf litter dry weight varies at each station and the length of time observed. Changes in dry weight loss of R. mucronata leaf litter can be seen in Figure 3.
Figure 2. The rest of the litter decomposed during 90 days. (a) 15th day, (b) 30th day, (c) 45th day, (d) 60th day, (e) 75th day and (f) 90th day.

Figure 3. Average decomposition of *R. mucronata* leaves after 90 days.

The highest weight loss of leaf litter from day 0 to day 90 was highest at station 1 with a salinity level of 0 - 10 ppt where the residual litter of *R. mucronata* leaves became 3.97 g with shrinkage of 46.03 g and percentage of decomposition rate 92.06% was followed by Station 3 with a salinity level of 21 - 30 ppt where the residual leaf litter was 5.91 g with a decrease of 44.09 g and a percentage of the decomposition rate of 88.18%. The lowest weight loss of leaf litter is at station 2 with a salinity level of 0 - 10 ppt where the remaining leaf litter becomes 7.50 g with a decrease of 42.50 g and a percentage decomposition rate of 85%. The value of the decomposition rate constant (k) of *R. mucronata* leaf litter from each station with various levels can be seen in Figure 4.
In Figure 4 shows the constant value of the highest decomposition rate of *R. mucronata* leaf litter at station 1 with a salinity level of 0 - 10 ppt is 10.2 while the lowest is at station 2 with a salinity level of 11 - 20 ppt which is 7.5. This shows that the level of salinity affects the rate of decomposition of *R. mucronata leaf* litter. According to [1] the level of sea water salinity affects the decomposition of mangrove leaf litter. The lower the level of salinity of sea water, the faster the decomposition process will occur.

It is known that the decomposition rate of station 3 is higher at 8.45 than station 2 which is 7.51. This is also influenced by the number of macrozoobenthos found in *R. mucronata leaf* litter bags at station 3 more than at station 2. Macrozoobenthos is an early composer which consumes *R. mucronata leaf* litter. This is in accordance with the statement of [6] which states that salinity will affect the number of macrobenthos that are in the waters because differences in salinity affect the diversity of species and the number of macrozoobenthos which serves to accelerate the rate of decomposition.

The application of *Aspergillus* sp. fungi also affects the decomposition rate of *R. mucronata* leaf litter because this type of fungi is known to be resistant in adverse environmental conditions than other microorganisms. According to [7] fungi are the organisms that produce the most degradative enzymes that directly attack all organic material. The existence of this degradative enzyme makes fungi a very important part in recycling natural waste and as a decomposer in the biogeochemical cycle. Suggested that fungi play an important role in the mangrove ecosystem, especially in relation to bacteria to accelerate leaf decomposition.

3.2. Macrozoobenthos

Macrozoobenthos are early composers who consume or chop up the remnants of leaf litter which are then re-released as impurities which are then processed by fungi and bacteria into proteins and carbohydrates. Macrozoobenthos species found in leaf litter bags *R. mucronata* can be seen in Table 2.

| Class     | Order      | Genus              |
|-----------|------------|--------------------|
| Bivalvia  | Venoroida  | *Polymesoda*       |
| Crustacea | Decapoda   | *Chiromantes, Penaeus* |
| Gastropoda| Mesogastropoda | *Telescopium*        |
| Turbellaria| Macrostromida | *Microstonum*     |
The existence of macrozoobentos will greatly affect the rate of decomposition. Litter bags placed at the observation station found many macrobenthos because they are a food source for macrobenthos. The number of macrozoobentos at each station is different from those of the Bivalvia class (shellfish), Crustacea (crab and shrimp), Gastropoda (snail) and Turbellaria (worm). This is consistent with the statement of [8] litter that fell to the forest floor did not immediately experience weathering by microorganisms, but needed the help of macrobentos. Macrzoobentos act as an early decomposer that works by chopping leaves into small parts followed by small organisms, namely microorganisms (bacteria and fungi) which break down organic matter into proteins and carbohydrates. The types of macrozoobentos contained in *R. mucronata* litter bags can be seen in Figure 5.

![Macrozoobenthos community](image)

**Figure 5.** Macrozoobenthos community: Gastropoda class (a), Crustacea class (b), Bivalvia class (c) Turbellaria class (d) and Crustacea class (e).

The highest number of macrozoobentos is found in the field, namely at station 3 with a salinity level of 20 - 30 ppt and the lowest at station 2 with salinity of 11 - 20 ppt which can be seen in Appendix 7. So this can affect the decomposition rate of *R. mucronata* leaf litter contained at station 3. According to [9] in mangrove forests macrozoobenthos themselves form a colony, some macrozoobentos require high salinity so that changes in salinity can make macrozoobentos die. Changes in salinity itself are affected by rainfall and tides.

### 3.3. Carbohydrate and protein content

The decomposition of *R. mucronata* leaf litter contributes organic material in the surrounding mangrove ecosystem. Fungi *Aspergillus* sp. which is applied to *R. mucronata* leaf litter can release enzymes that destroy complex organic molecules such as proteins and carbohydrates. The carbohydrate and protein content of *R. mucronata* leaf litter can be seen in Figure 6.

![Carbohydrate and Protein content](image)

**Figure 6.** Carbohydrate (A) and Protein (B) content in decomposed *R. mucronata* leaf litter.
The nutrient element C (carbon) is one of the forming of carbohydrate. Carbohydrates (CH$_2$O) include various organic ingredients, simple and complex sugars and sugar polymers, such as flour, cellulose and hemicellulose. During the short period of composition at various levels of salinity the levels of nutrient C have decreased, so this is related to a decrease in carbohydrate content in the leaf litter of *R. mucronata*. This is consistent with the statement of [10] that the nutrient C contained in mangrove leaf litter undergoing the process of decomposition at various levels of salinity also decreases with increasing levels of decomposition of leaf litter. From these results it can be seen that cellulose which is a component among various other components forming cell walls in the process of decomposition of litter breaks down and is released into the mangrove environment.

Application of *Aspergillus* sp. Fungi is known to release enzymes that destroy complex organic molecules such as proteins and carbohydrates from dead plants. The highest carbohydrate content in *R. mucronata* leaf litter is at station 1 with a salinity level of 0 - 10 ppt and the lowest is at station 2 with a salinity level of 11 - 20 ppt which has a slight difference with station 3 with a salinity level of 21 - 30 ppt. In Figure 6 it can be seen that the carbohydrate content of the *R. mucronata* leaf litter decreases during the short composition process.

The highest protein content of *R. mucronata* leaf litter during short composition was at station 3 with salinity 21 - 30 and the lowest was station 2 with salinity 11 - 20 ppt. From Figure 7, it is known that protein levels have increased during the short compositional period from the 15th day to the 90th day. The protein content of *R. mucronata* leaf litter is beneficial to the organism both in the process of tissue formation also plays a role in channeling energy and energy to breed or move for the organism. This is consistent with the statement of [11] that fat, protein, and carbohydrate are very beneficial for the organism because they are important food in the body, both used for growth and repair of tissues and are a source of energy.

4. Conclusion
The fastest decomposition rate of *R. mucronata* leaves is at station 1 with salinity 0 - 10 ppt which is 10.02 / year, followed by station 3 with salinity 21 - 30 ppt which is 8.45 / year and lowest at station 2 with a salinity of 11 - 20 ppt which is 7.51 / year. The highest average carbohydrate content in station 1 with salinity 0-10 ppt is 7.02%, followed by station 3 with salinity 21 - 30 ppt which is 5.19% and lowest station 2 with salinity 11 - 20 ppt which is 5.18%. The highest average protein content at station 3 ppt was 5.62%, followed by station 1 which was 5.26% and the lowest was station 2 which was 4.28%.

Acknowledgements
This study is funded by Kemenristekdikti DRPM Research Implementation under Contract Number: 93 / UN5.2.3.1/ PPM / KP-DRPM/2018.

References
[1] Yunasfi and Suryanto D 2008 Type - Types of Fungi Involved in Leaf Litter Decomposition Process Avicennia Marina at various level of salinity Research Journal of Mathematics 2 (1): 1-5.
[2] Naibaho R F, Yunasfi and Suryanti A 2015 *Avicennia Marina* Leaf Litter Decomposition Rate and its Contribution to Nutrition in Serambi Deli Waters in Labu Beach District The Aquacoastmarine Journal 7 (2): 13.
[3] Yulma Y, Ilshan B, Sunarti S, Malasari E, Wahyuini N and Mursyban M 2017 Identification of Bacteria in Littered Mangrove Leaves Decomposed in Mangrove and Bekantan Conservation Areas (KKMB) Tarakan City Journal of Tropical Biodiversity and Biotechnology 2 (1): 28-33.
[4] Fell, J.W., R. C. Cefalu, I. M. Masters dan A. S. Tallman. 1975. Microbial Activities in the Mangrove (Rhizophora mangle L.) Leaf Detrital Systems. Hlm. 661 – 679 dalam Proceedings of the International Symposium on Biology and Management of Mangroves. G.E. Walsh, S. C. Snedaker dan H. J. Teas (Peny.). Univ. Florida. Gainsville.

[5] Olson J S 1963 Energy Stroge and the Balance of Producer and Decomposer in Ecological System Ecology (44): 322-331.

[6] Panjaitan A, Djayus Y dan Siregar T 2015 The rate of decomposition of Rhizophora mucronata leaf litter and its contribution to nutrition in the waters of Serambi Deli beach, Pantai Labu sub-district Journal of Aquacoastmarine 10 (5): 1-11.

[7] Mc-Kane L 1996 Microbiology Applied and Practice Mc Graw Hill Book Company New York.

[8] Watumlawar Y, Sondak C, Schaduw J, Mamuaja J, Darwisito S and Andaki J 2019 Production and Decomposition Rate of Mangrove Litter (Sonneratia sp.) In the Bahowo Mangrove Forest Area, Tongkaina Village, Bunaken District, North Sulawesi Journal of Coastal and Tropical Seas 7 (1): 1-6.

[9] Thaher E 2013 Rhizophora mucronata litter decomposition rate with the application of Fungi Aspergillus sp. on various Salinities Essay University of Northern Sumatra.

[10] Yunasfi 2006 Leaf Litter Decomposition Avicennia marina by bacteria and fungi at Different Levels of Salinity Dissertation Bogor Agricultural Institute Bogor.

[11] Faldin, Nur A and Ramli M 2016 Study of detritus quality in the species of mangrove Rhizophora apiculata and Sonneratia albadi Lalowaru Village, North Moramo District, Konawe Selatan District Journal of Water Resource Management 2 (1).