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Original Article

Cellulose from elephant grass leaves *(pennisetum purpureum schumach.)* as an alternative of bioplastic material

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ABSTRACT

Bioplastic made by mixing cellulose pulp from elephant grass leaves, chitosan, and glycerol with variation of chitosan to cellulose pulp ratio3:10, 4:10, 5:10 (grams /grams), and 3 mL of glycerol / 2 grams of cellulose pulp, using inversion phase method. Characterization of bioplastic included analysis of functional group with FTIR, swelling test, density test, morphology analysis, melting point test, and mechanical properties test. The results of functional group analysis showed that there were no new functional groups compared to its origin material. The mechanical properties testing shown that bioplastic produced with variation of chitosan to cellulose pulp ratio 3:10, 4:10, and 5:10 had tensile strength 3.9 MPa, 7.2 MPa, and 5.0MPa; elongation at break 196.3%, 208.1%, and 329.7%; modulus of elasticity 3.5 MPa, 11.9 MPa, and 1.8 MPa respectively. Density values of those products were 1.1226 grams / cm^3, 1.1249 grams / cm^3, and 1.1273 grams / cm^3 while the water resistance value weresuccessively69.009 %, 27.822 %, and 14.136 %. The results shown that cellulose from elephant grass leaves could form bioplastic.

Keywords: bioplastic, elephant grass leaves, cellulose

INTRODUCTION

Elephant grass is plants belong to the Poaceae family[1]. Utilization of elephant grass now is still limited to fodder, even sometimes elephant grass regarded as the buggers [1;2]. Elephant grass leaves have 22 % - 40.85 % cellulose content[3;4;5]. Cellulose is the most abundant polysaccharide in nature[6]. Chain stiffness of cellulose can prevent the molecule hydration so its can improve water resistance[7]. High cellulose content on elephant grass leaves and its water resistance makes it potential to be utilized as bioplastic source[8]. Bioplastics are plastics from renewable sources, while plastics are made from petroleum or natural gas (9;10). At this time, plastic causing many environmental problems which its cannot be recycled perfectly and its non-biodegradable. Plastics released carcinogen compound such as dioxin. Dioxin in the body can cause health problems and cancer (11;12).

Conventional plastic problem cannot be resolved through restriction or reduction of the plastic use. This is providing opportunities to bioplastic product development. Benefit of bioplastic is biodegradable, so it's not become the environmental burden (13). Based on the above description, this research aims to find out whether the elephant grass leaves can be used as an alternative of bioplastic material.

MATERIALS AND METHODS

Apparatus: Apparatus were used are infrared spectrophotometer (Shimadzu, IR Prestige-21), digital analytical balance (Mettler Toledo), Dial Gauge (Toyoseki), Dumbbell Die Cutter, sieve mesh no. 50, melting point apparatus (Stuart Scientific), Universal Testing Machine (Orientec Co. Ltd, Type UCT-5T), scanning electron microscope (JEOL, JSM T330A), oven (Memmert),water bath stirrer (CIMAREC), caliper (Moore and Wright), grinding machines with screen sizes 2 (Hammer Mill),pH indicators, glass tubes, capillary pipe, and glass tools commonly used in the laboratory.

Materials: Materials were used are elephant grass leaves (*Pennisetum Purpureum* Schumach.), sodium hydroxide (NaOH) (Merck), hydrochloric acid (HCl) (Merck), acetic acid (CH3COOH) (Merck), glycerol (Technical), chitosan (PT Biotech Surindo), sulfuric acid (H2SO4) (Merck),

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potassium bromide (KBr) (Merck), sodium hipoklorit (NaOCl) (Technical), aquadestilata.

Methods: Methods included collection, determination and processing elephant grass leaves, analysis of cellulose content, production cellulose pulp, bioplastic production with inversion phase method, and bioplastic characterization.

Collection, determination and processing: Determination of elephant grass was done at Laboratory of Plant Taxonomy, Faculty of Matematic and Natural Science, Universitas Padjadjaran. Elephant grass that have been determinated, take the leaves then washed, dried, cut into size 2-3 cm, milled, and sieved with sieve mesh no. 50.

Analysis of cellulose: Analysis cellulose on elephant grass leaves was done using Chesson method(14). One grams drysample (weighta)added150 mL of distilled water and reflux at 100°C.The results were filtered, residues washed in 300mL hot water 60°C. Residues dried in oven at 75°C until constant weight (weight b). Residues added150 mL H_2SO_4 1 N and reflux at 100°C for 1 hour. The results were filtered, residues washed in ± 500mL distilled water until neutral and dried in oven at 75°C until constant weight(weight c). The dried residues soaked in 100 mL H_2SO_4 72% at room temperature for 4hours, then added 150 mL H_2SO_4 1 N and reflux at 100°C for1 hour. The results were filtered, residues washed in ± 500mL distilled water until neutral and dried in oven at 105°C until constant weight(weight d). Residues were ashed at 475°C for 3 hours and weighed (weight e).

Production of cellulose pulp: Production of cellulose pulp was done using method on Norashikin and Ibrahim research (2010) with modifications[15]. Cellulose pulp produced from elephant grass leaves powders by every 10grams elephant grass leaves powders soaked in 200 mL NaOH 4% at 80±5°C for 1 hour with occasional stirring. The results were filtered, residues washed with distilled water until neutral and dried in oven at 80±5ºC until constant weight. Each 2 grams result from the processadded36mLHCl 0.2Nand heated at 80±5°C for 2hours.The results were filtered, residues washed with distilled water until neutral then added NaOCl 12% until residues have white color and dried in oven at 80±5ºC until constant weight. The results were washed with distilled water until free of chlorine and dried until constant weight. Each2 grams product added 100 mL distilled water and heated at 90°C until cellulose pulp formed.

Production of bioplastic with inversion phase method: Production of bioplastic with inversion phase method started up with orientation phase, bioplastic made by mixing 3 mL of glycerol with variation cellulose pulp were 0.5grams, 1 grams, 1.5 grams, and 2grams. Each mixtured was printed on plate glass at50°C until layer formed and the best results were observed. Combination the best pulp cellulose pulp and glycerol used to make print solution. Printing solution made by mixing chitosan with the best cellulose pulp and glycerol form orientation phase with variation of chitosan to cellulose pulp ratio were 3:10, 4:10, 5:10 (grams /grams). The mixture stirred for 1hour until homogeneous printing solution. Each mixture was printed on plate glass dried in oven at 75°C50°C until bioplastic formed. Bioplastic discharge process from plate glass done with insert the plate glass to coagulation bath which contains NaOH 2% solution until bioplastic regardless from plate glass. Then dried bioplastic aerated.

Characterization of bioplastic:
Characterization of bioplastic which made by mixing cellulose pulp from elephant grass leaves, chitosan, and glycerol.

A. Physical properties test: These test include analysis of functional group with FTIR, density test, swelling test, melting point test, morphology test with Scanning Electron Microscope (SEM).
B. Mechanical properties test: These test include tensile strength, elongation at break, and modulus of elasticity with Universal Testing Machine (UTM).

RESULTS AND DISCUSSIONS

Collection, determination and processing of elephant grass leaves

Elephant grass harvested from Jatinangor, Sumedang, West Java. From the determination known that the elephant grass used in this research including:

Kingdom : Plantae
Phylum : Magnoliophyta
Class : Liliopsida
Ordo : Poales
Family : Poaceae
Genus : *Pennisetum*
Spesies : *Pennisetumpurpureum* Schumach.

After drying process, elephant grass weight shrinkage from 10kgto 4.44kg. Elephant grass leaves powders sifted to homogen.

Analysis of cellulose content: Analysis cellulose content on elephant grass leaves is done using Chesson method. Chesson method can provide cellulose content and lignin content on elephant grass leaves.

Elephant grass leaves had 25.208% cellulose content and 5.815% lignin content.

Production of cellulose pulp: Production cellulose pulp begins with NaOH 4% addition. NaOH addition caused delignification reaction, where lignin and hemicellulose cut off from cellulose [16]. Heating process aims to improve the reaction speed of NaOH molecules with lignin and hemicellulose[16].HCl0.2 N addition will complete hemicellulose removal. Hemicellulose degradation higher in acid than in alkaline[17]. Bleaching process aims to improve lignin removal.

Production of bioplastic with inversion phase method result: Bioplastic produced with inversion phase method. Inversion is polymer transformation process from liquid phase to solid phase [18]. Orientation phase done to get the amount of cellulose pulp that can produce best bioplastic. In orientation phase, glycerol addition function on cellulose pulp as plasticizers substance. Glycerol is a cheap substance, easily obtainable, renewable and biodegradable[19]. 3 mL glycerol addition produces bioplastics with the best mechanical properties[14;20]. The results orientation phase showed best bioplastic obtained from mixture of2 grams cellulose pulp and 3mL glycerol but the product is still very easily broken. Mixture of 2 grams cellulose pulp and 3 mL glycerol used to make bioplastic by adding chitosan. Chitosan addition can improve mechanical properties of bioplastic[21]. Chitosan is not toxic and biodegradable[22]. Bioplastic are made with variation of chitosan to cellulose pulp ratio were 3:10, 4:10, 5:10 (grams /grams). Printing solution was printed on plate glass and dried. Drying process observed periodically, so drying was not excessive that can affect the result. Bioplastic discharge from plate glass done with insert the plate glass to coagulation bath which contains NaOH 2% solution. NaOH 2% function as a non-solvent which diffuses into the bottom bioplastic so bioplastic regardless from plate glass and easily remove[20].

Analysis of functional group result: Analysis of functional groups bioplasticsis made to know what functional groups are present on bioplastics. Based on FTIR identification spectrum of bioplastic toward functional group its substance are not discovered new functional group formed. These signifies bioplastic produced through blending in physics process and bioplastics have properties such as constituting substances[9].

Melting point test result: In this research, melting point measurements done with melting point apparatus. Data on Table 3 shows that bioplastics tested damaged before it reaching its melting point. This is characterized by discoloration of bioplastic be dark brown. Melting point test result shows at temperatures 85.2 – 91.6°C observed steam which later form liquid on wall capillary pipe. Steam formation can occur because water component removal is not bound or absorbed moisture on bioplastics[23].At temperatures 144.1 – 204.5°C observed bioplastics color changes from yellow to dark brown. This condition can be caused by decomposition of bioplastic[23]. Decomposition temperature is temperature when bioplastic began to change composition constituent materials[24].

Density test result: Density can be defined as mass per unit volume of material. More number of chitosan added increase density values. More number of chitosan increasing possibility of chitosans to cover cavity between cellulose so cellulose structure become more tightly and bioplastic density values increase.

Swelling test: Swelling test aims to predict size of substances that can diffused into bioplastics. High water uptake indicates there are cavities voids in between bond in bioplastics[25]. Swelling test result shown in Figure 1. Highest value of water uptake on bioplastic by ratio chitosan and cellulose pulp 3:10 equal to 69.009%. This is suited with the density bioplastic values that showed the lowest density value.

Morphology test with SEM: SEM analysis of bioplastic done to determine the bioplastic morphology. Figure 2 shows that there are two parts which is part of a picture that is smooth and part of a picture that is rude. Smoothpart of the picture shows that bioplastic components has been mixed well, while rude part of the picture looked columns of coarse and a cavity authors shows that bioplastic components not mixed well.

Mechanical properties testing: Mechanical properties test include tensile strength, elongation at break, and modulus of elasticity. Elongation at break is maximum strain bioplastic when bioplastic be given energy until bioplastics time is finally broken[25]. Elongation at break test results can be seen in Figure 3. At Figure 3 can be seen that highest value of elongation at break on bioplastic by ratio chitosan and cellulose pulp 5:10 equal to 329.7%. More number of chitosan added increase probability of chitosan and cellulose interaction. Interactions between chitosan and cellulose in the bioplastics are hydrogen bonds[25]. More hydrogen bonds will increase the value of elongation at break of bioplastics produced. Tensile strength is maximum strength that can be attained until plastic survive before break[25].More number of chitosan added increase probability of chitosan and cellulose interaction. Increased interaction between bioplastics components has increased tensile strength values[26]. Tensile strength values decreased in bioplastics 5:10. Tensile strength decreased values may be caused by mixing method is still modestly so forming columns causes decreases bioplastic tensile strength. Modulus of elasticity indicating level of bioplastics stiffness. Similarly with tensile strength value, modulus of elasticity bioplastics values increased in number of chitosan added and decreased onbioplastic5:10. At Table 5 can be seen comparison between bioplastic resulting with some bioplastics and conventional plastics in market. Comparison data in Table 5 can be used as reference to produce bioplastic with better characteristics. For example, water uptake value of bioplastic resulting in this research are lower than water uptake value of some bioplastic, so mixing two kinds of bioplastic is expected can produce bioplastic that have better resistance water than previous bioplastic. At the other side, water uptake value is higher than conventional plastic give opportunity to produce products with mixture of bioplastic and conventional plastic more easily biodegradable[29].

CONCLUSION

The results of functional group analysis showed that there were no new functional group compared to its origin material. The bioplastic products could release its moisture and decomposed at different temperatures. The mechanical properties testing shown that bioplastic produced with variation of chitosan to cellulose pulp ratio 3:10, 4:10, and 5:10 had tensile strength 3.9 MPa, 7.2 MPa, and 5.0 MPa; elongation at break 196.3%, 208.1%, and 329.7%; modulus of elasticity 3.5 MPa, 11.9 MPa, and 1.8 MPa respectively. Density values of those products were 1.1226 grams / cm^3, 1.1249 grams / cm^3, and 1.1273 grams / cm^3 while the water resistance values were successively 69.009 %, 27.822 %, and 14.136 %. The results shown that cellulose from elephant grass leaves could form bioplastic.

Table 1. Cellulose content and lignin content on elephant grass leaves

No	Cellulose content (%)	Lignin content (%)
1	25.7913	6.1443
2	24.6249	5.4859
Average	25.208	5.815
Standard deviation	0.825	0.466

Table 2. The results of analysis of functional group with FTIR

Functional Group	Wavenumber (cm^{-1})				
	3:10	4:10	5:10	Chitosan-glyserol	Cellulose
$-NH_2$ (stretching)	3985.93 (w)	3887.61 (w)	3996.54 (w)	3886.11 (s)	-
-OH (stretching)	3387.03 (s)	3401.01 (m)	3386.06 (s)	3297.82 (s)	3347.49 (m)
-CH (stretching)	2943.88 (w)	2944.84 (w)	2943.88 (m)	2882.64 (m)	2904.82 (w)
C-O (stretching)	1411.42 (w)	1411.42 (w)	1414.43 (w)	1369.47 (s)	1443.64 (w)
C-N (stretching)	1045.43 (w)	1043.98 (w)	1044.46 (m)	1037.23 (s)	-

Description:
w = weak m = moderate s = strong

Table 3. Melting point test result

Changes that occurred	Bioplastic temperature change					
	3:10		4:10		5:10	
Water vapor appear	91.6^{0}C	94.6^{0}C	85.2^{0}C	89.0^{0}C	93.4^{0}C	96.6^{0}C
Yellow color plastic	144.3^{0}C	144.7^{0}C	144.1^{0}C	144.5^{0}C	146.7^{0}C	148.2^{0}C
Dark brown color plastic	204.3^{0}C	204.5^{0}C	203^{0}C	199.6^{0}C	197.8^{0}C	198^{0}C

Table 4 Density test result

Ratio chitosan and cellulose pulp	3:10		4:10		5:10	
	I	ii	I	Ii	I	Ii
Mass (grams)	0.0486	0.0613	0.0717	0.0711	0.0484	0.0389
Length (cm)	1	1	1	1	1	1
Width (cm)	1	1	1	1	1	1
Thickness (cm)	0.043	0.055	0.0635	0.0635	0.043	0.0345
Volume (cm^3)	0.043	0.055	0.0635	0.0635	0.043	0.0345
Density (g/cm^3)	1.1302	1.1152	1.1297	1.1202	1.1271	1.1275
Mean	1.1226		1.1249		1.1273	
Std dev (%)	0.0106		0.0067		0.0003	

Table 5. Comparison of plastic mechanical properties and physical properties [27;28]

Type	Density (g/cm^3)	Water uptake (%)	Tensile strength (MPa)	Elongation at break (%)	Modulus of elastisity (MPa)
Bioplastic 3:10	1.1226	69.009	3.9	196.3	3.5
Bioplastic 4:10	1.1249	17.822	7.2	208.1	11.9
Bioplastic 5:10	1.1273	14.136	5.0	329.7	1.8
PBAT	1,21	550	9	>500	52
PHBV	1.25	21	-	15	900
PCL	1.11	177	14	>500	190
PBSA	1.23	330	19	>500	249
PP	0.9	0.008	0.4 – 0.7	-	-

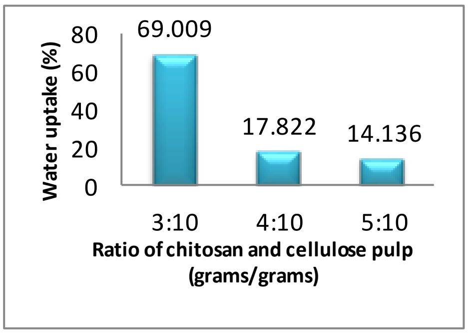

Figure 1. Effect variation of chitosan and cellulose pulp about water uptake value

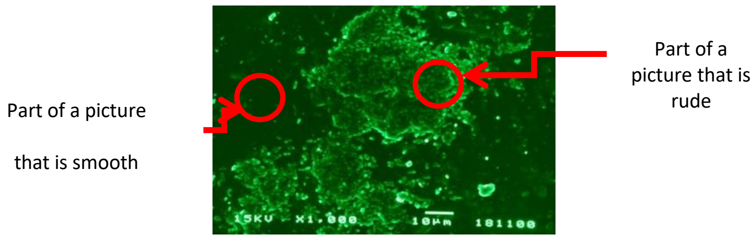

Figure 2. Cross-section of bioplastic by comparison of chitosan and cellulose pulp 5:10 on a scaled up 1000x

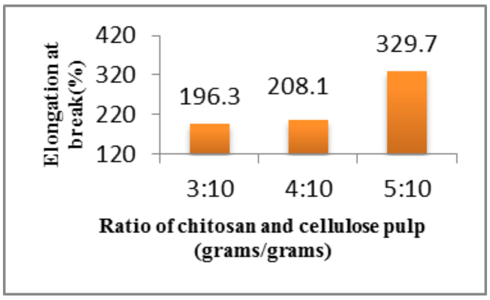

Figure 3. Effect variation of chitosan and cellulose pulp about elongation at break

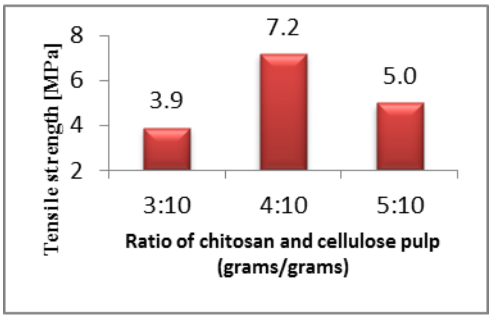

Figure 4. Effect variation of chitosan and cellulose pulp about tensile strength

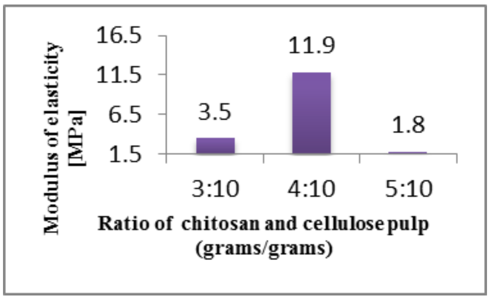

Figure 5. Effect variation chitosan and cellulose pulp about modulus of elasticity

REFERENCES

1 Gonggo, B. M., B. Hermawan, dan D. Anggraeni. 2005. Pengaruhjenistanamanpenutupdanpengolakantanahterhadapsifatfisikatanahpadalahanalang-alang. *Jurnalilmu-ilmupertanian Indonesia*.7(1):44-55.[in bahasa Indonesia]

2 Sari, N.K. dan K. Sumada. 2010. Kajianproduksi bioethanol darirumputgajah. Avaiable at:http://elib.pdii.lipi.go.id/katalog/index.php/searchkatalog/byId/276273 [Accessed 3 January 2012].[inbahasa Indonesia]

3. Samsir, M.I. 2010. Pembuatanbioetanoldaribatangrumputgajahdenganmetodefermentasisecaraberkesinambungan.[Skripsi]. Makassar: JurusanTeknik Kimia, PoliteknikNegeri Ujung Pandang. 4,7.[in bahasa Indonesia]

4. Ambriyanto, K.S. 2010. IsolasidanKarakterisasiBakteriAerobPendegradasiSelulosadariSerasahDaunRumput Gajah (*PennisetumpurpureumSchum*).[Paper].Avaiable at:http://digilib.its.ac.id/public/ITS-Undergraduate-13517-Paper.pdf [Accessed 3 January 2012].[inbahasa Indonesia]

5. Okaraonye, C.C. and J.C. Ikewuchi. 2009. Nutritional and antinutritional components of *Pennisetumpurpureum*Schumach. *Pakistan journal of nutritional* 8(1): 32-34.

6. Han, Y.J. and H.Z. Chen. 2007. Synergism between corn stover protein and cellulose. *Enzyme and microbial technology*.41:638-645.

7. Billmeyer, F.W. 1984. *Textbook of Polymer Science*.3rd Ed. New York: John Willey & Sons. 419-425.

8. Pramono, H. 2011. Pembuatanbioplastikdarilimbahrumahtanggasebagaibahan edible film ramahlingkungan.[Abstrak].Avaiable online at:http://lemlit.uny.ac.id/pembuatan-bioplastik-dari-limbah-rumah-tangga-sebagai-bahan-edible-film-ramah-lingkungan[Accessed 12 February 2012].[inbahasa Indonesia]

9. Darni, Y., H. Utamidan S.N. Asriah. 2009. Peningkatanhidrofobisitasdansifatfisikplastikbiodegradabelpatitapiokadenganpenambahanselulosaresidurumputlaut*Euchemaspin ossum*. [Paper]. Lampung: JurusanTeknik Kimia, FakultasTeknik, Universitas Lampung. 130,136.[inbahasa Indonesia]

10. Khoo, H.H., R.B.H. Tan, and K.W.L. Chng. 2010. Environmental impacts of conventional plastic and bio-based carrier bags. *Int J Life Cycle Assess*15:284–293.

11. Winarti, C. dan S.J. Munarso. 2005. KajianKontaminasiDioksinpadaBahanPangan.Dalam: *Prosiding Seminar NasionalTeknologiInovatifPascapanenuntukPengembanganIndustriBerbasisPertanian*. Bogor: BalaiBesarPenelitiandanPengembanganPascapanenPertanian. 1208.[in bahasa Indonesia]
12. Lucier, G.W., and M.A. Gallo. 1993. Receptor mechanism and dose-response models for the effects of dioxins. *Environ Health Perspect*101(1): 36-44.
13. Darni, Y., A. Chici, dan D.S. Ismiyati. 2008. Sintesabioplastikdaripatipisangdan gelatin denganplasticizer gliserol. [Abstrak].Avaiable at: http://repository.unila.ac.id:8180/dspace/handle/123456789/276?mode=full[Accessed 3 January 2012].[inbahasa Indonesia]
14. 14 Datta, R. 1981. Acidogenic fermentation of lignocellulose yield and conversion of components.*Biotechnology and Bioengineering*.23(9): 2167-2170.
15. Norashikin, M.Z. and M.Z. Ibrahim. 2010. Fabrication and characterization of sawdust composite biodegradable film. *World Academy of Science, Engineering and Technology*.65: 864-868.
16. Jalaluddindan S. Rizal. 2005. Pembuatan pulp darijeramipadidenganmenggunakannatriumhidroksida. *JurnalSistemTeknikIndustri*. 6(5): 53-56.[inbahasa Indonesia]
17. Casey. 1960. *Pulp and Paper Chemistry and Chemical Technology*. Volume I. New York: Pulping and Bleaching. Interscience Publisher.
18. Pinem, J.A. dan R. Angela. 2011. Sintesisdankarakterisasimembranhibrid PMMA/TEOT: pengaruhkonsentrasipolimer. [Prosiding].PekanBaru: FakultasTeknikUniversitas Riau. 3.[in bahasa Indonesia]
19. Yusmarlela. 2009. Studipemanfaatanplastisisergliseroldalam film patiubidenganpengisiserbukbatangubikayu. [Tesis]. Medan: Universitas Sumatra Utara. 17-19.[in bahasa Indonesia]
20. Sanjaya, I.G. dan T. Puspita. 2011. Pengaruhpenambahankhitosandan plasticizer gliserolpadakarakteristikplastik biodegradable daripatilimbahkulitsingkong. [Paper]. Surabaya: InstitutTeknologiSepuluhNopember. 3.[in bahasa Indonesia]
21. Chen, L., C.Y. Tang, N.Y. Ning, C.Y. Wang, Q. Fu, and Q. Zhang. 2009. Preparation And Properties Of Chitosan/Lignin Composite Films. *Chinese Journal of Polymer Science*.27(5) : 739−746.
22. Pasaribu, N. 2004. Berbagairagampemanfaatanpolimer.FakultasMatematika Dan IlmuPengetahuan. Universitas Sumatera Utara. 4-5. Avaiable at: *library.usu.ac.id/download/ft/tkimia-nurhaida.pdf (Accessed* 5 June 2012).[inbahasa Indonesia]
23. Dhanikula, A.B., R. Panchagnula. 2004. Development and characterization of biodegradable chitosan films for local delivery of paclitaxel.*The AAPS Journal*.6(3): 1-12
24. Sudirman, K.K. Aloma, I. Gunawan, A. Handayani, danE. Hertinvyana. 2002. Sintesisdankarakterisasikompositpolipropilena/serbukkayugergaji. *JurnalSainsMateriIndonesia*.4(1): 20-25.[inbahasa Indonesia]
25. Purwanti, A. 2010. AnalisisKuatTarikdanElongasiPlastikKitosanTerplastisasi Sorbitol.*JurnalTeknologi.* 3(2): 99-106.[inbahasa Indonesia]
26. Juari. 2006. Pembuatandankarakterisasibioplastikdari poly-3-hidroksialkanoat (PHA) yang dihasilkan*RalstoniaEutropha*padahidrolisatpatisagudenganpenambahandimetilftalat (DMF). [Skripsi]. Bogor: InstitutPertanian Bogor. 46.[in bahasa Indonesia]
27. Averous, Pr. L. 2008. Bioplastic.http://www.biodeg.net/bioplastic.html(Accessed 23 June 2012).
28. Pandey, A., P. Kumar, and V. Singh. 2010. Application of bioplastics in bulk packaging: a revolutionary and sustainable approach. Avaiable at: www.indiapackagingshow.com [Accessed 12 February 2012].
29. Bourtoom, T. 2008. Plasticizer effect on the properties of biodegradable blend film from rice starch-chitosan.*J. Sci. Technol*. 30: 149-155.