Optimization on Pulp Delignification from Nypa Palm (*Nypa fruticans*) Petioles Fibre of Chemical and Microbiological Methods

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**Abstract.** Demand for *handycraftpaper* is getting higher lately. This paper is commonly utilized as decorative paper, interior decoration, frames, and many more. Handycraftpaper making was one alternative to waste treatment and to reduce the use of wood fiber as paper raw material. Nypa palm (*Nypa fruticans*) petioles which is usually thrown away has the potency to be processed as pulp because it has 42.22% of cellulose and 19.85% of lignin. Delignification with chemical and microbiological methods has been executed to find out the pulp with the lowest lignin, highest cellulose and highest yield. The chemical method used NaOH 1 N (with concentration of 5, 10, 15, and 20%) and H₂O₂, while the microbiological method used effective microorganisms 4 (EM₄) which was economical and easy to find. Fermentation time (5, 6, 7 and 8 days) was implemented as treatment. For chemical method, the treatment that produced the optimum response of lignin and cellulose was 20% of NaOH concentration, while for the microbiological method was 8 days of fermentation. Orthogonal polynomial model showed that both chemical and microbiological methods produce cellulose had linier model that P-Value was less than 0.05. For lignin response, the chemical method obtained linier model, meanwhile the microbiological method obtained quadratic linier model where P-Value was less than 0.05.

**Keywords:** agricultural waste, delignification, handycraftpaper

**Introduction**

Nypa is one of the mangrove plants, which lives in tropical climates and spread evenly throughout Indonesia. Nypa or *Nypa fruticans* is one of the trees belonging to Arecaceae (palm) of which generally grows in the good waters of the coast, lake, river, swamp (Teo et al., 2010). However, this plant has not been fully utilized, as there are only certain areas which only use its NIRA to be made as sugar and its leaves to make the roof of the house.

Nypa palm plantations throughout Indonesia are estimated to reach 700,000 ha or 10% of the tidal area of 7 million ha, with an average tree population of 8,000/h. It is a estimated that the total nypa population in Indonesia reaches 5,600 million trees (Baharudin and Taskirawati, 2009). In the area of East Java Province, the area of nypa deployment is in the area around the coast in Bawean Island, Madura and partly on the southern coast of East Java, while for the area of Malang it is widespread on the southern coast of Malang.

Nypa plants are still used mostly for its fruit and leaves. The waste of the utilization resulted in unutilized waste, one of which was the rotted petioles/midrib. From one nypa tree, there is a midrib which has withered and weathered for about 3 Kg. The content of cellulose and lignin on palm petioles is

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42.22% and 19.85% (Akpakpan, 2011). According to Baharudin and Taskirawati (2009) in one hectare there found approximately 8,000 trees which is estimated to have 48 tons of fruit leaf waste and petioles per hectare per year.

Nypapetioles has the potential to be made into pulp. This waste contains cellulose, so it is very potential and will be more useful if it is processed into products that can be applied to small industries having high economic value such as handycraftpaper. The manufacture of handycraftpaper itself begins with the manufacture of pulp. This pulp can also be used to make other products such handycraft carton, cardboard, and other products. The color of the appearance of this materials type is very potential to be used as raw material for handycraftpaper pulp.

The process of preparing the pulp is to liberate cellulose from undesirable lignin (Pooja et al., 2010). The pulp process degrades lignin, and separates it from cellulose by minimizing damage to cellulose. In the pulping process there are several methods of manufacture namely mechanically, chemically and semi-chemically. The optimal pulping process for non-timber plant fibers is the alkali process using NaOH, because with the NaOH solution the lignin content of the fiber is soluble. Chemical process is done by developing a process of soda, which is an alkaline cooking process with NaOH as the solution. NaOH solution also serves as an intercellular fiber breaker so it aims at accelerating pulp formation (Malo, 2004). In general, if the resulting fiber is dark, bleaching can be applied using H_2O_2 solution.

The presence of NaOH, both in the pulp and paper industry is one of the most indispensable components. From the research conducted by Saenah (2002) and Masluklah (2002), the best conditions of NaOH use of pulp and kenaf characteristics are 12% and 14%. In addition to Onggo’s and Triastuti’s (2005) research, about the effect of pulping process on the color of handycraftpaper from reeds, it takes 10% NaOH as optimal result. Based on the above research, appropriate use of NaOH may vary depending on the type of material.

Meanwhile, in addition to physical and chemical methods, there is also biopulping process. Biopulping is one of the pulp-making processes with wood or non-wood raw material, with the aim of separation and degradation of lignin by using microbiology process. In general, the delignification process done by chemical processed products are not environmentally friendly. The problem can be solved by using the method of microbiological delignification or biodelignification. The use of biotreatment can save energy during the manufacture of pulp (Keller et al., 2003).

The application of biodelignification or removal of lignin makes the texture and appearance of the pulp is better than undegigned. The two feedstocks have different lignin and cellulose levels, so they may require different biodelignification times. Biodelignification can use a variety of microorganisms to degrade lignin. The selected organism must be able to deliberately delineate within a certain time required (Pooja et al., 2010). The microorganisms used in this study are EM4 (Effective Microorganism-4) agriculture. The bacteria in EM4 are not included into pathogenic bacteria. EM4 itself contains photosynthetic bacteria, Lactobacillus sp., Streptomycetes sp., Actinomycetes, and yeast that have the ability to degrade lignin and break down the bonds of lignin and cellulose. EM4 is a product that is easy to obtain, applicable, and low cost.

According to Kasim et al. (2002), the use of EM4 can effectively degrade lignin in oil palm empty bunches at 5-day incubation period of lignin degradation rate can reach 46.85% and cellulose degradation rate of 14.47%. Long observation of ripening need to be done to know the best time in lignin degradation on pulp making process with raw material of fruit skin and nypa bark. Based on these things it is important to do a research to find out the best raw materials used and the duration of the curing that will be associated with the ability of EM4 in degrading lignin and the extent to which degradation of cellulose in the manufacture of pulp.

The purpose of this research is to know the optimum treatment to produce pulp from nypa petioles with chemical and microbiological method. The result of this research is expected to be able to get the right process condition to produce the quality pulp so that it can become the alternative of paper industry raw material, reduce the exploitation of forest wood which has become the main raw material in papermaking industry and able to supply from total requirement of pulp for handycraft paper in Indonesia.
Materials
The main ingredients used in this research is nypa petioles obtained at Tamban Beach, South Malang, East Java, Indonesia.

1. Equipment and Materials on Chemical Methods
The equipment used in the process of making pulp in this research are beaker glass, hot plate, magnetic stirrer, stopwatch, blender, scissors, knife, measuring cup, digital scales and filter cloth. While the instruments used to test lignin and cellulose are water bath, refrigerant, scales, vacuum pump and oven.

The supplementary materials used include sodium hydroxide p.t (pro technic) with purity of 78%, well water and hydrogen peroxide p.t (pro technic) with purity 35%. While the material used for testing the levels of lignin and cellulose is H$_2$SO$_4$ p.a (pro analyst) with a purity of 98% and H$_2$O.

2. Equipment and Materials on Microbiological Methods
Equipment used in the manufacture of pulp are curing cabinet, plastic bottle, cutting knife, plastic bag for cover, container for material, digital scales, filter cloth, blender, stop watch for measuring time, and measuring cup. The tools used in pulp testing are cooling back, oven, hot plate, filter paper, digital scales, labuelsenmeyer, pumpkin measure, stopwatch.

Materials used in the manufacture of pulp are EM4 (Effective Mikroorganism 4 with concentration (10 mL EM4: 250 mL Water)) Agricultural production of PT Songgolangit Persada, and well water. Materials for pulp testing include H$_2$SO$_4$ (PA) and aquadest.

Methods
The research used quantitative method using complete randomized design and experimental analysis, followed by multiple attribute analysis. The data analysis used SPSS software. Repetition is done three times (Gomez, 1984). To know the model of the relationship between treatment and response (cellulose and lignin levels) then orthogonal polynomial analysis is used. Here are the research procedures with each method.

1. Chemical Method
The treatment used was the concentration of NaOH (N) solution, which consisted of 4 levels: The concentration of NaOH solution 5%, 10%, 15%, and 20% (v/v). The process of preparing pulp from petioles fiber chemically is as follows. Nypa bark is reduced in size to be chips in 2-3 cm then dried up to 10% moisture content. The dried nypa was weighed 20 grams. The material was delignified ie cooking using 500 ml of water and NaOH solution at concentrations of 5%, 10%, 15% and 20% for 1 hour with a temperature of 100°C. The subsequent treatment was the material of the delignification process carried out during blending 5 minutes to smoothen the fibers into a pulp form of fiber and afterwards the material can already be called pulp. Pulp was filtered and cleaned from the rest of the chemical solution. Dark pulp was enlightened using hydrogen peroxide and sodium hydroxide solution each 2% until the pH reached 8-12, since in this process it will be more optimal if done at alkaline pH, then heated for 1 hour at 80°C. Pulp temperature that has been enlightened then analyzed which includes lignin and cellulose with Chesson Method.

2. Microbiological method
The experimental design used was Completely Randomized Design with 5 days (24 hours), 6 days (72 hours), 7 days (120 hours), and 8 hari (168 hours) treatment. Nypa rupture is cut into small pieces between 3-5 cm, then dried with oven to 10% water content for optimum water content of the ingredients done by curing (Kasim et al., 2002). The next process is to make the EM4 mix used for the curing process. EM4 Agriculture used as much as 10 mL diluted to 250 mL water according to the best treatment (Kasim et al., 2002) was modified without adding sugar. The mixture of EM4 and water was mixed evenly and allowed to stand for approximately 12 hours. Once the raw material and starter EM4 is ready, it is continued for microbiological pulp making. Nypa bark is reduced to about 120 grams of chips 1-2 cm. Each type of fruit peel and palm leaf is divided into 30 grams. The diluted material and EM4 are mixed by spraying with a ratio of 120 grams of material: 100 mL starter EM4, then inserted in a container, sealed.
with a hollow fabric to keep the air exchange in place. The grating is carried out by the reversal and stirring process every 12 hours. The duration of curing was modified from Kasim's (2002) study using a long-lasting curing period of 5 days. Then it is continued by ripening in this research that is 5, 6, 7, and 8 days of ripening. Each curing takes the material and proceeds to make the pulp. The material is blended for 5 minutes with 250 mL water to separate the fiber bonds and separate the existing lignin. After that the pulp is washed with 1 liter water and filtered with a filter cloth until the water is clear or not red brownish. Then the material was tested for its lignin content, cellulose using Chesson Method.

**Results and Discussion**

1. **Chemical Method**

   The amount of cellulose produced by the pulping process using NaOH solution at concentrations of 5%, 10%, 15% and 20% was from 38.21 to 41.39%. Table 1 also shows that the higher concentration of NaOH solution the pulp cellulose content of the nypa fiber decreases. The main purpose of the pulping process is to degrade and dissolve the lignin as much as possible through the delignification process. However, during the delignification process, the possibility of damage and dissolution of cellulose components (polysaccharides) cannot be avoided (Sjostrom, 1995; Fengel, 1995 and Laksono, 2008).

   | Material     | Concentration of NaOH | Repetition 1 (%) | Repetition 2 (%) | Repetition 3 (%) |
|--------------|------------------------|------------------|------------------|------------------|
| Nypa Palm    | 5%                     | 41.39            | 40.77            | 40.26            |
|              | 10%                    | 39.82            | 39.61            | 39.71            |
|              | 15%                    | 38.86            | 38.95            | 38.53            |
|              | 20%                    | 38.42            | 38.81            | 38.28            |

   Pulp cellulose concentration in nypa bark after hydrolysis decreased, it is suspected due to hydrolysis of the amorphous portion of cellulose (polysaccharides) into oligo-saccharides and monosaccharides. According to Tsao et al., (1978), the amorphous part of cellulose is more easily hydrolysed than the crystallized portion. Cellulose is made up of 15% amorphous parts and 85% of the other is crystalline. Another thing that causes cellulose to degrade is due to the reaction of cellulose in the form of fiber when reacted with alkali, raw material fragments will swell or expand to a certain extent depending on the concentration of alkali and reaction temperature. According to Akhlus (2003), the NaOH solution as the ingredient develops into the crevices of fibers then binds to cellulose because cellulose having free hydroxide groups will have strong affinity with the polar solvent. In the process of developing cellulose, it causes hydrogen bonding between cellulose broken so that cellulose is degraded and the breaking of hydrogen bond with cellulose will be replaced with hydrogen bond from water and NaOH. Cellulose degradation occurs due to the hydrolysis process, and in the process of hydrolysis causes cellulose structures to fragment, break down and dissolve as the concentration of chemical solution increases.

   The average graph of kadaselulosa due to the influence of NaOH solution concentration with orthogonal polynomial method can be seen in Figure 1. In Figure 1 we can see the relationship between the concentration of NaOH solution and the cellulose content is linear.
According to Table 2, the value of pulp lignin content of nypa palm fiber as the concentration of NaOH solution was 6.87% to 9.24%. The higher concentration of NaOH is given, the lignin content obtained is lower. This is supported by Heradewi’s (2007) research, that the higher NaOH concentration will dissolve the larger lignin because NaOH catalyst serves to develop the pulp raw material structure, thus facilitating the penetration the cooking solution into the chips and the breaking of bonds between lignin molecules accelerates so that the lignin degradation is higher and dissolves quite a lot.

The lignin content of the midget fiber and the nypa fruit skin fibers resulting from the delignification process are closely related to the amount of concentration of the chemical solution in the delignification process. According to Sjostrom (1995), one that affects the success of the cooking or delignification process is the penetration of cooking chemicals into the structure of the chemical components of wood. Therefore, the possibility of other factors causing high levels of lignin pulp of nypa fiber is the lignin structure in the raw materials used.

Based on its structural elements, lignin can be divided into several groups namely guaiasil lignin and siringil lignin (Ahcmadi, 1990). Most woods with similar levels of lignin but have different rates of delignification. There is a difference in the rate of delignification in different types of wood because of the relationship that is closely related to the lignin-guided racillary ratio and the concentration of chemical solutions such as NaOH used in the pulping process. The more proportion of the lignin constituents, the higher the delignification rate (Gonzales et al., 1999 and Delrio et al., 2005).
The ease of the raw material in the pulping process is related to the proportion of the siringil lignin unit. The smaller lignin content of pulp, especially from the type of raw material of nypa fiber, means that the proportion of santyl lignin contained therein is considerable for the lignin degradation rate at the time of the delignification process as the NaOH solution concentration increases also higher.

In addition to lignin levels, the ease of pulping process is also influenced by lignin reactivity. High lignin reactivity will accelerate the rate of delignification and the greater the amount of soluble lignin. The rate difference of delignification during the pulping process is related to the type and proportion of lignin constituent monomers in bakupulp material (Gonzales et al., 1999 and Delrio et al., 2005). Differences in the value of the syrup and guaiasil ratios indicate the reactivity of different lignin constituent components and will affect the pulping process, especially in the delignification stage or the degradation and lignin dissolution process.

In the manufacture of the pods the cause of delignification is hydroxyl ions. Reactions during pulping are degradation and condensation of lignin and the first to be attacked are α ary ether (α-O-4) and aryl glycerol-β ary ether (β-O-4) (Fangel, 1995 in Wardoyo, 2001). Another factor that causes dissolved lignin is during enlightenment. The process in this study used a solution of Hydrogen Peroxide and NaOH at pH 8.87 as a catalyst. Hydrogen peroxide reacts optimum with lignin under alkaline conditions. The active ingredient in the pulp fiber enzyme brightening reaction and the nypa fruit leaf fiber is the ion of perhydroxyl (OH⁻) and the hydroxyl (OH).

The average graph of lignin content due to the influence of NaOH solution concentration by orthogonal polynomial method can be seen in Figure 2. In Figure 2 we can see the relationship between NaOH solution concentration and lignin level is linear.

![Figure 2. Average Graph of Lignin Content affected by NaOH Concentration](image_url)

Based on multiple attribute analysis, the optimum treatment was obtained at 20% NaOH concentration with average cellulose concentration of 38.50% and average lignin level of 7.02%. The yield of this treatment was 27.9%.
2. Microbiological method

The cellulose content of the nypa pulp can be seen on Table 3. It is known that the high cellulose content is with the duration of curing with EM4 for the 5th day of the lowest at 8 days of curing. This means that longer curing time is more likely to affect the decrease in end cellulose levels. This is due to Streptomyces and Actinomycetes bacteria in EM4 using cellulose as a source of energy (Thomas and Crawford, 1998).

Several types of Streptomyces sp. degrade lignin effectively between 5 to 12 days of curing, at that time cellulose is utilized as a substrate (Giroux et al., 1988). Atit (2005) adds that increasing curing time causing cellulase enzyme activity to increase until curing time optimum. At the time of curing the optimum pH is at 5 to 7. Cellulase enzyme activity reaches maximum at the time of curing the fourth day and its activity then decreases until it stops on the eighth day in line with the depletion of substrate availability.

| Material   | Fermentation Time | Repetition 1 (%) | Repetition 2 (%) | Repetition 3 (%) |
|------------|-------------------|------------------|------------------|------------------|
| Nypa Palm  | 5                 | 38.99            | 39.77            | 39.35            |
|            | 6                 | 37.8             | 36.89            | 36.31            |
|            | 7                 | 35.91            | 34.63            | 35.24            |
|            | 8                 | 35.15            | 33.57            | 33.91            |

Cellulose and hemicellulose is a constituent of plant tissue composed of different sugars. Cellulose is a linear polymer composed of D-glucose bound by β-1,4 glycosides to form cellobiosa. This compound is degraded by a microbial enzyme into an oligosaccharide and then into glucose. Cellulase enzyme is an extracellular enzyme, an enzyme produced in the cell and released into the medium so it can hydrolyze macromolecules such as cellulose (Sanchez, 2009).

The breakdown of cellulose is breaking up the anhydrous polymer into smaller molecules. Through these hydrolysis, oligosaccharides, trisaccharides and disaccharides such as selotriose, selobiose and glucose monomers or other solvents (alcohols, aldehyds, acids and ketones) form and produce CO₂ and water (Hardjo et al., 1989).

The average graph of cadecellulose due to the long effect of curing by orthogonal polynomial method can be seen in Figure 3. In Figure 3 we can see the relationship between the length of curing and the cellulose content is linear.
Figure 3. Average Graph of Cellulose Content Affected by Fermentation Time with EM4

From Table 4 it was found that the highest lignin content was obtained at 5 days of curing time, while the lowest was at 8 days. In this study showed that longer curing with EM4 resulted in decreased lignin levels. This is because the activity of bacteria in EM4 is able to degrade lignin. This is in accordance with research (Kasim et al., 2002) which states EM4 is able to degrade lignin. Giroux et al., (1988) added that the longer the curing, the activity of Streptomyces sp. dissolving lignin to acid precipitable polymeric lignin (APPL) is higher due to the availability of adequate substrate from lignocelluloses.

In EM4 there is a collection of several bacteria consisting of Photosynthetic Bacteria, Lactobacillus sp., Streptomyces sp., Actinomycetes, and Yeast (Ceppy, 2010). Microorganisms that play a role in lignin degradation in EM4 are from Actinomycetes sp. and Streptomyces sp. Hirsch et al., (2004) states Actinomycetes bacteria in the study generally produce extracellular enzymes peroxidase. Peroxidase resulting from Actinomycetes sp. and Streptomyces sp is useful for degradation of lignin materials (Nascimento and Silva, 2008).

Table 4. Result of Lignin Content from Microbiological Method

| Material    | Fermentation Time | Repetition 1 (%) | Repetition 2 (%) | Repetition 3 (%) |
|-------------|-------------------|------------------|------------------|------------------|
| Nypa Palm   | 5                 | 11.36            | 11.5838          | 11.7             |
|             | 6                 | 9.88             | 8.11             | 8.95             |
|             | 7                 | 7.57             | 7.91             | 7.64             |
|             | 8                 | 7.26             | 6.82             | 6.38             |

Both bacteria Actinomycetes sp. and Streptomyces sp. degrades lignin in a water-soluble form called acid precipitable polymeric lignin (APPL) by using polysaccharides from lignocellulose as the main source of carbon and energy source. The decrease in lignin levels using bacterial extracellular enzymes, the principal activity is depolymerization and solubilization and very little lignin mineralization activity. Not only peroxidase, a number of studies suggest that the combined enzyme peroxidase, xylanase, and cellulase help also in lignin degradation as it helps to break the crystalline cellulose bond (Thomas and Crawford, 1998).
The average graph of lignin content due to the effect of long curing with EM4 with the orthogonal polynomial method can be seen in Fig. 4. In Fig. 4 we can see the relationship between curing duration and lignin level is quadratic.

Based on multiple attribute analysis, the optimum treatment was obtained at 8 days of curing time with average cellulose level of 34.21% and average lignin level of 6.82%. The yield of this treatment was 18.35%.

Figure 4. Average graph of Lignin Content Affected by Fermentation Time with EM4

When it comes to a comparison, it was found out that chemical and microbiological treatment, have different results. The results of the chemical method appear to provide greater cellulose content and a much larger yield than microbiological methods, although the reduction of lignin levels is more prevalent in microbiological methods.

References
Achmadi, S. S. 1990. Kimia Kayu. Departemen Pendidikan dan Kebudayaan, Direktorat Pendidikan Tinggi Pusat Antar Universitas Ilmu Hayat Institut Pertanian Bogor. Bogor.
Akhlus, S. 2003. Delignifikasi Pulp Kraft secara Fotokimia Tak Langsung. Jurnal Kimia ITS Surabaya. Vol. 14 (2), 47-55.
Akpakpan, A. E., B. O. Ogunsiledan U. M. Eduok. 2011. Influence of Cooking Variables on The Soda and Soda- Ethanol Pulping of Nypafruticans Petioles. Australian Journal of Basic and Aspplied Sciences, 5 (12): 1202-1208.
Atit, Kanti. 2005. Actinomycetes Selulolitik dari Tanah Hutan Taman Nasional Bukit Duabelas, Jambi. Biodiversitas Volume 6, Nomor 2: 9-17.
BaharudindanTaskirawati. 2009. Hasil Hutan Bukan Kayu. Fakultas Kehutanan Universitas Hasanudin.
Ceppy, Nasahi. 2010. Peran Mikroba dalam Pertanian Organik. FakultasPertanianUnpad. Bandung.
Del Rio J. C., M. Hernando., P. Landin., J. Romero dan AT. Martinez. 2005. Determining The Effluence Of Eucalypt Lignin Composition In Paper Pulp Yield Using Py-GC/MS. Jurnal. Anal. Appl. Pyrolysis Vol. 74, 110-115.
Dence, C and P. W. Reeve. 1996. Pulp Bleaching Principle and Practice. TAPPI Perss. Atlanta. Page : 349-415.
Fangel, D. 1995. Kayu, Kimia Ultra strukturdanReaksi-Reaksi.Penerjemah H. Sastrohamidjojo. GadjahMada University Press,Yogyakarta.
Giroux H., Vidal P.,Bouchard J.,Lamy F. 1988.Degradation of Kraft Indulin Lignin by Streptomyces viridosporus T7A and Streptomyces badius.Application EnvironmentMicrobiology 54: 3064-3070
Gonzalez., G. Almendros., J.C. Del Rio., F. Martin., A. Gutierez., J. Romero. 1999. *Ease Of Delignification Assessment Of Wood From Different Eucalyptus Species By Pyrolysis (TMAH)-GC/MS And CP/MAS 13C-NMR Spectrometry*. Jurnal. Anal. Appl. Pyrolysis Vol. 49, 295-305.

Hardjo, S.N, Indrastuti dan T. Barbacut. 1989. Biokonversi: Pemanfaatan Limbah Industri Pertanian. PAU Pangan dan Gizi IPB, Bogor.

Heradewi.2007. Isolasi Lignin dari Lindi Hitam Proses Pemasakan *Organosolv* Serat Tandan Kosong Kelapa Sawit (TKKS). Skripsi. Fakultas Teknologi Pertanian. IPB. Bogor.

Kasim A, Aisman, Fitriani A. 2002. Uji Keefektifan *Effective Microorganism*-4 (EM-4) pada Delignifikasi Tandan Kosong Sawit pada Beberapa Tingkat Konsentrasi Inokulum. Andalas: Jurnal Penelitian 14:38:11.

Keller F.A., Hamilton J.E., Nguyen Q.A. 2003. Microbial Pretreatment of Biomass Potential for ReducingSeverity of Thermo-Chemical Biomass Pretreatment Application Biochemical Biotechnology. 105:27-41

Laksono, R. 2008. Kelarutan Komponen Kimia Kayu Reaksi Melinjo (*Gnetumgnemon*L) Selama Proses *Pulping* Kraft. Skripsi. Program Kehutanan. Fakultas Kehutanan. IPB. Bogor.

Malo, B. A. 2004. Membuat Kertas Dari Pelepah Pisang. Kanisius. Yogyakarta.

Masluklah, L. 2002. Studi Efektivitas Pulping Soda Antraquinon dan *Pulping* Natrium Asetat Etilen Glicol pada Serat Abaca. Skripsi. Jurusan Kimia. FMIPA. Universitas Brawijaya. Malang.

Onggo, H dan J. Triastuti. 2005. Pengaruh Sodium Hidroksida dan Hidrogen Peroxisidaterhadap Rendemen dan Warna Pulp dari Serat Daun Nenas. Pusat Penelitian Fisika-Lembaga Ilmu Penelitian Indonesia (LPI). Bandung. Jurnal Ilmu dan Teknologi Kayu Tropis Vol. 3 (1) : 37-43.

Pooja S., Othman S., Rokiah H., Rupani P.F., Leh Cheu P., 2010. Biopulping Of Lignocellulosic Material Using Different Fungal Species. Rev Environ Sci Biotechnol (2010) 9:141–151

Wardoyo, A. 2001. Pengaruh Bahan Kimia dalam Pelunakan Serpih Terhadap Sifat Pulp Semikimia *Acacia mangium*. Skripsi. Fakultas Teknologi Pertanian. IPB. Bogor.

Saenah, E. 2002. Pengaruh Dosis Soda terhadap Karakteristik Pulp Abaca dan Pulp Kenaf *Pulping* Soda-Antaquinon. Skripsi. Jurusan Kimia. FMIPA. Universitas Brawijaya. Malang.

Sanchez, C. 2009. Lignocellulosic Residues : Biodegradation and Bioconversion by Fungi. Biotechnology Advances 27:99-114.

Sjostorm, E. 1995. *Kimia Kayu : Dasar-Dasar Penggunaan*. Gadjah Mada University Press. Yogyakarta.

Teo S., W. F. Ang, A. F. S. L. Lok, B. R. Kurukulasuriya and H. T. W. Tan. 2010. The Status and Distribution of the Nipah Palm, *Nypafruticans* Wurmb. (Areaceae), in Singapore. Nature in Singapore 2010 3.: 45-52.

Thomas L., Crawford D.L. 1998. Cloning of Clustered *Streptomyces viridosporus* T7A Lignocellulose. Canadian Journal of Microbiology: 44, 4:12-23.

Tsao, G. T., M. Ladischdan T. Chou. 1978. *Fermentation Substrates from Cellulosic Materials Production of Fermentable sugars from Cellulosic Materials*. Di dalam D. Perlman (eds). Academic Press. New York.