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Flux and degradation rates of carbohydrates and lignin in a tropical wetland, West Java, Indonesia: a comparative study

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Abstract
A comparative study of lignin and neutral carbohydrate compositions was carried out on a sedimentary core collected from tropical wetland, west Java, Indonesia, to assess flux and diagenesis of these two important molecular fractions in sedimentary organic matter (SOM). Distribution of carbohydrates in sediments reflects both the lability of these compounds and their selective degradations as well as *in situ* production of secondary polysaccharides (e.g. microbial recycling). Among carbohydrates, cellulose-like fraction is relatively refractory and its degradation rate correlated with that of lignin in tropical wetland. The total carbon from lignin-derived phenols is higher in sediments than in tropical plants as a consequence of their rather refractory character. Nevertheless, evidences of cellulose-like materials and lignin decomposition were found to be followed first order reaction kinetics at different rates with relatively long half-life (7700 years for lignin and 3465 years for cellulose-like materials). Therefore, both lignin and cellulose-like fraction, derived from vascular plant debris, can be used together as a potential biomarker to study sources, depositional environments, and relative age of SOM.

Introduction

Large amount of terrestrial (vascular) plant organic matter (OM) are transported and preserved into lake, river, estuaries and coastal marine environment (Degens, 1991; Canuel et al., 1995; Goni et al., 2003).This terrestrial sources OM mostly composed of carbohydrates and lignin and small amount of other biomolecules (e.g. lipid). A significant portion of carbohydrates and lignin bound together by hydrogen and covalent bond (ester or ether) linkages (Fig.1). The analyses of lignin and carbohydrate compositions of these OM can be used for quantitatively estimating the flux and fate of vascular plants OM in the environment. To use these compounds as a quantitative tracer for such an application, knowledge of degradation rates relative to the associated OM and structural diagenetic changes is required. Diagenetic alternations of carbohydrates and lignin and related limitations of its suitability as quantitative tracer depend on the manifold environmental conditions in which diagenesis occur. Decomposition kinetics and molecular alterations of both carbohydrate and lignin under the diverse environments have been subject of several studies (Benner et al. 1990; Marchand et al., 2005).

Early diagenesis may measurably alter the original biochemical composition of sedimentary organic mixtures and thereby complicate applications of lignins (Hu et al., 1999; Dittmar and Lara, 2001) and carbohydrates (da Chunha et al., 2002) as source indicators. Although, it is traditionally believed that lignin is not degraded in a reducing environment and could influences carbohydrates preservation to some extent (Hedges et al., 1985; Mores et al., 1994). But recent

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laboratory and field studies showed that bacteria can anaerobically degrade/alter carbohydrates and to some extent lignin (Colberg and Young 1982; and Benner et al 1984) even at all under anaerobic condition. However, only few studies in the literature address the extent of early diagenetic changes of sedimentary organic matter (SOM) at molecular level for continental aquatic ecosystem such as tropical lake or wetlands (Bourdon et al., 2000). The flux and degradation rate of carbohydrates and lignin in a tropical wetland may provide a detailed records of early diagenetic changes and depositional environments. The comparative geochemistries of these compounds are needed to study degradation kinetics of different molecular components in the natural environment and to explore a new window of biomarker applications (i.e. possible advantages of applying these two biomarkers together).

The objective of the present study was to determine flux and degradation rate of lignin and carbohydrates in a sub aqueous tropical wetland. Molecular-level analyses for lignin and carbohydrates were performed on a core (RD1) samples, and the effects of diagenetic alterations of carbohydrates and lignin were identified. Long-term degradation rates of lignin and carbohydrates in a reducing environment were determined by using first order kinetics model. On the basis of these analyses, the suitability of the combined application of acid hydrolyzed carbohydrates and CuO-oxidation lignin products as diagenetic and source tracers were critically examined.

Materials and methods

The core (3.6 m length, 10 cm id) was taken by piston corer from the northwestern corner (6° 11` S and 105° 59′ E) of the Rawa Danau caldera where the greatest thickness of peat deposits was found. A subsurface shorter core (32 cm, 10 cm id) was taken using gravity corer to maintain sedimentary sequences. Both cores were kept frozen prior to analysis and were cut frozen into 2 cm slice using a stainless steel handsaw. Each slice was frieze dried, and then pulverized using mortar and pestle. After sieving through 300 μm, fine powder samples were stored in airtight, acid-washed brown glass vials for further analyses. A detailed description of the study area as well as core lithology and bulk geochemistry can be found in Tareq et al., 2007.

Total organic carbon and nitrogen of bulk homogenized sediment were analyzed using a Thermo Quest NA 2500 NCS elemental analyzer. The equipment was calibrated using alanine standards and half of the analyses were performed in duplicates. The mean deviation was less than ±5 % for both of organic carbon and total nitrogen based on duplicate analyses.

Samples were analyzed for lignin phenol concentrations using the alkaline CuO oxidation technique of Hedges and Ertel (1982) with minor modifications. Briefly, samples for lignin analysis (10 to 20 mg samples) were oxidized with CuO in 5 ml of 2 M NaOH for 3 hours in presence of $Fe(NH_4)_2(SO_4)_2.6H_2O$ (100 mg). After extraction and purification, lignin phenol monomers were silylated and quantified by GC using ethylvanillin as a internal standard. The reproducibility of the analytical procedure for all lignin phenols was better than 95 % except for the p-hydroxy phenols (90%). Detail of the method explains elsewhere (Tareq et al., 2004).

The procedure used for neutral sugar analysis is adapted from previous works (Mopper 1977; Cowie and Hedges, 1984; Bourdon et al., 2000) but mostly from Bourdon et al., 2000. Samples for neutral sugar analysis (approx. 100 mg samples) were hydrolyzed with 10 ml of 1.2 M HCl for 5 hours and more resistant sugar molecules (structural polysaccharides) were extracted using concentrated acid pretreatment prior to hydrolysis. After separation and purification, neutral sugars were silylated and quantified by GC using aditol as a internal standard. Analytical error (ranges from 5 to 12 %) was evaluated by triplicate analysis and is generally lower for more abundant sugars. Detail of the method explains elsewhere (Tareq et al., 2011).

Results and discussion

Carbohydrates and lignin flux: biogeochemical modification of SOM

Carbohydrates and lignin carbon accumulation [C(L)CA] rates (Carbohydrates: CCA and Lignin: LCA) for the core RD-1 were calculated using the following equation.

$$\text{CCA rate} = SR \times BD \times CC$$

Where CCA is in unit of $mgCcm^{-2}y^{-1}$, SR is the sedimentation rate (cm y^{-1}) given by the calibrated ^{14}C dates, CC is the total carbohydrates carbon (for lignin use lignin carbon) in mg/g-dry samples and BD is the bulk density in gcm^{-3} that was calculated according to Aucour et al., 1999. Values of carbohydrates and lignin carbon were calculated by multiplying contents of total carbohydrates (TCHO) and total lignin phenols (TLP) by factors of 0.44 and 0.62, which are equal or very close to, the molar C ratio of all the neutral monosaccharides and lignin phenols respectively. The CCA and LCA rates vary between 0.63 and 3.12 mg C cm^{-2} yr^{-1} (average 1.68) and 0.24 to 3.07 mg C cm^{-2} yr^{-1} (average 1.27) respectively (Fig. 1a). These rates generally increase with organic carbon accumulation rates (r^2_C = 0.42 and r^2_L = 0.62) and when TOC becomes more depleted in ^{13}C ($\delta^{13}C < -27$ ‰; Tareq et al., 2011). Biomolecules accumulation rates depend on the balance between net primary productivity and biogeochemical modifications including mineralization of original source biomolecules. The organic carbon accumulation rate at the study site (varying between 2.0 and 26.05 mg C cm^{-2} yr^{-1}) is much lower than the values of modern swamp net production (30–2000 mg C cm^{-2} yr^{-1}; Lieth, 1975). This is due to the fact that major portions of OM mineralized within short time periods and small refractory portion survived and deposited in the long run. However, deposited OM was further modified by sedimentary biogeochemical processes in natural environments.

The transformations involved during biogeochemical modifications strongly influence the molecular compositions of the organic matter that may be preserved in sediment. Analytical results showed that uncharacterized organic carbon increased with depth of the core (i.e. progress of diagenesis) in tropical wetland (Fig.1.b). At the upper few layers of the core, about 40% of TOC was characterized as three bimolecular fractions such as acid-hydrolysable carbohydrates, CuO-oxidation lignin phenols and extractable lipids whereas less than 20% of the TOC at the bottom of core was characterized as the initial input of biological macromolecules (e.g. carbohydrates, lipids, and lignin). A major portion of characterized OM comprise of lignin and carbohydrates (Fig.1a). The macromolecules from biological sources may undergo condensation,

polymerization, defunctionalization (e.g., dehydrolyzation, decarboxylation) and other biogeochemical alterations and finally yielding uncharacterized organic matter. Amounts of uncharacterized components increases as diagenesis precedes in SOM, suggesting either that they are lost from the SOM or that they are being permanently incorporated into the macromolecular structure of geopolymers (humin). However, the characterized organic matter was calculated depending on some theoretical assumptions (as explain in fig.1 caption) as well as a portion might be lost from the analytical window of the technique used for quantifications of each molecular fractions. In addition, carbon derived from nitrogenous organic compounds (eg. amino acid) was not estimated as characterized organic matter. These might be obscured absolute values of characterized components, nevertheless, relative values of characterized and uncharacterized components suggested significant biogeochemical modifications of deposited OM.

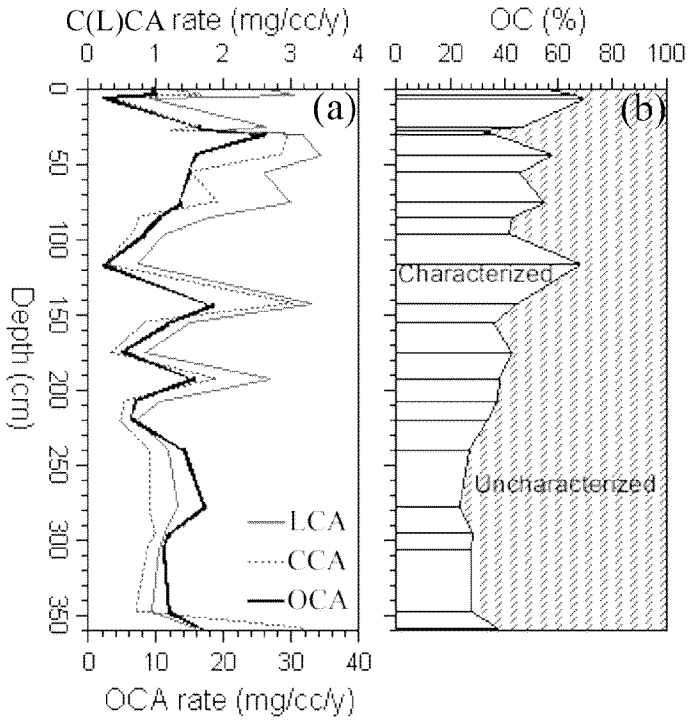

Fig.1. Vertical distributions of (a) carbohydrates and lignin carbon accumulation rate (b) characterized and uncharacterized fractions of carbon as percentage of total organic carbon (TOC). The corresponding carbon contents in lignin and carbohydrates were directly calculated using the hypothetical elemental weight percentages assigned to the carbohydrate ($C_{106}H_{177}O_{88}$: 44.4% C, 6.2% H, 49.4% O), and lignin ($C_5H_6O_2$: 61.6% C, 6% H, 31.5% O). Total carbon in lipids is experimentally determined.

Comparative geochemistry of carbohydrate and lignin

Carbohydrates are more labile compare to lignin. Thus, carbohydrates to lignin ratio (TCHO/TLP) could be used to distinguish between fresh and degraded SOM in a lake. Although, lignin occurrences only the higher (vascular) plants whereas carbohydrates occurrences in all sort of living organisms and in aquatic ecosystems a significant portion of OC derived from nonvascular plant sources. In general, TCHO/TLP ratios of Rawa Danau peat core decreasing with depth with elevated values at few sections due to source variations. The profile of this ratio is explained by the fact that although lignin partially decomposes during early diagenesis, carbohydrates loss is greater than that of lignin, resulting in a decrease of the TCHO/TLP ratio from an average initial value of 3.25 (±0.4) to less than 1.00. Fresh wood and corresponding buried wood have higher TCHO/TLP values than the average TCHO/TLP ratio of Rawa danau wetland surface sediments. The average TCHO/TLP ratio of three buried woods (spruce, alder and oak) is about half (3.54) of its fresh counterpart (7.14) (Hedges et al., 1985). Since buried woods dealt with relatively large fragments of woody debris, those results cannot be directly compared with our results on smaller fragments of vascular plant debris dispersed in sedimentary environment. However, lower TCHO/TLP ratio of surface sediments, compare to that of fresh and buried wood, indicated that a significant portions of carbohydrates especially nonstructural carbohydrates was lost after death of living organisms. There is a possibility of increasing this ratio due to *in situ* production of carbohydrate (e.g. microbial) or if major portions of SOM derived from nonvascular sources such as algae and planktons. The scatter nature with a week correlation between TCHO/TLP ratio and depth (Fig.2a) reflects this possibility of *in situ* secondary productions of carbohydrates and variation in sources.

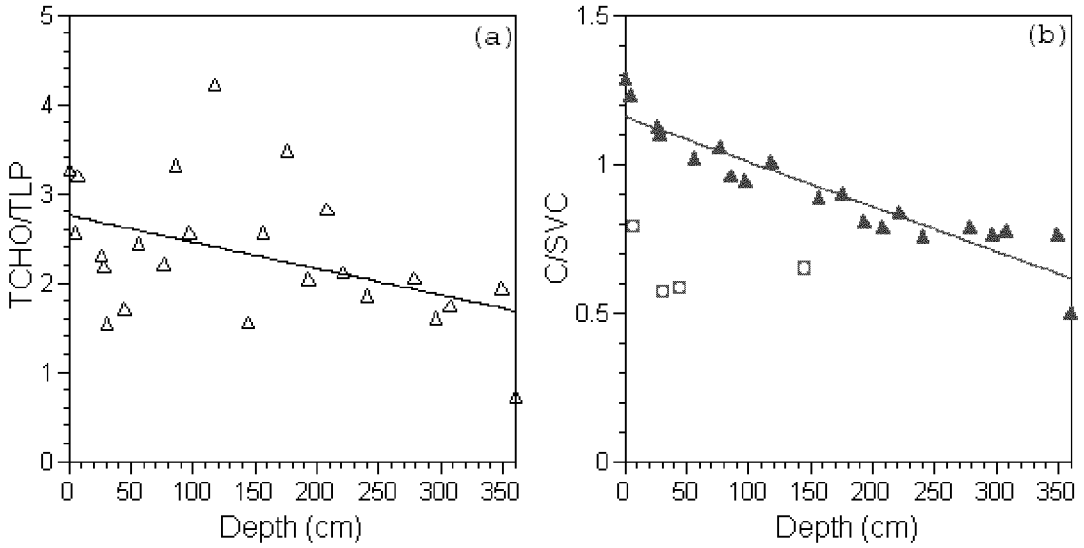

Fig.2. Vertical distribution of the ratios of (a) total carbohydrates and total lignin phenols (TCHO/TLP) (b) cellulose and sum of vanillyl, syringyl and cinnamyl phenols (C/SVC)

In a tropical wetland, angiosperm is mostly dominated and xylans (comprised of 7 acetate groups per 10 xylose) are the most abundant hemicellulose in angiosperms. The ester-bonded acetate groups in xylan can slowly hydrolyze under slight acidic condition, releasing acetic acid that could hydrolyzed glycosidic bonds in carbohydrates. Thus, the hemicellulose and some other fractions of TCHO might be degraded more easily in a tropical wetland through self hydrolysis (Hedges et al., 1985). Whereas cellulose is less degradable under weak acidic conditions compare to other fractions of carbohydrates (eg. hemicellulose). In addition, the lignified OM of higher plants is more resistant towards degradation and incorporated carbohydrates (mostly cellulose) can be protected. The strong correlation ($r^2 = 0.78$) between cellulose and lignin also supported the above assumptions. Thus, the ratio between cellulose and eight lignin phenols (C/VSC) except p-hydroxy group may reflect more precisely the degradation of terrestrial sources organic matter. The vertical profile of C/VSC also showed a decreasing trend with depth, and also suggesting degradation of terrestrial plant sources OM but relatively lower rate. The striking feature of the Fig 2(b) is a significant correlation between C/VSC and depths ($r^2 = 0.86$) except four major vegetation change events. This strong correlation indicated that degradation of terrestrial derived SOM related to their molecular distributions. Thus comparative degradation rates of cellulose and lignin reflect more consistent trend than that of absolute values. This consistent trend suggest a relative degradation rate of cellulose and lignin irrespective of sources and can be used as a supplementary proxy for age of sediment core in continental lake and marine coastal environments as like racemization process of amino acid in sedimentary environments. However, degradation rate of both cellulose and lignin depend on many factors like temperature, depositional environment and pre-depositional alternations which may be a potential problem for such applications.

Degradation rates of cellulose and lignin

Lignin and mild-hydrolysis resistant carbohydrates (mostly cellulose) in the sedimentary organic carbon pool are refractory in nature and survived long geological time scale without much degradation and could reflect the original biological sources (Mores et al., 1994; Tareq et al., 2004). However, Lignin and mild-hydrolysis resistant carbohydrates concentrations at Rawa Danau core (RD1) decreased slowly with large scale variation depending on its sources, indicating slow degradation rate and relative stability compare to other molecular fractions. If the slow degradation caused by *in situ* degradation after loss of more labile fraction and under conditions of steady carbon input irrespective of source variations then the degradation rates can be calculated. In general, most of the natural reactions follow first order kinetics and the slow degradation of lignin and carbohydrates in sedimentary deposits can be described with a first order decay equation (Berner et al., 1980).

$$\ln C_t = - kt + \ln C_o$$

Where C_t is the remaining organic constituent at time t, and C_o the initial concentration of the metabolizable organic matter, k is rate constants for the decay of the reactive fraction, and t is the mean age in years of the sample horizon as calculated from the linear regression line of depth vs. radiocarbon age (Tareq et al., 2004). Decay rate estimates for lignin and carbohydrates were determined for the 3.6 m core by calculating the best least squares fit of a line to a plot of ln C_t

Vs. t and best fit least squares solution of lignin and carbohydrates (Fig.3) can be explained by the following equations.

$\sum$Lignin = 15.89* exp [-9*10^{-5} t] (n = 24; r^2_l = 0.25)

$\sum$Cellulose = 18.85* exp [-2*10^{-4} t] (n = 24; r^2_c = 0.57)

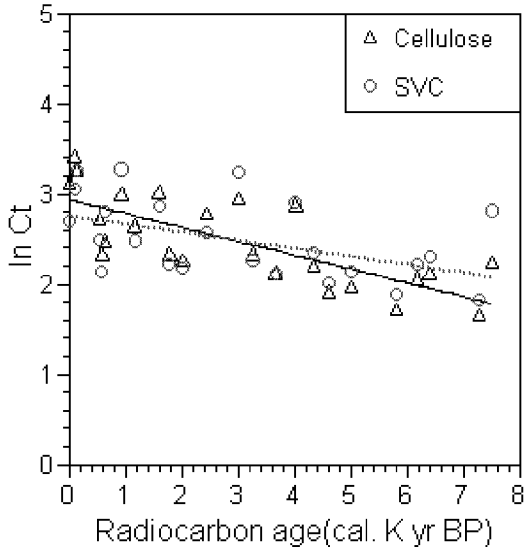

Fig.3. A plot of ln C_t vs. radiocarbon age (in k year) with linear regression line for both hydrolysis resistant carbohydrates (cellulose) and total lignin phenols (SVC).

The least square coefficient values (r^2_l = 0.25; r^2_c = 0.57) is not highly significant because of the variation of the sources of organic matter at Rawa Danau during the accumulation periods (Tareq et al., 2004). The slope of the line corresponds to the best estimate of first-order decay constants (k) correspond to 9*10^{-5} per year for lignin and 2*10^{-4} per year for carbohydrates; corresponding apparent half lives are 7700 and 3465 years for lignin and carbohydrates respectively. The apparent half life of lignin is more than double of cellulose and much lower than that of other lakes. (Lake Biwa, Japan; Ishiwatari and Uzaki, 1986, Lake Baikal, Siberia; Orem et al., 1997). This is in agreement with the traditional view that lignin is substantially more refractory than cellulose, especially under anaerobic conditions (Benner et al., 1984; Hedges et al., 1985). However, the inconsistent with the half lives of lignin in other lake sediments (Lake Biwa, Japan; Lake Baikal, Siberia) can be attributed to the deposition environment, nature and dispersion of terrestrial plant debris in the sedimentary environment as well as clay matrices in lacustrine deposits. The constant acid/aldehydate values for Vanilyll and Syringyl groups (Tareq et al., 2004) except few top layers indicate only the absence of *in situ* aerobic lignin degradation, but do not preclude anaerobic lignin degradation which may occur slowly without increasing these values. This slow degradation rate with relatively long half life of lignin indicate that the

composition of lignin phenol could still reflects its sources signatures without much deviations whereas, neutral sugar composition changes due to further modification and utilization of original molecules on the way of deposition. However, cellulose survived long time in a lignin enriched sedimentary environment without much degradation and could be used as a source indicator of terrestrial plant input.

Biogeochemical implications

The comparative study of lignin and carbohydrate composition gave an insight view of molecular-level diagenesis in a specific sub-aqueous environment such as tropical wetland and reveals a more refined assessment of organic matter sources. The flux and degradation rates of cellulose and lignin reveal that these components of SOM have different molecular stability in the natural environment. The labile nature and variable composition of carbohydrates in living organisms, compare to lignin phenols, make it difficult to use as an indicator of biological origin in a complex environment like tropical wetland. Even though vascular plant tissues have typical carbohydrate signatures (Cowie and Hedges, 1984), in natural media these signatures tend to disappear rapidly since degraders such as bacteria and fungi, in turn synthesize new polysaccharides. Consequently, different interpretations can be derived from carbohydrate composition. However the flux and degradation rate of cellulose showed that tropical plant origin cellulose is preserved in a subaqueous reducing environment. This refractory nature of plant origin cellulose could be used to estimate contribution of terrestrial plant to SOM of continental aquatic ecosystems as well as to marine coastal environments.

Total carbon from lignin derived phenols represented a significant portion TOC in tropical wetland and varied between 5.4% and 19.4% in sediments, thus highlighting the relative stability of lignin as previously observed (Benner et al 1984; Hedges et al., 1985; Cowie and Hedges, 1984; Lallier-Vergès et al., 1998). In addition, lignin oxidation product (LOP) is higher in sediments (SOM: 8.66 to 31.31 mg/100 mg OC with average 17.31 mg/100 mg OC; Tareq et al., 2004) than in tropical plants (Plant: 9.38 to 14.20 mg/100 mg OC with average of 11.12 mg/100 mg OC; Tareq et al., 2007) that also reflects their refractory character. Nevertheless, decomposition of lignin occurred and its degradation rates varied in different environments. Lignin oxidation products were lost at a lower rate than cellulose and total neutral sugars. The low degradation rate and stable acid/aldehyde ratios may result from another process of lignin decomposition that is aromatic ring cleavage. In contrast to phenyl-propyl chain oxidation, aromatic ring cleavage may be the predominant mechanism of lignin degradation under anaerobic conditions (Haddad and Martens, 1987; Dittmar and Lara, 2001). Lignin was thought to be refractory in anaerobic conditions, mainly because fungi, the most important wood decaying organisms are aerobes. However, the results of present works showed that lignin degradation in anaerobic conditions can occur without any changes in the (Ad/Al) of the vanillic and syringyl unit. Colberg and Young (1982) and Benner et al 1984 also demonstrated that lignin can be degraded at a significant rate by bacteria. Even under such circumstance lignin can be used as a molecular tracer to record paleoenvironmental changes due to it relatively long half-life.

Results of the present study provide an example of the extent to which terrestrial plants remains can be degraded in wetland sediments. The measured degradative rates have selectivity (upto 2

orders of magnitude differences in preservation for cellulose and lignin) for carbohydrates, cellulose and lignin components in sedimentary organic mixtures. However, these results are preliminary in many aspects and important questions remain to be answered, e.g., degradation mechanisms of different molecular components are not clear except that they are following first order reaction kinetics. In addition, absence of oxidative decomposition of lignin components (Ad/Al ratio remain constant) suggest that anaerobic condition prevailed during early diagenesis, some early exposure of the terrestrial plant debris to aerobic degradation can not be ruled out. This uncertainty is particularly key to the critical question of whether bio-polymer (e.g., lignin and cellulose) can be used as molecular tracer in natural environments.

Conclusion

On the basis of the above results, different mechanisms of carbohydrates and lignin degradation as well as mineralization of associated OC have to be considered before using as biomarkers. Lignin was evidently not affected by an initial step of fast aerobic degradation, and leaching did not remove a considerable proportion of lignin from the sediment because the lignin yield of aquatic organic matter was constantly low (Dittmar and Lara, 2001). Lignin and cellulose degradation within the tropical wetland was probably a constant anaerobic process obeying a first-order kinetic. Thus, lignin can be used as a potential biomarker together with other biogeochemical data such as bulk geochemistry, C/N, cellulose, stable carbon isotopes. On the other hand, the application of neutral monosaccharides is limited but plant origin cellulose has potential possibility to use as a biomarker.

References

Aucour, A.-M., Booefille, R., Hillaire-Marcel, C. 1999. Sources and accumulation rates of organic carbon in an equatorial peat bog (Burundi, East Africa) during Holocene: carbon isotope constraints, Palaeogeography Palaeoclimatology Palaeoecology, 150, 179–189.

Benner, R., Maccubin, A.E., Hodson, R.E. 1984. Anaerobic biodegradation of the lignin and polysaccharide components of lignocellulose and synthetic lignin by sediment microflora, Applied Environmental Microbiology. 47, 998–1004.

Benner, R., Weliky, K.,Hedges, J.I. 1990. Early diagenesis of mangrove leaves in a tropical estuary: Molecular-level analyses of neutral sugars and lignin derived phenols. Geochimica et Cosmochimica Acta, 54, 1991–2001.

Berner, R.A., 1980. Early Diagenesis: A Theoretical Approach, Princeton University Press, Princeton, NJ, pp 241.

Bourdon S., Laggoun-Defarge F., Disnar J.R., Maman O, Guillet B., Derenne S., Largeau C. 2000. Organic matter sources and early diagenetic degradation in tropical peaty marsh (Tritrivakely, Madagascar): Implication for environmental reconstruction during the sub-Altantic. Organic Geochemistry, 31, 421-438.

Bourdon, S., Laggoun-Défarge, F., Disnar, J.R., Maman, O., Guillet, B., Derenne, S., Largeau, C., 2000. Early diagenesis of organic matter from higher plants in a malagasy peaty marsh. Application to environmental reconstruction during the Sub-Atlantic. Organic Geochemistry, 31, 421–438.

Canuel, E.A., Cloern, J.E., Ringelberg, D.B., Guckert, J.B., and Rau, G.H. 1995. Molecular and isotopic tracers used to examine sources of organic matter and its incorporation into the food webs of San Francisco Bay, Limnology and Oceanography 40, 67–81.

Colberg, P.J., and Young, L.Y. 1982. Biodegradation of lignin derived molecules under anaerobic conditions, Canadian Journal of Microbiology, 28, 886–889.

Cowie, G. L., Hedges, J. I. 1984. Determination of neutral sugars in plankton, sediments, and wood by capillary gas chromatography of equilibrated isomeric mixtures. Analytical Chemistry, 56, 497–504.

da Cunha, L.C., Serve, L., Blazi, J-L. 2002. Neutral sugars as biomarkers in the particulate organic matter of a French Mediterranean river. Organic Geochemistry, 33(8), 953-964.

Degens, E.T., Kempe, S., Richey, J.E., 1991. In: Biogeochemistry of Major World Rivers, Wiley & Sons, SCOPE-42, New York.

Dittmar, T., Lara, R.J., 2001. Molecular evidence for lignin degradation in sulfate-reducing mangrove sediments (Amazônia, Brazil), Geochimica et Cosmochimica Acta, 65(9), 1417-1428.

Goñi, M.A., Teixeira, M.J., Perkey, D.W. 2003. Sources and distribution of organic matter in a river-dominated estuary (Winyah Bay, SC, USA). Estuarine, Coastal and Shelf Science, 57(5-6), 1023-1048.

Haddad, R.I., Martens, C.S. 1987. Biogeochemical cycling in an organic-rich coastal marine basin: 9. Sources and accumulation rates of vascular plant-derived organic material. Geochimica et Cosmochimica Acta, 51(11), 2991-3001

Hedges J.I. Ertel, J.R. 1982. Characterization of lignin by capillary gas chromatography of CuO products. Analytical Chemistry, 54, 174-178.

Hedges, J. I. Cowie, G. L. Ertel, J. R., BarBour, R. J., Hatcher, P. G. 1985. Degradation of carbohydrates and lignins in buried woods. Geochimica et Cosmochimica Acta. 49, 701-711

Hu, F.S., Hedges, J.I., Gordon, E.S., Brubaker, L.B., 1999. Lignin biomarkers and pollen in postglacial sediments of an Alaskan lake. Geochimica et Cosmochimica Acta, 63(9), 1421-1430

Ishiwatari R, Uzaki M.1987. Diagenetic changes of lignin compounds in a more than 0.6 million-year-old lacustrine sediment (Lake Biwa, Japan). Geochimica et Cosmochimica Acta, 51, 321-328.

Lallier-Vergès, E., Perrussel, B.P., Disnar, J.R., Baltzer, F. 1998. The relationship between environmental conditions and the diagenetic evolution of organic matter derived from higher plant in a present mangrove swamp system (Guadeloupe, French West Indies), Organic Geochemistry, 29, 1663–1686.

Lieth, H. 1975. Modeling the primary productivity of the world In: H. Lieth and R.H. Whittacker, Ed., Primary Productivity of the Biosphere, Springer-Verlag, New York, pp. 237–264.

Marchand, C., Disnar, J.R., Lallier-Vergès, E., Lottier, N. 2005. Early diagenesis of carbohydrates and lignin in mangrove sediments subject to variable redox conditions (French Guiana). Geochimica et Cosmochimica Acta, 69(1), 131-142.

Moers, M. E. C., de Leeuw, J. W., Baas, 1994. Origin and diagenesis of carbohydrates in ancient sediments. Organic Geochemistry, 21(10-11), 1093-1106.

Mopper, K., 1977. Sugars and uronic acids in sediment and water from Black sea and North sea with emphasis on analytical techniques. Marine Chemistry, 5, 585–603

Orem W.H, Colman SM, Lerch H.E. 1997. Lignin phenols in sediments of lake Baikal, Siberia: application to paleoenvironmental studies. Organic Geochemistry, 27(3-4), 153-172.

Tareq, S. M., Tanaka, N., Ohta, K. 2004. Biomarker signature in tropical wetland: lignin phenol vegetation index (LPVI) and its implications for reconstructing the paleoenvironment. Science of the Total Environment, 324(1-3), 91-103.

Tareq, S.M., Tanaka, N., Ohta, K. 2007. Isotopes and lignin signature in tropical peat core: An approach to reconstruct past vegetation and climate changes. *TROPICS* 16(2), 131-140.

Tareq, S.M., Ohta, K. 2011. Distribution of combined monosaccharides in sediments from the Lake Rawa Danau, West Java, Indonesia: sources and diagenetic fate of carbohydrates in a tropical wetland. Geochemical journal 45, 1-13.