

**Organic Matter Preservation in Marine Sediments:
A Bulk and Molecular Study**

Denis Brion

**A Thesis
In
The Department
of
Chemistry**

**Presented in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy at
Concordia University
Montreal, Québec, Canada**

July 2007

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ISBN: 978-0-494-37721-5
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ISBN: 978-0-494-37721-5

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ABSTRACT

Organic Matter Preservation in Marine Sediments: A Bulk and Molecular Study

Denis Brion, Ph.D.

Concordia University, 2007

Sedimentary organic matter (OM) composes 20% of all carbon preserved in the Earth's crust. Marine sediments constitute the major long-term sink of organic carbon (OC) on Earth, although only <0.3% of OM photosynthesized by plants is eventually preserved. While the burial of this small OM fraction affects the global cycles of atmospheric CO₂ and O₂, the mechanisms that control sedimentary OM preservation are still unclear. Recent studies have suggested a strong relationship between OM preservation and the OM physical forms, chemical compositions and cumulative exposure to O₂ during deposition and burial. However, although organic geochemistry has now progressed to the point where the major hydrolysable biochemicals (proteins, carbohydrates and lipids) can be measured using standard chromatographic methods, >75 % of sedimentary OM is still missed chromatographically and remains molecularly uncharacterized. We therefore combined bulk and molecular-level analytical approaches to target the mechanistic understanding of the effects of physical protection, molecular composition and O₂ exposure time (OET) on OM preservation. A wet chemical sequential extraction procedure was developed to quantify and separate OM from diverse marine sediments fractions having different chemical reactivity. This research project then focussed on one fraction believed to be determinant in OM preservation, i.e., non-hydrolysable oxygen-sensitive organic matter (OSOM). An optimized method based on the gentle chemical oxidation of OM using RuO₄ was developed in order to investigate the molecular structure of OSOM and its relationship to OM preservation under varying sedimentary conditions. The relative abundance of the OSOM fraction in marine sediments decreases exponentially with oxygen exposure time, in agreement with our working hypothesis. However, our data suggest a more complex relationship than previously thought in that inputs of terrestrially-derived OSOM most likely affect the degradation rate and relative abundances of the bulk OSOM fraction. RuO₄ treatment on the OSOM fractions revealed a composition consisting mostly of cross-linked aliphatics polyesters, and relative abundances of the oxidation products that are correlated with oxygen exposure time (OET). Further molecular, compound-specific isotopic (GC-IRMS) and spectroscopic (FTIR, solid-state HR-NMR) studies on the OSOM component will help verifying the hypothesis of the major role of oxygen exposure time in OM preservation.

Acknowledgements

Several people helped and supported me during this work that began in 2003. I would like to thank:

Dr. Yves G elinas for his availability even when his kids were sick, his confidence and the interest he had for my work. He shall stay a good supervisor example.

Dr.  milien Pelletier for his participation and his interest he has shown for my work.

Dr. Louis Cuccia, Dr. Justin Powlowski, Dr. Moritz Lehmann, Dr. Luc Tremblay and Dr. Peter Bird for their participation in this work as committee members.

All the LEGO and other lab members with whom I had a lot of fun and who were helpful (Alexandre Ouellet, Robert Panetta, Marie-H el ene Veilleux, Maggie Mouradjian, Nancy Austin, Lisa Barrazuol, Mina Ibrahim, Farah, and Madiha).

All crew members from different scientific missions

NGCC/CCGS Amundsen

NGCC/CCGS Teleost

R/V Coriolis II

R/V Alte Irizar

Argentinian naval base Jubany in Antarctica

My parents Marie-Odile and  ric Brion, and also my sister  milie Brion for their entire support from the beginning.

My friends, David Durand, Fran ois Yu, and others who supported me and helped me during this project.

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List of acronyms

δ: Difference in the stable isotope ratio of a sample versus that of a reference standard in per mil (‰)

Δ¹⁴C: Radioactive carbon (¹⁴C) isotope signature

δ¹³C: Stable carbon 13 isotope signature

δ¹⁵N: Stable nitrogen 15 isotope signature

ACN: Acetonitrile

C/N: Carbon to Nitrogen atomic ratio

CASES: Canadian Arctic Shelf Exchange Study

CCD: Carbonate Compensation Depth

CHN: Elemental analyzer for carbon, hydrogen and nitrogen

CHNS/O: Elemental analyzer for carbon, hydrogen, nitrogen, sulphur and oxygen

CP/MAS ¹³C-NMR: Cross Polarization Magic Angle Spinning ¹³C Nuclear Magnetic Resonance

DCM: Dichloromethane

DOC: Dissolved Organic Carbon

DIC: Dissolved Inorganic Carbon

dpm/g: disintegration per minutes per gram

EA: Elemental Analyzer

Et₂O: Diethyl ether

FAMES: Fatty Acid Methyl Esters

FID: Flame Ionization Detector

FTIR: Fournier Transform Infrared Spectroscopy

GC: Gas Chromatography

Gt: giga ton

i.d.: internal diameter

IAEA: International Atomic Energy Agency

IRMS: Isotope Ratio Mass Spectroscopy

MeOH: Methanol

MUC: Molecularly Uncharacterized Carbon

NMR: Nuclear Magnetic Resonance

OC: Organic Carbon
OET: Oxygen Exposure Time
OM: Organic Matter
OSOM: Oxygen-Sensitive Organic Matter
PDB: PeeDee Belemnite
Py-GC/MS: Pyrolysis-GC/MS
SPME: Solid Phase Micro-Extraction
tC: ton of carbon
TCD: Thermal Conductivity Detector
TFA: Trifluoroacetic acid
TOM: Terrestrial Organic Matter
UCM: Unresolved complex mixture
VPDB: Vienna PeeDee Belemnite
yr: year

CHAPTER I

GENERAL INTRODUCTION

1.1. Marine geochemistry

The field of organic geochemistry was born in 1936 when Alfred E. Treibs first discovered traces of organic molecules derived from living organisms in coal and petroleum. While in the early beginning organic geochemistry was limited to the field of applied petroleum research, it rapidly extended into many geochemical branches including the study of the Earth's and other planets chemical composition. The modern definition of geochemistry now encompasses: (i) the study of chemical processes that govern the composition of rocks and soils, (ii) the cycles of organic and inorganic matter, (iii) the energy that transports the Earth's chemical components in time and space, and (iv) their interactions with the hydrosphere and atmosphere. Some of the most important research areas in geochemistry are isotope geochemistry, the cycles of elements on Earth, cosmochemistry, biogeochemistry, and organic geochemistry. Organic geochemistry can be defined as the study of organic compounds derived from living or once-living organisms and of all the processes involved in their evolution in natural systems. Organic geochemical studies on sediments have allowed to deepen our understanding of carbon cycling, climate change and ocean processes, and also to understand the origins and sources of organic materials. Oceanographers are deeply involved in this type of research. Marine geochemistry is the direct offspring of this interest; it exploits several areas of geochemistry, each of which is applied to samples from aquatic environments. These

studies therefore make use of a wide array of traditional techniques in analytical, organic and inorganic chemistry. Because of the very complex chemical makeup of aquatic samples however, these techniques often fail to provide the level of information needed to decipher reaction histories and pathways. There is thus a great need for the development of new analytical approaches and tools to allow probing the detailed composition of organic materials and linking chemical makeup to global biogeochemical cycles on Earth.

1.2. Sediments

The ocean floor forms a natural sink that embodies all of the continent's degradation products, which are transported by different means (i.e., river, wind, coastal erosion and volcanic eruptions). Sediments are composed of particulate and dissolved products of continental weathering and aquatic production. These products undergo slow biological and geochemical processes and are deposited on the ocean floor. Therefore, sediments represent a unique source of information for understanding the origins of the continent and of the ocean.

Sedimentary material can be divided into three fractions including: (i) an organic fraction (i.e., living cells, life forms and their remains), (ii) a detrital inorganic component (minerals and metals), and (iii) a biogenic inorganic component (including carbonate or silica exoskeleton of plankton). In warm waters, most of the biogenic inorganic fraction consists of carbonates (shells of foraminifera and pteropodes), and, in cold waters, of silica (diatoms and radiolarians) (Lončarić *et al.*, 2007). Seas, oceans and lakes accumulate sediment over time. In the water column, because of its hydrophobic

properties, organic matter (OM) tends to associate into aggregates and onto mineral particles, and sink. Such accumulation, or sedimentation, occurs at different rates that are determined by several environmental parameters. Usually, in the deep ocean, the sedimentation rates are low (between $0.1 - 1 \text{ cm}\cdot\text{yr}^{-1}$) because of the very low terrestrial inputs and surface production. Near river mouths and on continental shelf areas, sedimentation rates can vary over a wide range (between $0.1 - 20 \text{ cm}\cdot\text{yr}^{-1}$) depending on surface productivity and the magnitude of the flux of particles transported from the land. Other parameters such as currents can also affect sedimentation rates (Henrichs, 1992; 1993).

Upon reaching the ocean floor, sediment-forming particles are progressively incorporated into the geological cycle of rocks and in the formation of fossil deposits (petrogenic kerogen) and fossil fuels. This incorporation starts with diagenetic processes occurring close to the interface of water and sediments. Diagenesis is defined as all physical, chemical and biological processes that transform sediments and their components such as organic matter, in the first meters before rock formation (Hodson *et al.*, 1983; Littke *et al.*, 1997). In other terms, it is one of the most important transitions for organic matter as it cycles through the global carbon cycle.

1.3. Carbon and oxygen cycles

1.3.1. The carbon cycle

On Earth, the global carbon cycle mainly comprises two parallel sub-cycles that share atmospheric CO_2 as their common source of carbon. The main distinction between

the organic and inorganic carbon sub-cycles is that in the latter, carbon is not incorporated into living biomass and its organic remains.

The organic carbon sub-cycle is in turn composed of two main components, namely: the biological and the geological organic carbon cycles. In the biological cycle, the carbon from atmospheric CO₂ or dissolved bicarbonate ion (HCO₃⁻) is taken up by plants and bacteria through photosynthesis (primary producers). This fixed carbon can then be transferred to higher animal species. All fixed organic carbon eventually ends up in metabolic or chemical oxidation pathways involved in the decaying biomass to oxidation products and CO₂. This turn-over usually takes place in a time frame of days or several thousand years, depending on the nature of OM produced by organism. The size of the carbon reservoir integrated into the geological cycle has been estimated in some studies and is always several orders of magnitude larger than that of the biological organic carbon cycle (6.4×10^{15} t C vs. 3×10^{12} t C, respectively) (see Rullkötter, 2000 and references therein). Its turn-over time is also several orders of magnitude longer, on the order of millions of years (Rullkötter, 2000). The cycle begins with the incorporation of biogenic organic matter into sediments or soils, and then leads to the formation of natural gas, kerogen, petroleum and coal or metamorphic forms of carbon, which may be reoxidized to carbon dioxide following uplifting and erosion of sedimentary rocks or by combustion of fossil fuels (Dickens *et al.*, 2004). Kerogen is known to be an insoluble mixture of organic material that makes up a large portion of the OM in sedimentary rocks; it represents the largest reservoir of OC in rocks (Table 1.1). When present in high concentration in rocks, kerogen may form oil shale deposit. Table 1.1 shows the

estimation from Hedges and Keil (1995) of the major reservoirs for inorganic and organic carbon.

Table 1.1. Major reservoirs of inorganic and organic carbon (Hedges and Keil, 1995)

Reservoir type	Amount of C in Gt
Inorganic	
Carbonates	60,000
Soil carbonates	1.1
Atmospheric CO ₂	0.66
Marine DIC	38
Organic	
Kerogen, Coal	15,000
Soil humus	1.6
Land plant tissues	0.95
Seawater DOC	0.60
Surface sediments	0.15

1.3.1.1. The inorganic carbon cycle

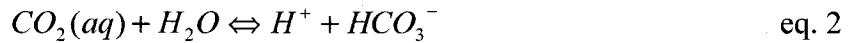
The inorganic carbon cycle describes the transfer of carbon between reservoirs in the inorganic form (CO₂, dissolved carbonate species, carbonate minerals). This cycle comprises several major components including the sources of CO₂, CO₂ chemistry in water, weathering of rocks, and release from combustion. At the water-atmosphere interface, CO₂ chemistry is based on the equilibrium of CO₂ in its solid, aqueous and gaseous forms.



Other areas on Earth can represent sources of CO₂ in this cycle, including areas of upwelling[†], although the ocean is in general considered as a global sink. Ocean

[†] See glossary

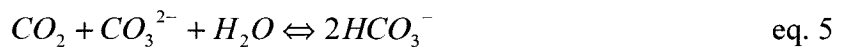
circulation, primary productivity and temperature are key factors that regulate the CO₂ chemistry in this cycle. When CO₂ dissolves in seawater, at pH > 7, bicarbonate ions are formed.



In basic conditions, it also leads to the formation of carbonate ions (CO₃²⁻), which can react with CO_{2(aq)}, thus producing more bicarbonate ions.

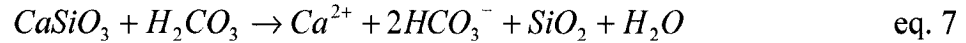


Through this last reaction, anthropogenic CO₂ can be titrated with oceanic carbonate until the maximum buffering capacity of the ocean is reached. The overall effect is a chemical equilibrium between CO₂, carbonate ions, and bicarbonate ions in the ocean water.

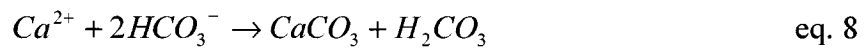


Carbonate (CaCO₃) and silicate (CaSiO₃) rocks react with CO₂ through weathering, also leading to the formation of HCO₃⁻ ions.





These products are released in rivers and flow toward the ocean. The SiO_2 can be incorporated into the skeletons of diatoms and radiolarian. Following its solubilisation through weathering processes, Ca^{2+} can be taken up with the bicarbonate ions present in water by organisms to form shells ($CaCO_3$). This reaction is the reverse of carbonate weathering.



Following the death of the organisms, sea shells then sink to the bottom of the ocean, where they can be preserved or re-dissolved depending on the saturation state of the water with respect to carbonates. Usually, at a depth of less than 4 km, water is saturated with $CaCO_3$, and the shells therefore do not dissolve (Conan *et al.*, 2002). However, at depths greater than 4 km, CO_2 (H_2CO_3) concentrations are higher and thus water is corrosive towards $CaCO_3$. The boundary between the two is called the carbonate compensation depth. Carbonate metamorphism is the last part of the cycle in which $CO_2(g)$ is re-generated by volcanoes and hydrothermal vents:

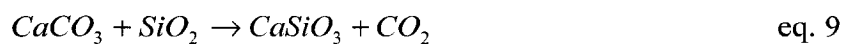


Figure 1.1 summarizes the global inorganic carbon cycle.

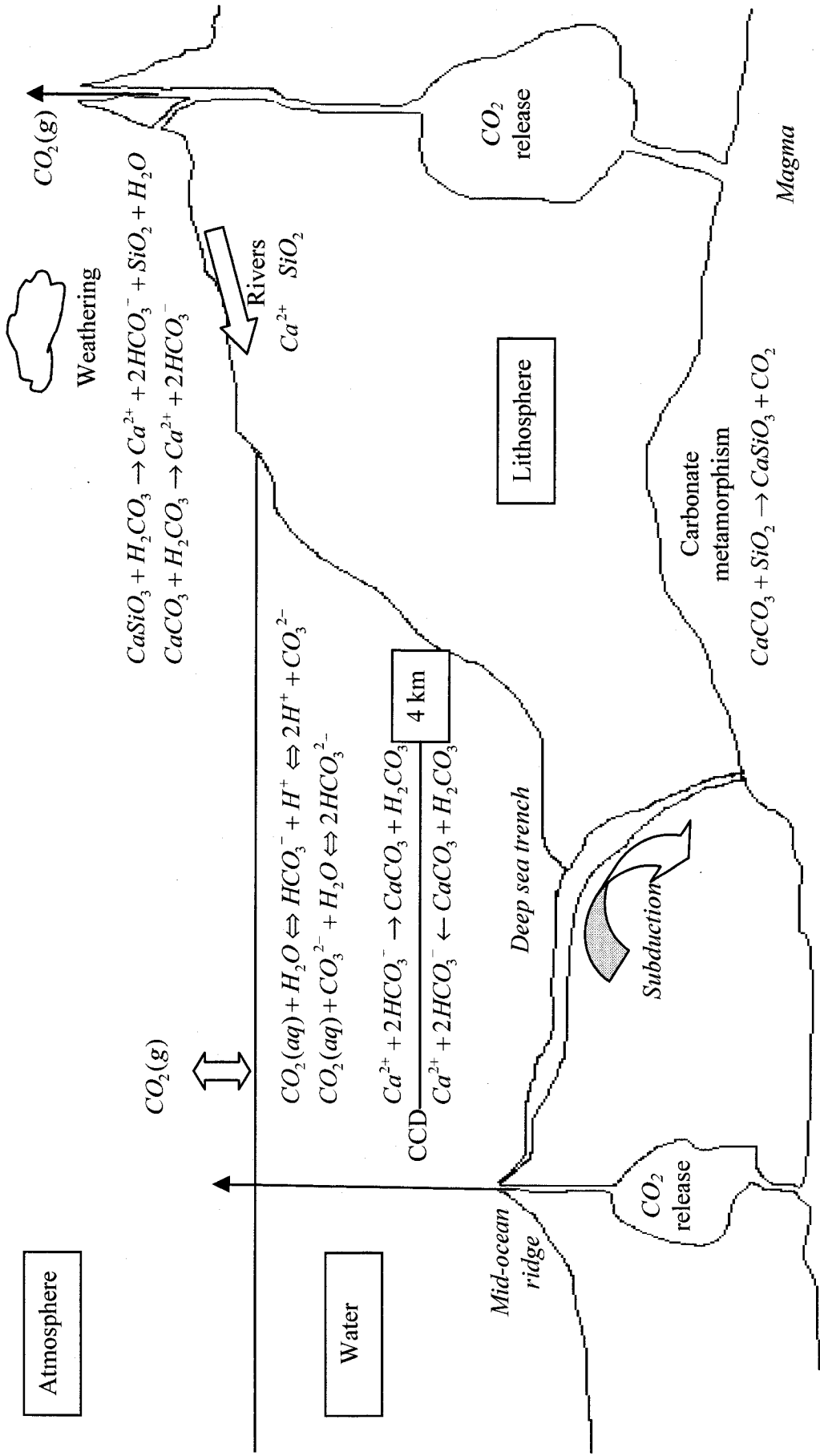
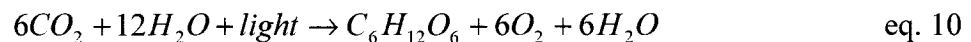


Figure 1.1. The inorganic carbon cycle (based on « Océanographie Générale » class notes by Bréthes *et al.*, 2000)

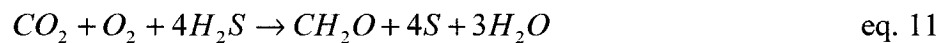
1.3.1.2. The organic carbon cycle

The organic carbon cycle is composed of four main carbon compartments which are the atmosphere, the biosphere, the lithosphere and the ocean. The cycle hinges on photosynthesis which consists of the incorporation of $\text{CO}_2(\text{g})$ or HCO_3^- by plants or bacteria. This carbon is then incorporated in either the terrestrial or the aquatic biomass. The respiration and decomposition of biomass produce $\text{CO}_2(\text{g})$. Some of the incorporated carbon can escape this decomposition loop to become part of either the soil or the sedimentary organic matter deposits. On geological time-scales, this OM can then be transformed into kerogen, coal, oil or gas, and if undergoing combustion, produce $\text{CO}_2(\text{g})$ that can then be incorporated into limestone (CaCO_3) and dolomite ($\text{CaMg}(\text{CO}_3)_2$) rock formations (Morse and Arvidson, 2002; Lu *et al.*, 2004). Figure 1.2 shows the different pathways involved in organic carbon fixation and transformation.

The chemistry involved in the organic carbon cycle is more complex than in the inorganic cycle. It includes the biochemistry of life forms and their degradation products. Photosynthesis (eq. 10) is of utmost importance because it is the source of organic carbon present in most organisms on Earth.



Chemosynthesis is another organic carbon production pathway. Instead of using light as a source of energy, chemosynthetic organisms satisfy their energy needs by oxidizing inorganic molecules such as H_2S , as shown in equation 11 (Corselli and Basso, 1996).



Carbon synthesized as carbohydrates ($C_6H_{12}O_6$ or CH_2O) in these reactions is used not only as an energy source but also for building major components of cells or living tissues. Most of this carbon eventually undergoes metabolic or chemical oxidation to CO_2 while a small fraction of it is buried in sediments and soils (see next chapter). Globally, the majority of the carbon present on Earth is stored in sedimentary rocks where one carbon out of five is organic in origin (Hedges and Keil, 1995). The organic carbon cycle is of primary interest for biogeochemists as it provides a detailed record of the biosphere's health and of the history of life on Earth. Moreover, since carbon fixation by plants over geologic times generated the O_2 present in today's atmosphere, and also because OM degradation and preservation have a strong impact on O_2 levels, the carbon cycle is tightly linked to the oxygen cycle.

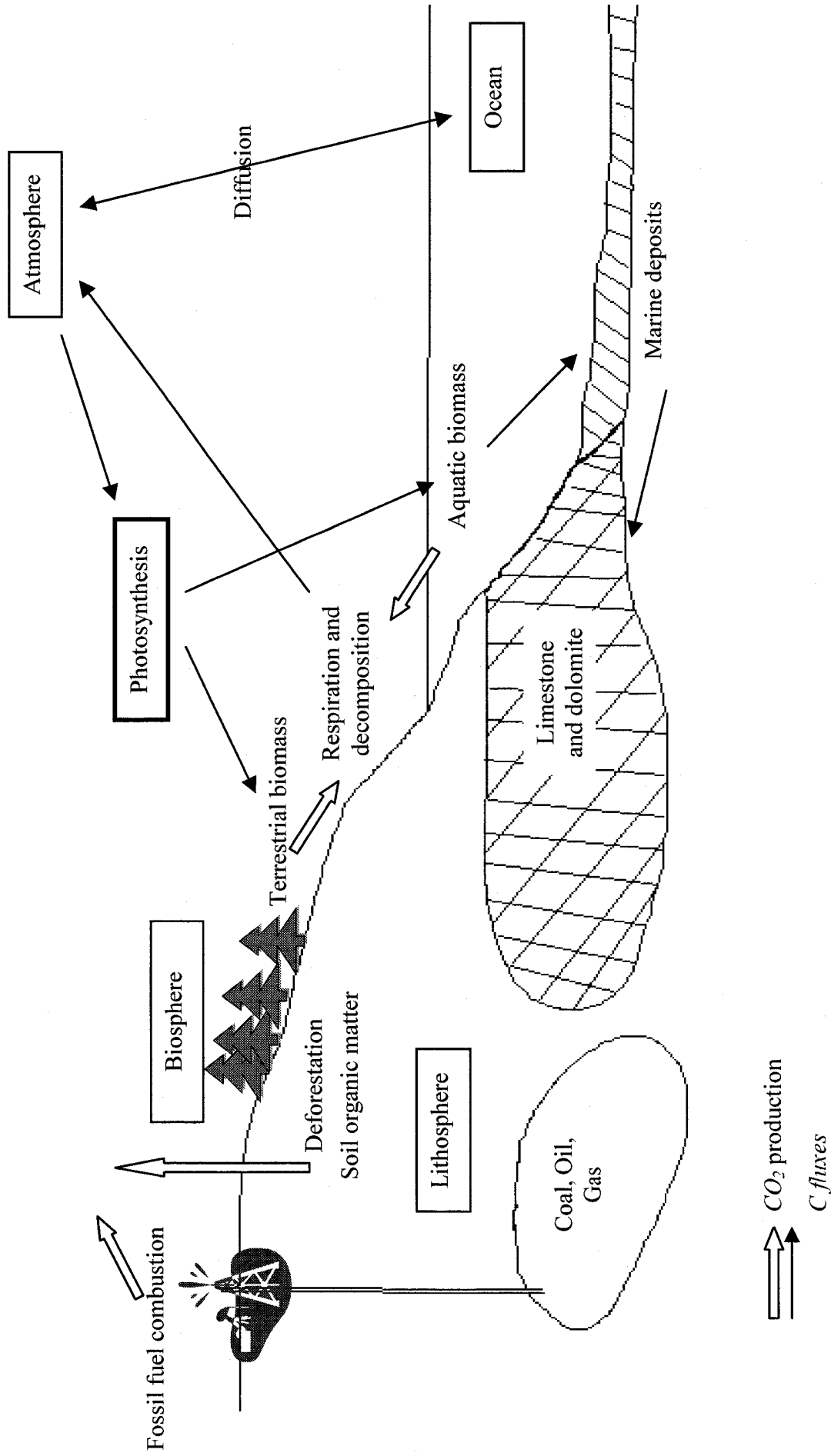


Figure 1.2. Simplified representation of the organic carbon cycle (based on « Océanographie Générale » class notes by Brêthes *et al.*, 2000)

1.3.2. The oxygen cycle

The oxygen cycle is the biogeochemical cycle that describes the fluxes of oxygen within and between the three main reservoirs of the Earth: the atmosphere, the biosphere, and the lithosphere. The vast majority of oxygen is contained in rocks and minerals within the Earth's crust (99.5%). Only a small fraction has been released as oxygen to the atmosphere (0.49%) (Walker, 1980). The main driving force in the oxygen cycle is photosynthesis, which is responsible for the Earth's modern atmospheric composition and life as we know it.

Oceanic photosynthesis contributes approximately 45% of the total oxygen to the oxygen cycle (Walker, 1980). An additional source of atmospheric oxygen comes from photolysis, whereby high energy ultraviolet radiations break down atmospheric water (H₂O) and nitrous compounds (N₂O) into simple components.



The main sink for atmospheric oxygen is via respiration and decay mechanisms in which animal life consumes oxygen and releases carbon dioxide. Because reduced lithospheric[†] minerals are oxidized with molecular oxygen, surface weathering and exposed rocks also consume oxygen. An example of surface weathering chemistry is the formation of iron oxides:

[†] See glossary



Oxygen also cycles between the biosphere and lithosphere. Marine organisms in the biosphere create carbonate shell material ($CaCO_3$) that is rich in oxygen. When organisms die their shells are deposited on the shallow sea floor and buried over time to create limestone rock in the lithosphere. Weathering processes initiated by organisms can also free oxygen from the lithosphere (Walker, 1980; Berner, 2005). In fact, some plants and organisms can release chelating agents such as peptides or sugars which may dissolve and break down minerals containing metal ions such as iron and aluminium present in rocks (Bland and Rolls, 1998).

The carbon and oxygen cycles have an important impact on the fate of OM, as they are directly related to the amount of OM present in each reservoir. Because of the limiting amount of oxygen in seawater and the slow rate of O_2 diffusion from the atmosphere to the deep ocean, OM is capable of decreasing the oxygen concentration through degradation processes and diagenesis (consumption of O_2) both in the water column and in the sediments. The fraction of the OM that is not degraded through oxygen consumption or reaction with other electron acceptors is eventually preserved on geological time scales in sediments and in sedimentary rocks, which are the largest reservoir for organic carbon on Earth. The extent to which preservation or degradation of OM is favored in different sedimentary settings has a critical impact on O_2 (and CO_2) level. Berner (2005) has for instance suggested that OM preservation and pyrite fluxes are the principal controls on O_2 . Because lands accumulate and preserve limited amounts of OM on a long-term basis, sedimentary OM thus represents the only link between the

active pool of carbon on the surface of the Earth and the slowly cycling (geological) organic carbon pool.

1.4. Organic matter in aquatic systems

Organic material or OM refers to any carbon-made material from living or once-living organisms and alteration products. It usually consists of the remains of a recently living organism, and it may also include still-living organisms as well as alteration products from biophysicochemical processes (UV radiation, combustion, metabolic products, etc.).

All OM that sinks in the water column may degrade progressively, be used by organisms or be preserved. Degradation processes are also active on the ocean floor, at the interface of water and sediments. Reduced molecules composing OM that still have a potential to release energy can be used by organisms (bacterial activity) or be degraded through abiotic chemical oxidation reactions. Throughout the Earth's history, almost all preserved OM has been incorporated in marine sediments deposited under oxygenated waters along continental margins (Hedges and Keil, 1995). Given oceanic productivity and sediment burial rates of 50×10^{15} and 0.16×10^{15} gC·yr⁻¹, respectively (Suess, 1980, Martin *et al.*, 1987; Berner, 1982), organic preservation in the marine environment is <0.3% efficient. Although empirical correlations often found in sediments suggest that productivity, sediment accumulation rate, bottom water oxicity, and OM source are key variables (Hedges and Keil, 1995), the exact nature and the relative importance of the mechanisms governing sedimentary OM preservation have remained unclear.

1.5. Isotopes and proxies

1.5.1. Carbon stable isotope ratio ($\delta^{13}\text{C}$)

Carbon is the principal element that living organisms utilize to build structural tissues, for replication and for energy-harvesting activities. Carbon is also an important component of the oceans, atmosphere, and crustal rocks. The physiologies of living organisms combine with chemical, physical, and geological forces to continually redistribute carbon between living and nonliving reservoirs in processes known as biogeochemical cycles. Such transfers of elements between reservoirs and biochemical species result in an isotopic fractionation[†] of elements that are composed of two or more stable (non-radioactive) isotopes. The measurement of isotopic fractionation is an important tool in oceanographic and limnological studies. Estimates of $\delta^{13}\text{C}$ [†] have become invaluable in biogeochemistry, in particular for determining the origin of compounds or evaluating diagenesis processes.

The stable carbon isotope signature of a sample, or $\delta^{13}\text{C}_{\text{sample}}$, is calculated using the stable isotope ratio of the sample compared to that of a reference. The value calculated in this way (see equation 15), expressed in per mil (the “delta” notation), indicates whether the sample is enriched (positive value) or depleted (negative value) in ^{13}C compared to the reference.

$$\delta^{13}\text{C}_{\text{sample}} = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{reference}}} - 1 \right] \times 1000 \quad \text{eq. 15}$$

[†] See glossary

The more negative the value the more the sample is depleted in the heavier isotope. The reference for carbon is based on VPDB (Vienna PeeDee Belemnite) which has a measured $^{13}\text{C}/^{12}\text{C}$ ratio value of $(11237.2 \pm 2.9) \times 10^{-6}$ (Craig, 1957).

An important process producing changes in the carbon isotopic composition in the geochemical cycle is the fixation of carbon from the carbon dioxide reservoirs (atmosphere and surface waters) in the form of complex organic molecules via photosynthetic reactions. Biologically fixed, carbon shows a high degree of enrichment in the light isotope compared to its source. The products of this process are subjected to rapid decay or to incorporation into sedimentary rocks, which occur there as solids, liquids or gases, either in dispersed form or in concentrated form, forming in the latter case often mineable deposits of fossil fuels, coal, petroleum or natural gas. The isotopic composition of carbon fixed in this manner is thus governed by photosynthesis and by the isotopic signature of CO_2 used in photosynthesis, as well as by any subsequent changes occurring during accumulation[†], diagenesis[†], catagenesis[†], and metamorphism[†].

Sources and sinks of carbon control the isotopic composition of total dissolved inorganic CO_2 in natural waters and result from isotope fractionation between solid, dissolved and gaseous phases, as well as from oxidation states. In natural waters, the carbon contributing to the total dissolved CO_2 is coming from several major sources, such as: atmospheric CO_2 , CO_2 derived from the decay of OM and dissolution of carbonate. Photosynthesis, carbonate precipitation and air-water exchanges are processes linked to the removal of dissolved inorganic carbon and thus also modify the isotopic composition of CO_2 .

[†] See glossary

Carbon fixation in photosynthesis may proceed by two pathways which differ by the number of carbon atoms in the first compound formed as an intermediate product (Bassham, 1971). The two pathways are known as the Calvin-Benson (C-3) and the Hatch-Slack (C-4) cycles. Terrestrial plants can be separated into three categories: C-3, C-4 and CAM (Crassulacean Acid Metabolism). The carbon isotopic composition of the plant material is highly correlated with the type of photosynthetic cycle used by the organism. Photosynthesis of C-3 type plants takes place via the enzyme ribulose-1,5-diphosphate carboxylase (RUDPC), while photosynthesis of C-4 type plants takes place via the enzyme phosphoenolpyruvate carboxylase (PEPC). In plants with crassulacean acid metabolism (CAM) both carboxylases are present. These plants alone are capable of net CO₂ fixation via the primary carboxylase reactions of C-3 and C-4 plants. Dark CO₂ fixation in CAM plants is functionally equivalent to photosynthesis in C-4 plants while CO₂ fixation in the light follows the C-3 pathway.

The enzymes involved in photosynthesis, RUDPC in C-3 cycle and PEPC in C-4 cycle, are responsible for the variations in isotopic composition. Ordinary C-3 plants form a 3-carbon compound called phosphoglyceric acid (PGA). The PGA is converted into another 3-carbon compound called phosphoglyceraldehyde (PGAL). Two PGAL molecules then combine to form a 6-carbon glucose molecule which is depleted in ¹³C ($\delta_{\text{PDB}}^{13}\text{C} \approx -25$ to -28 ‰). The Hatch-Slack cycle (C-4) combines CO₂ with phosphoenolpyruvate (PEP) to form a 4-carbon organic acid (oxaloacetic acid) which results in products less depleted in ¹³C than in the C-3 fixation pathway ($\delta_{\text{PDB}}^{13}\text{C} \approx -8$ to -18 ‰). The isotopic fractionation of ¹³C due to the CAM photosynthesis pathway is in

between C-3 and C-4 type plants isotopic fractionation ($\delta_{\text{PDB}}^{13}\text{C} \approx -14$ to -26 ‰) (Guilmette, 1983).

Between photosynthetic fixation and incorporation into the sediment, the OM is exposed to several oxidative processes and, as a result, only a very small fraction of the produced carbon compounds reach the sediments (Hedges and Keil, 1995). The main driving force that leads to isotopic fractionation in these processes is the kinetic effect. The ^{13}C isotope is heavier than the ^{12}C isotope, thus making its participation in chemical reactions slightly more difficult. Within the kinetic effect, the isotopic fractionation can be the result of: (i) isotope effects during bacterial degradation of OM with preferential use of ^{12}C (Kaplan and Rittenberg, 1964; Sackett and Thompson, 1963), and (ii) preferential elimination of certain functional groups with specific $\delta^{13}\text{C}$ through (bio)chemical alteration reactions (for example, carboxylic groups are usually enriched in ^{13}C compared to polymethylenic carbon) (Deines, 1989), and (iii) preferential preservation or degradation of molecules with an isotopic composition that differ significantly from that of the bulk OM pool (Deines, 1989).

In marine sediments, the $\delta^{13}\text{C}$ values of organic carbon vary from -10 to -30 ‰ (excluding environment where the methanogenesis[†] pathway is active and where the OM pool can be heavily depleted in ^{13}C) (Boehme *et al.*, 1996). In more than 90 % of all sediments sampled to date, the $\delta^{13}\text{C}$ isotopic composition of the OM pool varies over a very narrow range (between -17 and -27 ‰) (Deines, 1989; Liu *et al.*, 2007). Isotopic compositions heavier than -14 ‰ can be found in organic mud recently deposited in environments dominated by C-4 plants. The distribution of $\delta^{13}\text{C}$ isotopic compositions

[†] See glossary

for organic carbon from marine and terrestrial sediments differs considerably. While most marine sediments are grouped over a narrow $\delta^{13}\text{C}$ range of -17 to -22‰ (Table 1.2), soils vary over a much wider range with values that encompass those of marine sediments and extend from it both towards heavier and lighter isotopic compositions.

Table 1.2. End-member chemical and isotopic compositions of sources of organic matter to sediments (adapted from Burdige, 2006)

Organic matter type	$\delta^{13}\text{C}$ (‰)	Atomic C/N ratio
Vascular plants		20-500
C3 land plants	-25 to -28	
C4 land plants	-8 to -18	
Soil organic matter	-14 to -26 (approx.)	8-15
Marine phytoplankton	-17 to -22	5-10

1.5.2. Other proxies in geochemistry

Proxies are tools that allow identifying properties and processes through the measurement of certain molecules or parameters that have a specific source(s) and/or behaviour in the environment. In oceanography, proxies are often used and constantly needed in order to understand OM sources as well as reaction pathways and history. The very first tool commonly used as an OM source indicator is the bulk measurement of the atomic ratio of organic carbon to nitrogen (C/N). Atomic C/N ratios for OM of marine origin generally range from 5 to 10 because of the high content of nitrogen-rich proteins from lower organisms such as phyto- and zooplankton, as well as bacteria (Müller, 1977). Higher plant-derived OM has comparatively higher C/N values because of its high relative percentage of nonprotein materials (mostly cellulose, hemicellulose and lignin; Meyers and Ishiwatari, 1993). Despite its limitations, the atomic C/N ratio can thus

provide useful hints on the sources (marine vs. terrestrial) of OM along with historical variations in the relative importance of these sources in sediment cores.

Several other stable isotopes are commonly used in geochemistry, such as ^{18}O , ^{15}N and ^2H . Again, by knowing the isotopic fractionation due to kinetic or biophysical processes, an isotope ratio can provide valuable information on biogeochemical processes. For example, the $^{18}\text{O}/^{16}\text{O}$ isotope ratio, which is linked to the evaporation of water, can reveal changes in the past temperatures at the surface of the Earth when coupled to the measurement of time using the radioisotope ^{14}C . The major source of nitrogen used in terrestrial systems (N_2 fixation) is isotopically different from that used in marine systems (nitrate reduction; Kaplan, 1975). The $^{15}\text{N}/^{14}\text{N}$ isotope ratio is therefore often used for the determination of the source of organic materials entering the aquatic environment. The $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ isotope ratios are affected by meteorological processes that provide a characteristic fingerprint of their origin. This fingerprint is fundamental to investigating the provenance of groundwater in the hydrological cycle (Clark and Fritz, 1997).

Other proxies derive from molecular-level analyses and are used to pinpoint the sources of carbon in a sample. For example, molecules such as lignin are synthesized by vascular plants but are absent in marine plankton. Lignin is also relatively resistant toward oxidation and will degrade slowly under both aerobic and anaerobic conditions (Burdige, 2006 and reference therein). Hence, it is an excellent indicator for terrestrial OM sources and, when combined with $\delta^{13}\text{C}$ analysis of a sample, it can be used to estimate terrestrial OM contributions in aquatic sediments. A wealth of organic molecules such as sterols, aliphatic hydrocarbons, fatty acids, etc., and even contaminants can be

used as proxies in order to estimate sources, reaction histories, and even to identify transports pathway (marine or atmospheric currents). The field is growing rapidly, with several new proxies being proposed every year.

All these tools can be used to study OM in sediment; however, it is their combination that allows obtaining new insights into OM cycling and preservation. Presently, OM is more often characterized by bulk measurement approaches such as FTIR, NMR and elemental composition, which provide an assessment of the average chemical composition of OM in sediments. Unfortunately, these approaches do not provide detailed and specific molecular-level information. OM separation and/or fractionation schemes are therefore developed in many laboratories in order to identify and quantify specific molecules or OM fractions present in sediment. However, these schemes are often applied for the analysis of a single or a few organic compounds only (Li *et al.*, 2006; Deshmukh *et al.*, 2001; Stefanova and Disnar, 2000; Standley and Kaplan, 1998).

CHAPTER II

LITERATURE REVIEW

2.1. Organic matter composition in sediment

The accumulation of OM in ocean sediments results from terrestrial and marine inputs. It is mostly composed of biomolecular by-products of living organisms. Terrestrial OM (TOM) is carried by rivers and their tributaries as they drain emerged lands on their course to the ocean. TOM is mostly made of molecules from plants and their degradation products, as well as from microbial activity (including bacterial remains) (Wakeham *et al.*, 1997); in the last century however, molecules originating from anthropogenic activity, mainly contaminants from industrial and agricultural activities, have become increasingly present in the global OM pool. Before reaching ocean sediments, TOM can be partially degraded through (bio)chemical and photochemical processes in freshwater, estuaries and on local continental shelves and slopes, thus affecting the sediment's properties. The majority of marine OM inputs to sediment originate from the euphotic zone, where primary production takes place, most generally in the very first 100 m of water from surface. Marine OM is synthesized through the incorporation of atmospheric CO₂ by phytoplankton. This incorporation, or fixation, of CO₂ (atm) in the food web or through microbial activity generates a complex array of biochemical molecules (Rullkötter, 2000).

OM alteration and degradation first take place in the water column through biological and (photo)chemical processes (respiration, fermentation, and oxidation)

before entering the sediments (Figure 2.1). In the water column, a small fraction of the OM produced in the surface layer aggregates, sinks, and is eventually deposited onto the sea floor owing in part to mineral ballasting (Hedges and Keil, 1995), as some of the OM is intimately associated to biominerals such as carbonates and opal (biogenic silica) (Wüst *et al.*, 2002).

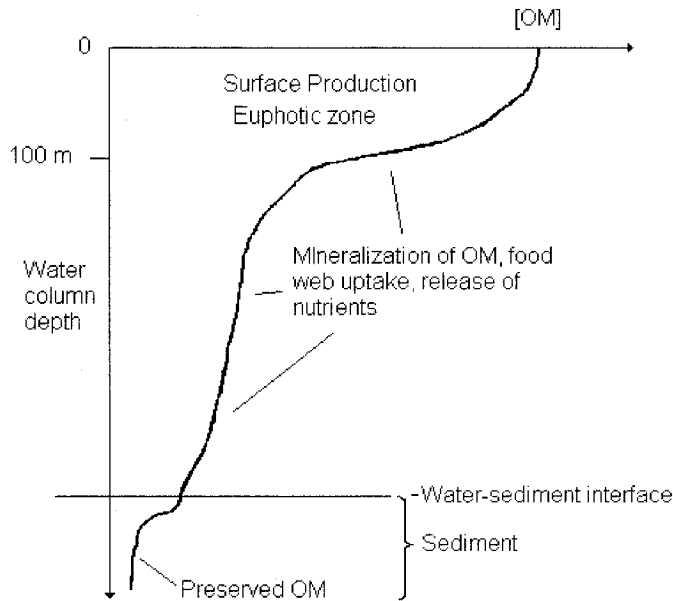


Figure 2.1. Organic matter flux from the surface to the ocean bottom (from Rullkötter, 2000)

As described above, diagenesis can be defined as all physical (current, diffusion, resuspension), chemical (consumption, precipitation) and biological processes (bioturbation) that affect sediment before rock formation. OM in sediment is altered by a complex suite of chemical and biological reactions included in diagenesis (Figure 2.2).

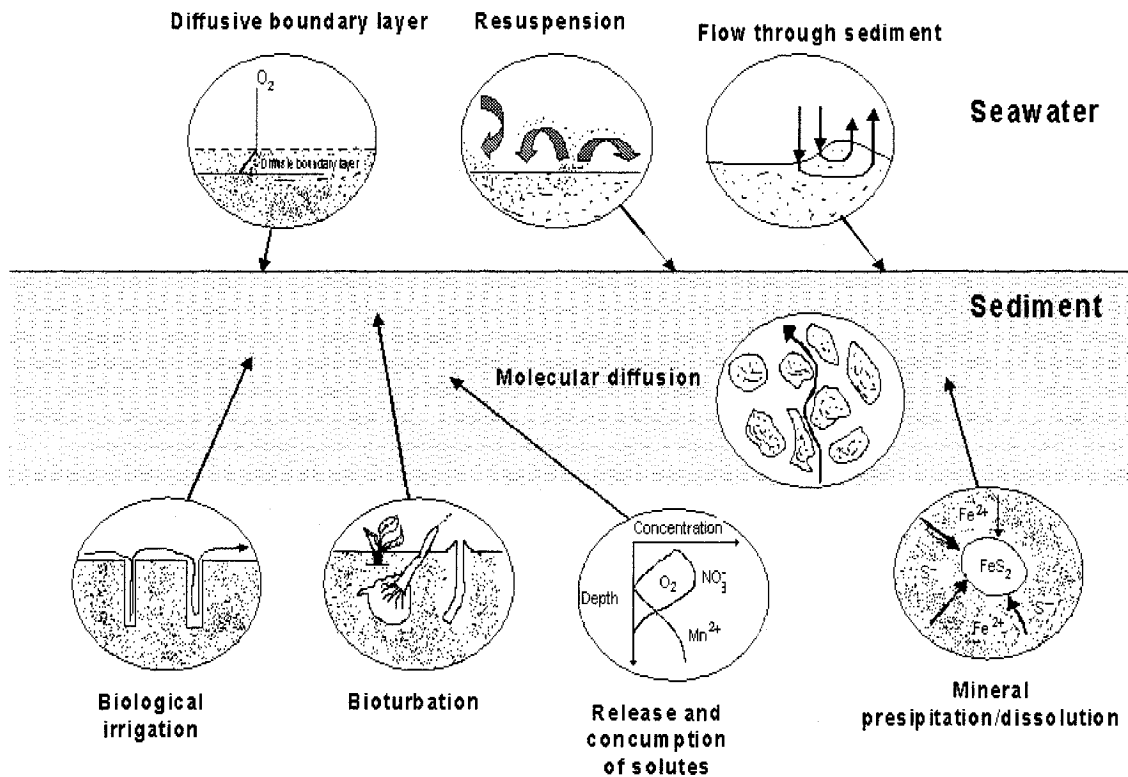


Figure 2.2. Typical diagenetic processes in marine sediments (from Rullkötter, 2000)

Most OM is very sensitive to oxygen and can be degraded in its presence. The amount of free oxygen at the sediment/water interface layer is one of the most important factors in determining the amount of OM eventually preserved and incorporated into the sediment matrix. OM follows a degradation pathway that depends on the presence of several oxidants (Hartnett *et al.*, 1998; Hedges *et al.*, 1999). A cascade of redox reactions takes place, based on the free energy yield (ΔG°_n) of the oxidation reaction (Table 2.1), the most favourable oxidant is first used until it is exhausted. The next one, again based on the ΔG°_n of the reaction, becomes the alternative oxidant until it is also exhausted. This series continues until the least favourable oxidant is used up, as detailed in Table

2.1, in which OM is represented in a simplified carbohydrate form (CH₂O) (Jickells and Rae, 1997).

Table 2.1. Sequence of redox equilibria in diagenesis

1. Aerobic respiration
$O_2 + CH_2O \rightarrow CO_2 + H_2O$
$\Delta G^\circ_n = -29.9 \text{ kcal/mol}$
2. Denitrification
$\frac{4}{5}NO_3^- + CH_2O + \frac{4}{5}H^+ \rightarrow \frac{2}{5}N_2 + CO_2 + \frac{1}{5}H_2O$
$\Delta G^\circ_n = -28.4 \text{ kcal/mol}$
3. Manganese reduction
$2MnO_2 + CH_2O + 2HCO_3^- + 2H^+ \rightarrow 2MnCO_3 + 3H_2O + CO_2$
$\Delta G^\circ_n = -23.3 \text{ kcal/mol}$
4. Nitrate reduction
$\frac{1}{2}NO_3^- + CH_2O + H^+ \rightarrow \frac{1}{2}NH_4^+ + CO_2 + \frac{1}{2}H_2O$
$\Delta G^\circ_n = -19.6 \text{ kcal/mol}$
5. Iron reduction
$4FeOOH + CH_2O + 4HCO_3^- + 4H^+ \rightarrow 4FeCO_3 + 7H_2O + CO_2$
$\Delta G^\circ_n = -12.3 \text{ kcal/mol}$
6. Sulphate reduction
$\frac{1}{2}SO_4^{2-} + CH_2O + \frac{1}{2}H^+ \rightarrow \frac{1}{2}HS^- + CO_2 + H_2O$
$\Delta G^\circ_n = -5.9 \text{ kcal/mol}$
7. Methane formation
$CH_2O \rightarrow \frac{1}{2}CO_2 + \frac{1}{2}CH_4$
$\Delta G^\circ_n = -5.6 \text{ kcal/mol}$

Figure 2.3 summarizes the diagenesis chemistry in the very first centimetres of sediments. These reactions produce different types of molecules which migrate by upward or downward diffusion in porewaters. These molecules can be re-oxidized, buried

(preserved) or released to the water column. Even though most originate from biomass, only a small fraction of sedimentary OM has been molecularly characterized to date.

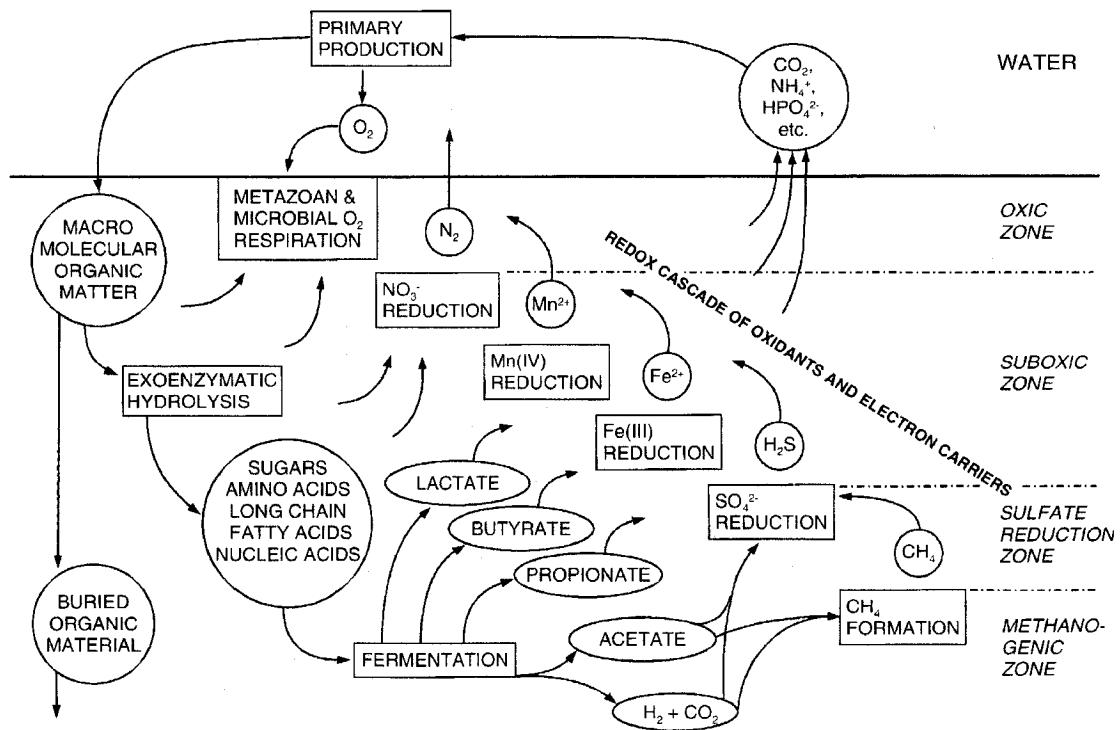


Figure 2.3. Pathways of organic carbon degradation in marine sediments and their relation to the geochemical zonation and the consumption of oxidants (from Fenchel and Jørgensen, 1977)

2.1.1. Characterized organic matter

The major vehicle driving the vertical redistribution of bioactive elements within the ocean is particulate OM (Svensen *et al.*, 2007). The sinking plankton-derived material from surface waters essentially supports all life in deeper waters, transfers carbon and nutrients to depth, and imprints a record of water column processes into underlying sediments. Sedimentary organic particles are extensively (> 99%) and selectively degraded during their transit to the deep-sea floor, leading to highly altered sedimentary records (Wakeham and Lee, 1993). Individual biochemicals, or their ratios, have been

used to measure this diagenetic status of OM in sinking particles and in sediments (Cowie and Hedges, 1992; Wakeham, 1996). Knowing the diagenetic status of OM is essential for employing biomarkers in source studies and understanding organic carbon preservation. OM reactivity, however, is a function of material matrix and its lability, so that chemical reactivities do not necessarily correspond with compound classes or structures in a predictable manner (Cowie and Hedges, 1992; 1994). Sedimentary OM originates from several possible sources and as a result, it comprises an array of biochemicals which can be divided into several broad categories.

2.1.1.1. Nucleic acids and proteins

All organisms have nucleic acids, which chemically consist in a regular sequence of phosphate, sugar and small variety of biosynthesized base units containing N. These nucleic acids can be transcribed into amino acid sequences, also termed proteins and enzymes. During the sedimentation of decayed organisms, nucleic acids and proteins are readily hydrolyzed chemically or enzymatically into smaller, water soluble units. Amino acids occur in rapidly decreasing concentrations in recent and sub-recent sediments, but may also survive in small concentrations in older sediments, particularly if they are protected (encapsulated) by the calcareous frustules or shells of marine organisms (Hedges and Keil, 1995). A certain amount of nucleic acids and proteins reaching sediment may be bound into the macromolecular OM network like kerogen or humic substances, which likely provide protection against hydrolytic and oxidative degradation (Aufdenkampe *et al.*, 2001).

2.1.1.2. Saccharides, lignin, cutin, suberin

Sugars are polyhydroxylated hydrocarbons that constitute an important fraction of biological material, particularly in plants. Polysaccharides serve as supporting units in skeletal tissues (cellulose, pectin, chitin), or as stored energy in seeds. Although polysaccharides are largely insoluble in water, they are easily converted into soluble C₅ and C₆ sugars by hydrolysis (Rullkötter, 2000).

Lignin is a structural component of plant tissues, formed in a three-dimensional network together with cellulose. Lignin is a product of macromolecular condensation of three different propenyl (C₃-substituted) phenols (i.e., p-coumaric acid, ferulic acid, and syringyl alcohol) (Figures 2.4 and 2.5). An important fraction of lignin is preserved during transport from land to ocean and during sedimentation to the sea-floor where it is found in humic OM of deltaic environments (Young and Frazer, 1987).

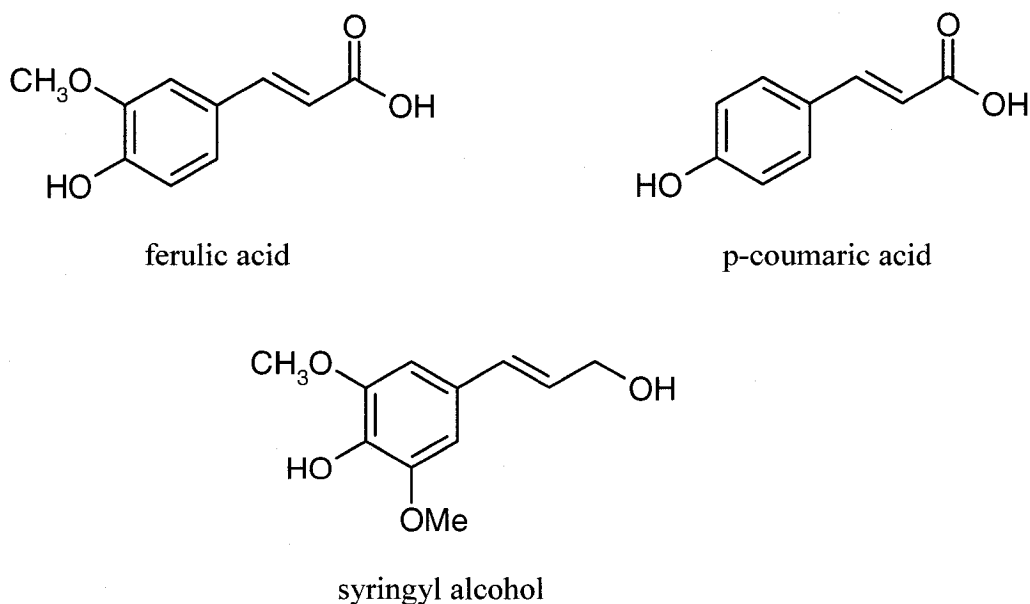


Figure 2.4. Monomers (substituted phenols) composing lignin

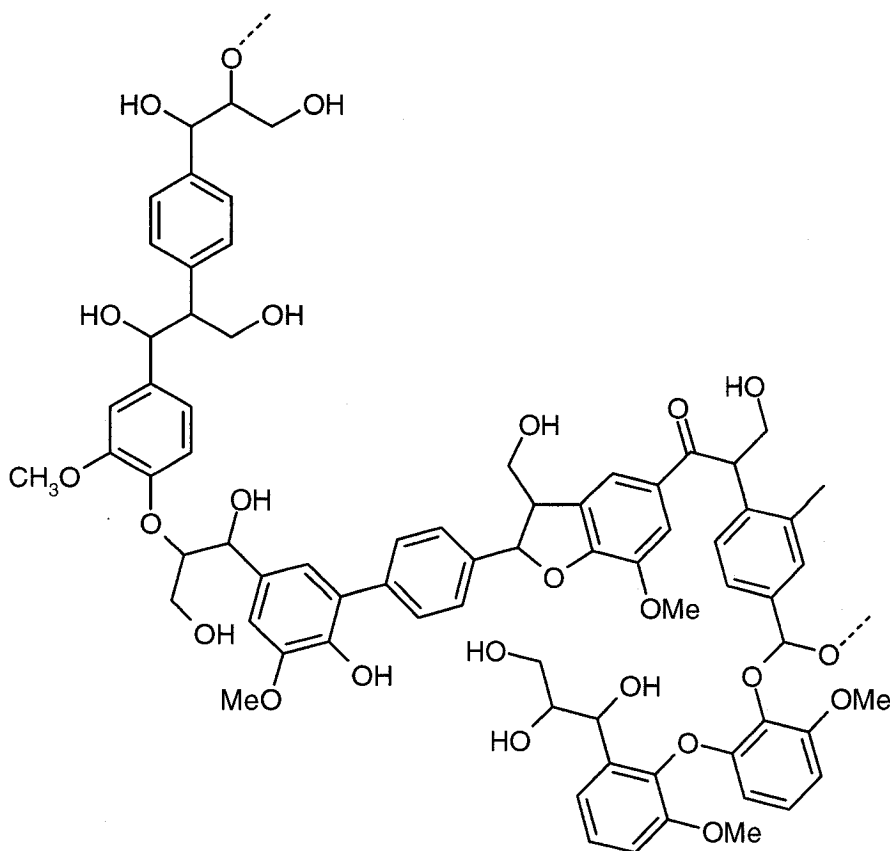


Figure 2.5. Possible structure of the lignin polymer (based on Young and Fraser, 1987)

Cutin and suberin are lipid biopolymers of variable composition. They are part of the protective outer coatings of all higher plants. Chemically, cutin and suberin are closely related polyesters composed of long-chain fatty and hydroxyl fatty acid monomers. Both types of biopolymers are labile, and are sensitive to hydrolysis, thus susceptible to further metabolism (Rullkötter, 2000).

2.1.1.3. Insoluble, non-hydrolysable highly aliphatic biopolymers

Insoluble, non-hydrolysable highly aliphatic biopolymers were discovered in the cell walls/membranes of algae and higher plant, as well as in their fossil remnants in sediments (Largeau and de Leeuw, 1995). These substances are called algaenan, cutan or

suberan, depending to their origin or co-occurrence with cutin and suberin in extant organisms[†]. They are composed of aliphatic polyester chains cross-linked with ether or carbon-carbon bridges (Blokker *et al.*, 1998), which render them very stable against degradation. Pyrolysis and other oxidative chemical methods are needed to decompose these highly aliphatic biopolymers into smaller units that are then amenable to direct analysis.

2.1.1.4. Lipids

Lipids are biologically produced molecules that are insoluble in water but are soluble in organic solvents, such as chloroform, ether or acetone (Rullkötter, 2000). Membrane components and certain pigments occurring in fats, waxes, resins, and essential oils are included in this broad, operationally-defined category. Lipids have a low solubility and a relatively good preservation potential during sedimentation despite their lability (Canuel and Martens, 1996), as opposed to other biogenic compounds classes such as amino acids or sugars (Rullkötter, 2000). Certain lipids are made up of saturated and unsaturated fatty acids bound to glycerol in the triglyceride esters of fats (Figure 2.6) (Rullkötter, 2000). Many forms of lipids exist depending on their origin and utility. Cell membranes, for example, consist in a large extent of fatty acid diglycerides with the third hydroxyl group of glycerol bound to phosphate or another hydrophilic group (Rullkötter, 2000). In waxes, fatty acids are esterified with long-chain alcohols instead of glycerol. Long-chain saturated hydrocarbons having predominantly odd carbon numbers usually come from plant waxes (Huang *et al.*, 2003). Some typical lipids often found in sediment are regrouped in Figure 2.6. The acyclic diterpene phytol is probably the most abundant

[†] See glossary

isoprenoid on Earth. It is usually esterified to chlorophylls or to bacteriochlorophylls and it is thus widely distributed in the green pigments of aquatic and subaerial plants. Sesterterpenes (C_{25}) are of relatively minor importance except in some methanogenic bacteria (Volkman and Maxwell, 1986). Hopane series are probably the geochemically most important triterpenes class. They are usually found in ferns, blue-green algae and bacteria having diploptene[†]-like structures (Figure 2.6) (Elvert *et al.*, 2001). Bacterial cell membranes contain hopanoids[†] which are a form of diploptene (Figure 2.6), such as bacteriohopanetetrol, synthesized for rigidifying the membrane (Wakeham, 1990). The widespread distribution of bacteria on Earth through time makes the hopanoids ubiquitous constituents of all OM assemblages (Rohmer *et al.*, 1992). Plants and animals have in common the most important sterol, which is cholesterol (C_{27}). Higher plants can contain sitosterol (C_{29}), which is the most abundant of this group (Jaffé *et al.*, 2006). Coupling steroids and terpenoids are useful as biological markers because they contain a high degree of information in the carbon skeleton even after sedimentation and often provide a chemotaxonomic link between the sedimentary OM and the precursor organisms in the biosphere (Poynter and Eglinton, 1991). Red and yellow pigments of algae and land plants composed of carotenoids are the most representative of the tetraterpenes (C_{40}). Due to their extended chain of conjugated double bonds (β -carotene) they are labile in most depositional environments (Reuss *et al.*, 2005). They are widespread in natural organisms; however they are found in low concentrations in marine sediments because of their rapid alteration by diagenetic processes. Chlorophylls and their derivatives are probably the second pigment type with the highest geochemical

[†] See glossary

significance. During diagenesis, chlorophylls are converted into the aromatized porphyrins coming from green plant pigment and bacteriochlorophylls (Rullkötter, 2000).

Although, a wide range of molecules listed above (e.g. previous sections) can be characterized at the molecular level using conventional chromatographic approaches, molecularly characterized OM represents a small portion of the total sedimentary OM. In fact, in normal coastal marine sediments, the identifiable amino acids, carbohydrates, lignin and lipids components only represent 10%, 5-10%, 3-5% and <5%, respectively, of total OM, thus leaving 70-80% of the OM mass unidentifiable (Burdige, 2006; Hedges and Oades, 1997). A large proportion of total OM is therefore still missed chromatographically and has been labelled the “molecularly uncharacterized carbon” (MUC) fraction (see next section).

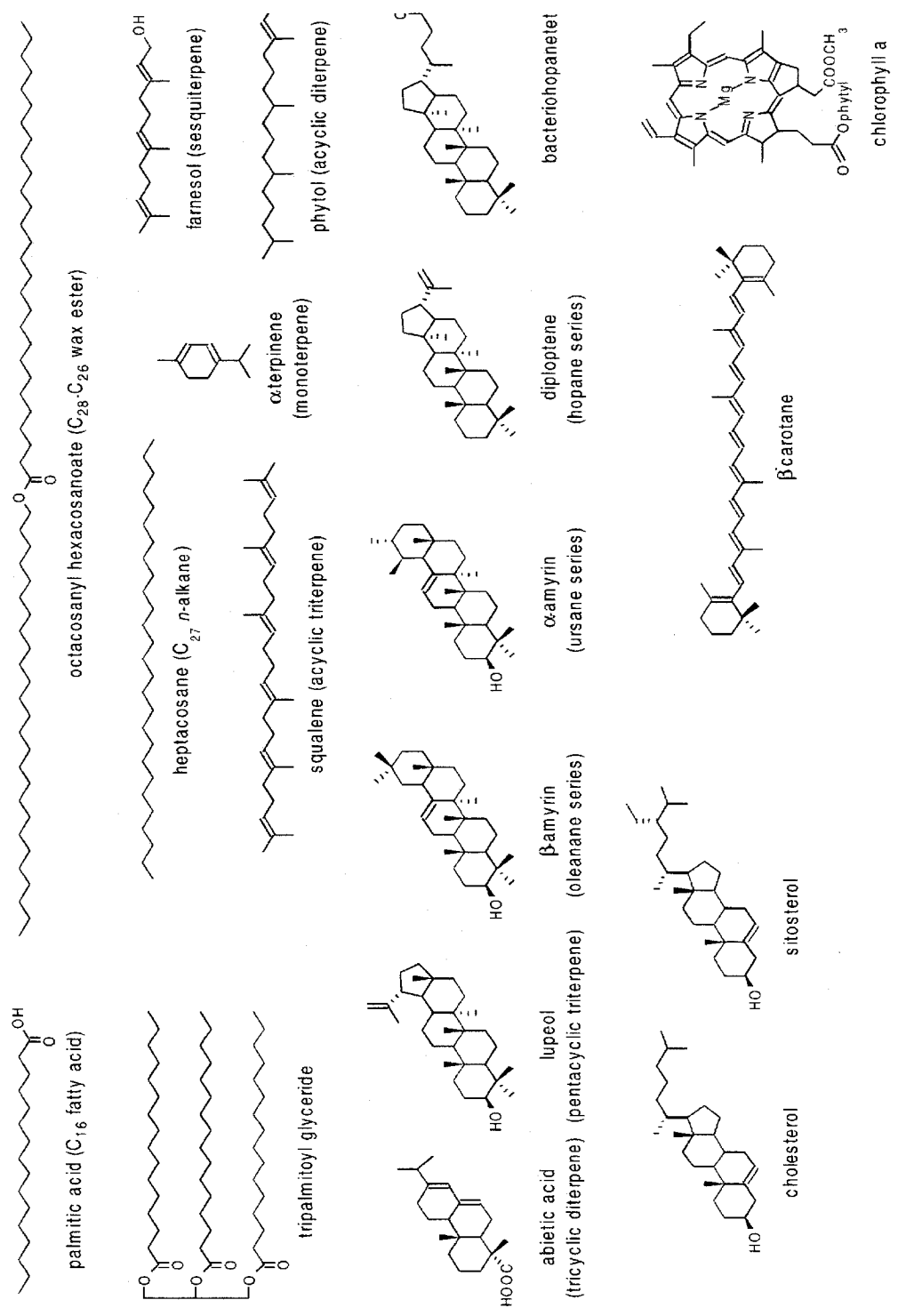


Figure 2.6. Molecular structures of representative low molecular weight lipids found in living organisms and surface sediments (Rullkötter, 1992)

2.1.2. Uncharacterized organic matter

Because of the high information potential of sediments for paleo[†]-reconstruction of past events or environmental conditions based on biomarkers (Macko, 1993), it is important to be able to characterize the composition of OM at the molecular level. Valuable organic geochemical information can be derived from the detailed structural and stereochemical analysis of biomolecules found in natural settings. In spite of knowing that sedimentary OM originates from the most part from the series of molecules listed above (previous sections), these characterizable molecules only represent a very small fraction of the total OM present in sediments.

In surface waters, near the euphotic zone, about 80% of total particulate OM can be chromatographically separated and characterized as plankton-derived material (Wakeham *et al.*, 1997). The remaining 20% has been labelled the molecularly uncharacterized carbon, or MUC (Hedges *et al.*, 2000). In deep ocean waters, this trend is reversed and the relative contribution of MUC, which increases with age, depth and diagenesis, to total OM reaches about 80% in the sediments (Brandes *et al.*, 2004).

The composition, formation pathways and information potential of this uncharacterized fraction are some of the most important unanswered questions in marine organic geochemistry (Hedges *et al.*, 2000). Understanding what comprises this molecularly uncharacterized OM is of great importance and interest, in part because this material may play a role in controlling carbon preservation in marine sediments and generate fossil fuels in the crust (Hedges *et al.*, 2000). In addition, these persistent, recalcitrant molecules may help modulating the global temperature and weathering rocks soils through their impact on the CO₂ cycle (Hedges *et al.*, 2000). MUC is generally

[†] See glossary

assumed to be derived from the degradation of labile sedimentary organic materials, which concurrently results in the concentration of resistant biochemicals. In this process, increasingly more recalcitrant organic compounds are preserved, until mostly large and highly cross-linked and/or highly condensed biomacromolecules remain; these giant macrobiomolecules are key contributors to MUC (Gatellier *et al.*, 1993; Zegouagh *et al.*, 1999). Black carbon, a broad class of highly condensed polyaromatic compounds derived from biomass burning or sedimentary rock metamorphism and comprising the operationally defined char, charcoal, coal, soot and graphitic carbon (Hedges *et al.*, 2000; Dickens *et al.*, 2004), also contributes significantly to the MUC component. These compounds have in common their resistance to hydrolysis and their very low solubility, which render them impossible to characterize at the molecular level using conventional bulk or separation (chromatographic) analytical techniques. The existence of the MUC fraction is thus intimately linked to the extraction, separation and identification methods used for molecularly characterizing sedimentary OM as only the compounds that fall into the analytical window of any given methods can be identified.

The inability to characterize the bulk of the particulate organic matter at greater depths reinforces the general paradigm of a transition from highly labile and relatively well-characterized OM in organisms and surface waters to biologically and analytically recalcitrant material in the deep-water column and sediments. The origin, reaction and fate of these compounds are still relatively obscure and detailed characterization studies of the important uncharacterized organic fraction are especially needed. (Hedges *et al.*, 2000).

2.2. Preservation mechanisms

The ocean is very efficient (> 99%) at recycling OC. The annual production of OC in the surface ocean is about 50-70 Gt of C·yr⁻¹, of which only about 0.1-0.2 % is buried and preserved in sediment and eventually enter the geological carbon cycle (Hedges and Keil, 1995; Burdige, 2006). Several studies carried out on the mechanisms responsible for OM preservation in sediment have led to competing hypotheses that are still under debate. Three major factors are known to affect C_{org} preservation; these are OM productivity, the presence of oxygen and other oxidants, as well as OM composition (Pedersen and Calvert, 1990; Demaison and Moore, 1980; Gélinas *et al.*, 2001a; Burdige, 2006; Aller and Mackin, 1984; Rimmer *et al.*, 2004; Cowie, 2005). Within these factors, several parameters, including primary productivity, sedimentation rate, shielding by mineral or organic matrices, particle winnowing, as well as the concentration of dissolved oxygen and other oxidants, could be responsible for the variations in carbon preservation in contrasting sedimentary environments. Because it is most often difficult to isolate one factor from the others, and also because factors may vary with locations and conditions, the study of a given preservation mechanism alone, with no influence from other parameters, is extremely challenging. The relative importance of each one of these mechanisms is thus still a subject of controversy.

2.2.1. Production, sedimentation and oxygen

A high OC productivity is related to a high photosynthetic production at the surface of the ocean. The OC produced in the euphotic layer eventually sinks to the deep ocean at a given rate, giving rise to a measurable sedimentation rate at the surface of the

sediment. A correlation exists between OC and the radioisotopes ^{210}Pb and ^{137}Cs , two metal ions with a high affinity for OM, showing that a high input of OC in sediment is due to its production from the surface waters (Wan *et al.*, 2005). The ^{137}Cs isotope originates exclusively from atmospheric sources and, upon entering the aquatic reservoir, it binds to particles. Therefore, sedimentation rates can be obtained from the radioactive decay (dpm/g) of this isotope. Mass balance calculations have shown that higher productivity in the surface layers leads to higher preservation of OC in the sediment, given constant sedimentation rates (Wan *et al.*, 2005; Rimmer *et al.*, 2004).

At constant primary productivity rate, sedimentation rate represents another factor that influences preservation. OM is better preserved when the sedimentation rate is high. Because degradation is a kinetically (time and oxidant availability dependent) controlled process, high sedimentation rates lead to relatively lower extents (shorter exposure time to oxidants) of OM degradation or mineralization. The result is the preservation of a fraction of the OC through a capping effect. Müller and Suess (1979) have shown that the percentage of OC preserved in sediments is correlated with sedimentation rates (Figure 2.7). However, rates greater than about $10 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{yr}^{-1}$ lead to a decoupling between sedimentation rate and OC preservation. Sandy deltaic organic-poor mineral sediments deposition explains the tailing off of the preserved % OC at high deposition rates (Doyle and Garrels, 1985).

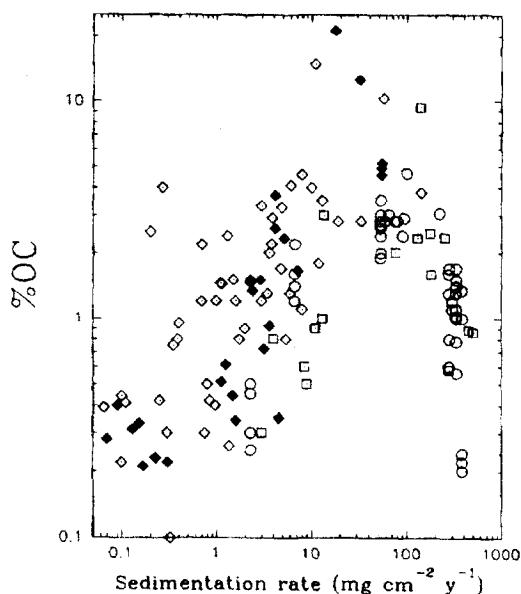


Figure 2.7. Relationship between organic carbon concentrations (wt% OC) and sediment accumulation rates for sediments from a variety of depositional environments coming from four different study (Hedges and Keil, 1995). The different symbols represent different sampling from these four studies

Such dilution effect at high sedimentation rates can be avoided by using burial efficiency, which also has been proposed as a proxy for OM preservation (Henrichs and Reeburgh, 1987; Betts and Holland, 1991; Cowie and Hedges, 1992a). Burial efficiency is defined as the OC accumulation rate below the diagenetically active surface layer, divided by the OC input rate at the sediment/water interface. A good correlation can be found between burial efficiency and sedimentation rate (Figure 2.8). However, this relationship must be used only if the preserved OM is freshly deposited for the first time because, generally, recycled organic materials[†] are more refractory than recently biosynthesized organic matter. Burial efficiencies can then be artificially inflated by the input of recycled OM to sediments (Hedges *et al.*, 1988; Hedges and Keil, 1995).

[†] See glossary

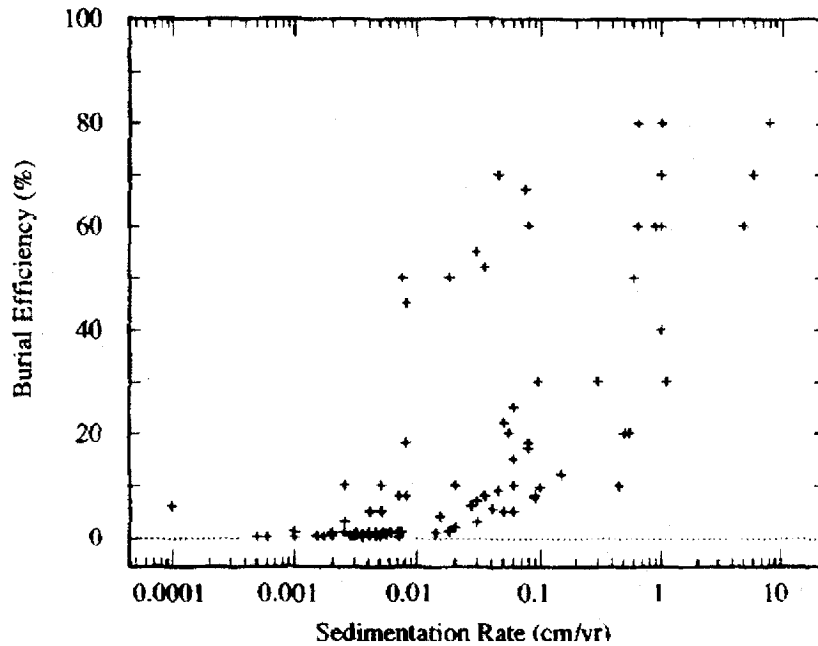


Figure 2.8. Relationship between percent burial efficiencies of sedimentary organic carbon and sediment accumulation rates at different sites (Hedges and Keil, 1995)

Dissolved oxygen concentrations in the ocean bottom waters have long been considered by petroleum geochemists to have an important effect on the percentages of OC found in marine sediments (Burdige, 2006 and references therein). In fact, some euxinic[†] areas such as the Black Sea where sediments are rich in organic material may offer strong clues for deciphering the relative importance of the different factors involved in OM preservation, such as (a) the requirement of consortia of interdependent bacterial communities for OM degradation linked to electron acceptors available in porewaters, (b) the increase in dissolved H₂S produced by sulphate reduction, (c) the decrease in biological mixing of the top layers of the sediments (irrigation and bioturbation) due to very low concentration of O₂ at the surface of the sediment, and (d) the presence of

[†] See glossary

highly insoluble fermentation-resistant material which is almost quantitatively preserved in euxinic area (Hedges and Keil, 1995; Rimmer *et al.*, 2004).

A factor among them is thought to play the most important role in OM preservation: the actual concentration of O₂ in sediment. It is known that anaerobic systems that can degrade OM are less efficient than aerobic organisms and because oxygen is essential for aerobic bacteria, better OC preservation is expected in anoxic and suboxic environments. Although bioturbation and sediment grain size distribution are factors that can modulate the O₂ penetration depth in sediments, a weak correlation is found between burial efficiency and bottom water oxygen concentration (Figure 2.9). Nevertheless, oxygen effects on short timescales have been clearly demonstrated (Benner *et al.*, 1984).

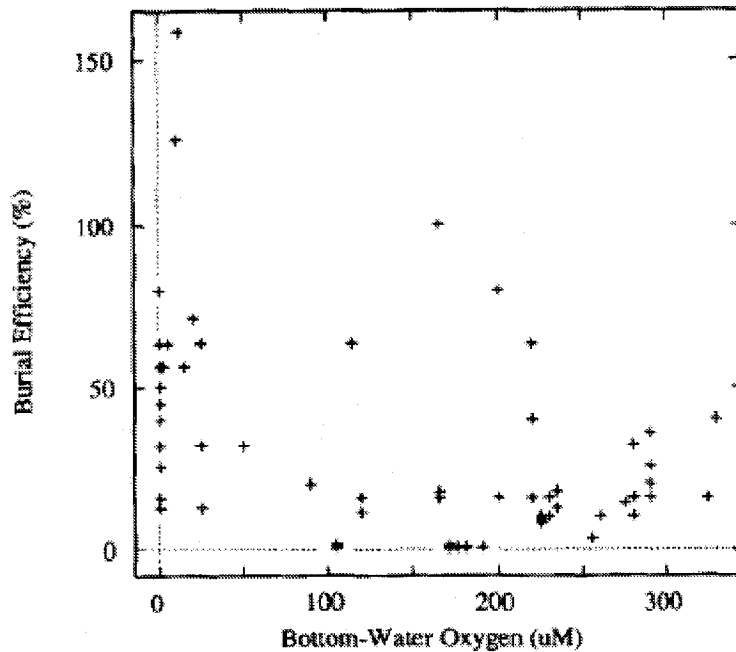


Figure 2.9. Relationship between burial efficiency and the corresponding bottom water oxygen concentration at different depositional sites (Hedges and Keil, 1995)

2.2.2. Hydrodynamics

The OC content of sediments is highly variable from one area to the other. This variability is mainly governed by *in situ* production or mineralization/degradation processes, but it could also be due to spatial displacement supported by hydrodynamic influences. Winnowing is a typical sporadic event of sediment turbidity, which occurs mainly because of hydrodynamic events (i.e., strong storms, earthquakes and landslides). Carbon-rich sediments can be hydrodynamically transported to an area where the sediment is impoverished in OC owing to lower primary production rates at the surface. In such case, the increased OM content in sediment is then explained by hydrodynamic sorting rather than because of a relationship between OC concentrations and bottom waters oxygen concentrations (Pederson *et al.*, 1992).

2.2.3. Mineral shielding

OM is deposited along continental margins and is generally concentrated in fine grained sediments having greater surface area. Organic material with hydrophobic properties tends to sorb on suspended particles in the water column. Suess (1973) was the first to show a direct correlation between the mineral surface area and the organic content of marine sedimentary particles. This relationship was found with sediment from various sites and is indicative of OM sorption (Figure 2.10). Mayer (1985) extended this study and demonstrated that OM in most marine sediments is sorbed to mineral surfaces, often in monolayer-equivalent coatings[†]. Recent studies have challenged the monolayer-equivalent coating hypothesis and now favour encapsulation by minerals such as clays as

[†] See glossary

the mechanism explaining OM preservation by mineral shielding (Mayer, 1999; Curry *et al.*, 2007).

Mineral surface area linked to clay content appears to be an important factor influencing the OM content of marine sediments accumulating along continental shelves and slopes. Organic materials can for instance be protected simply because they are shielded from enzymatic activity. Molecules trapped in mesopores formed by cavities on the mineral surface, or sorbed on the inner surfaces between the layers of sheet silicates, become inaccessible to emulsifiers that bacteria produce for dissolving material before ingestion and consumption (Park *et al.*, 2002). Silicate and clay are ubiquitous in marine environment and are thus good candidates for mineral shielding because of their multiple-sheet mineral matrix. The major reason for this type of preservation is sterical hindrance (Middelburg and Meysman, 2007; Rothman and Forney, 2007).

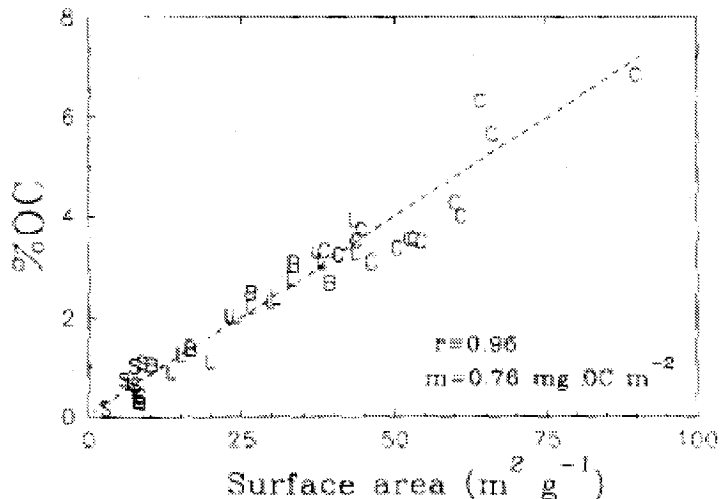


Figure 2.10. Relationship between organic carbon concentrations and mineral surface area for different grain size ((B)ulk, (S)and, (C)lay) fractions for suspended sediments from the Columbia River estuary and surface sediment. Four sites were sampled along the adjacent continental shelf and slope off Washington State (from Hedges and Keil, 1995)

Protective OM sorption is thought to play an important role in OM preservation. It agrees with the low OC contents of coarse sediments likely resulting from the low surface/mass ratio of the component particles. Such close association between organic matter and mineral surfaces facilitates burial of OM and allows a strong correlation between the rates of OC burial and sediment accumulation rates, and also to the observed reversal trends between OC concentrations and accumulation rate for sandy organic-poor sediments (Aller and Mackin, 1984).

2.2.4. An integrated hypothesis

All the above processes involved in OM preservation provide useful hints regarding factors that likely constitute important preservation mechanisms. Knowing that degradation of OM is directly modulated by redox conditions (e.g. $[O_2]$ linked to thermodynamics) and residence time (eg. sedimentation rate linked to kinetics) in the sediments, Hedges proposed a new hypothesis that merged all the processes believed to be involved in OM preservation (Hedges *et al.*, 1999). He suggested that sedimentary OM can be divided into three broad categories of organic compounds based on their reactivity in sediments, namely, (i) the (bio)chemically refractory fraction, (ii) the oxygen-sensitive fraction and (iii) the fermentable fraction. In surface sediments, where diagenesis takes place, refractory OC represents the fraction of OM that does not degrade under any redox conditions or any residence time (burial rate), or that degrades at a very slow rate. The fermentable fraction of OM is composed of hydrolysable OM that is completely mineralized within the first few mm to cm of the sediments, regardless of the redox conditions. The oxygen-sensitive fraction represents OM that degrades slowly in

the presence of dissolved O_2 in the porewaters. During deposition of solid particles in well-oxygenated deep waters, the fermentable OM fraction degrades rapidly, whereas the abundance of the oxygen-sensitive OM fraction (OSOM) slowly begins to decrease. The OSOM fraction is slowly altered or degraded with time, as long as the sediment remains oxic. Eventually, if all of the OSOM is consumed, the only remaining OM fraction is composed of highly refractory materials such as macromolecular, polyaromatic black carbon components, or OM protected through encapsulation within detrital or biogenic inorganic matrices. More rapid sedimentation rates and shallower O_2 penetration depths contribute to shorter exposure times to oxic conditions, leading to a decrease in the absolute extent of OSOM mineralization (Figure 2.11).

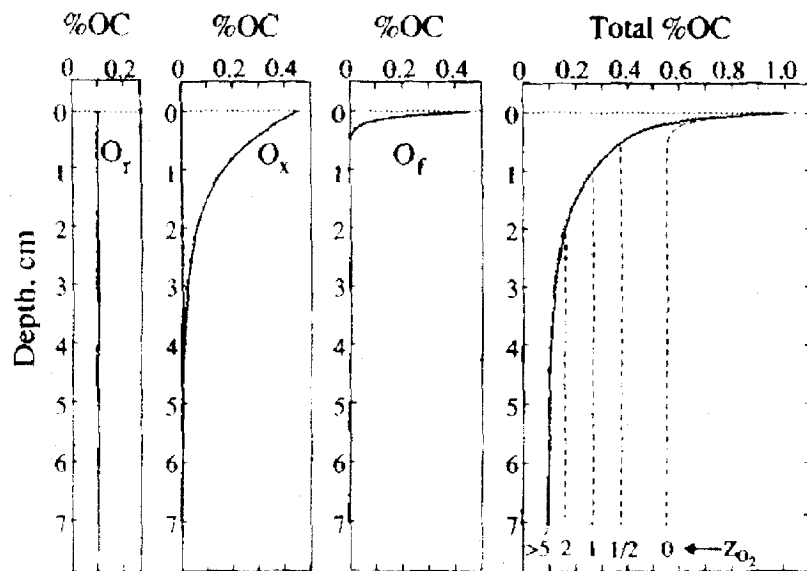


Figure 2.11. Hypothetical profiles for three different fractions of organic carbon in a continental slope sediment. O_r represents the refractory fraction, O_x the oxygen-sensitive and O_f the fermentable. The dashed vertical lines on the right hand side of the plot correspond to total % OC profiles obtained when the oxygen penetration depth (Z_{O_2}) decreases from >5 to 2, 1, $\frac{1}{2}$ and 0 cm below the sediment surface (from Hedges and Keil, 1995)

According to Hedges' oxygen exposure time (OET) hypothesis, OM preservation varies with bottom water oxygen content which can be affected by grain size and sedimentation rate. Oxygen exposure time is defined as the oxygen penetration depth in sediment (cm) divided by sedimentation rate ($\text{cm}\cdot\text{yr}^{-1}$), thus providing an estimate of exposure time in years. The total exposure time defined here corresponds to the time OM is exposed to molecular oxygen (oxic conditions) and others oxidants (suboxic conditions where all or any of the following electron acceptors are present: iron and manganese oxides, nitrates and sulfates) in the water column, the nepheloid layer[†] and the sediment. Longer OETs lead to more extensive OM degradation. The OET hypothesis explains why and where better OM preservation occurs, as shown in Figure 2.12.

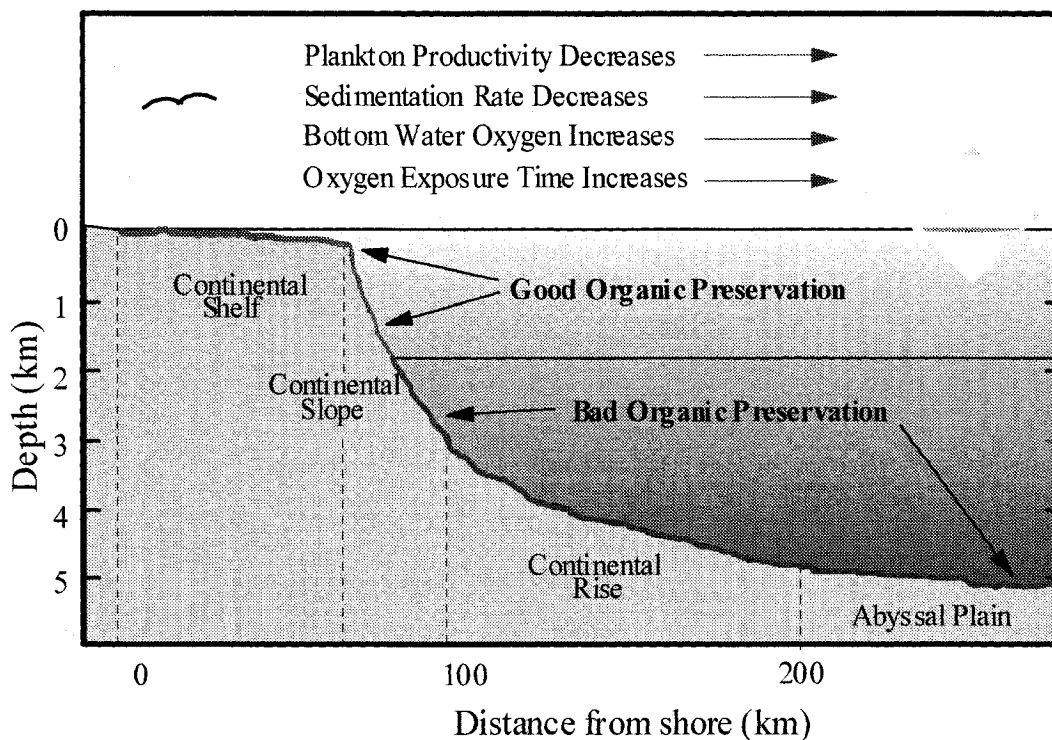


Figure 2.12. Preservation of carbon diagram with typical offshore trends (Hedges, pers. comm.)

[†] See glossary

This theory was positively tested in a previous work, in which data indicate that OC burial efficiency is strongly correlated with the length of time during which accumulating particles are exposed to molecular oxygen in sediment pore water (Hartnett *et al.*, 1998). Other studies conducted on the bulk chemical compositions of sedimentary OM have suggested that the abundance of the oxygen-sensitive fraction decreases exponentially with exposure time (Gélinas *et al.*, 2001a; Hartnett *et al.*, 2003). To increase our understanding of the importance of the OSOM fraction however, we now need to develop novel approaches to extract, quantify and molecularly characterize this fraction.

2.3. Analysing organic matter

In organic geochemistry, samples are analyzed with instrumental techniques commonly used in chemistry. Bulk OM analysis can be carried out by optical methods (identification of spores, pollen, algae, higher plant debris, etc...), or through the determination of its elemental content (C, H, N, S, and O analysis) and isotopic composition (^{13}C , ^{15}N , ^2H , ^{34}S , ^{14}C). These techniques are all inclusive, and do not fractionate samples. However, they give very little to no molecular-level information and are insensitive to subtle chemical variations. Bulk measurements of the OM chemical composition using common spectroscopic approaches such as FTIR and NMR have the advantage of being rapid, non-destructive, and quantitative (or semi-quantitative); however, they provide data of low resolution (Tremblay and Gagné, 2002; Gélinas *et al.*, 2001).

On the contrary, molecular-level measurements, including chromatographic separation coupled to mass spectrometry, have the potential to provide much more detailed biogeochemical information based on the molecular structure of the separated compounds. Generally, these measurement methods can simultaneously isolate and quantify compounds from more than one molecular family, and also allow determining the stable isotope composition of biomolecules from specific sources (compound-specific isotope analysis) (Yamada and Ishiwatari, 1999; Goñi and Eglinton, 1994). However, these assessments are slow and tedious, procedurally difficult (multistage isolation), and intrinsically selective. Because of the complexity and wide diversity of organic materials in sediments, it is impossible to identify, isolate and quantify all organic compounds using such molecular-level measurement methods. Novel approaches and/or more advanced analytical techniques are thus needed to complement bulk analysis for the characterization of OM in marine sediments, as it will be shown in the following chapters.

2.4. Working hypothesis and objectives

Sedimentary OM composes 20 % of all carbon preserved in the Earth's crust, influences atmospheric CO₂ and O₂ and generates fossil fuels (Hedges and Keil, 1995). Marine sediments constitute the major long-term sink of organic carbon (OC) on Earth where less than 0.3 % of OM photosynthesized by plants is preserved (Hedges and Keil, 1995). Recent studies have provided evidences that a relationship exists between OM preservation and the OM physical forms, chemical compositions and cumulative exposure to O₂ during deposition and burial (Hedges *et al.*, 2000; Burdige, 2007). By use

of bulk, molecular and compound-specific isotopic methods, the object of this thesis aims at the mechanistic understanding of the effects of these factors on OC preservation.

Most sedimentary organic substances are associated to inorganic materials that greatly influence their molecular reactivities and fates (Mayer, 1994; Hedges and Keil, 1995). Physical shielding of intrinsically reactive organic substances by mineral grains (Hedges *et al.*, 2001; Baldock and Skjemstad, 2000; Burdige, 2006) may provide a mechanism for the widely observed direct relationship between smaller particles sizes (increased surface area) and higher OM concentration in margin sediments (Mayer, 1994). In addition, exposure to oxic conditions during sediment deposition and accumulation is detrimental to OM preservation (Demaison and Moore, 1980). Recent evidence showed that OM burial efficiency (Hartnett *et al.*, 1998) and composition (Gélinas *et al.*, 2001a) are directly influenced by the time accumulating particles are exposed to oxic conditions. OM preservation in marine sediments likely represents a dynamic balance between the positive influence of protective mineral association and the destructive effect of cumulative exposure to oxic conditions. The overall effect of this dynamic balance is imprinted on the continuum of intrinsic refractoriness exhibited by the wide range of organic compounds accumulating in sediments (Keil *et al.*, 1994; Hedges *et al.*, 2001). Evidence is growing that this balancing mechanism may provide a sensitive negative feedback mechanism for control of the carbon and oxygen cycles over geologic times (Hedges *et al.*, 2001).

A mild HF/HCl demineralization method was recently developed to concentrate OM with minimal loss and alteration (Gélinas *et al.*, 2001), and was applied to characterize bulk sedimentary OM in 20 widely contrasting sediments by solid-state ^{13}C

nuclear magnetic resonance (NMR) (Gélinas *et al.*, 2001). The major findings of the NMR survey include: (i) a predominant component of non-hydrolysable, non-amino acid, aliphatic-rich OM that becomes more concentrated in sediments experiencing less exposure to O₂ (Gélinas *et al.*, 2001a), and (ii) a fraction of excess unsaturated carbon that may comprise charcoal and soot/graphitic black carbon. Parallel NMR experiments also suggested that a fraction of labile OM in plankton, sinking particles and sediments is shielded from degradation by their biomineral armour (Hedges *et al.*, 2001), although these results have since then been challenged (Hwang and Druffel, 2003).

Organic geochemistry has now progressed to the stage where most structural units of readily hydrolyzed major biochemical (amino acids, carbohydrates, lipids, lignin, etc.) can be measured using standard chromatographic methods. However, over 75 % of sedimentary OM is not chromatographically isolated and identified and remains molecularly uncharacterized (Hedges *et al.*, 2000, Wakeham *et al.*, 1997). The complete molecular characterization of sedimentary OM remains an impossible task and holistic tools (elemental analyzer, FTIR, NMR) are needed to probe the relative importance of the major OM preservation mechanisms in marine sediments. A new method that could fractionate sediment samples into different chemical reactivity classes would thus be very useful in organic geochemistry. Such method could allow quantifying and identifying new molecules that could then become proxies for a given reactivity class, thus facilitating the assessment of the role of chemical reactivity and other factors in the preservation of OM in marine sediments.

The Ph.D. project proposed here focuses on three major objectives. As part of the first objective, a method was developed to chemically separate and quantify different

reactivity classes of organic compounds in a series of contrasting sediments (St. Lawrence Gulf, Saguenay Fjord, as well as the Arctic, Mexican, Antarctic, and Washington coasts). Then, a new molecular-level and isotopic method was developed for the characterization of the oxygen-sensitive fraction of OM, which is expected to be important for carbon preservation. The third general objective consists in using these methods to test hypotheses about processes affecting oxygen-sensitive fraction transport, cycling, reaction history and preservation.

A strong emphasis was put on the molecularly uncharacterized oxygen-sensitive component, with particular consideration to the relative importance of the mechanism thought to be responsible for OM preservation (i.e. oxygen sensitivity). It is thought that oxygen-sensitive organic matter (OSOM) plays a major role in the chemical properties of marine sedimentary mixtures and their potential to form petroleum (Baldock and Skjemstad, 2000). The present research project draws heavily upon concepts, techniques, and samples utilized in recent works (Hedges *et al.*, 2001, Gélinas *et al.*, 2001, Gélinas *et al.*, 2001a, Gélinas *et al.*, 2001b, Hedges *et al.*, 2002). In particular, bulk analytical methods (CHN, stable isotopes) were used to characterize whole sediments and chemically-separated fractions. However, given the specific limitations of bulk analyses, molecular level characterization methods also had to be developed to assess the sources and dynamics of the different classes of components found in marine sediments.

CHAPTER III

SPECIATION OF ORGANIC MATTER PRESERVED IN AQUATIC SEDIMENT: A BULK METHOD

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Keywords: Organic matter, sediment, preservation, separation method, mineral protection, oxygen sensitivity, refractoriness

3.1. Abstract

Marine sediments constitute the major long-term sink of organic carbon (OC) on Earth, where <0.3% of organic matter (OM) photosynthesized by plants is eventually preserved. While the burial of this small OM fraction affects the global cycles of atmospheric CO₂ and O₂, the mechanisms that control sedimentary OM preservation are still unclear. Recent studies have suggested a strong relationship between OM preservation and the OM physical forms, chemical composition and cumulative exposure to O₂ during deposition and burial. In this study, a separation method designed to isolate different reactivity classes of sedimentary OM was developed in order to assess these different preservation mechanisms of OM in sediment. This new extraction technique comprises the successive removal of increasingly recalcitrant sedimentary OM fractions using dichloromethane extraction, fluorhydric/hydrochloric acid demineralization, trifluoroacetic and hydrochloric acid hydrolyses, as well as H₂O₂ and thermal oxidation. This method allows quantifying and characterizing different OM fractions (solvent-soluble, acid-soluble, hydrolysable, oxygen-sensitive and refractory) from one sample. Mass and total carbon balances were calculated throughout the procedure using elemental analysis and stable isotope ratio measurements on seven highly contrasting natural sediments with an organic carbon content ranging between 0.4 to 7.3 wt%. Our results suggest that the relative abundance of oxygen-sensitive OM (OSOM) is a good indicator of OM preservation in sediment ranging between 8 to 49% of total OC in these sediment samples. However, the relative contributions of marine and terrestrial OSOM fractions have to be assessed because differences in their chemical composition and degradation rate affects the relationship between OSOM abundance and OM preservation.

3.2. Introduction

Sediments represent the major long-term sink for organic carbon (OC) on Earth and are key players in the cycling of C and O₂ (Hedges and Keil, 1995). Marine OC, both dissolved and particulate, accounts for 22% of total carbon present on Earth (Hedges and Keil, 1995). About 5% of the carbon fixed in the surface ocean reaches marine sediments where a small fraction is eventually preserved on a long-term basis through its incorporation into sedimentary rocks (Hedges and Keil, 1995). Several studies have used productivity (Henrichs, 1992; Pederson and Calvert, 1990), hydrodynamics (Pederson *et al.*, 1992), burial efficiency (Betts and Holland, 1991; Cowie and Hedges, 1992) or thermodynamic parameters (Cowie *et al.*, 1995) to calculate the fraction of carbon preserved in local sediments; however, in all cases mechanisms governing organic matter (OM) preservation remained and still are unclear. In the World Ocean, Berner (1982) estimated that 94% of OM is preserved along continental margins and only 6% of total OM escapes degradation and is eventually deposited along continental rise and in the deep sea. With the increase in atmospheric CO₂ concentration since the onset of the industrial era and the associated climate change, it is becoming increasingly important to understand the mechanisms and pathways of OM preservation in sediments to get a better grasp on the fine controls of the global OC cycle.

Recent studies have reported a relationship between OM preservation and its physical forms, chemical composition and O₂ exposure during deposition and burial (Burdige, 2007 and references therein). The preservation mechanisms for sedimentary OM that have been proposed in these studies can be divided into three broad categories:

(a) protection of the OM through encapsulation, mostly by the mineral matrix but also within a macromolecular, most often hydrophobic, organic matrix, (b) refractoriness of certain classes of organic macromolecules, which makes them resistant to degradation in sediments, and (c) exposure time to oxic conditions, which modulates the extent of preservation of macromolecules that are degradable only under oxic conditions (selective preservation). The majority of sedimentary organic substances are closely associated to inorganic materials thus protecting them against chemical and biological re-mineralization (Mayer, 2004). Their reactivity is negatively influenced mostly through steric hindrance: a large fraction of OM associated to minerals is included in small pores or between layers of sheet silicate minerals (Luthy *et al.*, 1997; Ingalls *et al.*, 2004). Another mechanism by which OM is thought to be preserved is the existence of OC containing chemical structures that are intrinsically refractory towards degradation and thus, that are selectively preserved in the water column and in sediments (Nguyen *et al.*, 2003). Such organic materials can comprise highly degraded material that is only very slowly being further re-mineralized, or intrinsically resistant compounds such as polyaromatic black carbon particles (Gélinas *et al.*, 2001b).

The redox conditions of the deposition environment are also pivotal in determining the percentage of deposited OM that is eventually preserved in the sediments. Indeed, certain classes of macromolecular organic materials such as lignin and aliphatic-rich algaenans can only be degraded in the presence of oxygen (Hedges and Oades, 1997; Nguyen and Harvey, 2003). In 1995, Hedges and Keil proposed to blend all the parameters assumed to play a role in OC preservation based on the fact that the extent of degradation of OM directly depends on oxidation kinetics. In fact, Hedges suggested

that sedimentary OM can be separated into three main fractions based on their chemical reactivity in oxic and anoxic conditions (Hedges and Keil, 1995). These fractions are the chemically-refractory OM (efficiently preserved in both oxic and anoxic conditions), the oxygen-sensitive OM (biodegraded only in oxic conditions), and the fermentable OM (degraded in both oxic and anoxic conditions). According to this hypothesis, the extent of sedimentary OM preservation would thus result from the complex interplay between the abundance of these three fractions and sedimentary redox conditions. The concentration and penetration depth of dissolved oxygen act together to determine the amount of OM preserved in sediment. Key players in this OM preservation hypothesis are the oxygen-sensitive and the chemically-refractory OM fractions as they are the major components that might eventually be preserved in the sediment. Recent studies have shown that OM burial efficiency (Hartnett *et al.*, 1998) and chemical composition (Gélinas *et al.*, 2001a) are directly influenced by the time accumulating particles are exposed to oxic conditions, defined as the oxygen exposure time ($OET = \text{oxygen penetration (cm)}/\text{sedimentation rate (cm}\cdot\text{yr}^{-1})$). OET likely constitutes one of the most important factors that control the amount of OC preserved in sediments (Hartnett *et al.*, 1998).

The three mechanism categories are not mutually exclusive and most likely act in consort in marine sediments. The extent of OM preservation in marine sediments would be modulated through a dynamic balance between the positive influence of protective mineral association and chemical refractoriness, and the destructive effect of cumulative exposure to oxic conditions. As molecular oxygen is consumed during the oxidation of OC (in oxic conditions), such a balancing mechanism may provide a sensitive negative

feedback mechanism that helped control the carbon and oxygen cycles over geological timescales (Hedges *et al.*, 2001).

Determining the relative importance of each one of these mechanisms in contrasting marine sediments would significantly advance our understanding of the main controls on OM preservation and long-term burial in marine sediments. In the work presented here, we thus explored the possibility of fractionating sedimentary OM into different classes of compounds based on their chemical reactivity. As a result of the wide variety of biochemicals found in living organisms and early diagenetic processes in marine sediments, a multitude of types of organic molecules, often highly altered, accumulate to form the bulk of sedimentary OM which eventually contribute to the molecularly uncharacterized carbon (MUC) fraction (Rullkötter, 2000). These different types of molecules form a continuum of reactivities towards degradation (both oxic and anoxic), and can be separated into several operationally-defined classes corresponding to relevant biogeochemical processes occurring in sediments. Our work focussed on the insoluble and non-hydrolysable sedimentary fraction (oxygen-sensitive OM) which may contribute to MUC and which is thought to be determinant in OM preservation in sediment. We thus worked with seven sediment samples, collected in widely varying environmental conditions, and separated bulk OM into five broad classes of organic compounds: organic solvent-soluble, acid-soluble, hydrolysable, oxygen-sensitive, and refractory. A novel procedure involving the sequential chemical separation of bulk samples followed by elemental (OC, total N, atomic C/N ratio) and isotopic ($\delta^{13}\text{C}$) analysis was used to separate, quantify and characterize these different classes of OM.

3.3. Material and methods

3.3.1. Sediment and sampling

Location and depth for each sample are reported in Table 3.1. Surface sediments (0-5 cm) from Station SAG30 in the Saguenay Fjord (Québec, Canada), Station 16 in the St. Lawrence Gulf (Québec, Canada) and Station 200 in the Arctic Ocean (Franklin Bay, Beaufort Sea, Arctic Ocean; Canadian Arctic Shelf Exchange Study project) were sampled in May 2002, June 2005 and December 2003, respectively, using a box core. The Mexican sediment (0-5 cm) was collected with a multicorer on the western Mexican margin near Mazatlan in January 2000 (Station 306). The Antarctic sediment (0-5 cm) was collected in Potter Cove (Bellinghausen Sea) near the Argentinean naval base of Jubany using a van Veen grab in January 2005. Surface sediment (0-5 cm) of the Washington coast (Continental shelf near Washington State in the Pacific Ocean) and Eel River margin (Northern California, USA) were sampled in May 2001 using a boxcore. All sediments were sliced and/or sub-sampled for surface sample and lyophilized and stored in Ziploc[®] bags at -20°C.

These sediments represent different environmental conditions ranging from fully marine, suboxic samples to estuarine sediments bathed by oxic waters. The Mexican Margin sediment was sampled at a depth (375 m) corresponding to an intense oxygen-minimum zone with suboxic bottom water conditions. Surface productivity is comparable to that of other coastal upwelling zones off of Southern California and Western Africa (Longhurst *et al.*, 1995; Ganeshram *et al.*, 1999), leading to a high OM accumulation rate and OC content (7.30 ± 0.03 wt%; Table 3.1). OM at this site is considered well preserved (Dickens *et al.*, 2006) and essentially originates from marine sources, as

suggested by the atomic C/N ratio of 7.2 ± 0.9 and $\delta^{13}\text{C}$ stable isotope signature of -21.00 ± 0.03 ‰ measured for this sample. The Antarctic surface sediment (0-5 cm) has a very low OC content (0.38 ± 0.03 wt%) and was sampled in fully oxic bottom water conditions. The $\delta^{13}\text{C}$ signature of -24.00 ± 0.03 ‰ at this site suggests significant inputs from marine sources owing to the absence of vegetation on the Antarctic continent. This particular depleted ^{13}C isotopic signature for marine origin is explained by the temperature-dependent isotopic fractionation which in cold polar waters may lead to carbon isotope values of -26 ‰ or less for marine samples (Rau *et al.*, 1991). The Arctic sediment was sampled in Franklin Bay, near the discharge of the Mackenzie River, at 200 m depth in oxic conditions. The OC content is low (1.61 ± 0.03 wt%), and the $\delta^{13}\text{C}$ signature and atomic C/N ratio of -25.45 ± 0.03 ‰ and 9.1 ± 0.9 , respectively, reveals that OM at this site consists of a mix between terrestrial and marine organic materials. A recent study showed that about 60% of OM at this site can be attributed to terrestrial sources (Goñi *et al.*, 2005). The Saguenay Fjord sediment (SAG30) was sampled at a depth of 275 m. The sediment is bathed in oxic porewaters in the very first millimetres below the sediment–water interface as suggested by the presence of oxyhydroxides (Saulnier and Mucci, 2000; Deflandre *et al.*, 2002) and has a low OC content of 2.59 ± 0.03 wt%. The $\delta^{13}\text{C}$ signature of -25.84 ± 0.03 ‰ combined to an atomic C/N ratio of 12.6 ± 0.9 suggest strong terrestrial inputs to the sediment at this site (Louchouart *et al.*, 1997; 1999; Tremblay and Gagné, 2007). The sediment labelled Station 16 (St 16) was sampled in the Gulf of St. Lawrence at a depth of 300 m in a marine environment, as suggested by the low organic carbon content of 1.71 ± 0.03 wt% and a $\delta^{13}\text{C}$ signature of -21.79 ± 0.03 ‰. The Washington Coast sediment was sampled on the continental shelf

bordering Washington State (USA) in the oxygen minimum zone of the Margin. Its OC content of 2.58 ± 0.03 wt% and $\delta^{13}\text{C}$ signature of -22.15 ± 0.03 ‰ suggest low terrestrial contribution to OM despite the presence of the mouth of the Columbia River on the nearby coast. The Eel River sediment was sampled in the mixing zone between the Pacific Ocean and the Eel River in northern California (USA). The OC content at this site is 1.24 ± 0.03 wt%, with a $\delta^{13}\text{C}$ value of -23.96 ± 0.03 ‰ suggesting a low marine contribution to OM. Recent studies revealed high terrestrial contribution to sedimentary OM at this site (Blair *et al.*, 2003).

3.3.2. Separation method

The procedure developed in this work is shown on Figure 3.1. It involves a series of extractions that allow quantifying different OM chemical reactivity classes in sediments. First, a labile OM fraction, most likely composed of simple lipids, was removed using a typical dichloromethane (DCM) extraction technique (Bergman, 1963). Second, the DCM-extracted sediment was demineralised to expose the remaining OM to the following treatments. OM lost during the demineralization step corresponds to acid-soluble OM. The demineralised residue was then hydrolyzed with trifluoroacetic and hydrochloric acids (TFA and HCl, respectively) (Allard *et al.*, 1998), followed by sequential oxidation treatments with H_2O_2 (Eusterhues *et al.*, 2005). OM removed during these treatments corresponded to the hydrolysable (mostly sugars and proteins) and oxygen-sensitive (lignin and aliphatic-rich biopolymers) fractions, respectively. OM found in the hydrolyzed and oxidized residue is total refractory OM. Thermal oxidation was then used to quantify thermally-stable refractory OM, which roughly

corresponds to the petrogenic, graphitic-rich OM fraction (Dickens *et al.*, 2004b). Elemental and isotopic analysis was carried out following each treatment to obtain the relative abundance of each reactivity class and isotopic signature. Each step is detailed below.

The separation method was applied to these seven contrasting samples, using between 10-19 g of lyophilized sediment (representing between 50 and 800 mg of organic carbon). Because of the amount of sediment needed and the time involved to carry out the whole procedure, only one was analyzed for each sample, with the exception of the Mexican, SAG30, Arctic and Antarctic (Table 3.2) sediments that were analyzed once more each during method development and optimization.

The left branch of the method (Figure 3.1), which includes steps 6 to 11, was designed to quantify minerally-protected, or shielded, OM. It differs from the right branch in that the HCl/HF demineralization treatment was applied after the hydrolysis (step 6) and H₂O₂ oxidation (step 7) treatments, thus providing an assessment of non-protected hydrolysable OM, non-protected oxidizable OM, and total shielded OM. Unfortunately, in most cases there was not enough residual material left for the following steps (steps 9, 10, and 11).

3.3.3. Lipid fraction

Whole sediment was lyophilised for 48 h and then crushed into fine particles. Ten to nineteen grams of dry sediment (50 – 800 mg OC) was added to 15 mL of dichloromethane (DCM) in a Teflon® tube and was first sonicated for 30 min (Branson® 5210 Ultrasonic bath), then shaken overnight. The mixture was again sonicated for

30 min. Following centrifugation at ~1500 g for 10 min, the DCM solution was slowly decanted and the supernatant was discarded. The extract was then sonicated for 30 minutes with 15 mL of 1:1 (v/v) DCM:MeOH. The mixture was centrifuged once again at 1500 g and the supernatant was discarded as before. The sediment was then lyophilized and accurately weighed.

3.3.4. Demineralization

The lyophilized DCM-extracted sediments were then demineralised with HCl and a mixture of HF/HCl to remove carbonate and silicate minerals, respectively. Each sample was mixed with 22 mL of de-ionized water in a Teflon® tube. Two mL of 12N HCl were slowly added to obtain a final concentration of 1N HCl. The sediment slurry, in a solution ratio of 1:20 (m/v), was shaken for 1 h to dissolve carbonates and centrifuged as above. Twenty-five mL of a 1N HCl and 10% HF solution were then added to the slurry, which was shaken overnight to dissolve silica and harder oxide minerals. The slurry was then centrifuged as above before repeating the last two steps (HF addition with agitation and centrifugation) until most of the minerals were removed (upon visual inspection). However, mineral removal efficiency decreases with each step due to the presence of fluoride-containing minerals that form in the initial phase of the demineralization procedure through supersaturation and precipitation (Gélinas *et al.*, 2001). These minerals can only be solubilized and removed by complexing the fluoride ions they contain with boric acid. Ten mL of a 5% HF solution containing 1.33 g of H₃BO₃ solution were thus added to the residues recovered by centrifugation to obtain a solution-sample

ratio >10:1. The sample was then shaken overnight, centrifuged as above, lyophilized and weighed accurately.

3.3.5. Hydrolysis

The DCM-extracted and demineralised sediment was then hydrolyzed to sequentially remove carbohydrates and proteins. A sequential hydrolysis procedure was selected because when heated at high temperature in a concentrated HCl solution, carbohydrates and proteins react to form highly refractory condensation products through the Maillard reaction (Maillard, 1912). Ten mL of 2N TFA were first added to the samples to hydrolyze mostly polysaccharides (Allard *et al.*, 1998). The solution and headspace were purged with nitrogen gas for about a minute. The tube was then tightly capped and placed in a heating block at 100°C for 3 h. When cool, the tube was centrifuged at ~1500 g for 10 min and the supernatant was discarded. These steps were repeated twice with 10 mL of 4N TFA and 6N TFA solution, respectively. After discarding the supernatant from the last centrifugation, 10 mL of 6N HCl were added to hydrolyze material such as protein. The HCl solution and headspace were purged with N₂ gas, and the sample was heated at 95-99°C for 24 h in order to avoid water loss by evaporation and to hydrolyze the majority of peptides and proteins present in the samples. The sample was then centrifuged to remove the supernatant, rinsed with 10 mL of de-ionized water, and re-centrifuged to remove the rinsing water. The sample was then lyophilized and weighed accurately.

3.3.6. Oxidation

Harsh oxidizing conditions were used to quantify the oxygen-sensitive and thermally stable OM fractions. The demineralised non-hydrolysable fraction of the samples was treated with 1:1 solution ratio of water:H₂O₂ (30%), at a solid-to-liquid ratio greater than 1:10 (Eusterhues *et al.*, 2005). The Teflon® tube containing the sample was then loosely covered with aluminium foil and placed in a heating block at 50°C for 24 h. The same volume of H₂O₂ (30%) was added every day for a total of seven days. The tube was then centrifuged as above and the supernatant removed. The sample was rinsed with de-ionized water, dried in an oven set at 85°C, and weighed accurately. The dry sediment was then quantitatively transferred to a pre-weighed 1-dram glass vial and combusted at 375°C for 24 h to isolate the thermally stable OM fraction (Gélinas *et al.*, 2001b). The sample was again weighed accurately.

3.3.7. Elemental and isotopic analysis

Following each treatment, a small aliquot of the treated sediment was subsampled to conduct bulk analysis, such as elemental (OC) and stable isotope ($\delta^{13}\text{C}$) analyses. These aliquots were taken into account in the mass balance calculations. When relevant (non-demineralized samples), inorganic carbon was removed by treating the samples with HCl in the vapour phase. The OC content was analyzed with a Perkin Elmer Series II CHNS/O analyser 2400 calibrated with cystine. The analytical precision was $\pm 0.2\%$ for OC (Hedges and Stern, 1984). Bulk elemental (C and N), as well as ^{13}C analyses were also carried out in the same way but using a EuroVector 3028-HT elemental analyzer (EA) coupled to an isotope ratio mass spectrometer (IRMS)

(Isoprime, GV Instruments, UK). The EA-IRMS was calibrated with the laboratory standard β -alanine and with a pre-calibrated IAEA certified primary sucrose standard. The analytical precision was $\pm 0.02\text{‰}$ for ^{13}C (vs PBD) and $\pm 0.2\%$ for C and N.

3.4. Results and discussion

3.4.1. Separation method

The OC percentages, $\delta^{13}\text{C}$, and C/N ratios for all fractions are presented in Table 3.2. Using these results, carbon mass balance calculations were carried out to provide the relative contribution from each fraction to total OC in these samples (Figure 3.2). The different OM reactivity classes are labelled in their order of removal: (i) solvent-soluble, (ii) acid-soluble, (iii) hydrolysable, (iv) oxygen-sensitive, (v) thermally-sensitive and (vi) refractory.

We first used DCM (step 1) to remove soluble and solvent-available organic matter from our samples. DCM soluble material typically comprises compounds such as small lipids adsorbed onto particles or within the OM matrix by diffusion/solubility processes. These lipids normally are ^{13}C -depleted with respect to total organic matter in sediments and thus, we expected to find enriched ^{13}C signatures for the residues remaining following the DCM treatment. In most cases however, the DCM extracted residue was depleted in $\delta^{13}\text{C}$, suggesting that the extracted material was enriched, and sometimes considerably, compared to the whole organic pool. As these results go against most published evidence, a likely explanation for the depleted ^{13}C signatures of the DCM-extracted residues is that they still contained traces of the ^{13}C -depleted DCM. These measurements would have to be repeated to confirm this hypothesis. The

percentage of OC and the C/N ratios vary appreciably in all cases and the removal of carbon range is between 2 to 13% of initial OC for this fraction. The decreasing C/N ratios indicate the enrichment of N in residual sediment suggesting that the extracted OM was poor in N such as lipids. The organic-rich Mexican Margin sediment revealed a small increase of the C/N ratio suggesting that the residual sediment was slightly depleted in N compared to the initial sediment probably due to removal of OM freshly deposited and preserved in anoxic sediment. This highly labile, hydrophobic fraction is expected to account for a low proportion of the total OM in sediment (Rullkötter, 2000).

In step 2 (demineralization), HCl was first used to remove carbonates and solubilize the salts present in the sediment. The second treatment involved HF, which was used to dissolve most silicate and hard oxide minerals. Boric acid was added at the end of this step to remove of F-containing minerals which formed in the slurry upon reaching supersaturation conditions with respect to these phases. These treatments exposed most of the shielded OM to the following treatments and thus, shielded OM content cannot be estimated using the right branch of extraction tree alone (Figure 3.1). Demineralization using HF has been shown to very minimally alter sedimentary OM (Gélinas *et al.*, 2001; Rumpel *et al.*, 2006; Eusterhues *et al.*, 2007). More importantly, the small possible changes are not expected to modify the chemical reactivity of individual compounds to the point where they would be found in a different reactivity class. The OM fraction lost during demineralization corresponds to acid-soluble molecules protected from solubilisation either through direct sorption onto, or encapsulation within minerals. This fraction includes molecules such as small carboxylic acids and small, soluble peptides. In general, the measured $\delta^{13}\text{C}$ values were depleted for all samples,

which agree with the fact that peptides enriched in ^{13}C are removed at this step, except for the more terrestrial influenced samples such as the Eel River and the Saguenay Fjord sediments. These peptides preferentially sorb onto silicates (Aufdenkampe *et al.*, 2001). In these terrestrial sediments, lignin oxidation product such as benzene polycyclic aromatic acids which are usually depleted in ^{13}C (Goñi *et al.*, 2005) may have been extracted leading to an enrichment of ^{13}C in the residual sediment. The acid-soluble fraction varied appreciably between 7 and 47% of initial OC where Washington sediment had the highest value.

In step 3, TFA and HCl were used to separate hydrolysable OM (mostly polysaccharides and proteins, but also nucleic acids) from the non-hydrolysable OM remaining in the residue. During sedimentation of decayed organisms, polysaccharides, nucleic acids and proteins are readily hydrolyzed chemically or enzymatically into smaller water-soluble units. Simple sugars and amino acids exist in rapidly decreasing concentrations in recent and sub-recent sediments (because of their valuable energy potential), but may also survive in low concentration in older sediments, particularly if they are protected by encapsulation into calcareous frustules or shells of marine organisms (Aufdenkampe *et al.*, 2001). Proteins and amino acids with positive charge are also known to adsorb onto negatively-charged silicates (Aufdenkampe *et al.*, 2001).

First, TFA was used to remove molecules such as polysaccharides and other sugars remaining in the sediments, followed by the removal of proteins and nucleic acids using HCl. Ether bonds in polysaccharides are hydrolyzed by TFA, thus allowing for the removal of sugars in shorter chains or as monosaccharides. Polysaccharides are largely insoluble in water, but they are easily converted into soluble and highly labile pentoses

and hexoses by hydrolysis. Lipid biopolymers such as cutin and suberin (from higher plants), chemically similar to polyesters, and composed of both long-chain and hydroxy fatty acid monomers are also sensitive to hydrolysis and are considered easily metabolizable terrigenous OM. These lipids are used for building cell walls by organisms and are usually insoluble in solvents such as DCM (step 1). Following these two hydrolyses, the residue either became more depleted in ^{13}C because of the removal of ^{13}C -enriched compounds such as polysaccharides and proteins (SAG30, Eel, Antarctic and Washington), or remained about the same (Arctic, Mexico, Station 16), while the percentage of OC in all samples but the Antarctic sediment increases due to the carbon-rich nature of non-hydrolysable OM in the residues and the decreases in minerals content. Between 25 and 49% of initial OC (e.g. step 0) was removed during this treatment.

The residue at this step is thus composed of insoluble, non-hydrolysable OC. Recent studies using solid state CP/MAS ^{13}C -NMR techniques revealed a major contribution of aliphatic, esters (di-O-alkyl) and unsaturated carbon in the insoluble, non-hydrolysable fraction of OM in sediment, while the relative abundance of functional groups such as carbonyl, amide, O-alkyl, N-alkyl decreased dramatically following demineralization and hydrolysis (Figure 3.3) (Gélinas *et al.*, 2001a). Other studies have also suggested that insoluble and non-hydrolysable material originates from large biopolymers found in algae and higher plants (Largeau and de Leeuw, 1995). These substances are referred to as algaenan, cutan or suberan according to their origin or co-occurrence with cutin and suberin in extant organisms, but they also include lignin and its degradation products. They consist either of aliphatic polyester chains cross-linked with ether bridges which make them very stable toward degradation (Blokker *et al.*, 2000), or

of lignin derived structures which result from the macromolecular condensation of three different substituted phenols (see Chapter II).

Enzymatic catalysis is not essential to oxidatively degrade organic substances. Aerobic organisms produce small amounts of O_2^- as a metabolic by-product and can then generate hydrogen peroxide, or H_2O_2 (Tung and Sawyer, 1992). Oxidative agents such as H_2O_2 are sufficiently small to diffuse into nano-size pores on mineral surfaces where sorbed organic materials may be more easily preserved because microbial exoenzymes are too large to reach them (Mayer, 1994). H_2O_2 degradation (or O_2) is non-selective and generates mostly CO_2 , whereas enzymatic degradation is highly selective and generates CO_2 and a series of low molecular weight molecules, mostly carboxylic acids (Vollhardt and Schore, 1994). Knowing that low H_2O_2 concentrations are found in natural oxic sediments, we used peroxide to oxidize non-hydrolysable molecules such as polyaromatics, high molecular weight, substituted aliphatic chains and lignin (Leifeld and Kögel-Knabner, 2001) in order to assess the proportion of non-hydrolysable OM that can be oxidized in natural sediments. Because oxic degradation of non-hydrolysable OM by activated oxygen species at the very low concentrations found in sediments involves very long exposure times that are not compatible with laboratory experiments, we used a high concentration of H_2O_2 (15% V/V) and much shorter reaction times. The oxidizable fraction removed during this treatment thus most likely corresponds to the sum of all oxygen-sensitive materials, which would only be completely oxidized upon very long exposure times such as those found in deep sea pelagic sediments (>1000 years) (Hartnett *et al.*, 1998). Between 2 and 5% of initial OC remained after this treatment. The residual sediment is depleted in ^{13}C in all sediments, except for the Arctic and Antarctic samples

(Table 3.2). In all samples the concentration of OC decreases except for the Mexican Margin residue which increases most likely because the molecules that are not oxidized by H_2O_2 are mainly composed of carbon with a minor contribution from hydrogen (highly condensed polyaromatics or aliphatics) and also because of the presence of pyrite in this anoxic area that may react with this treatment (Gélinas *et al.*, 2001; Lefticariu *et al.*, 2006), thus reducing the relative contribution of minerals to the total mass of the residue. The overwhelming majority of hydrolysable, acid-soluble, solvent-soluble and oxygen-sensitive organic materials, which are typically enriched in oxygen- and nitrogen-containing functionalities, were removed during the treatments 1-4.

The last step consisted of a simple combustion in an oxygen-saturated atmosphere. It was used to remove organic compounds that are resistant to chemical oxidation, but that are sensitive to oxic degradation at high temperatures. The thermally-sensitive fraction represents refractory material that is still sensitive to oxygen but generally at lower rates than the materials oxidized with H_2O_2 . Organic material surviving this last treatment is expected to be highly aromatic, refractory molecules such as graphitic black carbon and pure graphite, which most likely derive from the alteration of organic materials at high pressures and high temperatures during rock metamorphism deep in the Earth's crust (Dickens *et al.*, 2004a; Haberstroh *et al.*, 2006). This highly refractory fraction, which accounts for a very small proportion of the initial OC in all sediment, is usually depleted in ^{13}C (Tables 3.2). All sediments, except the Eel River and Arctic samples, showed depleted ^{13}C value for this fraction compared to the value for the initial OC pool (non-treated). This result can be explained by the fact that petrogenic

graphitic carbon, exclusively delivered from terrestrial sedimentary rock sources (and thus ^{13}C depleted, is a major contributor to this fraction.

The steps from the left branch (steps 6 to 11) were planned to allow apportioning the total protected OM fraction into protected hydrolysable OM and protected oxygen-sensitive pools, thus providing clues on the type of OM that is minerally protected, and also on the controls on OM encapsulation into detrital and biogenic mineral matrices. The relative contribution OM entrapped into mineral particles to total OM was calculated for each sample and is shown in Table 3.3. In sediments with a large terrestrial contribution to total OM (>50%) such as the Eel River and the Arctic coastal margin sediments, the relative abundance of the minerally-protected OM fraction reaches a maximum values of 45.9% and 30.7% of initial OC, respectively (Table 3.3). This result could be explained by the fact that these sites are strongly influenced by riverine inputs from two rivers (McKenzie and Eel Rivers), which discharge particulate OM that comprise a large fraction of mineral-associated kerogen, as shown recently by Goñi *et al.* (2005) and Blair *et al.* (2003). The SAG30 sediment is also influenced by important terrestrial inputs; however, only a small proportion (8%) of total OM appeared protected by minerals. In this specific area, high sedimentation rates may explain the small proportion of protected OM by a mechanism that remains to be elucidated (e.g. mineral matrix-OM interactions). The Mexican sediment had the lowest relative abundance of minerally-protected OM at only 0.6% of initial OC. This may be explained by the very low contribution of terrestrially derived minerals at this site (Dickens *et al.*, 2006) and by the fact that the OM sedimentation rates are very high and OM well preserved (high OC content), thus decreasing the relative abundance of protected OM. In the OM-depleted Antarctic

sediment, 15.8% of the total OM pool is protected by minerals, similar to the value measured at Station 16 (18% of the initial OM pool). At these two stations, the sediment is characterized by a very low relative abundance of terrestrial OM and highly degraded marine OM. As explained in the next section (3.4.2) for the Antarctic sediment, the relative abundance of the minerally-protected OM increases with increasing degradation of the non minerally-protected OM. Note that OM protected by carbonate minerals would be missed using this method as the hydrolysis step is carried out in acidic conditions that result in the complete dissolution of carbonate minerals. While carbonate minerals are not expected to contribute much to total sedimentary inputs at the cold-water, biogenic silica dominated Antarctic site, the values calculated for all the others might be slightly underestimated and thus represent minimal estimates. Finally, the low relative abundance of protected OM measured for the Washington coast sediment (6.5% of total OM) was a little surprising in light of the above discussion; while the low relative abundance of terrestrial OM at this site agrees with this result, the long OET and low OM concentration should have resulted in a higher percentage for protected OM. This result could be explained by a different mineralogy at this site, although this or any other proposed explanation would be speculative at this point given the limited data set currently available.

3.4.2. Organic matter composition of different sediments

While the Antarctic sediment had the lowest OC concentration of all the samples in this study (0.3 wt%), it also had the highest proportion of hydrolysable OM (49% of initial OC). The values found for the other fractions were: 11% for solvent-soluble OM,

20% for acid-soluble OM, 18% for oxygen-sensitive OM, 2% for the thermally-sensitive OM, and less than 1% for the refractory OM fraction (Figure 3.2). The surface water primary production in this upwelling region was estimated at about 1-5 g OC m⁻²·yr⁻¹ (Isla *et al.*, 2004), with an OC accumulation rate of 0.066 mg C·m⁻²·yr⁻¹ (Isla *et al.*, 2004). The sedimentation rate of nearly 1 mm·yr⁻¹ obtained in this region by Isla *et al.* (2004), combined to the sediment OC concentration of 0.3 wt%, results in fully oxic conditions, as documented by Stein (1986, 1990). Sediments at this site therefore accumulate in fully oxic conditions and are exposed to oxygen for a long time (high OET, see Table 3.3). As a result, the relative contribution of the oxygen-sensitive organic matter fraction (OSOM) to total OM is one of the lowest (18% of initial OC) for this series of samples. The low OC concentration combined to a long OET and low relative abundance of the OSOM fraction suggests that the OM at this site is highly degraded. However, the relative contributions to total OM of the hydrolysable, acid-soluble and solvent-soluble fractions (49, 20 and 11% of total OM, respectively) are similar to those that are found in fresh, non-degraded OM such as marine plankton or bacterial cells (results not shown). As the Antarctic continent is either bare or covered by ice (no terrestrial primary production), the overwhelming majority of the OM inputs to sediments are derived from the marine primary producers in the surface waters. Hedges *et al.* (2001) recently used solid-state ¹³C NMR spectroscopy to estimate the relative proportion of the three major classes of biochemicals in marine plankton: total lipids, proteins and carbohydrates accounted for roughly 15-30, 50-60 and 20-25% of the total dry weight of the sample, respectively. These percentages are similar in fresh bacterial cells. In this sample, the solvent-soluble fraction accounted for 11% of initial OC, slightly lower than the relative contribution of

lipid structures to total OM in plankton biomass. This can be explained by the fact that the lipid component in plankton also included non-hydrolysable aliphatic materials that would be included in the “lipid” category as defined from NMR spectra, while the solvent extractable fraction mostly comprises low molecular weight lipids, which are degraded preferentially to acid-soluble materials and/or CO₂ upon OM degradation. Thus, it is expected that under oxic conditions prevailing at this site, the relative abundance of the solvent-soluble OM fraction decreases faster than those of the acid-soluble and hydrolysable fractions. It has recently been shown by Aufdenkampe *et al.* (2001) that positively-charged nitrogen side chains in proteins and amino acids are preferentially sorbed onto negatively-charged aluminosilicates clay minerals. Furthermore, in Antarctic sediment, the high abundance of biogenic silica (opal) plays the same role as clay minerals in protecting OM, only with an increased efficiency: indeed, in this case OM can either be sorbed onto opal particles or entrapped into the opal mineral matrix as phytoplankton use nitrogen-rich organic molecules as a scaffold for building their opal shells (Ingalls *et al.*, 2003). In that region of the world ocean, opal was found to contribute as much as 14 to 19% of the total dry weight of the sediment (Isla *et al.*, 2004). Preservation of OM predominantly through mineral protection thus offers a mechanism that can reconcile the above results: the low abundance of the oxygen-sensitive fraction, coupled to the very low OC concentration and long OET all suggest extensive degradation in oxic conditions. OM that survives oxidative degradation is protected through its association with minerals, either sorbed onto their surface or entrapped into their matrix. Most of this OM is released upon demineralization and subsequent hydrolysis. In this particular sediment, 15.8% of OM was protected by mineral

encapsulation which is relatively high considering that Antarctic sediment is well degraded (Table 3.3).

The Mexican sediment was sampled on the continental slope where the sediment accumulation rate is $6.87 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{yr}^{-1}$ (Hartnett *et al.*, 1998). The oxygen penetration depth at this site is only a few mm into the sediment resulting in an OET of only 0.032 year (Hartnett *et al.*, 1998). Sedimentary OM appears to be well preserved as indicated by the high OC concentrations of 7.31 wt% at this site (Table 3.2, see also Dickens *et al.*, 2006). The solvent-soluble OM fraction is low (4% of the total OC; Figure 3.2), while the acid-soluble and hydrolysable fractions were calculated to be 12% and 33%, respectively. Oxygen-sensitive OM is the most abundant OM fraction at this site, accounting for 48% of total OC. This value agrees fairly well with previous data obtained by Gélinas *et al.* (2001) and Dickens *et al.* (2006) in which they estimated that roughly 40% of total OM at this site consisted of insoluble and non-hydrolysable material characterized by highly cross-linked aliphatic molecules. The difference of 8% between these and our estimates was probably due to the presence of non-hydrolysable polyaromatic compounds such as combustion derived black carbon (e.g., soot) in our OSOM isolate. This Mexican sediment was sampled in an exclusively marine environment ($\delta^{13}\text{C} = -21.0\text{‰}$) where the terrestrial influence is expected to be very low.

The Washington coast sediment was sampled on the upper slope of the North Pacific continental margin close to the mouth of the Columbia River. However, the combined effects of local currents and hydrodynamic sorting of particles results in a low terrestrial contribution to total sedimentary OM at this site (Keil *et al.*, 1998). The OC content is 2.58 wt%, and its carbon stable isotope signature shows a strong predominance

of marine sources ($\delta^{13}\text{C} = -22.15\text{‰}$). The OET value of 100 years obtained by Hartnett *et al.* (1998) at this site is the highest among our samples (Table 3.3). The relative contribution of the oxygen-sensitive fraction accounts for 8% of total OM, which is the lowest value obtained in this study (Figure 3.2). The other fractions represented 2% (solvent-soluble), 47% (acid-soluble), 42% (hydrolysable) and 1% (thermally-sensitive and refractory) of total OM, respectively. The high sediment accumulation rate at this site ($102 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{yr}^{-1}$) may explain the initially high OC content in sediment while the long OET seems to be responsible for the very low abundance of oxygen-sensitive fraction. The OM repartition between the different reactivity classes is similar to that at Station 16 with, however, a higher contribution from the oxygen-sensitive fraction in the latter. Both sediments are typically marine, as indicated by their carbon stable isotopic ratios ($\delta^{13}\text{C}$) of -22.15‰ (Washington) and -21.79‰ (Station 16), respectively.

The Saguenay sediment was sampled in a region bathed by fjord conditions with a low renewal rate of bottom salt water owing to the presence of an important sill at the confluence of the fjord and the St. Lawrence River (Fortin and Pelletier, 1995). The sediment is relatively heterogeneous with OM inputs from both surface primary production and terrestrial sources from the Saguenay River. The sedimentation rate at this site ranges between 0.25 and $0.50 \text{ cm}\cdot\text{yr}^{-1}$, with an OC accumulation rate of $1.872 \text{ mg C}\cdot\text{cm}^{-2}\cdot\text{yr}^{-1}$ (St-Onge and Hillaire-Marcel, 2001). This extremely high rate translates into a very high water column and sediment oxygen demand. As a result, the oxic zone at the surface layer of the sediment is very shallow (about 1-2 mm; Mucci *et al.*, 2003). OM from primary production is known to be partially to almost entirely re-mineralized in the water column (Hedges and Keil, 1995), and thus OM inputs in this sediment are mostly

of terrestrial origin. The Saguenay Fjord sample had an OC concentration of 1.9 wt% and a high relative abundance of oxygen-sensitive OM (47% of total OM; Figure 3.2). In agreement with the high terrestrial inputs to total OM at this site, the oxygen-sensitive component has an exclusively terrestrial $\delta^{13}\text{C}$ signature (-28.32‰), which suggests that it is enriched in lignin and lignin degradation products. The refractory OM fraction accounted for about 1% of total OM. Its high atomic C/N ratio (27.37) and its typical terrestrial $\delta^{13}\text{C}$ signature of -29.37‰ suggests that recalcitrant material were also land-derived products.

The Arctic sediment used in this study had an intermediate OC concentration (1.6 wt%) and a high relative contribution from the oxygen-sensitive fraction (44% of initial OC; Figure 3.2). The relative contribution of the other fractions was 4% for the solvent-soluble, 17% for acid-soluble, 33% for hydrolysable, 1% for thermally-sensitive, and 1% for refractory OC. The McKenzie shelf, where this sediment was sampled, receives large inputs of terrestrial OM from the McKenzie River (Goñi *et al.*, 2005). The vast majority of sedimentary OM (>60%) in this area of the Arctic shelf is land-derived material with a high abundance of organic compounds such as cellulose as well as lignin and congeners, and also kerogen (Goñi *et al.*, 2005). Ligneous compounds and kerogen contribute significantly to the oxygen-sensitive fraction, thus partly explaining the high relative abundance of this fraction at this site. The sedimentation rate was calculated to be $\sim 0.1 \text{ cm}\cdot\text{yr}^{-1}$ (Gobeil *et al.*, 1991; Macdonald *et al.*, 1998; Goñi *et al.*, 2005), while the oxic layer was measured to vary between 1 and 2 cm (unpublished data collected during the CASES expedition of 2004). The calculated OET range is thus about 10 to 20 years.

Just like the McKenzie shelf sample, the sediment at this site receives high contributions of terrestrial OM from the Eel River (Blair *et al.*, 2003), which explains the large relative contribution of the oxygen-sensitive fraction to total OM (49%). The apportionment of OM in these last three sediments is very similar, which could be explained by the fact that they all receive high terrestrial OM inputs. Unfortunately, we were unable to find the data that would have allowed calculating OET for Eel River sediment collected on the California margin. In the Arctic margin, Eel River margin, and Saguenay Fjord sediments, terrestrial OM accounts for more than 50% of total OM (Goñi *et al.*, 2005; Macdonald *et al.*, 1998; Gobeil *et al.*, 1991; St-Onge and Hillaire-Marcel, 2001). In these last three sediments, the relative contributions from the oxygen-sensitive fraction were 44%, 49% and 47%, respectively (Figure 3.2), which included a significant proportion insoluble, non-hydrolysable molecules of terrestrial origin such as lignin.

3.4.3. Oxygen exposure time

Several studies have suggested that oxygen concentrations in the bottom waters play a strong role in OM preservation (see Tissot *et al.*, 1974, for instance). However, rather than examining the effect of bottom water O₂ concentrations, recent work proposed to factor in the average time of exposure of OM to oxic conditions in the water column and sediments (Hartnett *et al.*, 1998; Hedges *et al.*, 2002; Arnarson and Keil, 2007). Preliminary studies have shown a strong relationship between OET and the calculated OC burial efficiency (Hartnett *et al.*, 1998), which leads to the suggestion that preservation of OM is ultimately controlled by OET. Further work by Hedges *et al.* (1999) and Gélinas *et al.* (2001) reported that the sedimentary OM becomes increasingly

degraded with increasing OET, thus providing a direct mechanistic link between OM degradation and preservation in marine sediments.

The method developed in this work was designed in part to isolate the oxygen-sensitive fraction expected to be determinant for OC preservation in sediment. The oxygen-sensitive OM was isolated from the other OM fractions and quantified on an OC mass balance basis. As seen on Figure 3.4 there is a strong relationship ($r^2 = 0.9663$) between estimated OET and the relative abundance of the oxygen-sensitive fraction in sediments dominated by marine OM inputs. The relative contribution to total OM of the oxygen-sensitive fraction from both marine and terrestrial sediments decreases with OET, suggesting a kinetic control on the oxidative degradation of the oxygen-sensitive fraction. Although several more data points would be needed to better define the relationship, the correlation between the two parameters appears to be logarithmic. The same logarithmic relationship was found between OET and the relative abundance of the non-protein alkyl fraction in a different set of sediments by Gélinas *et al.* (2001), which roughly corresponds to the OSOM fraction measured here minus its polyaromatic, black carbon component. However, sediments that are influenced by large terrestrial inputs such as the Arctic and SAG30 samples plot above the linear regression and thus are enriched in oxygen-sensitive materials for the corresponding value of OET compared to marine sediments. This relative enrichment could be due to a kinetically slower degradation rate for terrestrial OM, and in particular its oxygen-sensitive component, compared to marine OM, or more likely, to the fact that the relative proportion of oxygen-sensitive material in the total OM inputs at these sites is higher at terrestrially influenced sites than for purely marine locations. Higher relative proportions of oxygen-sensitive materials are expected

in terrestrial OM delivered by rivers because of the high abundance of lignin and kerogen in these samples compared to those of algaenan-like material in marine OM. The relative abundance of the oxygen-sensitive fraction combined with OET could thus be an excellent predictor of OM preservation in estuarine and marine sediments.

3.5. Summary and conclusion

The aim of this work was to develop a wet chemical separation method to isolate different OM reactivity classes in natural sediments. The optimization of this method allowed the quantification as well as elemental and isotopic characterization of six main reactivity classes (solvent-soluble, acid-soluble, hydrolysable, oxygen-sensitive, thermally-oxidizable, and refractory) in seven different natural sediments. This method provides an efficient approach for testing the different hypotheses proposed to explain OM preservation in sediments.

OET thus appears to be a major factor in controlling OC retained in sediments. The OET hypothesis is consistent with molecular-level mechanisms requiring O₂ to degrade carbon-rich, hydrolysis-resistant OM (Hedges and Keil, 1995; Keil *et al.*, 1994; Kristensen and Holmer, 2001; Hulthe *et al.*, 1998; Dauwe *et al.*, 2001). It assumes that at least one fraction of total sedimentary absolutely requires oxygen to be degraded; however, neither OET nor any other single factor appears to control preservation under all conditions (e.g. inherent recalcitrance of terrigenous OM increases preservation). OM being deposited into sediments can be divided in three broad categories, namely (1) refractory OM, (2) oxygen-sensitive OM (OSOM and thermally-oxidizable) and (3) labile, or fermentable OM (which includes most of the solvent-soluble, acid-soluble and

hydrolysable. These broad categories are not mutually exclusive: labile material such as proteins are well preserved when entrapped into biogenic mineral structures or into non-hydrolysable (OSOM) materials, and thus artificially behave like refractory material. Such transfer of material from the labile to the more refractory pools (refractory and OSOM) however is minor and most likely plays a role only in highly degraded sediments such as the Antarctic samples, and in samples that receive large inputs from terrestrial sources. The OSOM fraction is a major player because (i) on a OC mass basis, it is one of the, if not the major OM component in all but the most degraded sediment samples, (ii) it is for the most part found on the surface, and not within, mineral particles; it is thus almost fully accessible to the low molecular weight activated oxygen species with which it reacts, and (iii) it will be degraded only in the presence of oxygen, thus providing a control feedback mechanism that links environmental conditions with OM preservation. At low sedimentation rates and in oxygenated sediments, OSOM is slowly but constantly being degraded and only refractory OM remains. Increasing the OC accumulation rate results in shallower O₂ penetration depths and thus shorter O₂ exposure times in the sediments; as the O₂ concentrations decrease, the degradation rate of the OSOM fraction decreases until it progressively stops. The fraction of the OSOM component remaining at this point is buried in the sediment and enters the very long-term geological cycle of carbon. Our data agree with the OET hypothesis as the relative abundance of the OSOM fraction in marine sediments decreases exponentially with OET. However, the results presented here also suggest a more complex relationship that must take into account the relative inputs of terrestrial and marine OM in a sample as the relative abundances, chemical composition and, most likely, degradation rate of the OSOM fraction in

terrestrial and marine OM differ considerably. What now needs to be done is the molecular-level characterization of the oxygen-sensitive OM in typical marine sediments and in terrestrially-influenced ones, and to assess their degradation rates in oxic conditions.

Table 3.1. Sample location and bulk characteristics of organic matter from the non-treated sediments

Sediment	Latitude	Longitude	Depth (m)	OC (wt%)	(C/N)_a	δ¹³C (‰)
Mexico	22°43.23N	106°28.91W	375	7.30	7.21	-21.00
Antarctic	62°14.00S	58°40.00W	70	0.38	3.91	-24.00
Washington	46°48.97N	125°00.45W	644	2.58	n/a	-22.15
Station 16	48°24.00N	60°44.00W	400	1.71	n/a	-21.79
Arctic	70°02.73N	126°18.06W	200	1.61	9.11	-25.45
Eel River	41°05.10N	124°22.09W	200	1.24	n/a	-23.96
SAG30	48°18.28N	70°15.44W	275	2.59	12.6	-25.84

Table 3.2. Carbon concentration, isotopic signature, and quantity in the residue at each step of the separation method

	Arctic				Mexico				Antarctic				SAG30			
	OC (wt%)	$\delta^{13}\text{C}$ (‰)	C (g) ¹	(C/N) _a	OC (wt%)	$\delta^{13}\text{C}$ (‰)	C (g)	(C/N) _a	OC (wt%)	$\delta^{13}\text{C}$ (‰)	C (g)	(C/N) _a	OC (wt%)	$\delta^{13}\text{C}$ (‰)	C (g)	(C/N) _a
	Step0 Initial	1.61	-25.45	0.403	9.1	7.30	-21.00	0.878	7.2	0.38	-24.00	0.095	3.9	2.59	-25.84	0.646
Step1 DCM	1.58	-25.75	0.388	8.4	7.18	-21.09	0.844	7.8	0.34	-25.62	0.084	3.0	2.41	-26.32	0.602	9.8
Step2 HCl/HF	6.06	-26.03	0.322	18.6	21.01	-21.53	0.739	8.9	0.96	-26.44	0.065	7.4	6.93	-25.91	0.556	16.6
Step3 TFA/HCl	6.13	-26.15	0.189	82.1	49.08	-21.85	0.450	9.3	0.35	-30.63	0.020	6.6	9.63	-26.92	0.319	18.7
Step4 H ₂ O ₂	0.47	-24.23	0.007	25.9	57.72	-23.12	0.031	12.2	0.06	-27.49	0.002	2.8	0.57	-28.32	0.010	27.0
Step5 Combust	0.17	-24.77	0.002	22.6	15.17	-23.02	0.000	1.7	0.03	-27.99	0.000	3.1	0.29	-29.37	0.004	41.5
Step6 TFA/HCl	1.83	-26.59	0.384	8.3	12.47	-21.49	0.688	9.0	0.32	-28.04	0.060	2.5	1.97	-27.36	0.434	17.9
Step7 H ₂ O ₂	0.80	-25.96	0.124	10.1	0.16	-27.48	0.005	9.0	0.10	-28.11	0.015	7.2	0.65	-21.7	0.052	9.2
Step8 HCl/HF	5.52	-26.82	0.094	17.7	n/a	n/a	n/a	9.5	1.89	-27.16	0.001	7.3	25.17	-27.28	0.010	15.5

¹C(g) values represent the quantity of carbon remaining following each step

Table 3.2. Continued

	Eel River			Station 16			Washington			Mexico*		Antarctic*		SAG30*		Arctic*	
	OC (wt%)	$\delta^{13}\text{C}$ (‰)	C (g) ¹	OC (wt%)	$\delta^{13}\text{C}$ (‰)	C (g) ¹	OC (wt%)	$\delta^{13}\text{C}$ (‰)	C (g) ¹	OC (wt%)	C (g) ¹	OC (wt%)	C (g) ¹	OC (wt%)	C (g) ¹	OC (wt%)	C (g) ¹
Step0 Initial	1.24	-23.96	0.342	1.71	-21.79	0.427	2.58	-22.15	0.534	7.31	1.244	0.30	0.056	1.95	0.447	1.63	0.281
Step1 DCM	1.08	-24.25	0.297	1.64	-22.10	0.409	2.55	-21.84	0.524	7.19	1.221	0.29	0.054	1.92	0.439	1.53	0.261
Step2 HCl/HF	2.51	-23.62	0.269	3.36	-22.30	0.261	5.84	-22.92	0.271	18.84	0.896	3.07	0.046	9.05	0.291	11.70	0.229
Step3 TFA/HCl	2.57	-24.03	0.182	4.22	-23.70	0.139	4.11	-23.55	0.045	25.15	0.605	0.90	0.002	28.46	0.280	37.94	0.195
Step4 H ₂ O ₂	0.29	-24.11	0.017	0.65	-25.32	0.013	1.05	-24.53	0.005	44.89	0.339	1.54	0.002	37.50	0.206	25.84	0.067
Step5 Combust	0.22	-22.69	0.011	0.09	-24.75	0.002	0.99	-24.84	0.003	0.75	0.001	0.59	0.000	1.73	0.005	13.73	0.020
Step6 TFA/HCl	1.03	-25.41	0.243	1.97	-24.66	0.376	2.05	-25.00	0.362	8.30	1.170	0.11	0.020	2.56	0.271	1.80	0.251
Step7 H ₂ O ₂	0.84	-26.24	0.157	0.59	-24.47	0.081	0.33	-26.41	0.035	8.60	1.166	0.35	0.019	2.49	0.249	1.70	0.230
Step8 HCl/HF	25.27	-25.53	0.073	4.11	-23.81	0.038	5.11	-24.86	0.016	23.84	0.918	0.37	0.019	6.87	0.237	5.82	0.200

¹C(g) values represent the quantity of carbon remaining following each step

*Data coming from another trial run carried out during the optimization of the method. Stable carbon isotope and total nitrogen values unfortunately are not available for these samples.

Table 3.3. Degradation status and inputs of terrestrial, protected and OSOM organic matter to seven estuarine and marine sediments

Sediment	OC (wt%)	Degradation	Terrestrial Contribution	Protected OM (% of initial OC)	OET (years)	OSOM (% of initial OC)
Mexico	7.30	--	---	0.6	0.032 ^a	47.76
Antarctic	0.38	+++	----	15.8	50 ^b	18.24
Washington	2.58	+++	-	6.5	100 ^a	7.61
Station 16	1.71	++	-	18	1.25 ^c	29.65
Arctic	1.61	+	++	30.7	20 ^d	45.11
Eel River	1.24	+	++	45.9	n/a	48.33
SAG30	2.59	-	+++	8	2 ^c	47.85

a: data from Hartnett *et al.*, 1998.

b: from unpublished data of the Argentinean Antarctic Institute.

c: data from Mucci *et al.*, 2003.

d: data from Goñi *et al.*, 2005.

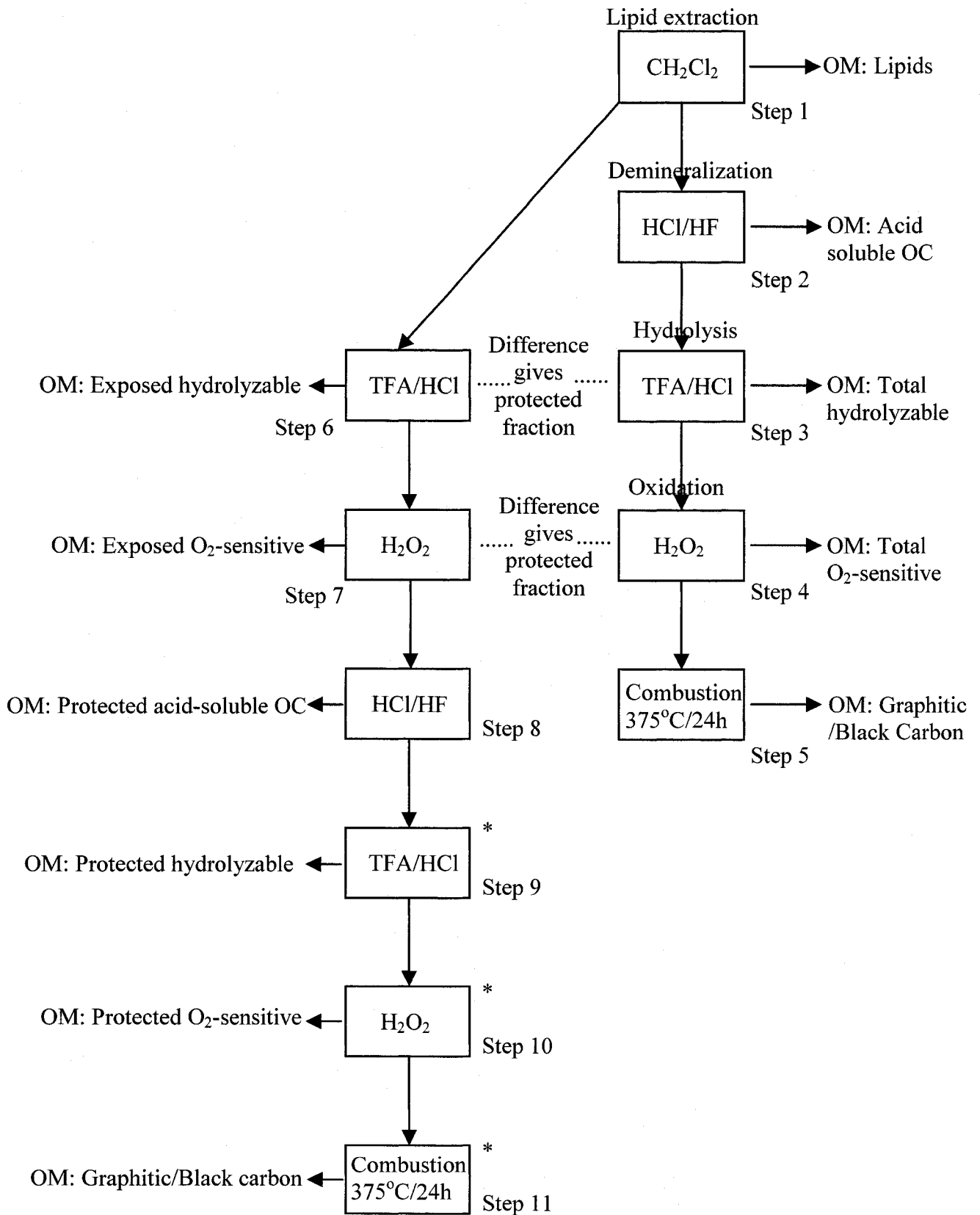


Figure 3.1. Diagram showing the separation method for the six reactivity classes of organic matter (solvent-soluble, acid-soluble, hydrolysable, oxygen-sensitive, thermal sensitive and refractory fractions). Steps marked with (*) were not applied because not enough material was left

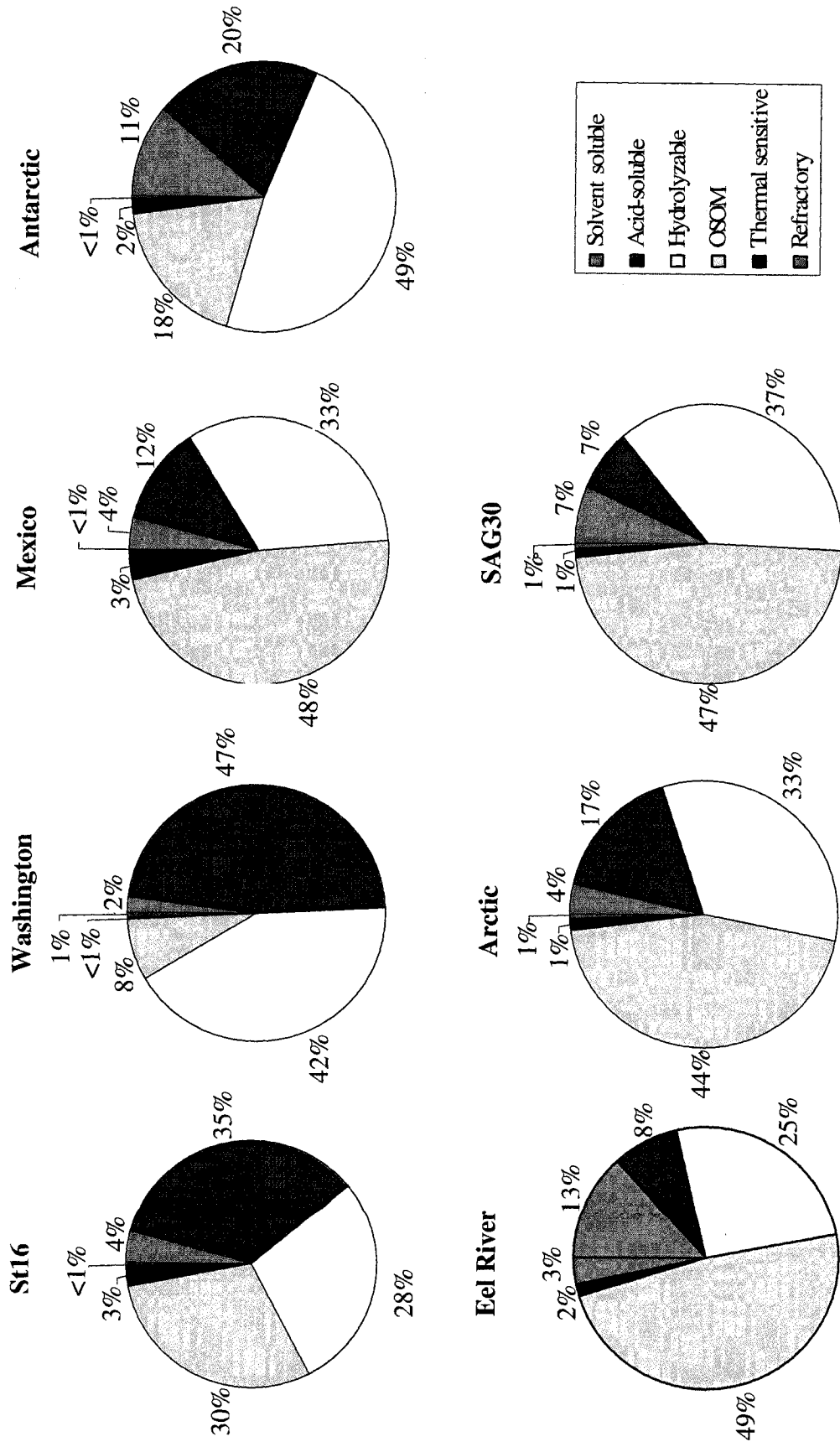


Figure 3.2. Repartition of the different fraction of OC in treated sediments

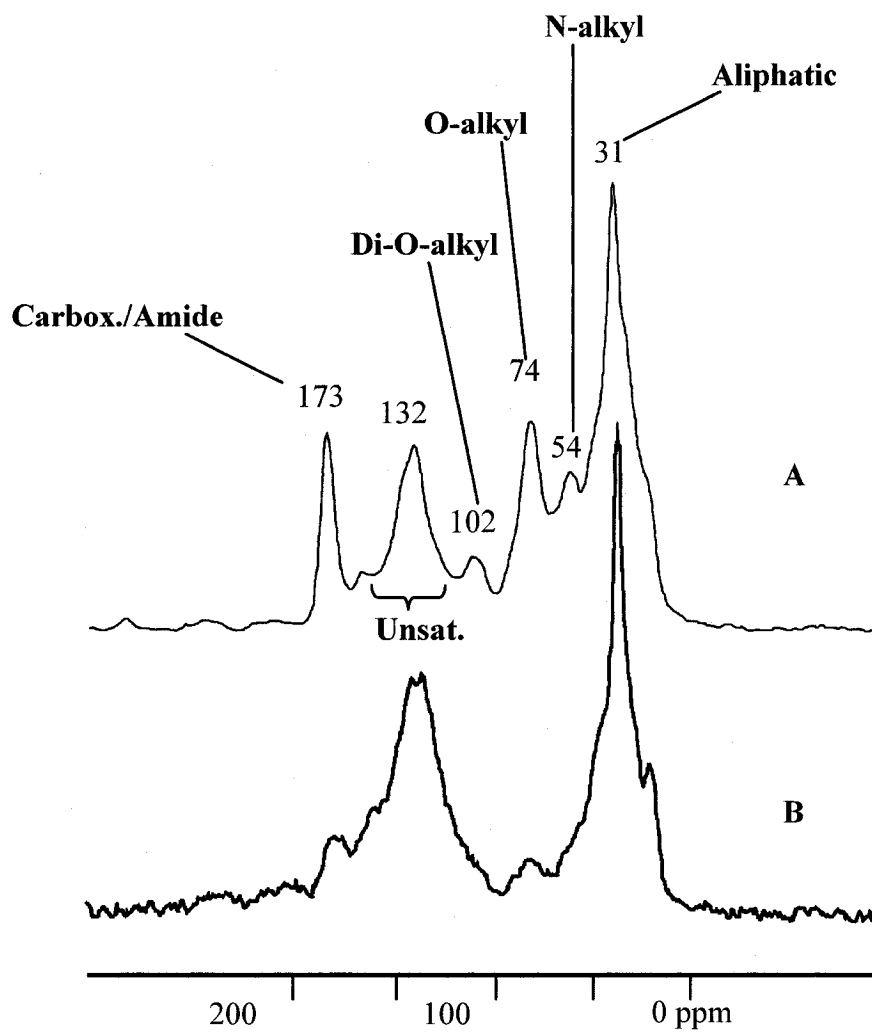


Figure 3.3. Solid-state CP/MAS ^{13}C -NMR spectra of organic-rich Mexican shelf sediment (A) demineralized and (B) insoluble and non hydrolysable isolated OM fraction (adapted from Gélinas *et al.*, 2001a)

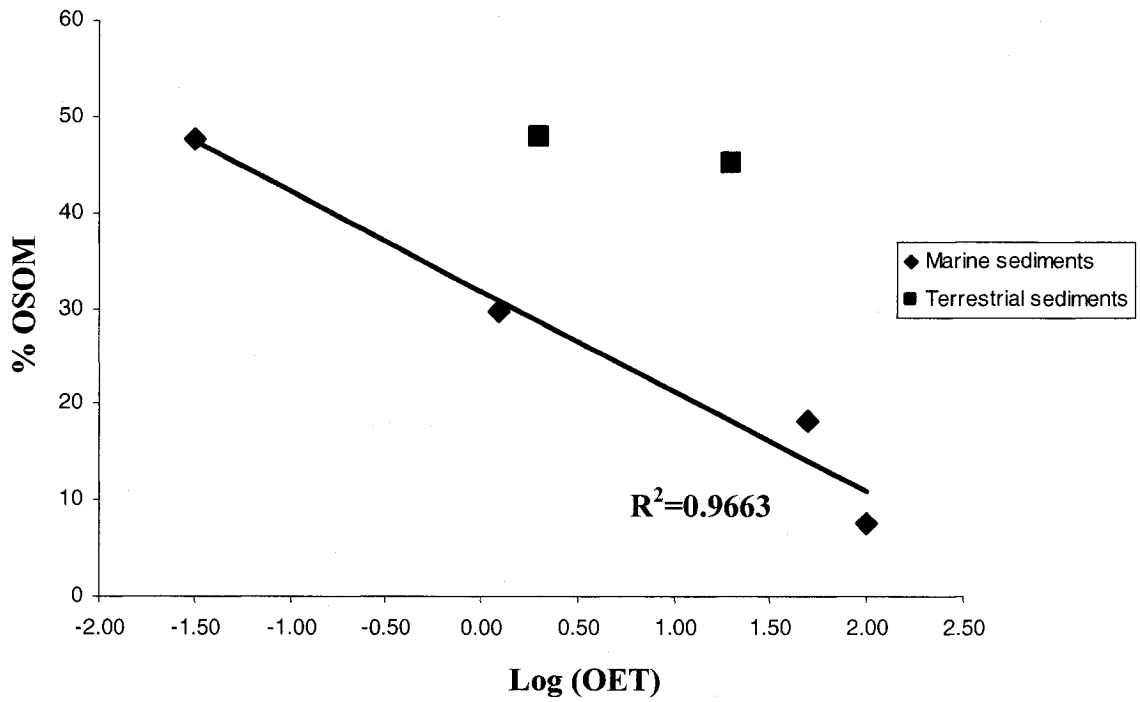


Figure 3.4. Relationship between oxygen exposure time and the relative contribution of the oxygen-sensitive organic matter fraction to total organic matter from marine-influenced (diamond) and terrestrially-influenced (square) samples except Eel River. The regression was calculated using the marine samples only

CHAPTER IV

MOLECULAR ASSESSMENT OF THE CHEMICAL STRUCTURE OF OXYGEN-SENSITIVE ORGANIC MATTER IN SEDIMENTS BY RuO₄ OXIDATION

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Keywords: Ruthenium tetroxide oxidation, organic matter, sediment, oxygen sensitivity, preservation.

4.1. Abstract

Understanding the mechanisms that control OM preservation in sediments requires a good molecular-level knowledge of the chemical composition of the sedimentary organic matter (OM). Organic geochemistry has now progressed to the point where the major hydrolysable biochemicals (proteins, carbohydrates and lipids) can be measured using standard chromatographic methods; however >75 % of sedimentary OM remains molecularly uncharacterized. The work presented in this paper focuses on a sedimentary OM fraction believed to be determinant in OM preservation, i.e., non-hydrolysable oxygen-sensitive organic matter (OSOM). The relative abundance of this fraction was found to decrease with increasing exposure time to oxic conditions. OSOM is expected to be mostly composed of insoluble, non-hydrolysable biomolecules such as algaenans. Ruthenium tetroxide (RuO_4) oxidation is known to cleave ether-linkages and has been successfully applied to elucidate the chemical structure of algaenans. An optimized method based on the gentle chemical oxidation of sedimentary OM using RuO_4 was used to investigate the molecular structure of the OSOM component in a range of contrasting sediments and its relationship to OM preservation under varying sedimentary conditions. Our results show that a series of C_6 to C_{24} alkanolic acids, and C_6 - C_{14} alkanedioic acids are produced upon oxidation, with a relative abundance distribution that reaches a maximum either at C_6 or C_{16} depending on the sample (alkanoic acids). The alkanedioic acids are more evenly distributed and peaked at either the C_6 , C_8 or C_9 alkanedioic acid. Also evidenced was a clear even-to-odd preference for both series of fatty acids, although more pronounced for the alkanolic acids, suggesting a small contribution from bacterial cell membranes to the OSOM pool, and also a relatively fresh OSOM pool. The OSOM component appears to consist of at least three distinct fractions,

although more work is needed to confirm this hypothesis: a first one that does not generate fatty acids or fatty acids with a chain length greater than C₆; a second one that generates fatty acids with a relatively low acids-to-diacids ratio, and a third one that is the most resistant to oxic degradation and that is enriched in alkanolic acids relative to the alkanedioic acids compared to the second fraction.

4.2. Introduction

Sedimentary organic matter (OM) is composed mostly of insoluble, highly complex macromolecules, often called protokerogen[†]. Protokerogen and kerogen are derived in large parts from the selective preservation of resistant, non-hydrolysable insoluble biopolymers found in the cell walls of marine and freshwater algae, and also in the protective layers of higher plants (Largeau and de Leeuw, 1995; de Leeuw and Largeau, 1993). Despite their low relative abundance in organisms compared to major biochemicals such as proteins, lipids and carbohydrates (1-2 % dry biomass), these biopolymers are selectively preserved upon sedimentation and burial because of their resistance against microbial and chemical degradation. Depending on the chemical conditions prevailing in the bottom waters and surface sediments, these macromolecules can thus constitute a major fraction of altered OM kerogen-rich in various OM-rich deposits (Tegelaar *et al.*, 1989).

Studies on the chemical structure of algal-derived cell walls using pyrolysis-gas chromatography/mass spectrometry (py-GC/MS) have shown that they are mostly composed of insoluble, non-hydrolysable, highly aliphatic material called algaenans (Goth *et al.*, 1988; Derenne *et al.*, 1992; de Leeuw and Largeau, 1993; Augris *et al.*, 1998; Blokker *et al.*, 2000). These biomacromolecules are highly resistant to hydrolysis by strong acids and bases, as well

[†] See glossary

as by enzymes. Their chemical structure consists of a network of long, mostly saturated, hydrocarbon chains that are cross-linked via ether linkages at a terminal position and one or more mid-chain positions (Blokker *et al.*, 2000) (Figure 4.1). In all algaenans investigated so far, ether and carbon-carbon bonds serve as important cross-links between the monomeric units, thus giving rise to a non-hydrolysable and insoluble biopolymeric network (Blokker *et al.*, 2000). Because of their size and lack of solubility in polar or non-polar solvents, molecular-level analysis by conventional chromatographic techniques such as GC-MS or LC-MS is possible only if these biopolymers first undergo a chemical treatment designed to depolymerise the network into smaller molecules.

Ruthenium tetroxide (RuO_4) is one of a number of oxidizing agents that have been applied to non-hydrolysable organic materials such as kerogen. Ruthenium tetroxide oxidizes alcohols, aldehydes, alkenes, alkynes, and some aromatic compounds to corresponding carboxylic acids similar to the action of permanganate (Vitorović *et al.*, 1988). However, an important difference is the chemoselectivity of RuO_4 towards substituted aromatic hydrocarbons. Permanganate generates aromatic acids as the main oxidation products from substituted aromatic hydrocarbons, in contrast to ruthenium tetroxide, which produces aliphatic acids from substituent (Smith and March, 2001; Nuñez and Martin, 1990). Besides, chemical structure of algaenans was successfully probed with ruthenium tetroxide known to cleave ether-linkages (Panganamala *et al.*, 1971; Sharpless *et al.*, 1981; Amblès *et al.*, 1996; Gelin *et al.*, 1997; Schouten *et al.*, 1998; Blokker *et al.*, 1998; Blokker *et al.*, 1999).

Previous work has shown that one of the major controls on OM preservation in marine sediments is the relative abundance of a fraction of the bulk OM pool that can only be degraded in the presence of oxygen, the oxygen-sensitive organic matter fraction, or OSOM

(Hedges *et al.*, 1999; Gélinas *et al.*, 2001; Arnarson and Keil, 2007). Because of the similarities between reactivity, chemical structure and relative abundance, this OSOM fraction and the organic pool believe to be precursor to kerogen could be one and the same. However, in terrestrially-influenced sediments from continental margins, non-hydrolysable and oxygen-sensitive lignin-derived products could also be included in this pool and influence OM preservation. RuO₄ oxidation followed by derivatization and GC-MS analysis of the oxidation products was thus carried out on the OSOM fraction isolated from a series of marine and/or terrestrially-influenced sediments. The structural details provided by this approach should provide clues on the sources contributing to the OSOM fraction and on its relative importance in OM preservation.

4.3. Material and Methods

4.3.1. Sediment sampling

Surface sediments (0-5 cm) from Station SAG30 in the Saguenay Fjord (Québec, Canada), Station 16 in the St. Lawrence Gulf (Québec, Canada), and Station 200 in the Franklin Bay (Beaufort Sea, Arctic Ocean; Canadian Arctic Shelf Exchange Study project) were sampled in May 2002, June 2005 and December 2003, respectively, using a box core. The Mexican surface sediment (0-5 cm) was collected with a multicorer on the western Mexican margin near Mazatlan in January 2000 at a 375 m depth (Station 306). The Antarctic surface sediment (0-5 cm) was collected in Potter Cove (Bellinghausen Sea) near the Argentinean naval base of Jubany using a van Veen grab in January 2005. Washington coast (Continental shelf near Washington State in the Pacific Ocean) and Eel River (Northern California, USA) were sampled in May 2001 for their surface sediment (0-5 cm). All

sediments were put in Ziploc bags, lyophilized and stored at -20°C. The bulk characteristics and location were reported previously in Table 3.1 (see Chapter III).

4.3.2. Removal of the solvent soluble fraction

Whole sediment was lyophilised for 48 h and then crushed into fine particles. Ten to nineteen grams of dry sediment (50 - 800 mg OC) was added to 15 mL of DCM in a Teflon® tube and was first sonicated for 30 min (Branson® 5210 Ultrasonic bath), then shaken overnight. The mixture was again sonicated for 30 min. Following centrifugation at ~1500 g for 10 min, the DCM solution was slowly decanted and the supernatant was discarded. The extract was then sonicated for 30 minutes with 15 mL of 1:1 (v/v) DCM:MeOH. The mixture was centrifuged once again at 1500 g and the supernatant was discarded as before. The sediment was then lyophilized and accurately weighed.

4.3.3. Demineralization

The freeze-dried DCM-extracted sediments were then demineralised with HCl and a mixture of HF/HCl to remove carbonate and silicate minerals, respectively. Each sample was mixed with 22 mL of de-ionized water in a Teflon® tube. Two mL of 12N HCl were slowly added to obtain a final concentration of 1N HCl. The sediment slurry, in a solution ratio of 1:20 (m/v), was shaken for 1 h and centrifuged as above. Twenty-five mL of a 1N HCl and 10% HF solution were then added to the slurry, which was shaken overnight. The slurry was then centrifuged as above before repeating the last two steps (HF addition with agitation and centrifugation) until most of the minerals were removed (upon visual inspection). However, mineral removal efficiency decreases with each step due to the presence of fluoride-

containing minerals that form in the initial phase of the demineralization procedure through supersaturation and precipitation (Gélinas *et al.*, 2001). These minerals can only be solubilized and removed by complexing the fluoride ions they contain with boric acid. Ten mL of a 5% HF solution containing 1.33 g of H₃BO₃ solution were thus added to the residues to obtain a solution-sample ratio >10:1. The sample was then shaken overnight, centrifuged as above, lyophilized and weighed accurately.

4.3.4. Hydrolysis

The DCM-extracted and demineralised sediment was then hydrolyzed to sequentially remove carbohydrates and proteins. A sequential hydrolysis procedure was selected because when heated at high temperatures in a concentrated HCl solution, carbohydrates and proteins react to form highly refractory condensation products through the Maillard reaction (Maillard, 1912). Ten mL of 2N TFA were first added to the samples to hydrolyze mostly sugars (Allard *et al.*, 1998). The solution and headspace were purged with nitrogen gas for about a minute. The tube was then tightly capped and placed in a heating block at 100°C for 3 h. When cool, the tube was centrifuged at ~1500 g for 10 min and the supernatant was discarded. These steps were repeated twice with 10 mL of 4N TFA and 6N TFA solution, respectively. After discarding the supernatant from the last centrifugation, 10 mL of 6N HCl were added to hydrolyze material such as protein. The HCl solution and headspace were purged with N₂ gas, and the sample was heated at 95-99°C for 24 h in order to avoid loss by evaporation and to hydrolyze a significant majority of polysaccharides. The sample was then centrifuged to remove the supernatant, rinsed with 10 mL of de-ionized water, and re-centrifuged to remove the rinsing water. The sample was then lyophilized and weighed

accurately, thus providing a non-hydrolysable OM fraction consisting of a mix of predominantly oxygen-sensitive OM with a minor refractory OM component (mostly polyaromatic graphitic and soot black carbon).

4.3.5. Ruthenium tetroxide treatment

Ruthenium tetroxide treatment was adapted from the method developed by Blokker (2000) and Yoshioka (2005). The freeze-dried non-hydrolysable fraction isolated above was subsampled to prepare an aliquot containing about 500 mg of OC. The aliquot was shaken with 10 mL dichloromethane (DCM), 10 mL deionised water and 10 mL acetonitrile (ACN) for 30 minutes in a 35-mL Teflon® tube. Then 5 g of sodium periodate (NaIO_4) was added to the mixture with 500 mg of $\text{RuCl}_3(\text{H}_2\text{O})_x$ (Blokker *et al.*, 2000). The slurry was shaken overnight then transferred into a separatory funnel. The reaction was quenched with 5 mL of diethyl ether (Et_2O) upon which the solution turned black, and extracted 3 times with 10 mL of DCM: Et_2O 4:1 (v/v). The aqueous phase was acidified to pH 1 with concentrated HCl and reextracted with 10 mL of DCM: Et_2O 4:1 (v/v). The organic extracts were combined and dried with Mg_2SO_4 . Prior to analysis, the organic extracts were concentrated under a gentle flow of nitrogen until few microliters was left, and then derivatized with 1 mL of a $\text{BF}_3/10\%\text{MeOH}$ solution purchased at Sigma-Aldrich. Derivatization was carried out at 100°C for 2 h in a one-dram vial and quenched with 6 mL of a 5% NaCl solution. The solution was then extracted with 3 mL DCM (3 times). The organic extracts were combined, evaporated to almost dryness and resuspended in 200 μL of DCM prior to injection.

4.3.6. Elemental and isotopic analysis

Following each treatment, a small aliquot of the treated sediment was subsampled to conduct bulk analysis, such as elemental (OC) and stable isotope ($\delta^{13}\text{C}$) analyses. These aliquots were taken into account in the mass balance calculations. When relevant (non-demineralized samples), inorganic carbon was removed by treating the samples with HCl in the vapour phase. The OC content was analyzed with a Perkin Elmer Series II CHNS/O analyser 2400 calibrated with cystine. The analytical precision was $\pm 0.2\%$ for OC (Hedges and Stern, 1984). Bulk elemental (C and N), as well as ^{13}C analyses were also carried out in the same way but using a EuroVector elemental analyzer (EA) 3028-HT elemental analyzer coupled to isotope ratio mass spectrometry (IRMS) (Isoprime, from GV Instruments). The EA-IRMS was calibrated with the laboratory standard β -alanine and with a pre-calibrated IAEA certified primary sucrose standard. The analytical precision was $\pm 0.02\text{‰}$ for ^{13}C (vs VPBD) and $\pm 0.2\%$ for C and N.

4.3.7. Gas chromatography analysis

Following the ruthenium tetroxide treatment and derivatization, the samples were analyzed by gas chromatography (GC 3800 Varian®) coupled to a Varian 2000 ion-trap mass spectrometer, and equipped with an AS8410 Varian autosampler. The column used was purchased from Restek Inc. (Rtx-5MS®) and measured 30 m, 0.25 mm (i.d.), 0.25 μm dF. The injector temperature was set at 310°C and a split mode with ratio of 5:1 was applied for the first five minutes and then decreased to 2:1. The column ramp temperature was first set at 50°C and held 5 min followed by increases to 140°C at 15°C/min, 214°C at 4°C/min, 216 at 0.5°C/min, 219°C at 4°C/min, 223°C at 0.5°C/min and finally to 300°C at 10°C/min, where

it was held for an additional 7 min. The mass spectrometer was in full scan mode (40-650 m/z). The identification of the peaks was based on fatty acid methyl ester standards purchased from Supelco (Supelco37 FAMES mix) and using the Varian and NIST mass spectral databases. Quantification was done using a gas chromatography coupled to a flame ionization detector (Agilent 6890N GC-FID) fitted with a 30 m long \times 0.32mm inner diameter column of HP-5 (polysiloxane with 5% phenyl) and film thickness of 0.25 μ m. This column was attached to a flame ionization detector set at 310°C. The injector (Gerstel CIS3) initial temperature was held for one minute at 70°C then increased at a rate 10°C per second up to 310°C and held there for 10 minutes. The column ramp and standards were the same as those used for the GC-MS analyses.

4.4. Results and Discussion

4.4.1. Oxygen-sensitive organic matter and oxygen exposure time

Seven highly contrasting sediment samples were collected on the Antarctic, Arctic, Mexican and East-Canadian coasts (Table 4.1) to document and quantify changes in the molecular makeup of the OSOM fraction, and to explore potential relationships between these changes and the deposition environment at each site, including OET. The OM in the Antarctic sediment is strictly derived from marine sources, as seen by the ^{13}C stable isotope ratio of -24.00‰ (typical of marine sources in Polar Regions), and also because of the absence of terrestrial production on the continent. The OSOM fraction at this site accounts for only 10% of the total OM pool, a reflection of the long OET (50 years) suggesting highly degraded OM (Table 4.1). The Mexican margin sediment is also a fully marine sediment ($\delta^{13}\text{C} = -21.00\text{‰}$); contrary to the Antarctic sample however, it has a high proportion of

OSOM (52% of the initial OM) and the shortest OET (0.032 year) suggesting good preservation of the OSOM fraction (Hartnett *et al.*, 1998). The sediment collected at Station 16 in the St. Lawrence Gulf also is characterised by OM bearing a marine signature ($\delta^{13}\text{C}$ of -21.79‰). The relative abundance of the OSOM component at this site is 34% of total OM and the OET is 1.25 year. The stable isotope signature of OM from the Washington margin sediment is also marine ($\delta^{13}\text{C} = -22.15\text{‰}$); however, the margin receives large fluxes of terrestrial OM from the Columbia River. The relative abundance of the OSOM component is 11%, with an OET of 100 years, suggesting highly reworked sediment. In the SAG30 sediment, the OSOM fraction accounts for 46% of total OM, which is mostly derived from terrestrial sources (Colombo *et al.*, 1996) ($\delta^{13}\text{C} = -25.84\text{‰}$). OET at this site is only about 2 years, suggesting a good preservation of the OSOM component. A high abundance of lignin-derived structures is expected to be present in the OSOM fraction of this sample. The Arctic shelf sediment is also receiving high inputs of terrestrial OM ($\delta^{13}\text{C}$ of -25.45‰). The OM at this site comprises the second highest relative abundance of OSOM (49% of total OM), and an OET of about 20 years. The total OM pool at Eel River site appears to be mostly marine in origin, as suggested by its ^{13}C isotopic ratio signature of -23.96‰, despite large inputs of terrestrial OM. However, the terrestrial OM being delivered by the Eel River consists in large part of kerogen material closely associated to mineral particles (Leithold *et al.*, 2005). The isotopic signature of the kerogen, which is derived from marine sedimentary material that has undergone a complete geological cycle at this site, is typically marine (-20 to -22‰) and explains why the signature of the total sedimentary OM pool at this site is more enriched in ^{13}C than expected (Leithold *et al.*, 2005). The high inputs of terrestrial OM also explain the high relative abundance of the OSOM fraction (45% of the total OM pool). Unfortunately,

we were unable to calculate the OET at this site because the oxygen penetration depth has not been measured. However, at a sampling depth of 300 m, the sediment surface is bathed in oxygen-depleted bottom waters (the oxygen-minimum zone of the California current). As a consequence, the O₂ penetration depth, and thus OET, is expected to be low (A.F. Dickens, *pers. comm.*). We therefore conservatively assumed that the OET at this site is about 1 year.

4.4.2. Ruthenium tetroxide treatment on oxygen-sensitive organic matter

Two major classes of oxidation products were found in the OSOM fractions: (i) saturated alkanolic acids and (ii) saturated alkanedioic acids. The quantification and characterization of the mono- (oic) and di-fatty acids (dioic) were carried out by GC-FID and GC-MS, respectively (Table 4.1). Despite the presence of a broad peak corresponding to an unresolved complex mixture (UCM) (Figure 4.2), several individual molecules can be positively identified through comparison with standards and direct GC-MS mass spectral analysis. The ruthenium tetroxide treatment commonly generates low yields of GC-amenable oxidation products when applied to complex macromolecular structures; however, it is assumed to provide a representative picture of the relative abundances of the main moieties that make up the bulk of these materials (Blokker *et al.*, 1998). Indeed, after previous treatment, the OSOM fraction, according to the NMR study of Gélinas *et al.* (2001a), is believed to be structurally similar to algaenans which is a macromolecular network of highly aliphatics with a presence of unsaturation, o-alkyl, di-o-alkyl and carboxylic functional groups. Despite the low yield of the ruthenium treatment, we assumed that the oxidation products were representative of the OSOM macromolecular network as seen by the work of Blokker (1998) with algaenans. Carbon mass balance calculation using the sum of the

concentrations of all the identified peaks listed in Table 4.1 results in an estimate of only 1-5 wt% of the starting OC that was oxidized by RuO₄ and quantified on the GC-FID.

The oxidation treatment on the OSOM fractions released a series of alkanolic acids with a chain length from C₆ to C₂₄ and a series of alkanedioic acids from C₆ to C₁₄ (Table 4.2). Note that it is highly probable that shorter alkanolic and alkanedioic acids (C₂-C₅) were also produced during the oxidative treatment; however, these acids are highly volatile, particularly when derivatized, and have thus been lost during sample preparation. An alternative method, based on head-space solid-phase micro-extraction followed by GC analysis (Huang *et al.*, 2007), must be used to target these volatile compounds.

In all samples, the alkanolic acids series peaked at C₁₆ or C₆ followed by C₁₄ compounds, whereas the alkanedioic acids series showed no apparent maximum in the abundance of the short chains of diacids found in the samples (C₆ to C₁₀) (Table 4.2 and Figure 4.3). An unresolved complex mixture (UCM) was apparent on all chromatograms. The UCM results from the co-elution and peak overlap of a great number of molecularly similar compounds that cannot be resolved using the column and chromatographic conditions from this work. Although the intensity of the UCM relative to that of the sum of all alkanolic and alkanedioic acids varied between samples (highest for the Mexican margin and SAG30 sediments, lowest in the Antarctic sediment, and intermediate in the Washington, Station 16, Arctic and Eel River Samples), no clear trend was found in this series of samples.

Table 4.2 lists the concentrations of the two series of ruthenium tetroxide oxidation products found in the samples. The results are expressed in $\mu\text{mol}\cdot\text{g}^{-1}$ of OC in the OSOM fraction. The Washington sediment was the sample that showed the highest total concentration of alkanolic and alkanedioic acids followed by the Antarctic, SAG30, Eel

River, St16, Mexico and Arctic. In this last sample, the GC signal of individual alkanedioic acids was so low that this sample had to be dropped when calculating correlation and indexes that make use of alkanedioic acids (see below).

The relative abundances of all alkanolic (FA) and alkanedioic (diFA) acids are plotted on Figure 4.3 (relative abundances in mole percentages). Large differences were found between samples. Sediments with a strong terrestrial influence such as the Eel River and the SAG30 samples, but also for the Washington coast sample, showed a high relative abundance of the low molecular weight alkanolic and alkanedioic acids compared to the other four samples. In these later ones, a strong predominance of C₁₂ to C₁₈ alkanolic acids, and C₈ to C₁₂ alkanedioic acids was apparent. Note that the low signal intensities measured for the Arctic margin samples significantly skews such relative distribution profile in favour of the most abundant molecules as several alkanolic and alkanedioic acids could not be quantified on the GC chromatogram. The low intensities measured for this sample were due to the very low mass of OSOM material available to run the RuO₄ treatment.

A clear predominance of even-numbered carbon alkanolic and alkanedioic acids is evident from Figure 4.3. This is true for all samples, as shown by the ratio of the sum of all even-numbered alkanolic acids over the sum of all odd-numbered ones (Even/Odd) varying between 3.9 and 20.7 (Table 4.2). Such strong predominance of the even-numbered alkanolic acids suggests that the fraction of the OSOM pool that is structurally characterized using this approach (RuO₄ oxidation) is mostly marine plankton in origin, and that it has not been significantly assembled with bacterial molecules. The absence (or very low abundance) of iso- and anteiso- alkanolic acids as oxidation products also suggest a very low contribution of bacterial cell component to the OSOM pool (Gonzalez-Vila *et al.*, 1994). However, more

work would be needed in order to identify a signal from bacteria that may disappear in our analysis in order to assess the bacterial contribution in the OSOM fraction.

This fraction of the OSOM pool is further evidenced in Figure 4.4. Although we expected that the relative abundances of alkanolic and alkanedioic acids would be related to the sources of OSOM in these samples and to the extent of alteration/degradation, the constant ratio between the sum of all alkanolic acids over the sum of all alkanedioic acids suggests otherwise. Despite OETs that vary over three orders of magnitude, the Σ_{FA} and Σ_{diFA} values fall almost exactly on a straight line for all samples ($r^2 = 0.9943$, Figure 4.4). This strong correlation suggests that despite the differences noted in the relative abundances of the alkanolic and alkanedioic acids (Figure 4.3), the abundance of cross-links and/or unsaturated carbon-carbon bonds relative to the straight chains is comparable in all samples. It further suggests either that the fraction of the OSOM pool characterized using this approach originates from a common source (e.g. bacteria or phytoplankton), or that different source organisms are able to synthesize biopolymers with a similar chemical structure. Further insight could be gained regarding the structure and the source(s) of the fraction of the OSOM component that generates fatty acids by reducing the number of functional groups that are cleaved upon RuO_4 oxidation in the OSOM component; using H_2 to reduce the double bonds present in the aliphatic chains of the fatty acids would most likely reduce the relative abundances of alkanedioic acids, which could then all be ascribed to cross-links and substituted aromatic structures. This could allow defining a cross-linking, or degree of condensation index of the OSOM component in each sample.

Significant variations in the total yields of alkanolic and alkanedioic acids, as well as in the ratio of the relative abundances of the mono- and diacids were found between the

samples. When plotted against OET (Figure 4.5), these variations seem to be linked to OET, although in a non-linear fashion. The total yield of fatty acids generated upon RuO_4 oxidation clearly increases with an increase in OET linked to % OSOM (see previous chapter Figure 3.4), suggesting that the fraction of the OSOM component that generates fatty acids is relatively more resistant towards oxidative degradation than the rest of the OSOM component. The ratio between the sum of all alkanolic acids and all the alkanedioic acids also varied with OET. Fresh materials (short OET) had the highest contributions from the diacids (e.g. Mexican sample), which relative abundance decreased quickly with OET to reach a minimum (or maximum acids over diacids ratio) for exposure times of 20-60 years. The relative abundance of the diacids then appears to increase again at long OETs although more samples would be needed to confirm this trend.

Taken together, these results suggest that at least two OSOM pools generate fatty acids upon treatment with RuO_4 ; first, a diacid-rich component that is more susceptible to oxidic degradation is present in the samples that have not been exposed to oxygen for a long time (short OETs). The abundance of this fraction would quickly decrease with OET owing to its higher degradation rate in oxidic conditions, as suggested by decrease in the measured yields between OETs of <1 year and 20 years. The relative abundance of the second component would then increase as the abundance of the diacids-rich fraction and that of the fraction of the OSOM component that does not generate fatty acids would decrease. Upon longer exposure to oxidic conditions, the more highly cross-linked fraction of the OSOM component would then be selectively preserved, leading to an increase in the relative abundance of the diacids.

Although clearly speculative at this point, the explanation provided above makes good geological sense and does not disagree with what is currently known regarding the non-hydrolysable fraction of sedimentary marine OM. The alkanedioic-depleted and alkanolic acid-rich OSOM fraction that is highly resistant to oxic degradation is analogous to algaenans (Blokker *et al.*, 1998), while the diacid-rich OSOM fraction that show a lower resistance to oxic degradation could be derived from polyphenolic structures such as lignin polymers in the case of terrestrial influenced sediment. As non-hydrolysable lignin polymers are less hydrophobic than highly cross-linked, aliphatic-rich algaenans, they are more accessible to dissolved oxidants and thus would be expected to be more susceptible to oxic degradation in oxic aqueous conditions. Despite the its marine origin and its OET of ~100 years, the OSOM fraction from Washington Coast sediment generated a large fraction of alkanedioic acid upon oxidation, which could be attributed to the relatively higher degree of cross-linking of the OSOM materials that are surviving long exposures to oxic conditions. At such long exposure times, bacteria would have had enough time to rework the OM originally deposited in the sediment, to produce a highly recalcitrant bacterially-derived OSOM fraction. Note that most of the ligneous materials are not detected using this approach as the very short-chain fatty acids and fatty diacids produced upon oxidation with RuO_4 would be lost because they are highly volatile, or would not be detected on the GC because they co-elute with the solvent. Lignin also generates C_6 alkanolic and alkanedioic acids upon oxidation, which most likely explains why terrestrially-influenced samples (Eel River, SAG30) are highly enriched in these compounds (Figure 4.3); however, other sources of C_6 alkanolic and alkanedioic acids must be present in the OSOM fraction as they are the most abundant fatty acids in the marine Washington coast samples.

The contrasting distribution of short-chain and longer-chain alkanolic and alkanedioic acids in the samples seemed paralleled by the extent to which even-numbered fatty acids predominate over the odd-numbered ones (Figure 4.6). A chain length index was defined by adding the micromolar concentrations of the C₆ to C₁₂ alkanolic acids divided by the sum of the concentrations of the C₁₃ to C₂₄ alkanolic acids. For the alkanedioic acids, the ratio corresponds to the sum of the C₆ to C₉ diacids, divided by the sum of the C₁₀ to C₁₄ diacids. The choice of the cut-off for the short-chain and long-chain fatty acids was based solely on the range of chain lengths encountered in the samples – it was not guided by any biogeochemical rationale.

When the defined chain length index is plotted against the even-to-odd preference index (Figure 4.6), a very strong relationship between these two parameters is evidenced for both the alkanolic and alkanedioic acids series (correlation coefficients of 0.9965 and 0.8459 for the alkanolic and alkanedioic acids, respectively). The relationships suggest that samples with fatty acids of shorter chain length also have higher even-to-odd preference indexes usually corresponding to fresh organic material. Although the relationships are strongly dependent on the relative abundance of the C₆ molecule (which contributes only to the short chain pools and the even-numbered fatty acids pool), the relationship remains strong for the alkanolic acids ($r^2 = 0.9433$) even when not taking into account the C₆ fatty acids in the calculation of both indexes. For the alkanedioic acids however, the correlation coefficient falls to 0.2492.

The strong relationship between the two indexes also agrees with our interpretation of the variations in fatty acid abundances and acid-to-diacid ratios with OET (Figure 4.5). The diacid-rich fraction of the OSOM pool (see above) is more sensitive to oxic degradation and

thus its degradation rate is faster. It would thus be found only at short OET, when the OSOM fraction is “fresher”, or less altered/degraded. Even-to odd ratios are also higher in fresh OM than in highly reworked materials in which the ratio is closer to one. As the relative abundance of the alkanedioic acids decreases with the oxidation of the OSOM fraction with the fastest oxidation rate, the slowly reacting OSOM fraction could be slowly reworked by bacterial and chemical forcing, thus reducing the even-to-odd preference index. Alternatively, it could also be that the slowly reacting OSOM fraction is synthesized with a lower even-to-odd preference index. These hypotheses are very speculative and the data acquired in this work does not allow us to discriminate between these two possibilities and others. It deserves further analysis in order to better assess the origin and distribution of these materials composing the OSOM fraction.

4.4.3. Ruthenium tetroxide oxidation products

Oxygen-sensitive materials constitute an OM fraction that degrades slowly in the presence of dissolved O₂ in the water column and in porewaters. The OSOM fraction is slowly altered and degraded with time, and for as long as the sediment remains under oxic conditions. Eventually, for extremely long exposure times (>1000 years), most of the OSOM fraction is consumed, leaving only an OM fraction composed of highly refractory materials such as macromolecular, polyaromatic black carbon components (Gélinas *et al.*, 2001), or compounds entrapped into a diagenetically stable mineral matrix. What is surprising is the fact that the rate at which the OSOM fraction is degraded in marine sediments seems to be independent of sampling location worldwide, and thus on the environmental and geological conditions prevailing at the sampling sites (see Chapter III above and Gélinas *et al.*, 2001).

The only exception is for sediments that receive high inputs of terrestrial OM, for which the relative contribution of the OSOM fraction appears higher at any given OET than for marine sediments (Chapter III). Getting insights into changes in the chemical makeup of the OSOM with increasing OETs or increasing inputs of terrestrial OM would thus help understanding the dynamics of the OSOM fraction.

Ruthenium tetroxide oxidizes aromatic carbon structures, such as found in lignin, by releasing the alkyl substituents of aromatic rings, producing short-chain alkanolic acids and alkanedioic acids. The carboxyl carbon atom in these acids originates from the junction of the alkyl substituent to the ring. Non-substituted aromatic carbon atoms are oxidized to CO₂, so that isolated aromatic rings are entirely degraded. On the contrary, polyaromatic structures and polysubstituted aromatic rings can be transformed into benzene polycarboxylic acids. Recently, alkanedioic acids were obtained with the ruthenium tetroxide oxidation of refractory OM in which diacids were formed by the oxidation of cross-linking of unsaturated lipids (Quénéa *et al.*, 2005). The diacids are most probably originating from the ether cross-links and alkyl bridges within the macromolecular network similar to that of Algaenans (Figure 4.1), or from a polymethylenic chain bordered by an unsaturation and/or a cross-link. Therefore, the number of cross-links and unsaturation within the macromolecular network is reflected in the relative abundance of short-chain diacids. The relative abundances of alkanedioic acids with different chain lengths depend mostly on sources of OSOM, as double bonds and esters bridges occur in different proportions in biolipids from different sources (Almendros *et al.*, 1991). Source information could thus be extracted from the analysis of the chemical makeup of the OSOM. RuO₄ oxidizes esters, vicinal ethers, primary alcohols and olefinic double bonds to produce carboxylic and dicarboxylic acids (Schouten *et al.*, 1998).

Thus, lignin, a polymer comprising carbon-carbon or ether bound propyl phenol structures, is oxidized to low molecular weight alkanolic acids (mostly C₄ to C₆) and hydroxyl-alkanoic acids, while aliphatic compounds such as cutin and suberin are oxidized to a variety of alkanolic and alkanedioic acids of different chain lengths (Amblès *et al.*, 1996; Blokker *et al.*, 1998). Algaenans, consisting of aliphatic polyester chains cross-linked with ether bridges, are broken down into alkanolic acids coming from the periphery of the network while alkanedioic acids originate from cross-links and saturated aliphatic chains located within the network, between two functional groups (cross-link, unsaturation). Therefore, the oxidation by RuO₄ of the OSOM fraction from marine sediment is expected to produce a wide variety of alkanolic and alkanedioic acids whereas terrestrial OSOM fraction, mainly composed of propyl phenol polymers, should generate a higher proportion of low molecular weight alkanolic acids mixed with longer alkanolic and alkanedioic acids resulting from the oxidation of cutin- and suberin-like compounds. Ruthenium tetroxide oxidation was successfully applied in the past to molecularly characterize kerogen, algaenans, the insoluble alkyl carbon fraction from soils, and the aliphatic side chains of aromatic carbon in Chondritic meteorites (Dragojlović *et al.*, 2005; Blokker *et al.*, 1998; Winkler *et al.*, 2005; Huang *et al.*, 2007), and thus shows a great potential to provide answers to the question of the exact origin of the OSOM pool in these sediments.

4.5. Conclusion

We have isolated and characterised the insoluble and non-hydrolysable OSOM fraction from seven sediment samples using a wet chemical separation method (Chapter III), and ruthenium tetroxide oxidation. Our results show that C₆ to C₂₄ alkanolic acids, and C₆-C₁₄

alkanedioic acids are produced upon oxidation, with a relative abundance distribution that reaches a maximum either at C₆ or C₁₆ depending on the sample (alkanoic acids). The distribution of the alkanedioic acids is more even, with a maximum at the C₆, C₈ or C₉ alkanedioic acid. Also evidenced was a clear even-to-odd preference for both series of fatty acids, although more pronounced for the alkanoic acids, suggesting a small contribution from bacterial cell membranes to the OSOM pool, and also a relatively fresh OSOM pool. The OSOM component appears to consist of at least three distinct fractions, although more work is needed to confirm this hypothesis: a first one that does not generate fatty acids or fatty acids with a chain length greater than C₆; a second one that generates fatty acids with a relatively low acids-to-diacids ratio, and a third one that is the most resistant to oxic degradation and that is enriched in alkanoic acids relative to the alkanedioic acids compared to the second fraction.

More work is however needed to test this hypothesis. First, the results obtained for the RuO₄ oxidation of the OSOM pool should be coupled to data obtained using a chemical OSOM degradation scheme that is specific to terrestrial OM. A lot of information could be gained on the composition and dynamics of the terrestrial OSOM pool by using a method specifically optimised for the analysis of lignin (cupric oxide oxidation, see Goñi *et al.*, 2005). Also needed is the analysis of the OSOM fraction using the modified RuO₄ oxidation method coupled to SPME analysis, as suggested by Huang *et al.* (2007). Finally, in future studies, the separation method that was used to isolate the OSOM components from the samples studied here should be upscaled to increase the mass of OSOM material isolated for further analysis. By doing so, spectroscopic analyses such as FTIR and solid-state ¹³C NMR could be applied to the OSOM components to assess the relative abundances of the major

chemical functionalities in the bulk OSOM component. Higher abundances of the different fatty acids generated upon oxidation would also allow measuring the carbon stable isotope composition of individual fatty acids, thus providing clues on the origin of the bulk OSOM fraction, and, if our hypothesis is true, of the three OSOM sub-fractions.

The work presented in this paper is the first molecular-level assessment of the composition and dynamics of the OSOM fraction in marine sediments. Until very recently, the non-hydrolysable, molecularly uncharacterized OM component was considered a homogeneous black box with very little knowledge on its molecular composition. Although a lot more work is needed before we can pretend that we fully understand the dynamics of the OSOM component in marine sediments and its role in OM preservation, efforts such as this one should be pursued and intensified as they represent steps in the right direction.

Table 4.1. Sample OC content and oxidation products obtained after RuO₄ treatment on different OSOM fractions

Sediment	OC (wt%)	$\delta^{13}\text{C}$ (‰)	OSOM ¹ (%)	OET (yr)	FA ² ($\mu\text{g/gC}$) ⁴	diFA ³ ($\mu\text{g/gC}$) ⁴
Mexico	7.30	-21.00	48	0.032	259.8	45.72
Antarctic	0.38	-24.00	18	50	4301	507.4
Washington	2.58	-22.15	8	100	15865	2379
Station 16	1.71	-21.79	30	1.25	861.6	143.7
SAG 30	2.59	-25.84	48	2	3073	531.0
Arctic	1.61	-25.45	45	20	210.1	23.47
Eel River	1.24	-23.96	48	1 ⁵	1169	187.7

¹OSOM fraction expressed as a percent of total OC in the untreated sediment

²FA = alkanolic acids

³diFA = alkanedioic acids

⁴Normalised to OC of the OSOM fraction

⁵estimated (Dickens, *pers. comm.*)

Table 4.2. Quantification of the oxidation products from the RuO₄ treatment

Alkanoic acids	Mexico μmol/gC ¹	Antarctic μmol/gC	Washington μmol/gC	Sta. 16 μmol/gC	SAG30 μmol/gC	Arctic μmol/gC	Eel River μmol/gC
C ₆ ²	0.11	3.86	39.11	0.05	6.75	-	5.70
C ₈	0.00	-	1.27	-	1.29	-	0.13
C ₁₀	0.04	0.03	17.02	0.02	1.30	-	0.41
C ₁₁	0.04	0.65	2.09	0.11	0.49	0.02	0.12
C ₁₂	0.09	2.11	4.57	0.36	1.13	0.11	0.30
C ₁₃	0.04	1.12	1.73	0.24	0.38	0.04	0.13
C ₁₄	0.17	3.87	5.99	0.72	1.19	0.26	0.43
C ₁₅	0.07	1.14	2.27	0.26	0.47	0.07	0.11
C ₁₆	0.32	6.37	12.72	1.38	2.46	0.38	0.63
C ₁₇	0.03	0.24	0.73	0.13	0.23	-	0.01
C ₁₈	0.12	0.74	4.14	0.33	0.86	0.02	0.07
C ₂₀	0.02	-	-	0.01	0.17	-	-
C ₂₁	0.01	-	-	-	0.05	-	-
C ₂₂	0.02	-	-	-	0.21	-	-
C ₂₃	0.00	-	-	-	-	-	-
C ₂₄	0.02	-	-	-	0.05	-	-
Σ _{FA}	1.11	20.14	91.64	3.60	17.04	0.89	8.04
Even / Odd	4.5	5.4	12.4	3.9	9.5	n.a. ³	20.7
Σ _{C6-C12} /Σ _{C13-C24}	0.35	0.49	2.32	0.18	1.80	n.a.	4.83
Alkanedioic acids							
C ₆	0.02	0.12	4.81	0.01	1.50	-	0.45
C ₇	0.03	0.37	2.82	0.05	0.63	-	0.26
C ₈	0.04	0.39	1.38	0.08	0.24	0.05	0.08
C ₉	0.02	0.53	1.37	0.20	0.28	0.02	0.07
C ₁₀	0.02	0.57	0.67	0.13	0.28	0.02	0.05
C ₁₁	0.04	0.33	1.34	0.14	0.10	0.02	0.05
C ₁₂	0.02	0.21	0.39	0.06	0.09	0.01	0.15
C ₁₃	0.02	-	0.40	0.04	0.04	-	-
C ₁₄	0.01	0.11	0.45	0.01	0.03	-	-
Σ _{diFA}	0.23	2.63	13.63	0.72	3.19	0.12	1.10
Even / Odd	0.89	1.14	1.30	0.66	2.02	n.a.	1.89
Σ _{C6-C9} /Σ _{C10-C14}	1.01	1.16	3.19	0.92	4.91	n.a.	3.41

¹Data are given in μmol per g of OC in the OSOM fraction²Indices indicate the number of carbon atoms in the non-derivatized FA and diFA³n.a. = not available; the GC signal measured for this sample was too low to allow calculating these ratios

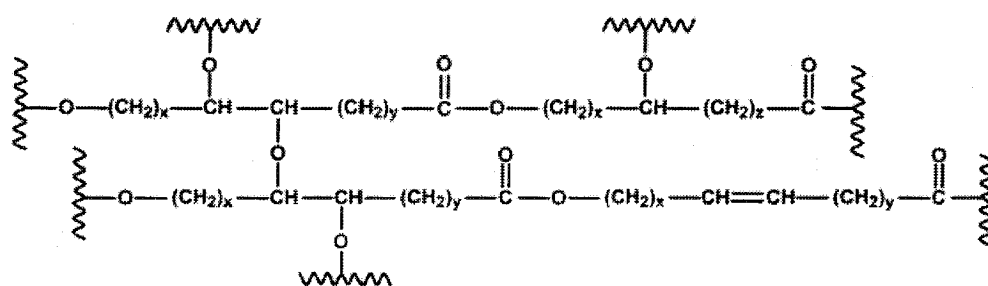


Figure 4.1. Proposed simplified structure of algaenans consisting of linear polyester chains cross-linked via ether bonds (from Blokker *et al.*, 1998)

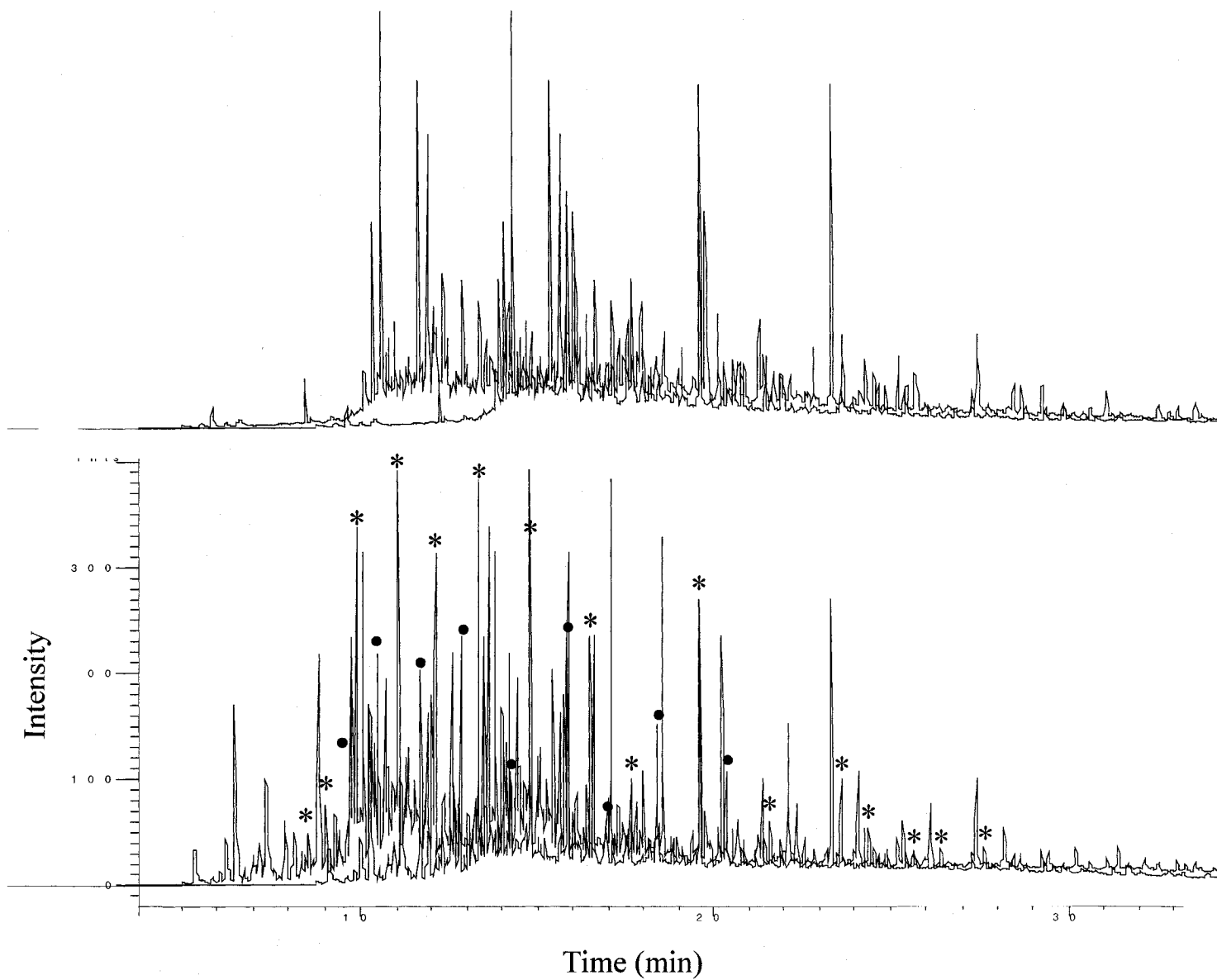


Figure 4.2. GC-MS chromatogram of the derivatized RuO_4 oxidation products of the oxygen-sensitive organic matter fraction isolated from Mexican margin sediments * alkanolic acid; • alkanedioic acid.

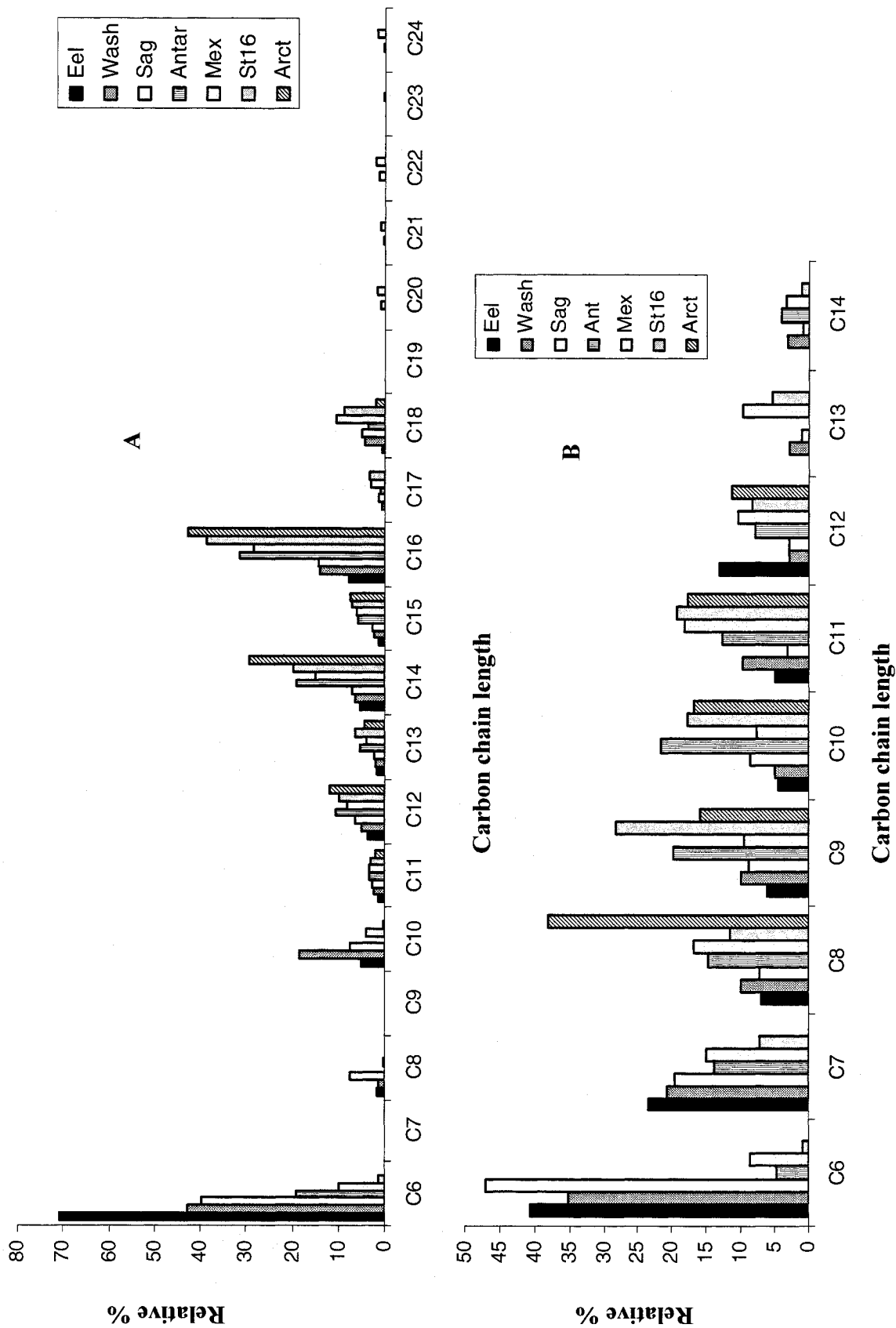


Figure 4.3. Distribution of alkanedioic and alkanedioic acids obtained following the RuO₄ treatment of the different oxygen-sensitive organic matter fractions. Data are expressed in their relative abundance % of the total moles of alkanedioic (A) and alkanedioic (B) acids present

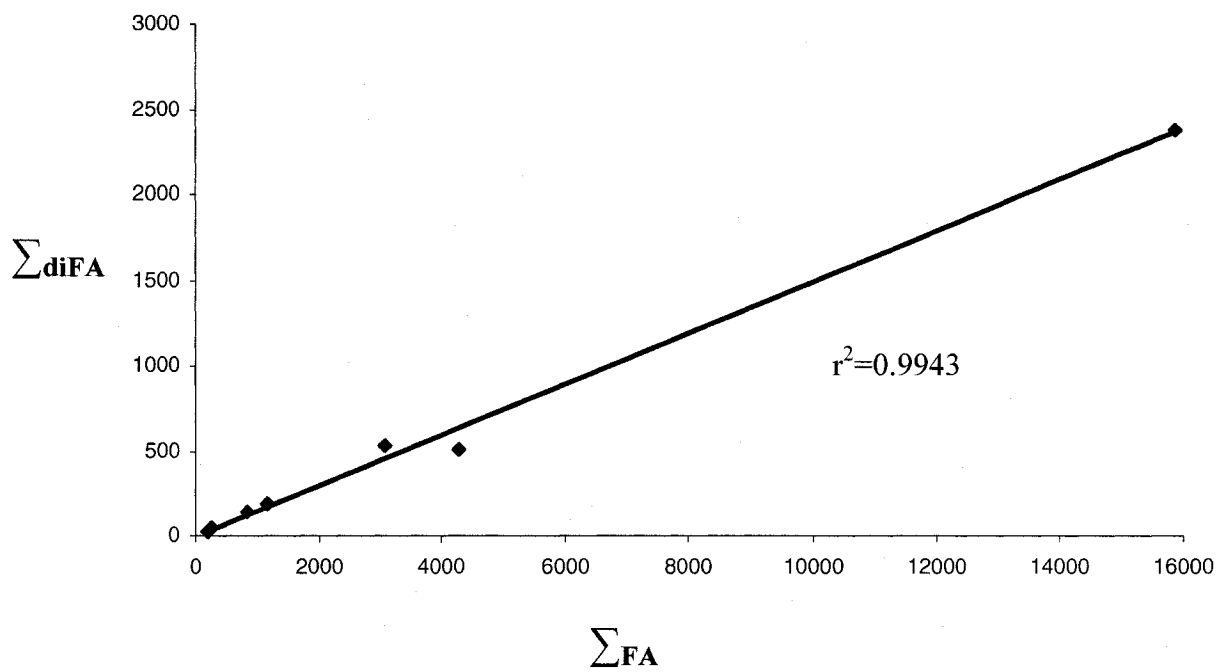


Figure 4.4. Relationship between the abundance of alkanolic and alkanedioic acids in the oxygen-sensitive organic matter fraction of the sediments under study

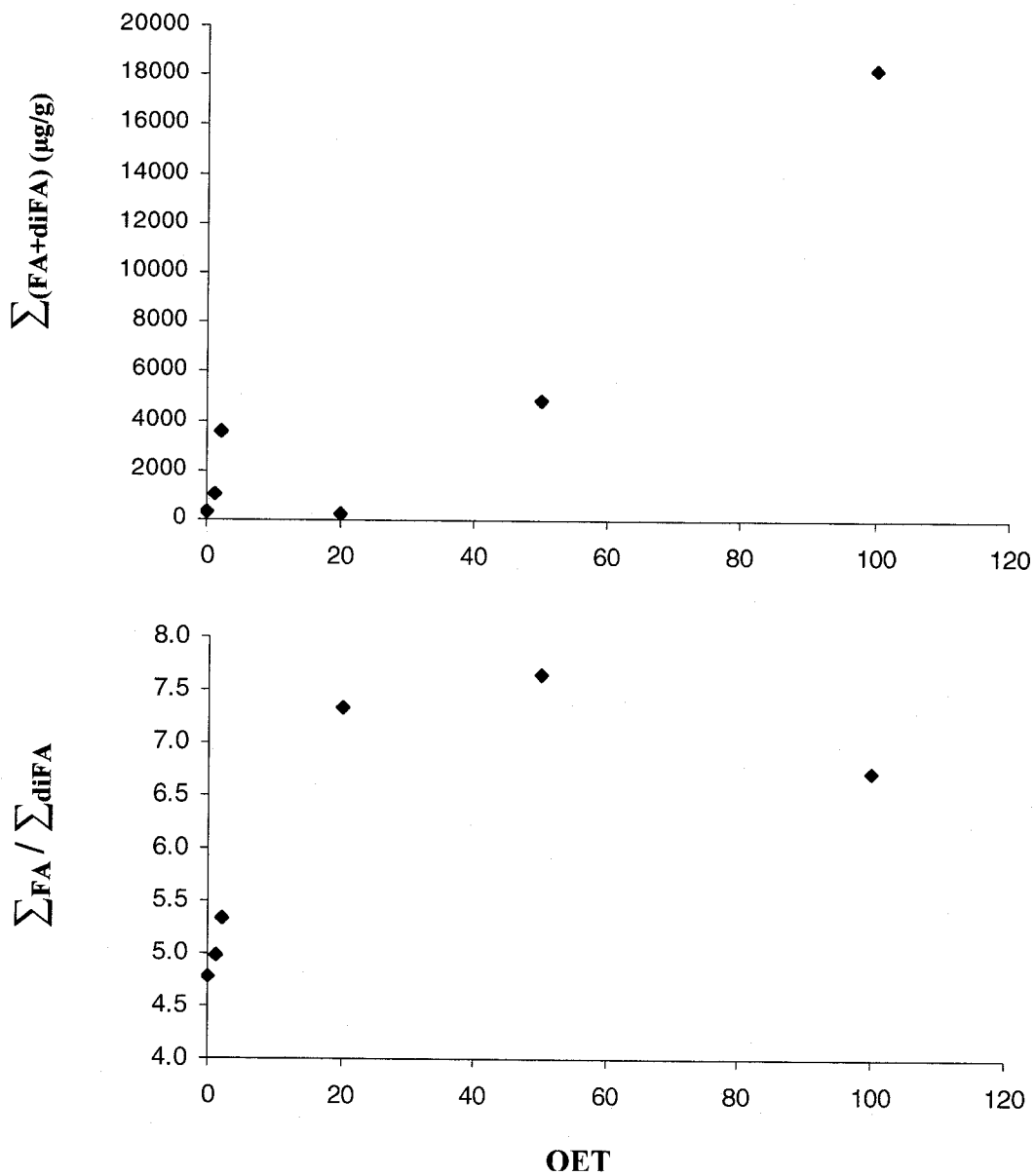


Figure 4.5. Relationship between the abundance of the alkanolic and alkanedioic acids and their ratio with oxygen exposure time

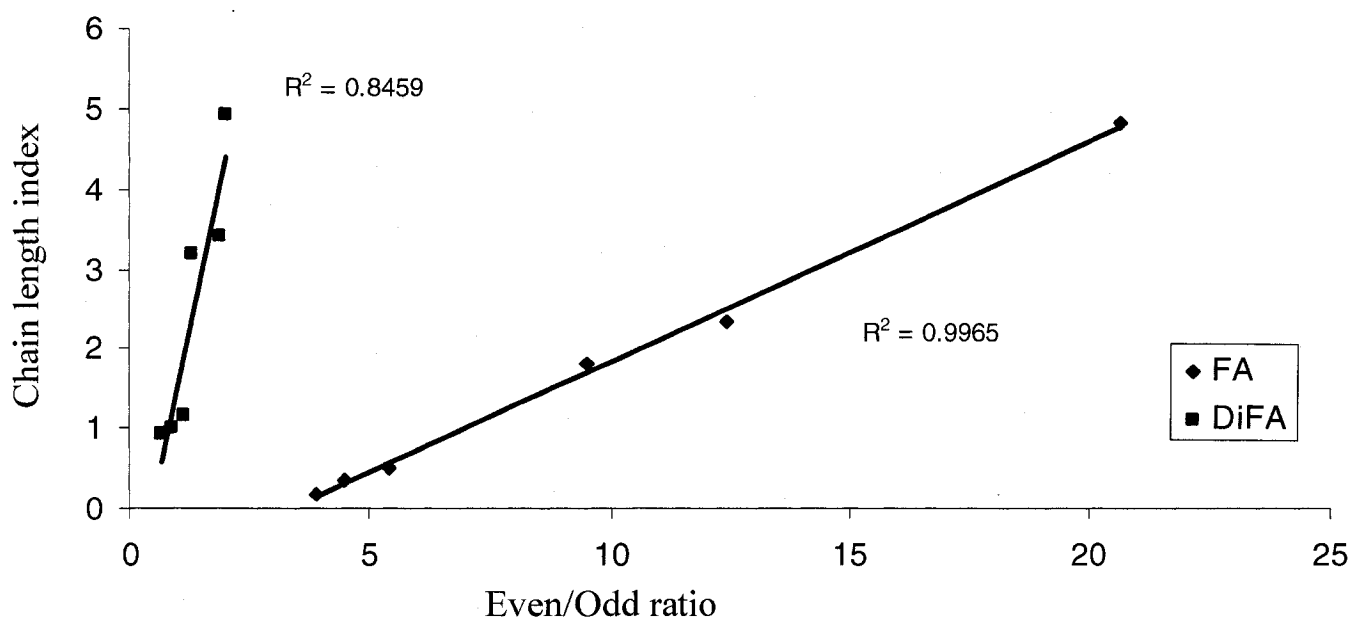


Figure 4.6. Relationship between the even-to-odd preference index and the chain length index. The chain length index is defined in this work as the sum of the molar concentrations of the C_6 to C_{12} alkanolic acids divided by the sum of the molar concentrations of the C_{13} to C_{24} alkanolic acids. For the alkanedioic acids, the the sum of the C_6 to C_9 alkanedioic acids was divided by the sum of the C_{10} to C_{14} alkanedioic acids

CHAPTER V

GENERAL CONCLUSION

In marine sediments, OM is currently quantified through the measurement of the concentration of organic carbon, which is usually determined using high-temperature combustion (elemental analyzer) and total conductivity detection of the resulting CO₂ following the removal of inorganic carbon. Although sensitive, quantitative and diagnostic (C/N ratios), elemental measurements cannot discriminate OM from the five reactivity classes that correspond to different intrinsic reactivity potentials: labile and hydrolysable (both degraded in oxic and anoxic conditions), as well as oxygen-sensitive (degraded only in oxic conditions), chemically refractory (not significantly degraded in any conditions over long time scales), and shielded (not degraded because of their mineral or organic 'capsule'). The development of the wet chemical separation procedure (Chapter III) involving chemical fractionation of bulk samples, followed by elemental and isotopic analysis (EA, EA-IRMS), is better suited and is representative of the whole sample rather than just the extractable/hydrolysable sub-fractions. The optimised extraction procedure is tedious and takes about three months to complete per series of samples. Elemental analysis following each treatment is carried out to obtain (by difference) the abundance of each reactivity class. This method allowed the quantification as well as elemental and isotopic characterization of six main reactivity classes (solvent-soluble, acid-soluble, hydrolysable, oxygen-sensitive, thermally-oxidizable, and refractory) in seven different natural sediments. It provides an efficient approach for testing different hypotheses that have been proposed to explain OM preservation in sediments. It also allowed the isolation of the insoluble, non-hydrolysable

oxygen-sensitive organic matter in sediments assumed to be degraded only in presence of oxygen. The relative abundance of this fraction found in our samples agrees with the OET hypothesis as it decreases exponentially with OET. We have also found that this OSOM fraction degrades at different rates depending on chemical composition and the relative contribution of terrestrial marine OM to the bulk OSOM pool.

We have isolated and characterised the insoluble and non-hydrolysable OSOM fraction from seven sediment samples using a wet chemical separation method (Chapter III), and ruthenium tetroxide oxidation. The OSOM component appears to consist of at least three distinct fractions, although more work is needed to confirm this hypothesis: a first fraction that does not generate fatty acids or fatty acids with a chain length greater than C₆; a second fraction that generates fatty acids with a relatively low acids-to-diacids ratio, and a third fraction that is the most resistant to oxic degradation and that is enriched in alkanolic acids relative to the alkanedioic acids compared to the second fraction. What is needed now is to combine data obtained using RuO₄ oxidation on the OSOM fraction of terrestrial samples to the results obtained here for mixed OSOM pools. Also needed is the analysis of the OSOM fraction using the modified RuO₄ oxidation method coupled to SPME analysis, as suggested by Huang *et al.* (2007), to recover the low molecular weight mono- and di-carboxylic acids generated by lignin. Finally, in future studies, the separation method that was used to isolate the OSOM components from the samples studied here should be up-scaled to increase the mass of OSOM material isolated for further spectroscopic analysis such as FTIR and solid-state ¹³C NMR. These types of analysis could help assessing the relative abundance of the major chemical functionalities in the bulk OSOM components. In addition, higher abundances of the different fatty acids as oxidation products would allow the measurement of

the carbon stable isotope composition and thus, providing clues on the origin of the bulk OSOM fraction, particularly in terrestrially-influenced samples.

Because molecular studies are difficult to achieve for macromolecular compounds present in sediments and also because of the molecular complexity of the sedimentary organic matrix, advanced techniques are needed to gain insight into their detailed composition. Powerful new approaches based on compound-specific isotope analysis have only recently introduced in the arsenal of organic geochemists. In future studies, compound-specific radiocarbon analysis (by GC-fraction collector followed by acceleration mass spectrometry), and compound-specific stable isotope analysis by GC-IRMS for the characterization of the RuO₄ oxidation products of the OSOM fraction could be exploited. The ¹⁴C is a complementary proxy that is used extensively to determine the age of a sample based on the natural radioactive decay of this isotope. For example, recent studies have shown that old kerogen eroded from sedimentary rocks from the drainage basin and highly degraded soil carbon can compose a large fraction of OM delivered by estuaries to shelf sediments (Goñi *et al.*, 2005). This ancient carbon could be derived from a reservoir (soils or permafrost) in which it aged for a long time before being released to the continental shelf (Goñi *et al.*, 2005). Compound-specific ¹⁴C analysis allows distinguishing between ancient soil-derived OM and modern OM produced by plants. This technique would be instrumental in apportioning the OSOM fraction between young algaenan-like macromolecules and old kerogen-derived material.

Another new approach that may be used is high-resolution solid-state ¹³C NMR on sub-samples recovered at each step of the extraction procedure, and especially before RuO₄ oxidation, to obtain complementary information on the chemical make-up of the OM

removed and preserved at each step. The advantage in this type of work is that it becomes possible to distinguish with a high resolution typical of liquid NMR, different types of carbon (alkyl, aromatic, etc.) within each isolated solid OM fraction. The combination of these relatively new techniques will provide strong clues on the molecular makeup of the different classes of OM and a better understanding of the major preservation mechanisms in marine sediments.

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Glossary

Accumulation: In oceanography, accumulation represents the successive deposits of material coming from the surface on the ocean floor for different periods of time.

Algaenan: An insoluble, non-hydrolysable, mostly aliphatic, macromolecular network composed of cross-linked polymer of molecules present in algae.

Catagenesis: Catagenesis is a term used in petroleum geology to describe the conversion of organic kerogens into hydrocarbons.

Diagenesis: In geology and oceanography, diagenesis is any chemical, physical, or biological change undergone by a sediment after its initial deposition and during and after its lithification, exclusive of surface alteration (weathering) and metamorphism.

Diploptene: Pentacyclic compounds indicative of bacterial source and are produced for rigidity-imparting constituents of their cell membranes (see Figure 2.6 for structure).

Euxinic: Refers to an area or a zone with no oxygen.

Extant organisms: In this study, extant organism refers to complex organisms as the opposite of micro-organisms.

Hopanoid: Hopanoid is a pentacyclic molecule of the diploptene family, similar to sterols, whose primary function is to improve plasma membrane fluidity (see Figure 2.6 for structure).

Isotopic fractionation: The mass differences, due to a difference in the number of neutrons, result in partial separation of the light from heavy isotopes of an element during chemical reactions is defined as isotope fractionation.

Lithospheric: Lithospheric refers to lithosphere which is the solid outermost shell of a rocky planet.

Metamorphism: Metamorphism can be defined as the solid state recrystallisation of pre-existing rocks due to changes in heat and/or pressure and/or introduction of fluids i.e without melting.

Methanogenesis: Methanogenesis is the formation of methane by micro-organisms known as methanogens.

Monolayer-equivalent coatings: Material/molecules attached to a mineral surface with a thickness corresponding to the size of the material/molecule.

Nepheloid layer: A bottom water particle-rich layer that sits just above the ocean floor.

Paleo-(studies): Refers to studies in the ancient time (geological time).

Protokerogen: Protokerogen is the precursor of kerogen, it is mainly composed of insoluble, non-hydrolysable, highly refractory organic matter but not incorporated to sedimentary rocks as opposed to kerogen.

Recycled organic materials: Organic material that have already passed through a complete diagenetic process or degradation process.

Upwelling: Upwelling is an oceanographic phenomenon that involves wind-driven motion of dense, cooler, and usually nutrient-rich water towards the ocean surface, replacing the warmer, usually nutrient-deplete surface water.

$\delta^{13}\text{C}$: Defines the isotope ratio of a sample between ^{12}C and ^{13}C compared to a standard in the (δ) unit which is ‰.