DISPERSED OIL FINGERPRINTING IN MARINE ENVIRONMENTS

by

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ABSTRACT

The occurrence of offshore oil spills can induce various negative effects on marine environments. Oil fingerprinting is a key technology to identify the sources of crude oil and associated refined products spilled into the environments. Spill oil fingerprinting can be achieved by investigating the diagnostic relationships among specific hydrocarbons, known as biomarkers. Biomarkers in oils can be uniquely distributed to pinpoint the oil geographic source and weathering status.

Dispersants are widely used marine oil spill treatment agents, containing surfactants and solvents. It can reduce interfacial tension between oil and seawater by enhancing the generation of small and stable oil-surfactant micelles (i.e., oil-in-water emulsion). By using dispersants, the spilled oil in a water emulsion bridged by surfactants, called chemically dispersed oil (CDO), can dwell in seawater for a longer period under proper conditions. CDO fingerprinting is essential for assessment of its environmental impact, selection of further response countermeasures, and for a better understanding of the fate and behaviors of CDO in marine environments.

However, dispersant application could change the physicochemical properties of spilled oil, which is challenging for the applicability of current environmental forensics for CDO fingerprint and limits the research on the topic reported. To address this challenge, this thesis carried out investigations on dispersed oil fingerprinting in marine environments in the following aspects: 1) investigation of the applicability of existing typical biomarkers for fingerprinting of short-term weathered CDO, 2) identification of relatively long-term

weathered CDO through screening eight types of aliphatic and aromatic biomarkers, 3) differentiation of CDO from non-dispersed oil using principal component analysis, 4) assessment of the impacts of biodegradation of weathered dispersed oil (treated by a shrimp-waste based new dispersant) on fingerprinting of CDO, and 5) comprehensive evaluation of environmental factors on CDO fingerprinting. The research outputs lead to a group of identified biomarkers for effective dispersed oil identification and oil weathering assessment, a better understanding of the characteristics of spilled oil treated by dispersants, and a more robust means for tracking fate and behaviors of CDO in marine environments.

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LIST OF ABBREVIATIONS

Ada	Adamantane	iso	Methl shifted on structure
β-oxidation	Oxidation in β carbon	IS	Internal Standard
BaP	Benzo[*a*]pyrene	MA	Mono-aromatic
BTEX	Benzene, Toluene, Ethyl Benzene and Xylene	MAS	Monoaromatic steranes
CA	Cluster analysis	MCA	Multiple correspondence analysis
CDO	Chemically dispersed oil	NL	Newfoundland and Labrador
Ci-B	Alkylated- benzothiophene	ppm	Part Per Million
Ci-C	Alkylated-chrysene	ppmv	Part Per Million in Volume
Ci-D	Alkylated- dibenzothiophene	PCA	Principal component analysis
Ci-F	Alkylated-fluorene	PCs	Principal components
Ci-N	Alkylated-naphthalene	RSD	Relative standard deviation
Ci-P	Alkylated-phenanthrene	Ses	Sesquiterpane
Ci-Py	Alkylated-pyrene	SIM	Selected Ion Monitoring
DCM	Dichloromethane	SD	Standard deviation
Dia	Diamantane	TA	Tri-aromatic
FTIR	Fourier-Transform Infrared Spectroscopy	TAS	Triaromatic sterane
homo	Additional carbon on structure of biomarkers	TER	Terpane
GC-MS	Gas Chromatograph – Mass Spectrometer	WCO	Weathered crude oil
ICP-MS	Inductively Coupled Plasma Mass Spectrometry		

LIST OF SYMBOLS

t	weathering time (e.g., second, minute, day
α	Asymmetric carbon with functional groups (H) equatorial (axial) down
β	Asymmetric carbon with functional groups (H) equatorial (axial) up
ω	Omega carbon
d	Deuterium (heavy hydrogen)
k	First-order reaction rate constant (min^{-1})
e	Mathematical constant
n	Number (of samples, of structures)
V	Volume (e.g., mL, L)
R^2	Coefficient of determination
π	pi bonds
i	Numbers (i = 0,1, 2,…)
C	Carbon
R	Right-handed configuration (chirality)
S	Left-handed configuration (chirality)

CHAPTER 1 INTRODUCTION

1.1 Background

Crude oils and refined petroleum products are the dominant energy fuel sources and play a pervasive role in modern society. As world population increases and developing countries become more industrialized, the increasing oil demand and use are projected to remain so over the next two decades (Council 2003). Oil thus is an essential chain of the global economy and business cycle, and the prices of crude oils significantly influence global economy activity, capacity, and prices (Cashin et al. 2014, He et al. 2010, Odularu and Okonkwo 2009, Rasche and Tatom 1977). Canada has the 3rd largest crude oil reserves (169 billion barrels of oil, 10% of world total). Oil and gas have generated over $108 billion to Canada's gross domestic product in 2018. Offshore oil activity, especially in Newfoundland and Labrador, produces over 4.3% of Canadian oil production in 2018 (Stantec 2019).

Intentional and accidental marine oil spills occur regularly worldwide with the offshore exploration, production, storage, transportation and utilization of petroleum products (Figure 1.1). The spilled oils can induce marine and coastal oil pollution, and consequently threaten the health of human-being and ecosystems (Esbaugh et al. 2016, Frantzen et al. 2016). For example, the Deepwater Horizon oil spill releases over 4.9 million barrels of oil into the Gulf of Mexico (Griggs 2011). Over 8,000 species are killed or affected (Biello 2010). The economic impact over the next 7 years of BP oil spill could be 8.7 billion US$ (Sumaila et al. 2012).

Quantitative analysis of the concentrations of crude oils is important for tracking the

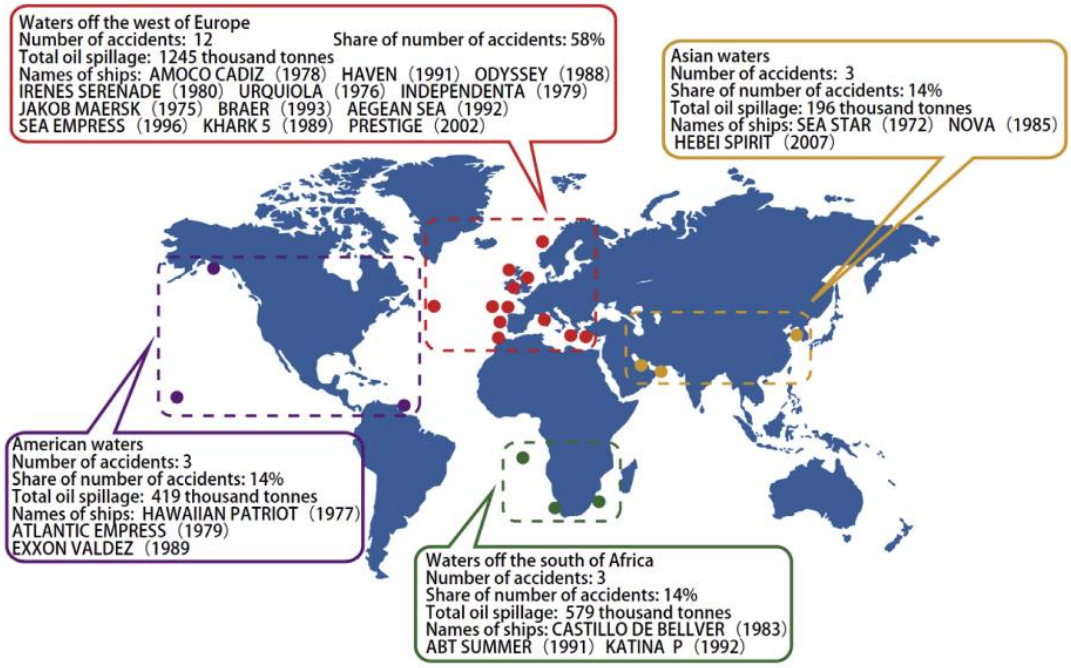


Figure 1.1 World map of major oil spill 1970-2017

Source: (Chen et al. 2019b)

occurrence of oil contaminants in the marine environment, evaluating the impacts of an oil spill on ecosystems, and demonstrating the performance of various response techniques (Stout et al. 2005a). Nevertheless, challenges exist regarding crude oil quantitative measurements (Wang et al. 1999). The first one is that many compounds in crude or refined oils have not been clearly identified yet (Arey et al. 2007b). Many un-decrypted hydrocarbons in various types of oils cannot be directly quantified using current standard analytical methods. Secondly, spilled oil result in a heterogeneous distribution in horizontal and vertical directions in the ocean especially after the application of dispersants as spill treating agents (McCay and Payne 2001). Thirdly, oil weathering can bring difficulties in decisively defining the similarity between weathered spilled oil and probable sources of oil for litigious purposes (Douglas et al. 2016). Complex weathering processes restrict the tracing of the fate and behaviors of spilled oil in the marine environment. Different hydrocarbons are always weathered in various degrees driven by their physicochemical properties and the different selectivity in biodegradation by unique indigenous microbial community. Many circumstances, such as the fast dilution of dispersed oil in seawater, can further perplex the biodegradation process (Prince et al. 2017). Therefore, advancement of analytical methodologies is highly desired to reliably evaluate the oil weathering status, to accurately monitor fate and behaviors of spilled oil, and to precisely track the spill source of oil released to the marine environment.

Oil fingerprinting is a key methodology to identify and differentiate the sources of unknown crude oils and associated refined products spilled into the marine

environment (Bayona et al. 2015). The categories of spilled oil are evaluated by the diagnostic relationships among specific hydrocarbons, known as biomarkers (Hostettler et al. 2007). Biomarkers in certain oils could be uniquely distributed to imply the specific geographic source, oil processing, and even the weathering status (Wang et al. 2006a). Current oil source identification is effectively realized through forensic fingerprinting of samples from oil spills and suspended sources.

Oil fingerprinting becomes more challenging due to the wide usage of chemical dispersants for oil spill response (Fingas and Banta 2008). They are a group of chemical agents that can emulsify spilled oil and disperse emulsion into water for promoting natural or artificial attenuation processes (Tsutsumi et al. 2000). Chemical dispersants are currently popular marine oil spill treating agents due to their high oil dispersion efficiency and less restrictions to environmental and site conditions (Fuller et al. 2004). In the Deepwater Horizon oil spill in 2010, more than 1.7 million gallons of chemical dispersants (i.e., Corexit 9500A and 9527A) was applied as a critical countermeasure (United States Coast Guard 2011). The usage of chemical dispersants led to the generation of chemically dispersed oil (CDO). The fingerprinting of CDO using existing biomarkers, however, has limited focus.

Moreover, the appearance of oil-in water emulsion in CDO can dramatically decrease the interface tension, change certain oil properties such as oil viscosity, and further affect the behaviors of CDO (Macnaughton et al. 2003, Swannell and Daniel 1999). Particularly, chemical properties of dispersants can diversify the weathering of CDO and crude oil (Zhuang et al. 2016). In previous studies on the effects of weathering on CDO, only a few

existing biomarkers identified for crude oil fingerprinting were directly adopted, such as terpanes and steranes. Overall, the applicability of existing oil biomarkers for CDO fingerprinting and oil tracing need to be further examined.

1.2 Statement of Problems

Dispersants can significantly affect oil physicochemical properties (e.g., oil viscosity and solubility) and further influence the weathering of dispersed oils. Current biomarkers and associated methodologies are thus not directly applicable and inaccurate for CDO fingerprinting in marine environments. Several research gaps have been identified and listed below:

1) Lack of applicable biomarkers for CDO fingerprinting during physiochemical weathering

Evaporation rate of dispersed oil is slower than non-dispersed oil as the formation of emulsions can hinder the release of oil to vapor phase (Aranberri et al. 2002, Macnaughton et al. 2003, Swannell and Daniel 1999). In previous studies regarding the effects of weathering on dispersed oil, only a few of existing biomarkers identified for crude oil fingerprinting were directly adopted, such as terpanes and steranes.

The change in oil viscosity by emulsion can affect photo-oxidation rate as well (Payne and Phillips 1985). The increased dissolution of hydrocarbons, coupled with the enhancement driven by photo-oxidized products, can make the biodegradation rate of CDO different (Genuino et al. 2012). Although some studies, for example, Wang *et al*.

(2013), indicated that the distributions of TA- and MA-steranes in weathered crude oil (WCO) are unaffected by weathering (Wang et al. 2013a), the relative concentrations of TA-steranes to hopanes in weathered Deepwater Horizon (DWH) oil were found to decrease due to photo-oxidation (Radović et al. 2014). Weathering of biomarkers may be affected by the application of dispersants in the marine environment. Stout *et al*. also observed a decrease in the relative concentration of TA-steranes to hopanes in weathered floating Macondo oils, which was treated by dispersants (Stout et al. 2016). Therefore, reliable biomarkers for CDO fingerprinting during physiochemical weathering need to be screened and comprehensively evaluated.

2) Limited knowledge on differentiation of CDO from WCO

Possible candidate biomarkers for fingerprinting of different CDO have been investigated through some experiments (Olson et al. 2017, Song et al. 2016, Song et al. 2018). However, whether the application of dispersants can affect the weathering of biomarkers is unknown. Some low molecular weight biomarkers were found as degradable and influenced by multiple weathering processes, such as sesquiterpanes and diamondoids (Wang et al. 2006b). Alkylated PAHs are important and degradable hydrocarbons in spilled oil, and hence widely used to trace oil physiochemical weathering and biodegradation (Douglas et al. 1996, Stogiannidis and Laane 2015, Wang et al. 1998a). The variations of the ratios of such biomarkers may be clearly linked to the effects of dispersants. As such, multivariate analysis methodologies, such as principal component analysis (PCA),

will play a valuable role to objectively differentiate chemically dispersed oil (CDO) from WCO or non-dispersed oil. To our knowledge, differentiation of CDO from WCO using PCA has not been reported. The changes in degradable biomarkers can be used to trace and differentiate the weathering degrees of CDO from WCO, which are helpful for unravelling the correlation among the fate of different weathering processes, decision making of application of countermeasures in marine environments, and environmental damage assessment.

3) Unclear role of oil biodegradation in CDO fingerprinting

The ratios of different biomarkers can be adopted as the crucial diagnostic index for diverse oil characterization. However, it may counter the difficulties in tracing the biodegradation rates of CDO using current biomarkers, especially when new dispersants are applied. Dispersants influence the biodegradation rate and selectivity in degradable hydrocarbons involving biomarkers for a few reasons. Firstly, the transformation of smaller oil droplets driven by dispersants is theoretically more biodegradable due to the increase in the surface areas of oil that contact bacteria in the marine environment (Brakstad et al. 2015, Lessard and DeMarco 2000, Prince et al. 2013). Secondly, dispersants significantly change the physicochemical properties of oil, such as viscosity and solubility, which are of scientific importance for oil biodegradation (Haus et al. 2000, Khelifa et al. 2007). These properties can simultaneously alter biodegradation through influencing other relevant weathering, such as photo-oxidation (Payne and Phillips 1985). Thirdly, dispersants have clear impacts on the population, composition, and activities of the microbial community (Kleindienst et al. 2015a, Kleindienst et al. 2015b). Therefore,

the diagnostic ratios containing susceptible biomarkers to biodegradation may be affected by the application of dispersants. Among all the identified biomarkers, terpanes are the most recalcitrant to biodegradation and are relatively stable for semi-quantification and source identification, even with the application of chemical dispersants. They are still degradable in marine environments when oil is heavily biodegraded (Bost et al. 2001, Seifert and Moldowan 1979). However, other biomarkers with lower resistance to biodegradation in CDO can have diverse degrees of biodegradation. Therefore, the stable diagnostic index for tracing biodegradation of CDO, especially for new dispersants, are essential for accurate monitoring of biodegradation of CDO.

The biodegradation of alkylated PAHs have undoubtedly diverse preferences clearly led by many reported factors, such as the numbers of aromatic rings and structures, nitrogen and phosphate levels, the enhancement of photo-oxidation, and selections of PAH-degrading organisms, the ratios of different types of PAHs are thus reasonably applied to trace the degrees of oil biodegradation (Cerniglia and Heitkamp 1989, Haritash and Kaushik 2009, Maki et al. 2001, Prince et al. 2003). Nevertheless, few investigations have studied the correlation between the diagnostic ratios composed by alkylated PAHs and biodegradation of CDO.

In the development of new dispersants, current studies have been mainly focused on the efficacy rather than the biodegradability of oil treated by new dispersants. Therefore, no research has tackled the differences and correlations of the diagnostic index of biodegraded oil treated by CDO, especially involving new dispersants, and non-dispersed oil. It seems that the different types of dispersants can considerably affect oil

biodegradation results, including alkylated-PAHs (Bruheim et al. 1999, Makkar and Rockne 2003, Zolfaghari-Baghbaderani et al. 2012). Current oil tracing methodology is thus susceptible to biodegradation treated by traditional and new dispersants. An advanced oil tracing method is needed to address these challenges.

4) Unrevealed environmental and weathering conditions on CDO fingerprinting

More attention needs to be paid on identifying the importance of different environmental and weathering conditions on the variations of biomarkers for CDO fingerprinting. Many factors can change the weathering processes and pathways of spilled crude oil in the marine environment, such as temperature, salinity, oil to dispersant ratios (ODR), and oil concentrations in marine environments (Campo et al. 2013, Daling et al. 2003, MacNaughton et al. 1999, Okpokwasili and Odokuma 1990, Payne et al. 1991). These factors can influence the stability of biomarkers for CDO source identification.

Meanwhile, current correlations between the changes in the diagnostic ratios of spilled oil and weathering processes were insufficient to explain the impacts of weathering factors on the changes of biomarkers in CDO under many conditions. For example, the degradation of alkylated-PAHs could be mainly attributed to photo-oxidation as stated in previous studies (Bacosa et al. 2015, Dutta and Harayama 2000, Prince et al. 2003). While the changes in the ratios may be a general result of the combination of multiple weathering processes in real seawater, especially including photo-oxidation and biodegradation (Vergeynst et al. 2019).

However, the effects of environmental conditions on the weathering of current biomarkers for CDO fingerprinting and weathering tracing have not been well examined. Weathering processes, including evaporation, dilution, photo-oxidation, and biodegradation, could adversely affect different groups in biomarkers at a specific duration. The environmental factors probably plays critical roles in the CDO fingerprinting, environmental impact assessment, oil fate and behavior investigation, spill response and cleanup actions (Gong et al. 2014). Therefore, the effects of the application of dispersants on the fingerprinting of dispersed oil, involving oil identification and weathering status functions, need to be comprehensively investigated.

1.3 Objectives and Tasks

This thesis work was aimed at filling the above identified research gaps through advancement of CDO fingerprinting methodologies in marine environments for oil source identification and dispersed oil fate and behavior analysis. The main research tasks include (Figure 1.2):

(1) To examine the physio-chemical weathering of biomarkers in CDO samples and screen the stable biomarkers for oil source identification using a short-term weathering simulation;

(2) To evaluate the stability of eight types of aliphatic and aromatic biomarkers in weathered dispersed oil using a long-term weathering simulation;

(3) To investigate the impact of the of dispersant application and weathering duration on variances of biomarkers using PCA;

(4) To track how a biodegradation process could interfere the behaviors and variations of biomarkers in CDO samples, and trace oil biodegradability using a newly generated green dispersant; and

(5) To discuss impact of crucial environmental and weathering conditions on CDO source identification under multiple weathering simulation scenarios.

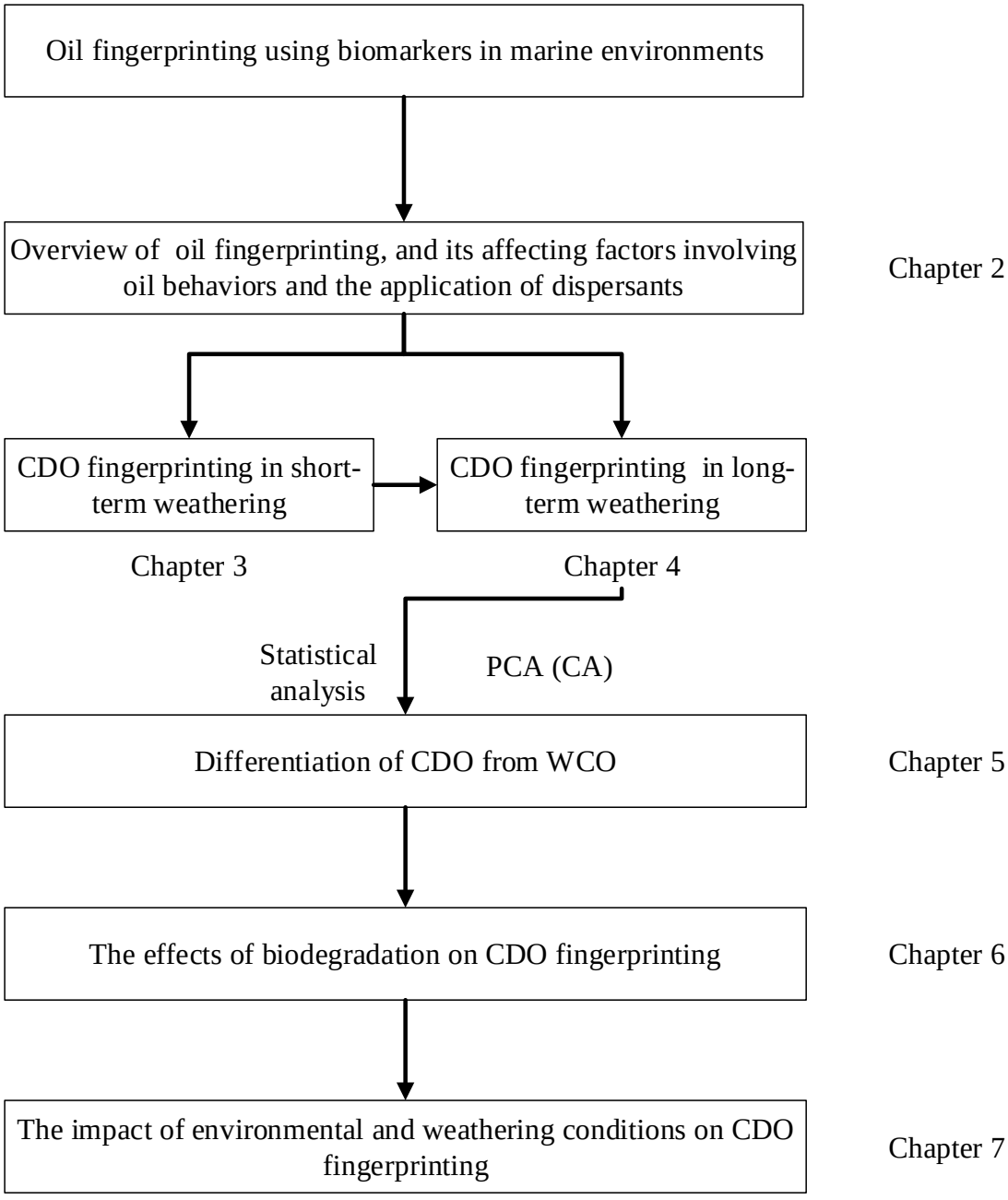


Figure 1.2 Flow chart of this thesis

1.4 Structure of This Thesis

This thesis was aimed at developing the scientific knowledge of CDO fingerprinting and the advancement of CDO fingerprinting methodologies in marine environments. Chapter 2 gives a comprehensive review of relevant knowledge and technologies, including current oil fingerprinting methodologies, factors affecting oil fingerprinting, usage of chemical dispersants for marine oil spill response, as well as behaviors of spilled oil and CDO in marine systems.

Chapter 3 investigates the stability and suitability of three groups of biomarkers (i.e., sesquiterpanes, steranes and terpanes) for CDO characterization with a short-term weathering. The applicable diagnostic ratios and biomarkers for oil identification are summarized.

Chapter 4 studies the applicability of eight types of biomarkers (namely, adamantanes, diamantanes, sesquiterpanes, steranes, terpanes, TA-steranes, MA-steranes, and alkylated-PAHs) to characterize CDO after long-term weathering. The stability of diagnostic ratios, especially those from different types of biomarkers, are evaluated and summarized.

Chapter 5 applies several PCA to differentiate weathered CDO from weathered crude (non-dispersed) oil (WCO) using 103 diagnostic ratios of the same type of biomarkers and those of two types of biomarkers as input data. The effects of dispersant usage and weathering duration on biomarkers in CDO and WCO are studied through statistical analysis.

Chapter 6 studied the influence of biodegradation in CDO fingerprinting using identified biomarkers. The biodegradability of two types of oils treated by a newly generated green dispersant based on shrimp waste (SW) is examined.

Chapter 7 investigates the possible influence of environmental and operational factors on CDO fingerprinting. The importance of different weathering conditions in the variations of biomarkers is analyzed and discussed.

Chapter 8 summarizes the major research findings, scientific and practical contributions arising from this thesis work. Recommendations are made for future work based on the current research outcomes.

CHAPTER 2 LITERATURE REVIEW

2.1 Behaviors of Spilled Oil in Marine Environments

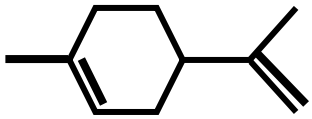
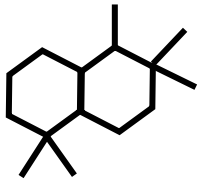
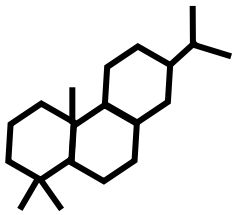
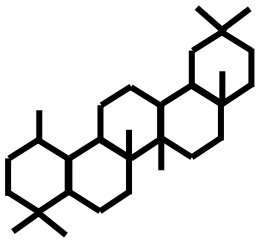
2.1.1 Oil composition

Oil is a necessary source of energy in modern society. Although new energy resources are being developed, the energy usage depending on oil does not significantly change when demand of oil increases. Crude oil refers to a group of natural petroleum products composed by the mixture of hydrocarbons with a wide variety of molecular weights. Oil composition varies with the geological formation of the locations where oil is found. Oil includes multiple classes of compounds; many of them are still not clearly identified. Basically, oil can be classified into four groups: saturates, aromatics, resins, and asphaltenes. Basically, oil components can be classified into four groups: saturates, aromatics, resins, and asphaltenes according their physiochemical properties.

Saturates are organic compounds that contain only single covalent carbon-carbon bonds. The primary saturates in oils are alkanes, that are comprised of carbon and hydrogen with the maximum numbers of hydrogen atoms for each carbon. Alkanes, or called as paraffins, can be characterized as straight-chain alkanes, acyclic isoprenoids, and cycloalkanes. Acyclic isoprenoids are long-chain structures formed by single isoprenoids. Cycloalkanes represent ring-based alkanes, including monocyclic and polycyclic alkanes. Polycyclic alkanes are mainly terpanes and steranes with different isoprene units (Table. 2.1).

Aromatics are unsaturated cyclic compounds that contain one or more benzene rings. Benzene rings are relatively persistent in the environment due to the stability arising from their strong resonance energy (i.e., aromaticity). Aromatics in petroleum are

Table 2.1 Classification of isoprenoids identified in crude oils

Terpanes name	Composition	Typical structure
Monoterpenoids	10 carbon numbers/ 2 isoprene unit	
Sesquiterpenoids	15 carbon numbers/ 3 isoprene unit	
Diterpenoids	20 carbon numbers/ 4 isoprene unit	
Triterpenoids	30 carbon numbers/ 6 isoprene unit	

mono aromatic (BETX), polycyclic aromatic hydrocarbons (PAHs), and aromatic isoprenoids.

Polar compounds are hydrocarbons bonding with other elements mainly include nitrogen, oxygen, and sulfur atoms (NOSs). The small polar compounds are described as resins, and larger ones as asphaltenes. The presence of polar compounds is the major reason for the polarity and adhesivity of oil. Oil composition decides the properties of oil, such as density, viscosity, specific gravity, solubility, volatility, vapor pressure, and distillation fractions.

2.1.2 Fate and behaviors of spilled oil in marine environments

Crude oil, as a complicated mixture, undergoes a range of physiochemical, and biological processes when released into marine environments (Fig. 2.1).

2.1.2.1 Oil Transport

The oil spreading occurs when oil is immediately spilled. The early spreading theory hypothesized that oil spreads as insoluble chemicals on calm seawater (Lehr 2001). The gravity thus was assumed as the major driving force for the spreading at the beginning stage (Blokker 1964). However, the gravity-based model did not agree well with the real observations (Stolzenbach et al. 1977). More factors were considered, including the relationships among gravity, interfacial tension, and viscosity, to better predict the variance of oil slick thickness (Fay 1971, Fingas 2015a). Since some models did not match the real spill areas in case studies either, Lehr *et al*. assumed that the shape of an initial oil slick was an ellipse as a result of the direction of winds (Lehr et al. 1984, Murray 1972).

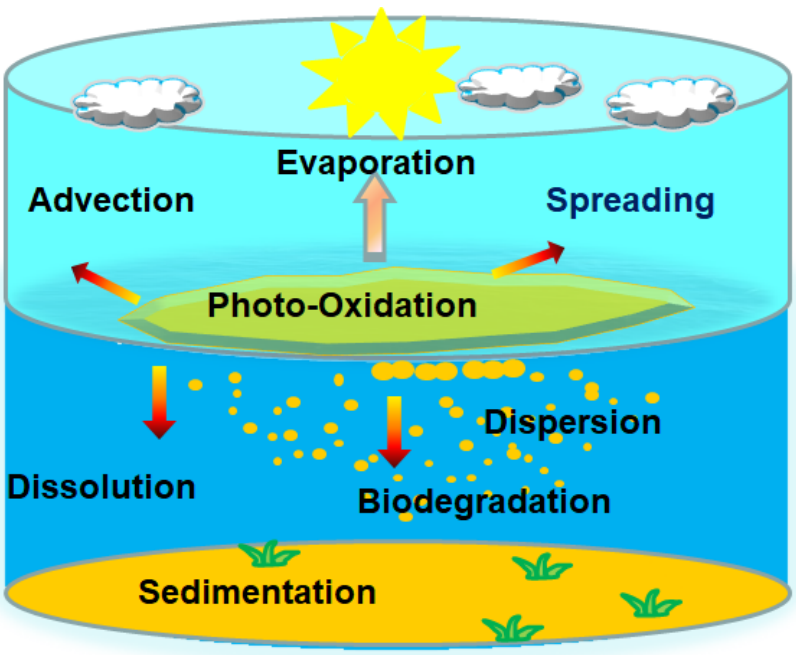


Figure 2.1 The fate and behaviors of spilled oil in marine environment

However, the accuracy of this model was still unsatisfactory due to the lack of consideration of more environmental factors and conditions of the initial oil spill (Lehr 2001). More theories were then focused on environmental factors, especially winds and waves, as well as the effects of Langmuir circulation (Simecek-Beatty and Lehr 2017, Thorpe 2000). An oil spreading model based on radar multiangle methods had a more accurate prediction (Matveev et al. 2016). Although the mechanisms of oil spreading have been well explored, accurate prediction of oil spill is still complicated and incomplete so far. The movement of an oil slick at seawater surface can be seen as the advection generated by surface currents and wind effects. The diffusion of oil is a result of random processes.

2.1.2.2 Oil weathering

(1) Evaporation:

Evaporation is an important weathering process. It can eliminate massive amounts of spilled oil from aquatic environments. The straightforward measurement of oil evaporation in the marine environment is difficult so far because uncertainties are always inevitable and would significantly affect the results, such as the variation of oil composition as a mixture, uncontrollable weather conditions and time delays (Fingas 2016, Kotzakoulakis and George 2018). The pseudo-component modeling became an reasonable pathway at one time to monitor and predict the evaporation after oil spills (Reed et al. 1999). The most accepted theory is based on the concept of the gas boundary layer. For liquid with low evaporation capacity, the evaporation rate is limited, because the vapors can saturate in the gas layer and slow the evaporation, especially under low turbulence conditions. The evaporation rate is thus directly

relevant to the diffusion rate, affected by liquid concentration, area of the evaporation, wind, and turbulence (French-McCay 2004, Stiver and Mackay 1984, Sutton 1934). It was found that spilled oil may not perfectly fit the gas boundary layer assumption, because the evaporation rate of oil mixture was observed to be independent of wind velocity, though some specific hydrocarbons were strictly correlated with wind speed (Fingas 2016, Fingas 2004). Diffusion-limited evaporation model is applied to explain the evaporation partially related to the gas-boundary layer theory. A recent modification was made to complex the assumption of the rate of diffusion by treating oil slick as a diffusive layer with concentration gradients for different components (Kotzakoulakis and George 2018). Multiple-scale experiments or field observations are still needed to verify the current model.

The evaporation degrees of spilled oil highly depends on oil composition (French-McCay 2004). For oil containing a high proportion of light and volatile compounds, such as gasoline and light crude oil, evaporation can lose 30% or more substances in a few days after an oil spill occurs (Stiver and Mackay 1984). However, heavy oil, such as lubricating oil and dilbit, is barely affected by the evaporation (Fingas 2015b). The evaporation rate is clearly affected by temperature. However, some observations indicate that evaporation rate does not changes significantly with the increase in evaporation area nor variation of wind (Fingas 2004). After the removal of small-molecular weight compounds, the remaining components are abundant of resins and waxes, which change the properties of oil.

(2) Dispersion and dissolution

Energy provided by wave and turbulence, especially breaking waves, drives the

natural oil dispersion (Delvigne and Sweeney 1988). Either oil slick or subsurface spilled oil can be broken into small oil droplets. Vertical mixing process plays an imperative role in the formation of small oil droplets (Delvigne 1993, Lonin 1999, Tkalich and Chan 2002). The efficiency of oil dispersion can be attributed to many factors, including the properties of oil (e.g., viscosity, oil composition, and oil thickness), and environmental conditions (e.g., temperature, mixing energy, and water density) (Delvigne and Sweeney 1988, Farwell et al. 2009, Lehr et al. 2002). Oil droplets with appropriate sizes can stay in water column for a while, and larger ones will resurface rapidly (Mackay 1977).

Dissolution is the process that the water-soluble fractions of a spilled oil are dissolved into the seawater. The diffusion coefficient of dissolution of a crude oil in seawater is linearly correlated with water temperature, implying a diffusion-controlled process (Hamam et al. 1988). Oil dissolution may be enhanced by an oil dispersion process (Hansen et al. 2011). Soluble components in oil are commonly soluble aromatics, such as BETX, naphthalene, and alkylated-naphthalenes, alkylated-phenanthrenes, and some other PAHs with low molecular weights (Essaid et al. 2003, González et al. 2006). Since many of these compounds are ecotoxic, dissolution of them in seawater therefore poses a significant thread to various marine lives (Carls et al. 2008, Neff et al. 2000).

(3) Photo-oxidation

When spilled oil is exposed to sunlight, the chromophoric parts of dissolved hydrocarbons can be excited by sunlight. The excited compounds interact with dissolved oxygen, water, and radicals, to produce reactive oxygen species and organic

radicals. Hydrocarbons can be oxygenated by these active species and hence become more susceptible to microbial degradation. PAHs are more easily affected by photooxidation in aquatic environment (Bacosa et al. 2015).

Spilled oil can react with free radicals initiated by light. The energy provided by sunlight can facilitate the attack of free radicals (Schwarzenbach and Gschwend 2016). Many hydrocarbons can be oxidized to C-OH, COH, and COOH initialized by free radicals (Choe and Min 2006). The intermediary products can be further oxidized by microorganisms and photo-oxidation. The photo-oxidation process can be affected by radicals, which is highly sensitive to aerobic environments (Shankar et al. 2015). In an aerobic environment, oxygen radical will become an important but probably a minor initiator to oxidize hydrocarbons (Payne and Phillips 1985). Typically, for alkanes, oxygen radical will attack the hydrogen in the tail. For PAHs, they will react with radicals in the methyl groups of some hydrocarbons and break the aromatic rings. Photo-oxidation has a more significant influence on alkylated PAHs than alkanes in crude oil (Bacosa et al. 2015, Dutta and Harayama 2000).

(4) Biodegradation

Biodegradation is one of the most crucial processes that can remove petroleum from the marine environment. Biodegradation is realized oil-degraders that are commonly recognized as microorganisms, such as *Rhodococcus* and *Pseudomonas* (Espuny et al. 1995, Hasanuzzaman et al. 2004). These microorganisms always have diverse capacities of degrading different classes of hydrocarbons and their immediate products in spilled oil (Venosa and Zhu 2003). Alkanes always have a relatively higher priority to be biodegraded in a spilled oil (Atlas 1981). The most common mechanism of

biodegradation is the preliminary terminal attack, involving monoterminal and diterminal oxidation, via a β-oxidation (Gottschalk 2012). The monoterminal attack to the terminal methyl or the methylene group in the alkanes firstly forms an alcohol, which is subsequently converted into an aldehyde and then a monocarboxylic acid. The carboxylic acid is then changed into a fatty acid by β-oxidation. Some bacteria, such as *Pseudomonas*, can attack either end in a hydrocarbon chain and form a mixture of acids (Thijsse and Van der Linden 1961). If no β-oxidation happens, esters with high molecular weights can be generated (Heringa et al. 1961). Another pathway to generate fatty acids from alkanes is the ω-oxidation when terminal oxidation occurs (van Beilen and Funhoff 2005). The involved enzyme is a mixed oxidase system (single-protein component type) with a multicomponent electron transfer system (Figure 2.1).

Although PAHs are normally genotoxic and have low solubility, a wide range of organisms, such as bacteria, fungi, and algae can utilize PAHs as the carbon sources for metabolism (Cerniglia and Heitkamp 1989). The metabolic pathways of PAHs are usually initialized by the formation of diols with the catalysis of molecular oxygen and enzymes that includes monooxygenases and dioxygenases (Varanasi 1989). A hydroxylated intermediate (usually catechol) is formed with the catalysis of dehydrogenase. The aromatic ring cleavage subsequently occurs either via *ortho* fission *meta* fission to form a di-carboxylic acid.

Resins and asphaltenes are recalcitrant to biodegradation in crude oils (Westlake et al. 1974). While they are partially degradable in low percentages (Pineda-Flores and Mesta-Howard 2001, Rontani et al. 1985, Tavassoli et al. 2012). Several

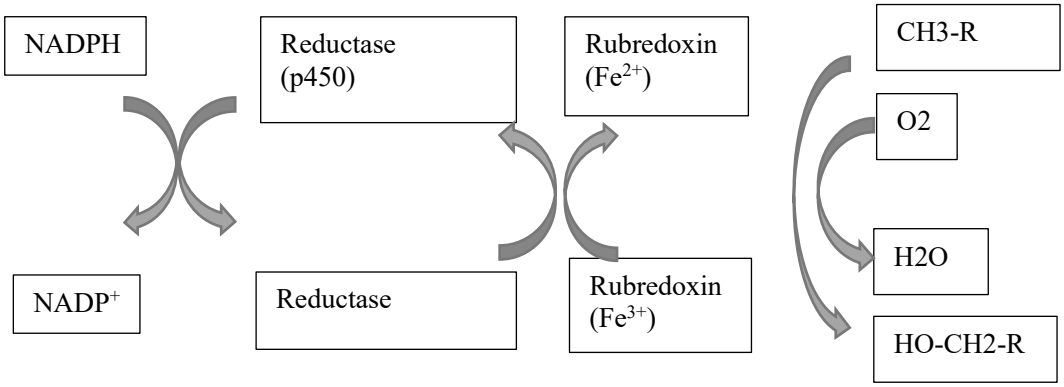


Figure 2.2 The mixed-function oxidase system (Abbasian et al. 2015, Cederbaum 2014)

microorganisms, such as *Corynebacterium* and *Bacillus*, have been identified to utilize asphaltenes for biotransformation (Gao et al. 2017, Pineda-Flores et al. 2004, Pineda-Flores and Mesta-Howard 2001). Limit knowledge is known on the mechanisms of resins and asphaltenes biodegradation so far. More pieces of evidence pointed to microbial degradation of resins. The difficulties of detection and complex structure limit the studies on the mechanisms and kinetics.

(5) Sedimentation and interactions of spilled oil with suspended particles

Spilled oil can attach to fine particles and suspended solids in seawater, further forming oil-mineral aggregates (OMAs) (Stoffyn-Egli and Lee 2002). OMAs, as a stable structure, can transport in seawater, and some of them could be settled on the bottom (Wincele et al. 2004). Both organic and inorganic particles can interact with oil to form OMAs (Lee 2002).

2.2 Dispersants as Oil Spill Treating Agents

Application of dispersants is an effective and important response to oil spill in open water (Chapman et al. 2007). Dispersants include surfactants and solvents. Surfactants usually have two ends: a hydrophobic end to attach water and a lipophilic end to attach oil. Solvents including chemicals and petroleum distillates perform as a reducer of surfactant viscosity to facilitate the mixture and attachment between oil and surfactants. Dispersants could reduce oil-water interfacial tension, leading to the formation of small and stable oil-surfactant micelles in seawater (Tkalich and Chan 2002). Small oil droplets can easily spread in marine environments, and further facilitate natural biological depletion of petroleum hydrocarbons (Brakstad et al. 2015, Lee et al. 2013, Prince 2015). The mechanism of dispersants was shown in Fig. 2.3.

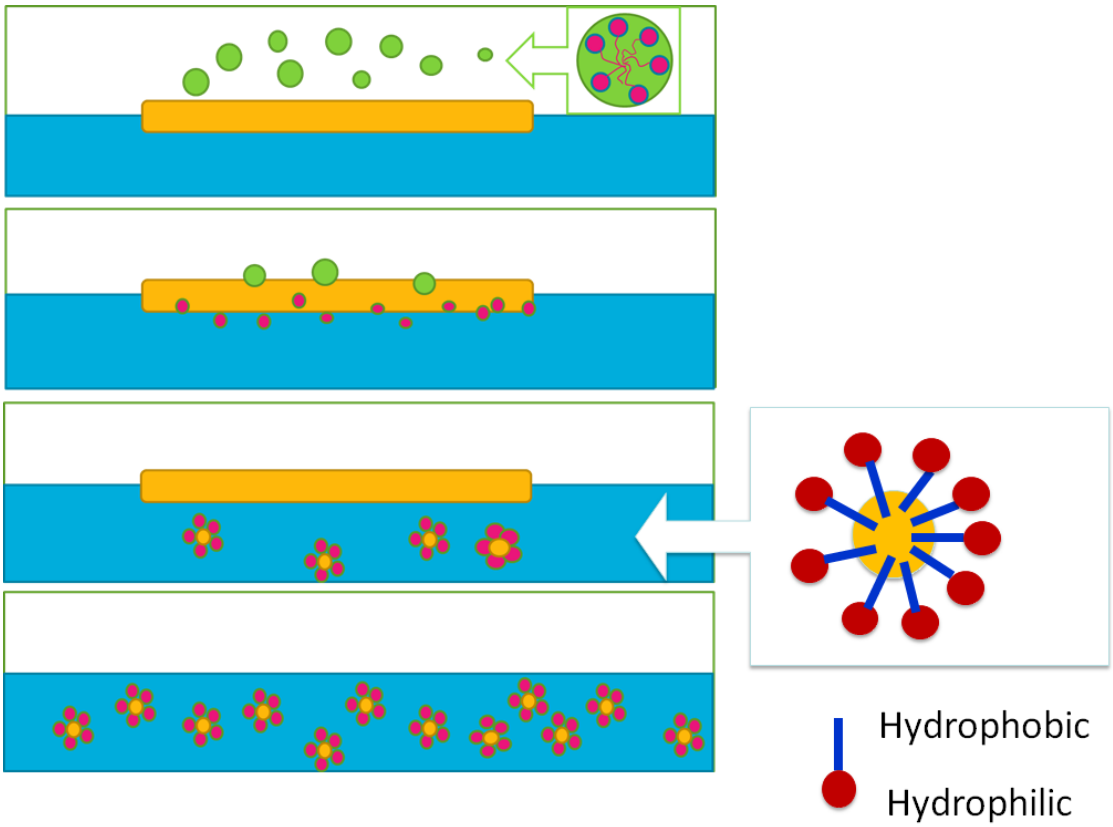


Figure 2.3 Mechanism of chemical dispersion

2.2.1 History

Since the 1960s, increasing oil transport through large vessels in the sea has increased the risk of accidental oil spills. In 1967, around 10000 bbl of surfactants, as "detergents", were used to clean around 1 million bbl of crude oil released from the "*Torrey Canyon"* tanker (Smith 1968). Unfortunately, surfactants failed to disperse the oil, but form stable oil-dispersant emulsions (Board and Council 1989). Many marine lives are severely affected by the toxic complexes, such as fish, barnacles' shells, and mussels (Corner et al. 1968). The observation reflected the strongly negative impacts on the application of dispersants. Second generation dispersants were developed using less acutely toxic formulations, but they were less effective due to predilution. The third-generation dispersants were further produced to decrease the volume for better storage and transport of dispersants (Etkin 1998).

2.2.2 Composition of modern dispersants

The third-generation dispersants are composed of several surfactants with glycol and petroleum distillate solvents. The most used surfactants can be classified as non-ionic and anionic surfactants, such as ethoxylated fatty acid ester and alkyl sulfosuccinate. The main compositions of surfactants are summarized in Table 2.2.

2.2.3 Effectiveness of dispersants and its affecting factors

Effectiveness is a major indicator for evaluating the performance of dispersants. Multiple-scale tests are designed to measure the degree of dispersion or the stability of dispersed oil in the water column. Ideal dispersion effectiveness (DE) of commercial dispersants depends on the type and properties of oil. For example, dispersion effectiveness of Corexit 9500 on Alaskan North Slope (medium oil) can reach an

efficiency of 80% or even a higher level, but with only 40-50% in case of heavy oil and diluted bitumen (Belore et al. 2009, King et al. 2015). To reach acceptable effectiveness, appropriate operation conditions should be correlated with various scenarios, such as seawater temperature, mixing energy driven by winds and waves, and dispersant-to-oil ratio (Belore et al. 2009, Chandrasekar et al. 2006, Li et al. 2008, Li et al. 2009).

Many uncontrollable factors can affect the testing results, such as types of oil, oil weathering status, and the salinity of seawater. The effectiveness of dispersants could be significantly enhanced through increasing the dose of dispersants and mixing energy (Kaku et al. 2006, Mukherjee and Wrenn 2009, Pan et al. 2017). Low seawater temperature results in increased viscosity of oil and slightly reduced effectiveness (Egbogah and Ng 1990, Lehtinen and Vesala 1984, Roelands et al. 1963). The variation of salinity can influence the effectiveness as well since the properties of emulsion and viscosity can change with salinity (Chandrasekar et al. 2006).

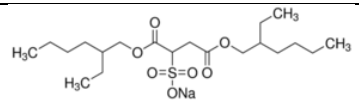
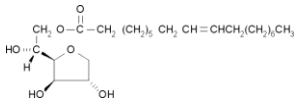
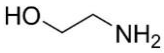
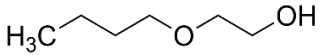
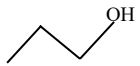
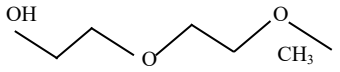
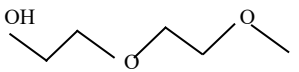
Current well-recognized effectiveness tests include laboratory-scale, meso-scale, and large-scale methodologies. Laboratory and meso-scale tests mainly generate a controllable mixing environment in a reactor for the dispersion of oil and dispersants. The diverse tests differ mainly in terms of the energy sources used (the shaking approach of mixing energy) and the levels of mixing energy (non-breaking waves or breaking waves). The main laboratory-scale tests include Baffled Flask Test (BFT), Warren Spring Laboratory test (WSL), batch scale method developed by French Institute of Petroleum (IFP), and Mackay-Nadeau-Steelman test (MNS) (Gillot et al. 1986, Lewis et al. 1985, Mackay et al. 1984).

A large-scale experiment is important for testing dispersion effectiveness. A large-scale tank can mimic more realistic waves and performance of dispersants. Major wave tanks for effectiveness determination include the Ohmsett wave-tank in the United States, and the large wave tank at the Centre for Offshore Oil, Gas and Energy Research (COOGER), Canada.

2.2.4 Behaviors of dispersants in marine environments

The fate and behaviors of dispersants are crucial for assessing environmental damage, monitoring of the effects of dispersants, and achieving effective decision-making of oil spill response. When dispersants enter aquatic environments, the key components can be utilized by organisms. Dioctyl sodium sulfosuccinate (DOSS) can be photo-oxidized under the catalysis of sunlight and generate some by-products, such as an octyl group by a hydroxyl group (Batchu et al. 2014). From the observations of the BP oil spill, DOSS, the key dispersant components are detected in deep water conditions after the subsea injection. The results indicated probable long-term effects of dispersants on ecological systems and the biodegradation of oil. The most substantial impact of dispersants is that whether the dispersants could stimulate or inhibit biodegradation of the dispersed oil. However, historical investigations showed conflicting observations regarding the stimulation or inhibition of dispersed oil biodegradation. In some cases, oil biodegradation could be either enhanced or not significantly affected by the addition of dispersants, especially when a low concentration of dispersed oil was dealt with (Brakstad et al. 2018, Prince et al. 2017). Meanwhile, inhibition is likely correlated with increased dissolution of polycyclic aromatic hydrocarbons (PAHs) after dispersion and chemical components of dispersants (Hamdan and Fulmer 2011, Rahsepar et al. 2016).

Table 2.2 Composition of common dispersants

Category	Ingredient	Structure layout (example)
Surfactant	Dioctyl sulfosuccinate sodium salt (Canevari 1974)	
Nonionic surfactant	Fatty acid esters (Hessel et al. 1995)	
Surfactant	Ethanolamine (Pei and Zheng 2016, Yoon and Choi 2008)	
Surfactant/mutual solvent	2-Butoxyethanol (Wise et al. 2014)	
Solvent	Propanol (Major et al. 2012)	
Solvent	Hydrocarbons (Alkanes, aromatics, Kerosene)	
Solvent	dipropylene glycol monomethyl ether (Lepain and Charlier 1984)	

Whether the biodegradation of dispersed oil is enhanced or restrained may highly depend on various scenarios, such as the concentration of dispersed oil, nitrogen and phosphorus nutrients, and diverse and indigenous microbial community. Since these non-negligible concerns are of great importance to the ecological system, especially with a large amount of the application of dispersants, many countries have implemented strict control over the approval and use of dispersants (Belkina et al. 2015, Guevarra 2011).

2.2.5 New dispersants

The demand for both high effectiveness of dispersants and bioavailability of dispersed oil stimulate the generation of new dispersants after the ecological concerns of the third generation of dispersants. Two main directions of new dispersant generation are new formulations and bio-technological surfactant-based dispersants. New dispersant formulations focus on the modification or the development of new surfactants and solvents. The primary efforts for new formulations are the development of low-toxic substrates with acceptable effectiveness compared to chemical dispersants. Nonionic surfactants, a typical type of low-toxic surfactants, are found to have sufficient capacity to decrease surface tension and generate oil-in-water emulsion for oil spill agents. The typical nonionic new surfactants are polymeric surfactants. The essential performance of this type of surfactants indicates high effectiveness. Polyisobutylene succinic anhydride adduct (PIB-SA) can be modified by esterification and amidation to form ethoxylated and amidated polyisobutylene succinate (Al‐Sabagh and Atta 1999). This series of polymeric surfactant-based dispersants had larger than 60% DE for asphaltenic and waxy crude oil in WSL tests. Recycled poly waste (ethylene

terephthalate) can generate nonionic surfactants to decrease surface tension and efficiently disperse oil (Atta et al. 2006). Polymerizable nonionic nonyl phenol ethoxylates have good performance to form emulsion obtained from a preliminary experiment (Atta et al. 2013). Gel-like mesophase combining with surfactants can generate stable oil-in-water emulsions as dispersants (Owoseni et al. 2018). Besides, amphiphilic ionic surfactants have a high potential for oil dispersion as well (Atta et al. 2016). Meanwhile, some efforts have been made to introduce low-toxic solvents to reduce environmental damage.

Some new oil dispersion approaches are attractive since they assist the dispersion or enhance the effectiveness. For instance, the combination of eco-friendly surfactants and nanoparticles may become a new direction for future oil dispersion method based on the high stability of emulsions (Al-Sabagh et al. 2012). Mineral fines can increase the suspended particle concentration and droplet stability, and the coexistence of dispersants and mineral fines can enhance oil dispersion into water column (Li et al. 2007). Halloysite clay nanotubes can load different anionic and nonionic surfactants for oil dispersion and remediation with robust performance (Nyankson et al. 2015b). Natural surfactants may be potentially useful for treating oil spill as well. Soybean lecithin (a biodegradable natural surfactant-containing phosphorus and nitrogen to accelerate biodegradation) have potential to be an alternative of traditional dispersant with good dispersion performance (Nyankson et al. 2015a).

Biotechnological application in oil spill response is commonly referred to as the usage of biosurfactant based oil treating agents. Biosurfactants are comprised of surface-active molecules generated by organisms found in polluted areas (Ayed et al.

2015). These biosurfactants are produced for oil-eating or oil-adapting functions for living organisms in polluted marine environments, oil refinery factories, and other oil-contaminated sites. The biosurfactants can reduce interfacial tension and promote the formation of oil-in-water emulsions. The biosurfactants for oil dispersion have complicated structures with biopolymers, lipopeptides, esters, and fatty acids. Biosurfactants for oil treatment are often the mixture of several compounds. The Northern Region Persistent Organic Pollution (NRPOP) Control Laboratory at Memorial University in Canada has developed a series of green dispersants with compatible dispersion effectiveness including biosurfactant-based dispersants produced from *Rhodococcus* (Cai et al. 2016). Concentrated lipopeptides created using hydrophilic-lipophilic deviation conception was effective in dispersing oil according to baffled flask tests (Rongsayamanont et al. 2017). Some biosurfactants consist of polymeric compounds as hydrophilic parts and fatty acids as hydrophobic parts (Crescenzi et al. 2002). Glycolipid protein produced from biofilm-forming bacteria may have a good potential in dispersing oil due to the stable emulsification (Peele et al. 2016). Notably, some new bio-related surfactants are water-soluble, which decreases the environmental risks induced by solvents. The sophorolipid biosurfactant is helpful in dispersing weathered oils under various conditions (Saborimanesh and Mulligan 2018). Another sample is the hydrolysis of shrimp waste, which is capable of dispersing different types of oils in several scenarios (Zhang et al. 2018b).

There are some other attempts of mixing different surfactants to generate new candidates of dispersants (Athas *et al*., 2014). Chen et al. (2019a) synthesized palygorskite and rhamnolipid to obtain a new dispersant. The new formulations

enabled efficient dispersion of oil hydrocarbons in artificial seawater under certain conditions. Another interesting combination is the combination of a nonionic surfactant named lactonic sophorolipid (LS) and a surface-active ionic liquid: choline laurate ([Cho][Lau]) (Shah et al. 2019). The mixture of surfactants has > 80% DE with proper situations, such as mixture proportion of LS to [Cho][Lau] and DOR. The droplet size of dispersed oil can even be decreased to 100 nm. Although current mixture procedures of surfactants still based on the trials of binary changes of doses, design of experiments (DOE), such as mixture design and uniform design, can be effectively added into the design of new dispersants for new surfactant functions, especially when more types of surfactants are to be involved (Brandvik and Daling 1998, Song et al. 2013).

Although it is known that production costs still restrict the real application of new dispersants, these new ideas give us new directions for more ecologically friendly and effective dispersants. Their industrial applicability is desired to be improved through extra processing, such as the purification and optimization of production for facilitating oil biodegradation and cost reduction (Mukherjee et al. 2009, Mulligan and Gibbs 1990). Besides, there are few reports that evaluate the applicability of new formulations on dispersing oil in SSDI system. The composition and properties of live oil is different from crude oil released to the surface of seawater. Another critical scientific question for new dispersants is that: will the application of dispersants stimulate or inhibit microbial degradation of spilled oil in the ocean? Few studies were focused on this essential environmental risk after the production of dispersants and determination of effectiveness. The response of the bacterial community in oil-infested sea water to the application of a surfactin produced by *Bacillus* and a

chemical dispersant (Ultrasperse II®) have been investigated (de Almeida Couto et al. 2016). A mixture of a glycolipid-based biosurfactant and ionic surfactant has been proved to be less toxic than some commercial dispersants recently (Shah et al. 2019). Although the addition of surfactin enhanced oil-degrading bacteria, biodegradation rates in addition to surfactin do not significantly increase. We may partially speculate the possible effects from the structure and bioavailability of new dispersants. The real tests are still needed because the actual conditions are more complicated.

2.3 Forensics Fingerprinting of Spilled Oil in Marine Environments

2.3.1 Fingerprinting using biomarkers

Oil fingerprinting is one of the key technologies to identify and differentiate the sources of unknown oil and associated refined products spilled into the environments (Bayona et al. 2015). The categories of spilled oil are evaluated by the diagnostic relationships among specific hydrocarbons, known as biomarkers (Hostettler et al. 2007).

Biomarkers in certain oils can be uniquely distributed to pin-point the specific geographic source, oil processing method, and weathering status (Wang et al. 2006a). The most commonly and widely identified biomarkers applied in oil spill environmental forensics are aliphatic and aromatic hydrocarbons (Table 2.3). Aliphatic biomarkers mainly include diamondoids, sesquiterpanes, steranes and terpanes. Diamondoids are known as three-dimensional cyclohexane-ring alkanes naturally existed in petroleum (Gao et al. 2016). Diamondoids, especially adamantanes and diamantanes, are also used as a tool to evaluate the maturity of crude oil and to differentiate oil types due to their

differentiable distributions. However, diamondoids are not widely applied in case studies in comparison with terpanes and steranes (Chen et al. 1996, Springer et al. 2010, Wang et al. 2006b). Sesquiterpanes are cyclic saturates commonly discovered in different types of oils. Diverse oils with different weathering degrees, such as light oil, diesel, and heavy fuel (Wang et al. 2005), can be differentiated by the diagnostic ratios of sesquiterpanes. Steranes and terpanes are more recalcitrant to weathering processes than other biomarkers (Bost et al. 2001). Various sources of mysterious oil spilled offshore have been successfully unraveled by the quantitative comparison of steranes and terpanes among "unknown" oil with the group of identified crude oil, for example, oil flume that floated to other places derived from the BP oil spill (Bayona et al. 2015, Wang and Fingas 2003b) (Chandru et al. 2008). Aromatic biomarkers are normally TA-steranes, MA-steranes. The aromatic biomarkers are key indicators to characterize the source of oil from sediments and the aquatic environment (da Silva and Bícego 2010, Romero-Sarmiento et al. 2011). Some other hydrocarbons, such as alkylated PAHs, have also been applied for fingerprinting of crude oils (Wang and Fingas 2003b).

2.3.2 Methodologies for oil fingerprinting

The concept "fingerprinting" was developed to reduce the spills of petroleum into the sea from the perspectives of legislation and execution (Ehrhardt and Blumer 1972). The early source identification was realized through the differences of various patterns of alkanes in oil using gas chromatograms (GC) coupled with mass spectrometry (MS) (Albaiges and Albrecht 1979, Ehrhardt and Blumer 1972, Reed 1977). The accuracy and effectiveness of oil fingerprinting methodologies have been improved with the

application of analytical instruments, such as capillary GC (Stout and Wang 2016). The analysis strategies become more robust and accurate nowadays after a few modifications and standardizations (Daling et al. 2002, Wang et al. 1994b, Wang et al. 2006a). Although EPA and ASTM developed and improved some analytical methods applicable for oil identification in the 1990s, these methods have low sensitivity and specificity for analyzing the mixture of petroleum (Wang and Fingas 2003a). Modern robust oil fingerprinting method requires proper sampling approaches, sample pre-treatment and analytical methodologies, and data analysis and interpretation (Wang and Fingas 2003a). Tiered strategies were applied as well to define the degrees of the analytical fineness for various requirements (Wang et al. 1998b, Wang et al. 1997). To achieve a successful analysis, quality control and quality assurance are always required in oil fingerprinting technologies. Main requirements follow the standard protocols from USEPA and ASTM with some improvement of the accuracy (Wang et al. 1994a, Wang et al. 2006a).

Recently, many advanced instruments became available for identification biomarkers, such as comprehensive two-dimensional gas chromatography mass spectrometry (GC×GC)-MS, isotopic resolution mass spectrometry (IRMS), electrospray ionization liquid chromatography-mass spectrometry (ESI-LC-MS), ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICRMS) (Gaines et al. 1999). Among these technologies, GC×GC is a widely recognized method in recent years with better-resolving power to separate groups of hydrocarbons, especially for the isomers and hydrocarbons with similar retention time (Adahchour et al. 2006, Ventura et al. 2010). The effects of physio-chemical weathering to biomarkers was successfully evaluated based on (GC×GC)-MS, indicating a reliable option for

semi-quantification and quantification analysis for oil identification and characterization (Arey et al. 2007a). Besides, the $\delta^{13}C/^{12}C$ value of spilled oil is an important indicator of oil source. Since the $^{13}C/^{12}C$ value varies with weathering, high-resolution analysis is desired (Bayona et al. 2015). GC-IRMS is useful in oil fingerprinting and determination of weathering status. The weathering of *n*-alkanes can be linked to the variations of $^{13}C/^{12}C$, although specific analysis of individual biomarkers may not be accurately detected using current methods (Cortes et al. 2010, Mansuy et al. 1997).

Diagnostic ratios are the main indicators for oil fingerprinting to realize the functions of oil source identification, characterization, and weathering tracing. The changes of diagnostic ratios of biomarkers are always seen as the key elements that oil differentiation achieves, and some ratios can be used to evaluate the different weathering processes.

The unique distribution of hydrocarbons can form diverse but unique diagnostic ratios, and the differences in these ratios can be used to differentiate oils (Wang et al. 2006b). Bi-plot of ratios is a commonly applied tool for straightforward differentiation. The most commonly applied diagnostic ratios include the internal ratios of terpanes and steranes, such as Ts/Tm, C29/C30, $C_{27}\alpha\beta\beta R$ and $C_{27}\alpha\beta\beta S$ (Osadetz et al. 1992, Ventura et al. 2010, Wang et al. 2004, Yim et al. 2011).

In addition to the numerical analysis of the diagnostic index of biomarkers, multivariate analysis techniques, particularly principal component analysis (PCA), has been introduced to fingerprint spilled oil (Kaufman et al. 1997). It is a powerful technique to differentiate different oils, because oils with different distributions of

detected hydrocarbons or their diagnostic indices can be separated into different components (Stout et al. 2001). PCA methods are widely used in the oil fingerprinting field to identify oils and their weathering status using the combination of component patterns of oil (Christensen et al. 2004, Prata et al. 2016). The diagnostic ratios of *n*-alkanes, terpanes, and steranes are effectively applied as variables in PCA to differentiate oil types, such as light and heavy fuel oil, diesel, lubricants and crude oils (Christensen et al. 2005, Ismail et al. 2016, Sun et al. 2018). Weathering degrees of crude oils could be evaluated by the application of diagnostic ratios of biomarkers, such as diamondoids, sesquiterpanes, terpanes, steranes, and alkylated-PAHs (Azevedo et al. 2008, Sun et al. 2015). Two to three principal components are commonly obtained. A bi-plot is then used to visualize these differences to assist in the interpretation the PCA results. Each vector represents the combinations of the contributions of two components. PCA can be combined with other statistical techniques and chemometric data analysis tools to decrease the possibility of making faulty decisions, such as discriminant analysis to maximize the distances among different categories, and warping methods to minimize noises from chromatograms (Christensen et al. 2005, Ismail et al. 2016, Sun et al. 2015, Tomasi et al. 2004). These techniques decrease the signal noise of instruments, statistically narrowing down the differentiation processes, and directly increase the validation accuracy of oil fingerprinting.

2.3.3 Biomarkers for tracing weathering status

The weathering processes affecting oil fingerprinting mainly include evaporation, dissolution, photo-oxidation, and biodegradation. The degradation of biomarkers caused by weathering processes probably has specific preference

and could be distinguished by the variation trend of special biomarkers.

Evaporation can cause considerable oil weight loss (Daling et al. 2014, Wang et al. 1998a) especially at the early stages of an oil spill. Evaporation can be reasonably recognized through the difference of concentrations between hydrocarbons with less carbon numbers and hydrocarbons with more carbon numbers. Alkanes with different carbon numbers are often applied to deduce the occurrence of evaporation, such as $(\Sigma C_8\text{-}C_{14})/(\Sigma C_{22}\text{-}C_{28})$ and $(\Sigma C_{10}\text{-}C_{25})/(\Sigma C_{17}\text{-}C_{25})$ (Boehm et al. 1982, Wang and Fingas 1995, Wang et al. 1994b). The diagnostic ratios of sesquiterpanes are changed in oil weathering including evaporation (Wang et al. 2005). The diagnostic ratios of adamantanes and diamantanes in crude oil can be affected by evaporation involved biodegradation experiment as well (Wang et al. 2006b).

Photo-oxidation can deplete many hydrocarbons in oils, such as some alkylated PAHs and TA-steranes (Prince et al. 2003, Radović et al. 2014). Furthermore, photo-oxidation can gradually transfer oil saturates and aromatics to oxygenated ones (Aeppli et al. 2012). Few studies correlated pure photo-oxidation to aliphatic biomarkers due to a higher resistance (Radović et al. 2014). Alkylated-PAHs have different weathering degrees with the presence of photo-oxidation. Alkylated phenanthrenes (9/4-methyl-phenanthrenes and methyl-anthracene), methyl-pyrenes, benzofluorenes, and methyl-chrysenes, are more readily degraded by the solar radiation (Radović et al. 2014). Benzo[*a*]pyrene is degraded faster than benzo[*e*]pyrene with photo-oxidation (Douglas et al. 2002). These diagnostic ratios are potentially applicable in

fingerprinting of dispersed oil for weathering status tracing.

Biodegradation is a dominant way to degrade most of the hydrocarbons in oils. The distribution of *n*-alkanes could tell a long story about the preference of biodegradation. Famous indicators included pristane/phytane, *n*-C_{17}/pristane, *n*-C_{18}/phytane, and (Σ odd alkanes)/(Σ even alkanes). The changes in these ratios reveal the preference of biodegradation for different types of hydrocarbons, such as a preference for straight-chain alkanes over branched alkanes (Atlas and Bartha 1992, Prince 1993, Wang et al. 2006a). Meanwhile, the diagnostic ratios of different types of biomarkers can be altered by biodegradation. Diamantanes have a higher resistance to biodegradation than adamantanes (Grice et al. 2000, Wang et al. 2006b). Isomers of steranes have different sensitivity to biodegradation (Seifert and Moldowan 1979). A wide range of alkylated PAHs can be microbial utilized by bacteria, fungi, algae, and other organisms in several pathways driven by the oxygen (Haritash and Kaushik 2009). Some ratios were altered in biodegradation but remained stable in physiochemical weathering, such as (3+2-methylphenanthrenes) / (4-/9+1-methylphenanthrenes) and (1,3+1,6-dimethylnaphthalene)/total of C_2-Naphthalene (Wang et al. 1998a). Besides, the different weathering degrees of alkylated-PAHs point to the potentiality of alkylated PAHs as important indicators for recognition of different weathering processes.

2.4 Summary

This chapter summarizes the core knowledge, technologies, and researches directions relevant to CDO fingerprinting, involving the fate and behaviors of spilled oil; the

application, impacts, and behaviors of dispersants; and fingerprinting methodologies for crude oil source identification and oil weathering status analysis. The application of dispersants may affect oil fingerprinting because the important physiochemical properties of spilled oil and the formation of oil droplets can lead to different behaviors of CDO in comparison to non-dispersed oil. Therefore, a comprehensive study on fingerprinting of dispersed oil needs to be conducted.

CHAPTER 3 USE OF SESQUITERPANES, STERANES AND TERPANES FOR FORENSIC FINGERPRINTING OF CHEMICALLY DISPERSED OIL[1]

The contents of this chapter are based and expanded on the following paper:

Song, X., Zhang, B., Chen, B., & Cai, Q. (2016). Use of sesquiterpanes, steranes, and terpanes for forensic fingerprinting of chemically dispersed oil. Water, Air, & Soil Pollution, 227(8), 281, DOI: 10.1007/s11270-016-2981-1
Role: Xing Song solely worked on this study and acted as the first author of this manuscript under Dr. Baiyu Zhang and Dr. Bing Chen's guidance. Most contents of this paper were written by him and further edited by the other co-authors. Dr. Cai helped conduct parts of the experiments and data analysis.

3.1 Introduction

Previously, limited studies on the behavior of weathered CDO in offshore environment were focused on the investigation of the biodegradation effects. Alkanes and aromatic hydrocarbons of CDO in smaller droplet sizes (10μm) were biodegraded faster than those in larger sizes (30μm) (Brakstad et al. 2015). Chemical properties of different surfactants in dispersants could affect the biodegradation of CDO, which it is therefore different from the biodegradation of crude oil (Zhuang et al. 2016). Although biodegradation is the most important effect on the concentration changes and composition changes of hydrocarbons in CDO, the impact of physicochemical weathering cannot be ignored. At the earliest stage after an oil spill, the evaporation can cause considerable weight loss of CDO (Daling et al. 2014, Wang et al. 1998a). Photo-oxidation could deplete certain inordinate hydrocarbons, such as methyl-phenanthrenes and methyl-chrysenes (Prince et al. 2003, Radović et al. 2014). Besides, it was Aeppli et al. (2012) found that oil weathering can increase oxygenated components and deplete saturates and aromatic hydrocarbons simultaneously in oil. Therefore, the concentrations of compounds in CDO may be susceptible to physiochemical weathering. Although the weathering of crude oil may produce some potential biomarkers, such as steranes and terpanes, there have been no experimental studies on the effect of physiochemical weathering on the stability of biomarkers in CDO. In this study, three groups of potential oil biomarkers (i.e., sesquiterpanes, steranes, and terpanes) were selected as the targeting candidates for fingerprinting of CDO in seawater. The stability of biomarkers in CDO and their effectiveness as biomarkers were examined using a batch-scale weathering system. The

diagnostic ratios of biomarkers were calculated. The performance of biomarkers in CDO during physicochemical weathering was also compared with those in naturally weathered crude oil.

3.2 Materials and Methods

3.2.1 Chemicals and samples

Surrogate solution: four common surrogates used in oil fingerprinting for quality control including acenaphthalene-d_{10}, phenanthrene-d_{10}, perylene-d_{12}, and terphenyl-d_{14} were dissolved in dichloromethane (DCM) to form the surrogate solution (Wang et al. 2000). The concentrations of them were 3.0 μg/mL, 3.0μg/mL, 11.2 μg/mL, and 3.0 μg/mL, respectively.

Internal standard (IS): C_{30} 17β(H) 21β(H)-hopane was used as the internal standard (IS). The concentration of the IS was 0.1 mg/mL. All chemicals used in the study were acquired from Sigma Aldrich Canada.

Crude oil samples: crude oil applied was taken from a Canadian oil company. The main physiochemical properties were summarized: (1) API was 34.2; (2) the viscosity at 20 C was 14 cSt; (3) the vapor pressure was 34.2, and (4) the volume of saturates and aromatics were 34.9% and 32.4% (vol %) respectively. Each crude oil sample was prepared through dissolving 0.8g of crude oil in 10 mL of hexane. One hundred μL of dissolved crude oil was syringed from crude oil sample, and the crude oil sample was spiked with the internal standard and diluted by *n*-hexane till the volume became 1 mL.

Weathered crude oil (WCO) samples: four artificial seawater samples were prepared by

well mixing 120 mL distilled water with 4.32 g of sea salt (36%) in each sample and were placed in four 250 mL Erlenmeyer flasks, respectively. One hundred μL of crude oil was introduced to the surface of each seawater sample with a pipette. The flasks were placed on a shaker at the speed of 120 rpm at 30 °C for 2, 5, 9, 12 days of oil weathering, respectively. The oil solutions in the four flasks were treated as the WCO samples.

CDO samples: four artificial seawater samples were prepared by well mixing 120 mL of distilled water with 4.32 g of sea salt (36%) in each sample and were placed in four 250 mL Erlenmeyer flasks, respectively. One hundred μL of crude oil was introduced to the surface of each seawater sample with a pipette. Corexit 9500A was chosen as the testing chemical dispersant. Ten μL of Corexit 9500A (1:10, $v_{Corexit}/v_{oil}$) was added into the oily seawater samples for oil dispersion. The flasks were placed on the shaker under the same conditions for 2, 5, 9, 12 days of oil weathering, respectively. The oil solutions in the four flasks were treated as the CDO samples.

3.2.2 Sample analysis

Both WCO and CDO samples need to be pre-treated before GC-MS analysis. Five hundred μL of the surrogate solution was added into each WCO sample. The surrogates were used to examine the recovery of the pretreatment process. The sample was then transferred into a separation funnel. DCM was used to transfer all residual oil attached to the surface of each flask containing WCO into the funnel. Forty mL of DCM was used each time and the process was repeated for five times. The organic phase in the funnel was then separated after the extraction by DCM. The extraction processes were repeated two more times with 200 mL of DCM applied each time. Water left in the collected

organic phase was removed by sodium sulfate anhydrous. The organic phase was then concentrated to 10 mL using a rotary evaporator followed by nitrogen flow. One hundred and fifty μL of concentrated organic phase was accurately introduced into a 150 μL microvial by a 500 μL syringe. Two μL of the IS was introduced into each microvial.

Twelve mL of each CDO sample was transferred from the flask to a separation funnel using a liquid dropper. Fifty μL of the surrogate solution was added into the funnel. The sample was extracted by DCM for three times in the funnel, with 120 mL of DCM applied each time. Water left in the collected organic phase was removed by sodium sulfate anhydrous. The organic phase was then concentrated to 1 mL using a rotary evaporator followed by nitrogen flow. One hundred and fifty μL of concentrated organic phase was accurately introduced into a 150 μL microvial by a 500 μL syringe. Two μL of the IS was introduced into each microvial.

The crude oil samples and the pre-treated WCO and CDO samples were then ready for GC-MS analysis. The sesquiterpanes, steranes and terpanes in the samples was characterized. The GC-MS system (Agilent model 6890) was equipped with a DB-5ms capillary column (30 m). The GC operation conditions were determined based on a method of oil fingerprinting, as shown in Table 3.1 (Mulabagal et al. 2013). Each sample was injected into GC using a splitless mode and the injector temperature was 280 °C. Helium was applied as carrier gas. The GC oven temperature was set at 50 °C for 2 min, then ramp at 6 °C/min to 300 °C for 20 min. The temperature of GC-MS interface conditions was: 300 °C. MS detection were using electron ionization mode with 70 eV and ion source temperature 300 °C. Full scan mode and SIM mode were applied for

Table 3.1 Settings of GC-MS operation

Name of Parameters	Operation
Column inner diameter	0.25 mm
Phase thickness	0.25 μm
Injection type	Splitless Inlet
Splitless Time	0.5 minutes
Inlet pressure	16 psi (constant)
column head pressure	16 psi (constant)
Carrier gas flow rate	1.9 mL/min
Injector temperature	280°C
MS mode	SIM mode
Temperature of the transfer line	300°C
Temperature of ion source	300°C
temperature of manifold	200°C
Software for data acquisition and peak integration	Agilent MSD ChemStation software MSD ChemStation E.02.01.1177

identification and characterization of biomarkers. The main m/z value for three types of biomarkers, involving sesquiterpanes, steranes, and terpanes, was 123, 217, and 191, respectively.

3.2.3 Quality assurance and quality control (QA/QC)

QA/QC measurements were applied to determine the reliability of the experiment results. Four crude oil samples were used for providing the original distribution patterns of biomarkers. The preparation of WCO and CDO samples were in duplicate. Two aliquots of 150 μL of extracted organic phase were selected from a well-pretreated WCO or CDO sample for duplicate GC-MS analysis. Therefore, the data were expressed by the average of 8 runs with their standard deviation (SD). The coefficient of determination (R^2) of calibration of surrogates was > 0.9940. The average recoveries (%) of four surrogates ranged from 84% to 108%.

3.3 Results and Discussions

3.3.1 Identification of sesquiterpanes, steranes, and terpanes in crude oil samples

The sesquiterpanes, steranes, and terpanes in crude oil were identified and the results are shown in Figure 3.1 (a-c). Individual peak of each biomarker was confirmed based on retention time, m/z value, corresponding carbon number, and the chromatograms of reference oils (Wang et al. 2005, Wang et al. 2006b, Yang et al. 2009). The information of identified peaks in this thes, including the codes (abbreviation) of peaks, compound names, experimental formula, and target ions referred to literature data, were listed in Table 3.2. Ten bicyclic sesquiterpanes were found and identified at m/z 123 as C_{14}

Table 3.2 Characteristics of petroleum hydrocarbons and biomarkers in this thesis

Peak	**Compound**	**Formula**	**Target ions**
Alkanes			70, 85
C8-alkane	Octane	C_8H_{18}	70, 85
C9-alkane	Nonane	C_9H_{20}	70, 85
C10-alkane	Decane	$C_{10}H_{22}$	70, 85
C11-alkane	Undecane	$C_{11}H_{24}$	70, 85
C12-alkane	Dodecane	$C_{12}H_{26}$	70, 85
C13-alkane	Tridecane	$C_{13}H_{28}$	70, 85
C14-alkane	Tetradecane	$C_{14}H_{30}$	70, 85
C15-alkane	Pentadecane	$C_{15}H_{32}$	70, 85
C16-alkane	Hexadecane	$C_{16}H_{34}$	70, 85
C17-alkane	Heptadecane	$C_{17}H_{36}$	70, 85
Pri	Pristane		70, 85
C18-alkane	Octadecane	$C_{18}H_{38}$	70, 85
Phy	Phytane		70, 85
C19-alkane	Nonadecane	$C_{19}H_{40}$	70, 85
C20-alkane	Eicosane	$C_{20}H_{42}$	70, 85
C21-alkane	Docosane	$C_{21}H_{44}$	70, 85
C22-alkane	Tricosane	$C_{22}H_{46}$	70, 85
C23-alkane	Tetracosane	$C_{23}H_{48}$	70, 85
C24-alkane	Pentacosane	$C_{24}H_{50}$	70, 85

C25-alkane	Heneicosane	$C_{25}H_{52}$	70, 85
C26-alkane	Hexacosane	$C_{26}H_{54}$	70, 85
C27-alkane	Heptacosane	$C_{27}H_{56}$	70, 85
C28-alkane	Octacosane	$C_{28}H_{58}$	70, 85
C29-alkane	Nonacosane	$C_{29}H_{60}$	70, 85
C30-alkane	Triacontane	$C_{30}H_{62}$	70, 85
C31-alkane	Hentriacontane	$C_{31}H_{64}$	70, 85
C32-alkane	Dotriacontane	$C_{32}H_{66}$	70, 85
C33-alkane	Tritriacontane	$C_{33}H_{68}$	70, 85
C34-alkane	Tetratriacontane	$C_{34}H_{70}$	70, 85
C35-alkane	Pentatriacontane	$C_{35}H_{72}$	70, 85
Adamantanes			
1	Adamantane	$C_{10}H_{16}$	136
2	1-methyladamantane	$C_{11}H_{18}$	135
3	1,3-dimethyladamantane	$C_{12}H_{20}$	149
4	1,3,5-trimethyladamantane	$C_{13}H_{22}$	163
5	1,3,5,7-tertramethyladamantane	$C_{14}H_{24}$	177
6	2-methyladamantane	$C_{11}H_{18}$	135
7	1,4-dimethyladamantane, *cis*-	$C_{12}H_{20}$	149
8	1,4-dimethyladamantane, *trans*-	$C_{12}H_{20}$	149
9	1,3,6-trimethyladamantane	$C_{13}H_{22}$	163
10	1,2-dimethyladamantane	$C_{12}H_{20}$	149
11	1,3,4-trimethyladamantane, *cis*-	$C_{13}H_{22}$	163
12	1,3,4-trimethyladamantane, trans-	$C_{13}H_{22}$	163
13	1,2,5,7-tetramethyladamantane	$C_{14}H_{24}$	177
14	1-ethyladamantane	$C_{12}H_{20}$	135
15	1-ethyl-3-methyladamantane	$C_{13}H_{22}$	149
16	1-ethyl-3,5-dimethyladamantane	$C_{14}H_{24}$	163
17	2-ethyladamantane	$C_{12}H_{20}$	135
Diamantanes			
1	diamantane	$C_{14}H_{20}$	188
2	4-methyldiamantane	$C_{15}H_{22}$	187
3	4,9-dimethyldiamantane	$C_{16}H_{24}$	201

4	1-methyldiamantane	$C_{15}H_{22}$	187
5	1,4- and 2,4-dimethyldiamantane	$C_{16}H_{24}$	201
6	4,8-dimethyldiamantane	$C_{16}H_{24}$	201
7	trimethyldiamantane	$C_{17}H_{26}$	215
8	3-methyldiamantane	$C_{15}H_{22}$	187
9	3,4-dimethyldiamantane	$C_{16}H_{24}$	201
Sesquiterpanes			
1	C_4-decalin	$C_{14}H_{26}$	123, 179
2	C_{14} sesquiterpane	$C_{14}H_{26}$	179
3	C_{15} sesquiterpane	$C_{15}H_{28}$	123, 193
4	C_{15} sesquiterpane	$C_{15}H_{28}$	123, 193
5	8β(H)-drimane	$C_{15}H_{28}$	123, 193
6	C_{15} sesquiterpane	$C_{15}H_{28}$	123
7	C_{16} sesquiterpane	$C_{16}H_{30}$	123
8	C_{16} sesquiterpane	$C_{16}H_{30}$	123, 193
9	C_{16} sesquiterpane	$C_{16}H_{30}$	123, 193
10	8β(H)-homodrimane	$C_{16}H_{30}$	123, 207
Steranes			
DIA27S (1)	C_{27} 20S-13β(H),17α(H)-diasterane	$C_{27}H_{46}$	217, 218
DIA27R (2)	C_{27} 20R-13β(H),17α(H)-diasterane	$C_{27}H_{46}$	217, 218
C27S (7)	C_{27} 20S-5α(H),14α(H),17α(H)-cholestane	$C_{27}H_{48}$	217, 218
C_{27}αββR (8)	C_{27} 20R-5α(H),14β(H),17β(H)-cholestane	$C_{27}H_{48}$	217, 218
C_{27}αββS (10)	C_{27} 20S-5α(H),14β(H),17β(H)-cholestane	$C_{27}H_{48}$	217, 218
C27R (11)	C_{27} 20R-5α(H),14α(H),17α(H)-cholestane	$C_{27}H_{48}$	217, 218
C28S(13)	C_{28} 20S-5α(H),14α(H),17α(H)-ergostane	$C_{28}H_{50}$	217, 218
C_{28}αββR(14)	C_{28} 20R-5α(H),14β(H),17β(H)-ergostane	$C_{28}H_{50}$	217, 218
C_{28}αββS(15)	C_{28} 20S-5α(H),14β(H),17β(H)-ergostane	$C_{28}H_{50}$	217, 218
C28R(16)	C_{28} 20R-5α(H),14α(H),17α(H)-ergostane	$C_{28}H_{50}$	217,

			218
C29S	C_{29} 20S-5α(H),14α(H),17α(H)-stigmastane	$C_{29}H_{52}$	217, 218
C_{29}αββR	C_{29} 20R-5α(H),14β(H),17β(H)-stigmastane	$C_{29}H_{52}$	217, 218
C_{29}αββS	C_{29} 20S-5α(H),14β(H),17β(H)-stigmastane	$C_{29}H_{52}$	217, 218
C29R	C_{29} 20R-5α(H),14α(H),17α(H)-stigmastane	$C_{29}H_{52}$	217, 218
Terpanes			
C23	C_{23} tricyclic terpane	$C_{23}H_{42}$	191
C24	C_{24} tricyclic terpane	$C_{24}H_{44}$	191
C25	C_{25} tricyclic terpane (a)	$C_{25}H_{46}$	191
C26	C_{26} (S + R) tricyclic terpanes	$C_{24}H_{42}$ + $C_{26}H_{48}$	191
TR28a	C_{28} tricyclic terpane (a)	$C_{28}H_{52}$	191
TR28b	C_{28} tricyclic terpane (b)	$C_{28}H_{52}$	191
TR29a	C_{29} tricyclic terpane (a)	$C_{29}H_{54}$	191
TR29b	C_{29} tricyclic terpane (b)	$C_{29}H_{54}$	191
Ts	18α(H),21β(H)-22,29,30-trisnorhopane	$C_{27}H_{46}$	191
Tm	17α(H),21β(H)-22,29,30-trisnorhopane	$C_{27}H_{46}$	191
H29 (C29)	17α(H),21β(H)-30-norhopane	$C_{29}H_{50}$	191
C29TS	18α(H),21β(H)-30-norneohopane	$C_{29}H_{50}$	191
M29	17α(H),21β(H)-30-norhopane	$C_{29}H_{50}$	191
H30 (C30)	17α(H),21β(H)-hopane	$C_{30}H_{52}$	191
H31S (C31S)	22*S*-17α(H),21β(H)-30-homohopane	$C_{31}H_{54}$	191
H31R (C31R)	22*R*-17α(H),21β(H)-30-homohopane	$C_{31}H_{54}$	191
H32S (C32S)	22*S*-17α(H),21β(H)-30,31-bishomohopane	$C_{32}H_{56}$	191
H32R (C32R)	22*R*-17α(H),21β(H)-30,31-bishomohopane	$C_{32}H_{56}$	191
H33S (C33S)	22*S*-17α(H),21β(H)-30,31,32-trishomohopane	$C_{33}H_{58}$	191
H33R (C33R)	22*R*-17α(H),21β(H)-30,31,32-trishomohopane	$C_{33}H_{58}$	191
H34S (C34S)	22*S*-17α(H),21β(H)-30,31,32,33-tetrakishomohopane	$C_{34}H_{60}$	191
H34R (C34R)	22*R*-17α(H),21β(H)-30,31,32,33-tetrakishomohopane	$C_{34}H_{60}$	191
H35S (C35S)	22S-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane	$C_{35}H_{62}$	191

H35R (C35R)	22*R*-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane	$C_{35}H_{62}$	191
TA-steranes			
1	C20 triaromatic-sterane	$C_{10}H_{16}$	231
2	C21 triaromatic-sterane	$C_{11}H_{18}$	231
3a	C22 triaromatic steroids (a)	$C_{12}H_{20}$	231
3b	C22 triaromatic steroids (b)	$C_{13}H_{22}$	231
4	C26 triaromatic-chloestane (20S)	$C_{14}H_{24}$	231
5	C26 triaromatic-chloestane(20R) + C27triaromatic-ergostane(20S)	$C_{11}H_{18}$	231
6	C28 triaromatic-stigmastane (20S)	$C_{12}H_{20}$	231
7	C27 triaromatic-ergostane (20R)	$C_{12}H_{20}$	231
8	C28 triaromatic-stigmastane (20R)	$C_{13}H_{22}$	231
MA-steranes			
1	C21 5ß monoaromatic steroid	$C_{21}H_{30}$	253
2	C21 5a monoaromatic steroid	$C_{21}H_{30}$	253
3a	C23 monoaromatic steroid (20S)	$C_{22}H_{32}$	253
3b	C23 monoaromatic steroid (20R)	$C_{22}H_{32}$	253
4	C27 monoaromatic 5ß(H)-cholestane (20S)	$C_{27}H_{42}$	253
5	C27 monoaromatic diacholestane (20S)	$C_{27}H_{42}$	253
6	C27 monoaromatic 5ß(H)-cholestane(20R) +diacholestane (20R)	$C_{27}H_{42}$	253
7	(C27 monoaromatic 5α (H)-cholestane (20S)) + C28 monoaromatic 5ß(H)-ergostane(20S) +diaergostane (20S)	$C_{27}H_{42+}$ $C_{28}H_{44}$	253
8	C27 monoaromatic 5α (H)-cholestane (20R)	$C_{27}H_{42}$	253
9	C28 monoaromatic 5α (H)-ergostane (20S)	$C_{28}H_{44}$	253
10	C28 monoaromatic 5ß(H)-ergostane (20R) +diaergostane (20R)	$C_{28}H_{44}$	253
11	C29 monoaromatic 5α (H)-stigmastane (20S)	$C_{29}H_{46}$	253
12	C28 monoaromatic 5α (H)-ergostane (20R)	$C_{28}H_{44}$	253, 193
Alkylated PAHs			
C1-N	C1-naphthalenes	$C_{11}H_{10}$	142
C2-N	C2-naphthalenes	$C_{12}H_{12}$	156
C3-N	C3-naphthalenes	$C_{13}H_{14}$	170
C4-N	C4-naphthalenes	$C_{14}H_{16}$	184
C1-F	C1-fluorenes	$C_{14}H_{12}$	180
C2-F	C2-fluorenes	$C_{15}H_{14}$	194
C3-F	C3-fluorenes	$C_{16}H_{16}$	208
C1-P	C1-phenanthrenes	$C_{15}H_{12}$	192
C2-P	C2-phenanthrenes	$C_{16}H_{14}$	206
C3-P	C3-phenanthrenes	$C_{17}H_{16}$	220

C4-P	C4-phenanthrenes	$C_{18}H_{18}$	234
C1-D	C1-dibenzothiophenes	$C_{13}H_{10}S$	198
C2-D	C2-dibenzothiophenes	$C_{14}H_{12}S$	212
C3-D	C3-dibenzothiophenes	$C_{15}H_{14}S$	226
C4-D	C4-dibenzothiophenes	$C_{16}H_{16}S$	240
C1-Py	C1-pyrenes	$C_{17}H_{12}$	216
C2-Py	C2-pyrenes	$C_{18}H_{14}$	230
C3-Py	C3-pyrenes	$C_{19}H_{16}$	244
C1-B	C1-benzo[b]naphthothiophenes	$C_{16}H_{10}S$	248
C2-B	C2-benzo[b]naphthothiophenes	$C_{17}H_{12}S$	262
C3-B	C3-benzo[b]naphthothiophenes	$C_{18}H_{14}S$	276
C1-C	C1-chrysenes	$C_{19}H_{14}$	242
C2-C	C2-chrysenes	$C_{20}H_{16}$	256
C3-C	C3-chrysenes	$C_{21}H_{18}$	270

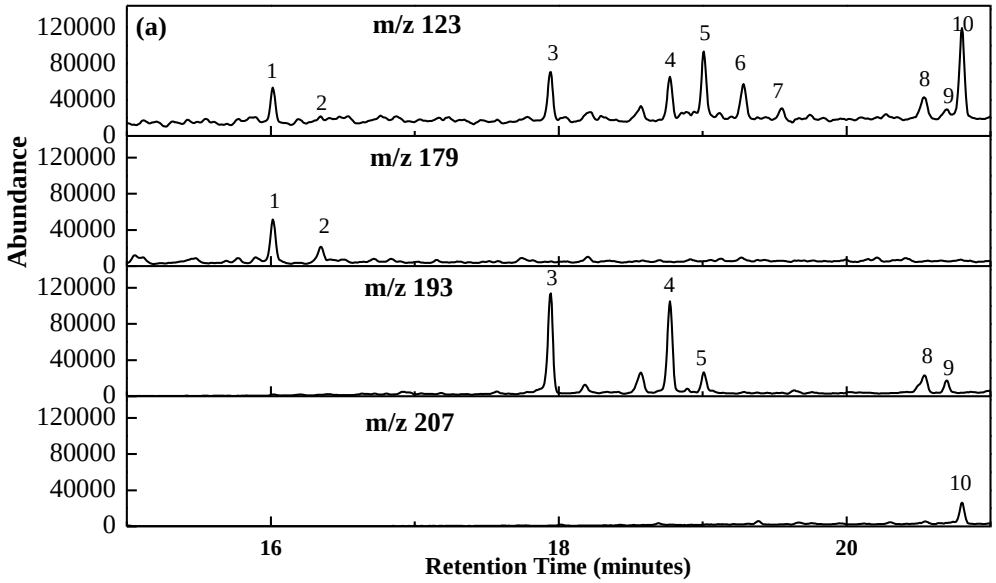
(Peaks 1 and 2), C_{15} (Peaks 3 to 6), and C_{16} (Peaks 7 to 10) sesquiterpanes. The sesquiterpanes were further confirmed at target m/z 179, 193, and 207, according to the distribution patterns of sesquiterpanes in reference oils (Wang et al. 2005, Yang et al. 2009). Diagnostic ratios of sesquiterpanes in crude oil were calculated at m/z 123 and compared with those (at m/z 123) in some reference oils (Table 3.3). The results indicated that crude oil samples applied in this study were differentiated from other oils using sesquiterpanes as biomarkers. Main steranes identified in crude oil samples were stereoisomers from C_{27} to C_{29}. Ionic steranes (C_{27}αββR/S, C_{28}αββR/S, C_{29}αββR/S) were major biomarkers in steranes at m/z 217 and 218. Major diagnostic ratio in steranes was C_{27}αββ(R+S)/C_{29}αββ(R+S). All the recognized peaks of terpanes were hopanes from C_{27} to C_{35} at m/z 191. Major diagnostic ratios of steranes and terpanes in crude oil samples were calculated and compared with those in reference oils (Table 3.4). It was further confirmed that the crude oil could be differentiated from other reference oils using steranes and terpanes as biomarkers.

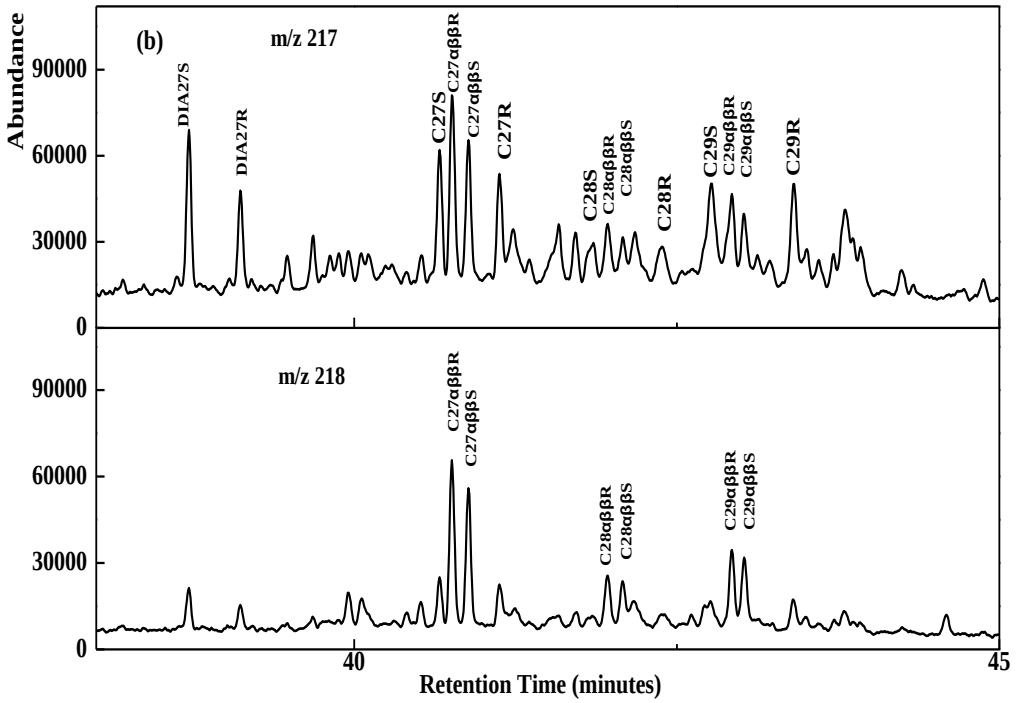
3.3.2 The performance of sesquiterpanes in fingerprinting of CDO and WCO samples

Ratios of peak areas of sesquiterpanes with the same carbon numbers, such as peak 1:2 (C_{14} sesquiterpanes), 3:4 (C_{15} sesquiterpanes), and 8:10 (C_{16} sesquiterpanes), were commonly used as diagnostic indices (Stout et al. 2005b, Wang et al. 2005). Additionally, sesquiterpanes with different carbon numbers, such as peak 1:10 (C_{14}: C_{16} sesquiterpanes) and 5:10 (C_{15}: C_{16} sesquiterpanes), were implemented to identify the source of crude oil (Stout et al. 2005b, Wang et al. 2005). In this study, the base peaks of sesquiterpanes in

CDO and WCO samples were illustrated in Figure 3.2 (a-b). The sesquiterpanes patterns in CDO and WCO samples changed significantly with days. The average diagnostic ratios ±SD (n=8), and relative standard deviations (RSD = SD/average diagnostic ratios × 100%), were shown in Table 3.5. The RSD value was applied to examine the variability of diagnostic ratios based on the evaluation method for characterization of weathered oil (Daling et al. 2002, Wang et al. 2013a, Wang et al. 2013b). The RSD values of a diagnostic ratio of C_{15} (peak 4:5) and two diagnostic ratios of C_{16} sesquiterpanes (peak 8:9 and peak 8:10) in CDO and WCO samples were all less than 10%. The diagnostic ratios in WCO and CDO samples show slight changew compared with those in crude oil samples. These relatively stable ratios indicate that the presence of Corexit 9500A did not affect identification of CDO or WCO samples using C_{15} (peak 4:5) and C_{16} (peak 8:9 and peak 8:10) sesquiterpanes as biomarkers. These three diagnostic ratios can be considered as candidate biomarkers for CDO fingerprinting. The RSD values of all other diagnostic ratios than the three ones in WCO and CDO samples were also calculated (Table 3.5). The results show that those RSD values are all more than 10% so that these relevant diagnostic ratios cannot be used for CDO or WCO identification. Therefore, the two ratios 4:5 and 8:10 were determined as primary candidate sesquiterpanes for selected CDO fingerprinting.

Most of the diagnostic ratios of sesquiterpanes in CDO and WCO in this study are not stable compared to those in Wang's study (Wang, 2005). This is because, given the differences in weathering methods, previous weathering primarily considered evaporation of crude oil using rotatory evaporator within 48 hours (Wang et al. 2003).


(a)
m/z 123
m/z 179
m/z 193
m/z 207
Abundance
120000
80000
40000
0
1
2
3
4
5
6
7
8
9
10
16
18
20
Retention Time (minutes)


(b)
m/z 217
m/z 218
Abundance
90000
60000
30000
0
D1A27S
D1A27R
C27S
C27αββR
C27αββS
C27R
C28S
C28αββR
C28αββS
C28R
C29S
C29αββR
C29αββS
C29R
40
45
Retention Time (minutes)

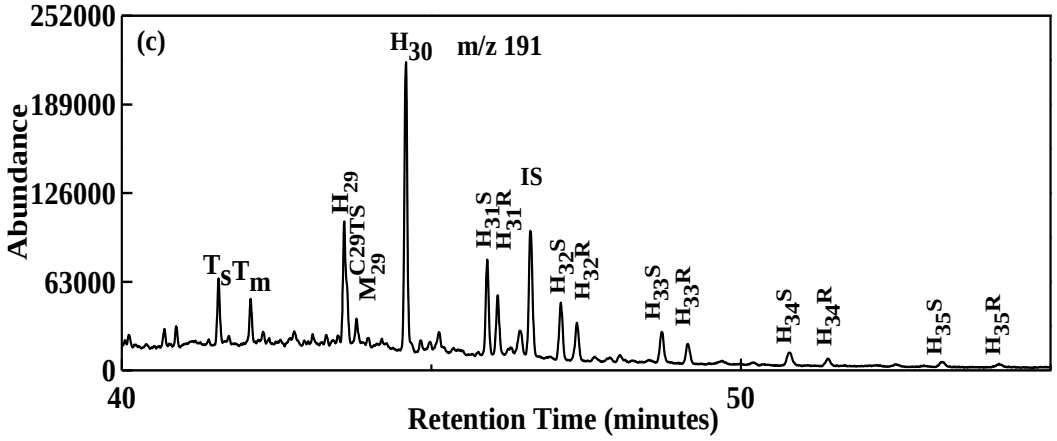


Figure 3.1 Identification of selected families of biomarkers in crude oil using GC-MS chromatograms of (a) sesquiterpanes (m/z 123, 179, 193 and 207), (b) steranes (m/z 217 and 218), and (c) terpanes (m/z 191)

Table 3.3 Comparison of major diagnostic ratios of sesquiterpanes in selected crude oil and reference oils

Oil	Weathering conditions	Diagnostic ratios of sesquiterpanes								References
		peak1:2	3:5	4:5	5:6	8:10	1:5	3:10	5:10	
Crude oil	Crude [a]		0.69	0.62	1.80	0.35	0.53	0.53	0.77	
Cook Inlet	Crude and evaporated weathered	0.96	1.25	1.06	0.92	0.31	0.76	0.83	0.67	(Wang et al. 2005)
Maya	Crude and evaporated weathered	3.47	0.42	0.26	2.56	0.19	0.42	0.37	0.90	(Wang et al. 2005)
Liao River crude	Crude	2.02	1.06	0.58	1.89	0.31	0.59	0.94	0.88	(Wang et al. 2013b)
Prudhoe bay	Crude and evaporated weathered	1.15	1.25	1.04	2.33	0.26	0.63	1.15	0.92	(Yang et al. 2009)

[a] Crude: Crude oil when weathering time is 0

Table 3.4 Comparison of major diagnostic ratios of steranes and terpanes in selected crude oil and reference oils

Oil	Weathering conditions	diagnostic ratios of steranes and terpanes								References
		Ts/Tm	Mc_{30}/Hc_{30}	C_{29}/C_{30}	$C_{31}S/R$	$C_{32}S/R$	$C_{33}S/R$	$C_{34}S/R$	$C_{27}\alpha\beta\beta/C_{29}\alpha\beta\beta$	
Crude oil	Crude [a]	1.50		0.47	1.47	1.36	1.48		1.35	
BIOS	Crude and weathered	0.25		0.95		1.58	1.58			(Wang and Fingas 1997)
Spilled oil (lube and diesel fuel)	Weathered	1.05		0.87	1.13	1.44	1.58	1.56	0.72	(Wang et al. 2004)
Spilled oil (light)		0.72		0.86	1.26	1.36	1.57		0.95	(Yang et al. 2012)
Kirkuk oil	Crude	0.25	0.08	1.39						(Mohialdeen et al. 2013)
Mississippi Canyon Crude	Crude and weathered			0.60					0.81	(Yang et al. 2013)
DH oil	Evaporated	0.91		0.38	1.56	1.86	1.50	2.03		(Mulabagal et al. 2013)

mousse	Emulsified	0.92	0.37	1.70	1.86	1.56	1.70		(Mulabagal et al. 2013)
Tar Ball	Weathered	0.93	0.37	1.70	1.70	1.70	1.70		(Mulabagal et al. 2013)
Quebec Oil	Relative fresh	0.96	0.84	1.29	1.70	1.52		0.68	(Wang et al. 2001)
Alberta Oil sand	Oil sand	0.29	0.84	1.37	1.36	1.54	1.57	1.13	(Yang et al. 2011)

[a] Crude: Crude oil when weathering time is 0

Biomarkers are not high-volatile compounds and could be continuously affected by weathering for a longer time relative to volatile organic compounds. The evaporation duration in literature (2 day) may not be sufficient to thoroughly monitor the evaporation of biomarkers with low molecular weights, as well as further photo-oxidation and oxygenation. Contrarily, the stability of diagnostic ratios for WCO fingerprinting is similar to those in weathered Liao river crude oil (Wang et al. 2013b). The diagnostic ratios, including 4:5 (C_{15} groups), 5:6 (C_{15} groups), 8:9 (C_{16} groups) and 8:10 (C_{16} groups) in Liao river crude oil were demonstrated to be relatively resistant to biodegradation. In this study, the ratios 4:5 and 8:10 of WCO and CDO are still stable (RSD < 10%) and with significant peaks. Williams et. al (Williams et al. 1986) found that the depletion of sesquiterpanes in different biodegraded crude oils depleted irregularly. Bicyclic sesquiterpanes in different oils may have definitely different degradation during weathering processes.

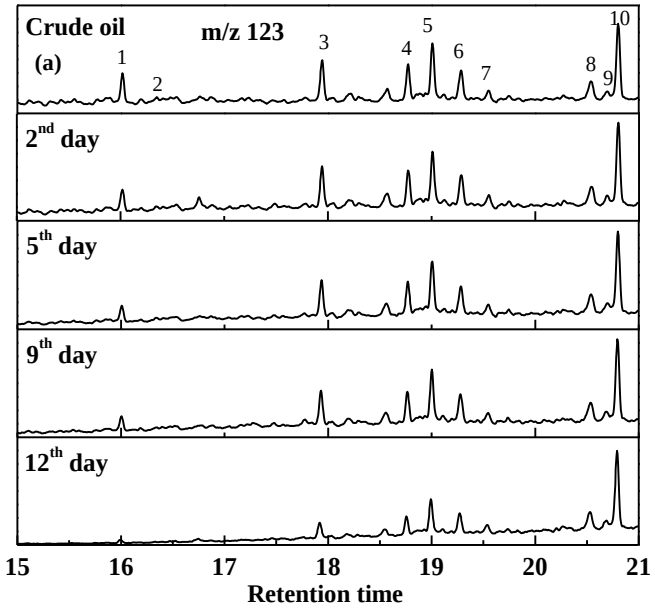
Crude oil samples could be distinguished from other oils by seven diagnostic ratios of sesquiterpanes (i.e., 3:5, 4:5, 5:6, 8:10, 1:5, 3:10, 5:10) based on the comparison of diagnostic ratios (Table 3.3). Nevertheless, only two ratios (i.e., 4:5 and 8:10) can be applied for CDO fingerprinting. The results confirmed that there were less available biomarkers resulting from the presence of Corexit 9500A and weathering made the CDO identification a challenging task.

ANOVA was taken to analyze the differences of all ratios among days for CDO and WCO fingerprinting, and the difference of each ratio between two types of oil. The results indicated that all the ratios show the significant difference at a 95% confidence interval with variations of days and oil types.

To estimate whether the statistical differences would create difficulty in differentiating oils, double ratio plots of the selected 4:5 and 8:10 in CDO were thereby used to compare with the same plots in some oils in the literature (Figure 3.3), including Cook Inlet (Wang et al. 2005), Diesel (Yang et al. 2009), ND diesel fuel (Stout et al. 2005b), Maya (Wang et al. 2005), Prudhoe bay (Yang et al. 2009), and a biodegraded crude oil from Liao River oil field (Wang et al. 2013b). In Figure 3.1, the two oils with different sources, the CDO and the biodegraded oil from Liao River oil field in China, could not be clearly differentiated, though the original crude oil without dispersion treatment and the Liao River crude oil before weathering could be identified using the same ratio plots (Table 3.3). The overlap of the range of diagnostic ratios further increased the difficulty in CDO identification. A conclusion could thus be drawn that sesquiterpanes could not be used as the stable biomarkers for CDO fingerprinting.

3.3.3 The performance of steranes in fingerprinting of CDO and WCO samples

Although most ratios among different steranes might be applied as biomarkers (e.g. $C_{27}\alpha\beta\beta R$/ $C_{27}\alpha\beta\beta S$, DIA27S/27R, and $C_{27}S$/ $C_{27}R$), few references used them to identify oils. In this study, the performances of the possible diagnostic ratios were examined. The peaks of steranes at m/z 217 in CDO and WCO samples were shown in Figure 3.4 (a-b). Diagnostic ratios values were summarized in Table 3.6. Most of the diagnostic ratios show low RSD (< 5%) values. The RSD of four diagnostic ratios DIA27S/27R, $C_{27}\alpha\beta\beta R/C_{27}\alpha\beta\beta S$, $C_{28}\alpha\beta\beta R/C_{28}\alpha\beta\beta S$, and $C_{27}S/C_{27}R$, are even lower than 3%. The low RSD values mean that steranes show high stability and strong resistance to the weathering in CDO and WCO samples, which is consistent with the high stability of steranes in other weathered oils.



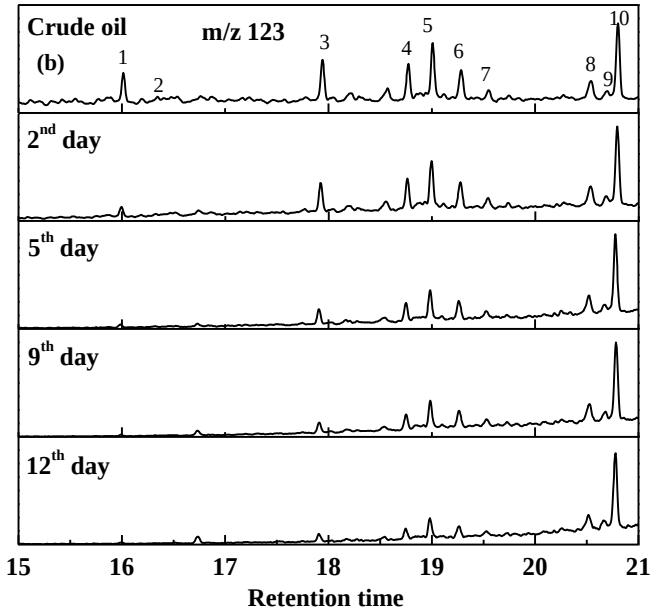


Figure 3.2 Comparison of GC-MS chromatograms of sesquiterpanes (m/z 123) in crude oil and CDO/WCO samples at the 2th, 5th, 9th, and 12th day, respectively: (a) CDO, and (b) WCO

Table 3.5 Diagnostic ratios of target sesquiterpanes in CDO and WCO samples

Time (day)	Oil	Diagnostic ratios of sesquiterpanes (average ± SD)[a]							
		3:4	3:5	4/5	5/6	8/10	8/9	3/10	5/10
2	CDO	1.19±0.08	0.72±0.06	0.61±0.04	1.70±0.12	0.35±0.03	2.50±0.19	0.49±0.03	0.68±0.05
	WCO	0.97±0.07	0.59±0.04	0.60±0.03	1.61±0.13	0.33±0.02	2.33±0.15	0.34±0.01	0.58±0.05
5	CDO	1.06±0.10	0.64±0.07	0.60±0.01	1.69±0.11	0.33±0.04	2.30±0.75	0.40±0.02	0.63±0.05
	WCO	0.80±0.10	0.48±0.06	0.61±0.05	1.34±0.06	0.29±0.06	2.32±0.54	0.15±0.03	0.31±0.04
9	CDO	1.07±0.09	0.67±0.07	0.63±0.03	1.59±0.23	0.33±0.03	2.41±0.44	0.39±0.01	0.59±0.07
	WCO	0.78±0.07	0.45±0.07	0.58±0.07	1.37±0.14	0.29±0.04	2.50±0.56	0.13±0.02	0.30±0.04
12	CDO	1.07±0.13	0.66±0.08	0.61±0.05	1.47±0.10	0.33±0.03	2.31±0.24	0.35±0.08	0.53±0.08
	WCO	0.78±0.22	0.43±0.11	0.57±0.09	1.21±0.29	0.28±0.08	1.95±0.56	0.08±0.03	0.19±0.04
Average		0.96	0.58	0.60	1.50	0.32	2.33	0.29	0.48
RSD (%)		16.70	19.32	3.37	11.83	7.46	7.48	51.04	38.17

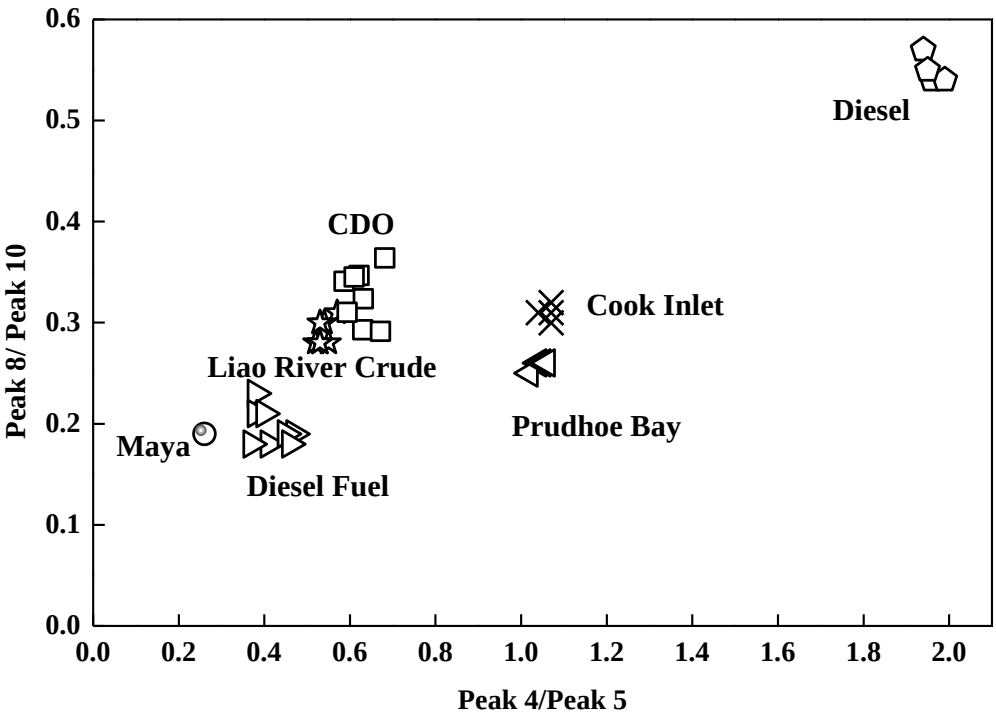
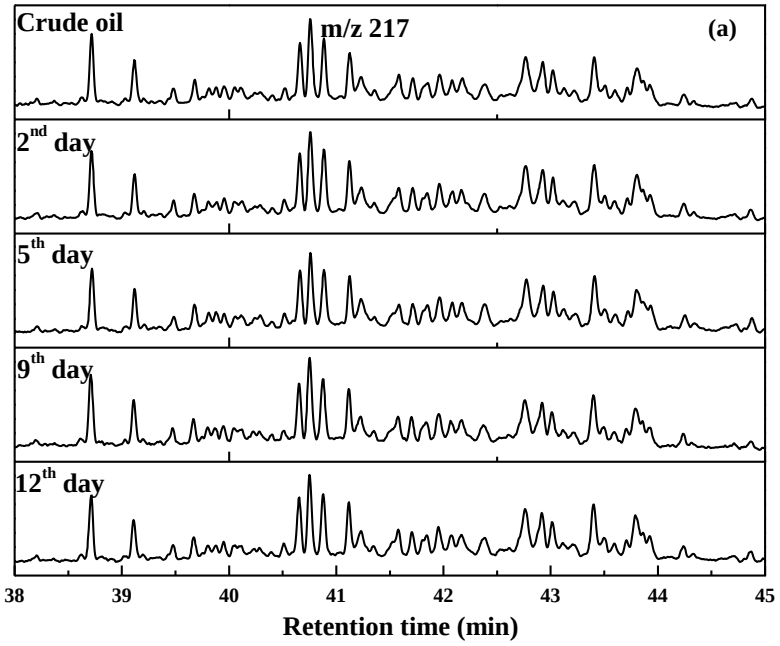


Figure 3.3 Differentiation of CDO from reference oils using double ratio plots of Peak4/Peak5 and Peak8/Peak10

The presence of these stable diagnostic ratios could avoid the overlap of the ranges of diagnostic ratios in different oils. Therefore, the family of steranes in crude oil could be used to possibly identify CDO. According to the stability of diagnostic ratios of steranes, the most fitful steranes for the selected oil are DIA27S/27R > C_{27}S/C_{27}R> C_{27}αββR/C_{27}αββS > C_{28}αββR/ C_{28}αββS> C_{29}S/C_{29}R > C_{27}S/C_{27}αββR. The suitability of steranes as biomarkers for CDO fingerprinting were further examined with the combination of terpanes in Chapter 3.3.4 owing to the lack of sufficient diagnostic ratios of steranes in reference oils.

3.3.4 The performance of terpanes in fingerprinting of CDO and WCO samples

The GC chromatograms of terpanes at m/z 191 in CDO and WCO samples were shown in Figure 3.5 (a-b). The average diagnostic ratios ±SD (n=8), and relative standard deviations were shown in Table 6. Almost all the diagnostic ratios have shown low RSD (<5%) values. All the diagnostic ratios were shown stable due to the stable characteristics of terpanes in water phase during the weathering process. Diagnostic ratios in terpanes, such as Ts/Tm and C29/C30, were usually used to examine the practical difference among different oils (Joo et al. 2013). Double ratios plots (Ts/Tm vs. C29/C30) thus were applied (Figure 3.6) to compare the ratios in CDO with those in references oils, including a spilled oil(Wang et al. 2001), BIOS (Wang and Fingas 1997), spilled oil from Detroit River (Wang et al. 2004), Kirkuk Crude oil (Mohialdeen et al. 2013), Quebec oil (Wang et al. 2001), Alberta oil sand (Yang et al. 2011), Tar Ball (Mulabagal et al. 2013), and a light refined oil (Yang et al. 2012). Results indicated that the CDO was definitly distinguished from other reference oils. Since the low RSD value of applied biomarkers in CDO and WCO samples, the differentiation of CDO from other samples did not imply that there are


Crude oil
m/z 217
(a)
2nd day
5th day
9th day
12th day
38
39
40
41
42
43
44
45
Retention time (min)

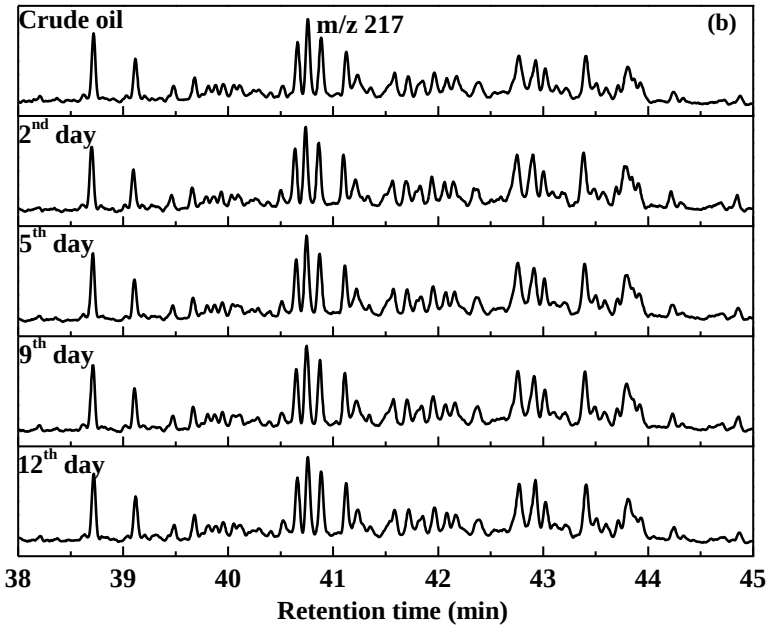
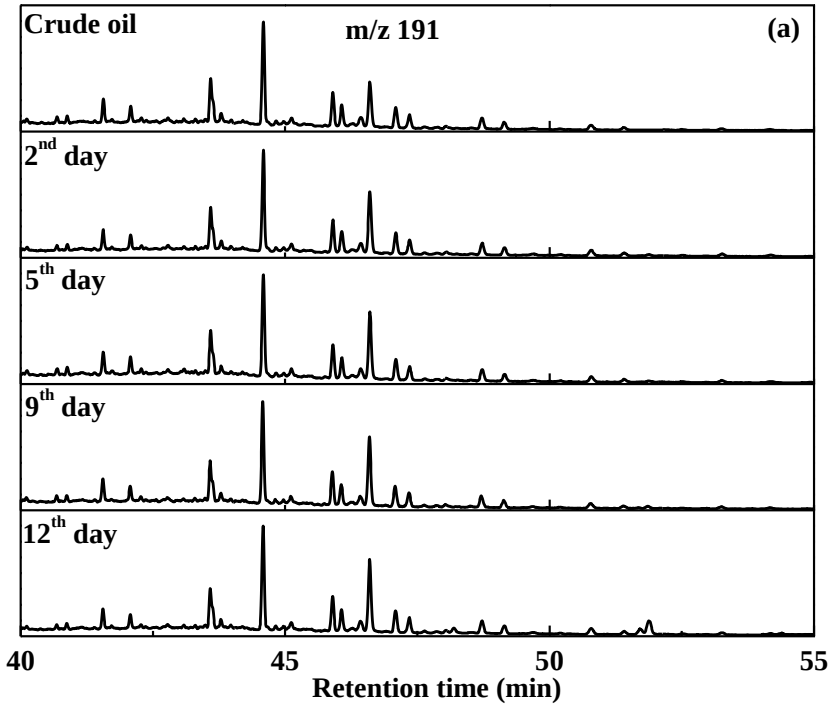


Figure 3.4 Comparison of GC-MS chromatograms of steranes (m/z 217) in crude oil and CDO/WCO samples at the 2th, 5th, 9th, and 12th day, respectively: (a) CDO, and (b) WCO


Crude oil
m/z 191
(a)
2nd day
5th day
9th day
12th day
40
45
50
55
Retention time (min)

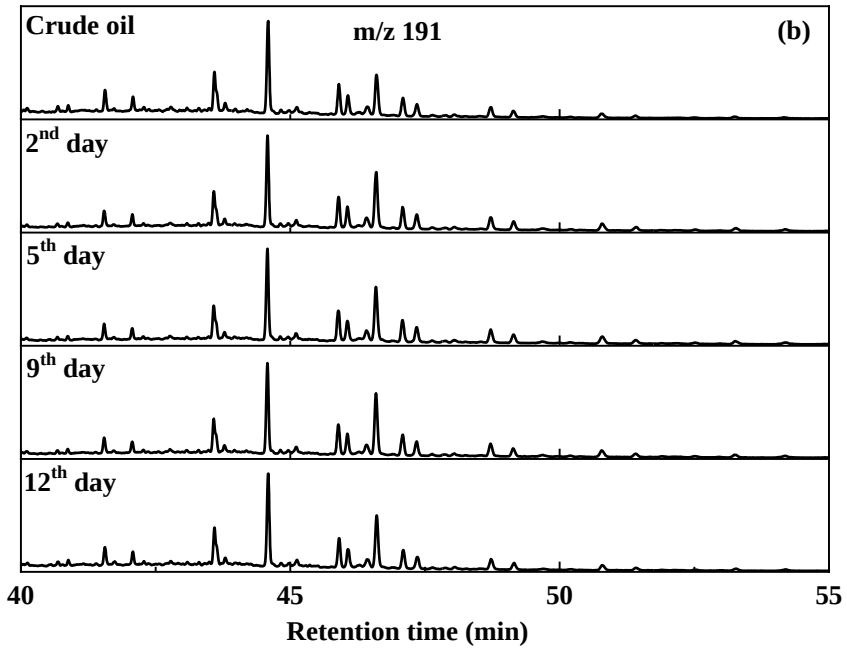


Figure 3.5 Comparison of GC-MS chromatograms of terpanes (m/z 191) in crude oil and CDO/WCO samples at the 2th, 5th, 9th, and 12th day, respectively: (a) CDO, and (b) WCO

Table 3.6 Diagnostic ratios of target steranes in CDO and WCO samples

Time (d)	Oil	Diagnostic ratios of steranes (average ± SD) [a]							
		DIA27S/27R	$C_{27}S/C_{27}\alpha\beta\beta R$	$C_{27}\alpha\beta\beta R/C_{27}\alpha\beta\beta S$	$C_{28}\alpha\beta\beta R/C_{28}\alpha\beta\beta S$	$C_{29}\alpha\beta\beta R/C_{29}\alpha\beta\beta S$	$C_{27}S/C_{27}R$	$C_{28}S/C_{28}R$	$C_{29}S/C_{29}R$
2	CDO	1.54±0.08	0.69±0.02	1.28±0.04	1.35±0.07	1.53±0.05	1.14±0.06	0.83±0.08	1.32±0.08
	WCO	1.56±0.07	0.71±0.02	1.26±0.04	1.29±0.06	1.85±0.08	1.13±0.02	0.98±0.06	1.24±0.08
5	CDO	1.53±0.07	0.79±0.03	1.22±0.03	1.36±0.06	1.47±0.06	1.19±0.04	0.92±0.06	1.32±0.08
	WCO	1.59±0.08	0.68±0.03	1.27±0.03	1.35±0.06	1.62±0.04	1.11±0.07	1.01±0.03	1.31±0.09
9	CDO	1.49±0.08	0.68±0.03	1.33±0.04	1.39±0.06	1.50±0.08	1.11±0.07	1.04±0.04	1.39±0.10
	WCO	1.57±0.08	0.69±0.03	1.26±0.03	1.32±0.05	1.71±0.08	1.13±0.06	1.02±0.04	1.27±0.07
12	CDO	1.50±0.06	0.69±0.02	1.30±0.04	1.39±0.10	1.49±0.03	1.15±0.07	1.01±0.07	1.36±0.08
	WCO	1.57±0.09	0.69±0.01	1.25±0.03	1.29±0.05	1.82±0.10	1.12±0.04	1.05±0.03	1.29±0.10
Average		1.55	0.70	1.27	1.34	1.62	1.13	0.98	1.31
RSD (%)		2.21	5.39	2.50	2.77	9.35	2.30	7.47	3.69

[a] n=8

Table 3.7 Diagnostic ratios of target terpanes in CDO and WCO samples

Time (d)	Oil	Diagnostic ratios of terpanes (average ± SD)[a]				
		Ts/Tm	C_{29}/C_{30}	$C_{31}\alpha/C_{31}\beta$	$C_{32}\alpha/C_{32}\beta$	$C_{33}\alpha/C_{33}\beta$
2	CDO	1.57±0.04	0.49±0.01	1.49±0.02	1.36±0.05	1.53±0.07
	WCO	1.52±0.15	0.45±0.01	1.46±0.04	1.33±0.03	1.43±0.06
5	CDO	1.32±0.08	0.46±0.00	1.46±0.02	1.31±0.04	1.39±0.07
	WCO	1.58±0.11	0.46±0.01	1.45±0.03	1.37±0.03	1.49±0.07
9	CDO	1.51±0.03	0.48±0.01	1.47±0.03	1.37±0.04	1.48±0.07
	WCO	1.49±0.12	0.46±0.01	1.46±0.02	1.34±0.06	1.53±0.07
12	CDO	1.50±0.07	050±0.01	1.50±0.03	1.40±0.04	1.46±0.06
	WCO	1.53±0.07	0.48±0.01	1.48±0.03	1.38±0.08	1.52±0.06
Average		1.50	0.47	1.47	1.36	1.48
RSD (%)		5.33	3.81	1.15	2.16	3.44

[a]n=8

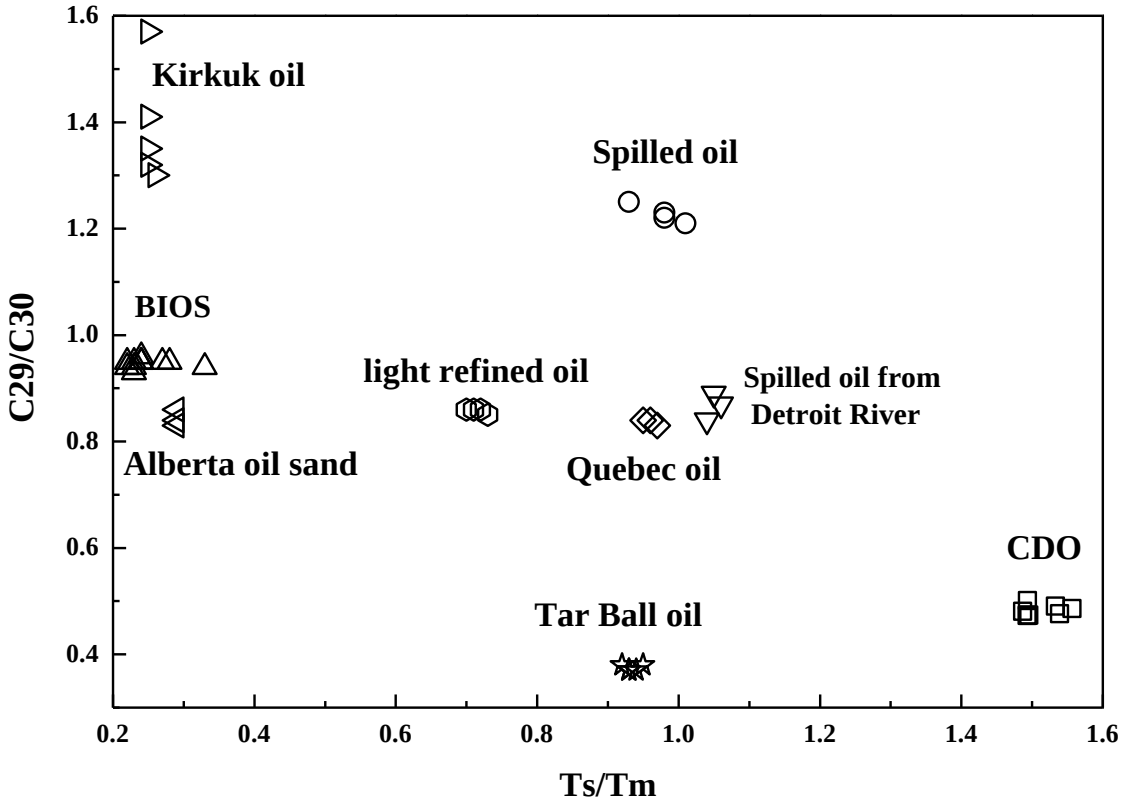


Figure 3.6 Differentiation of CDO from references oils using diagnostic ratios of terpanes (Ts/Tm, vs. C29/C30)

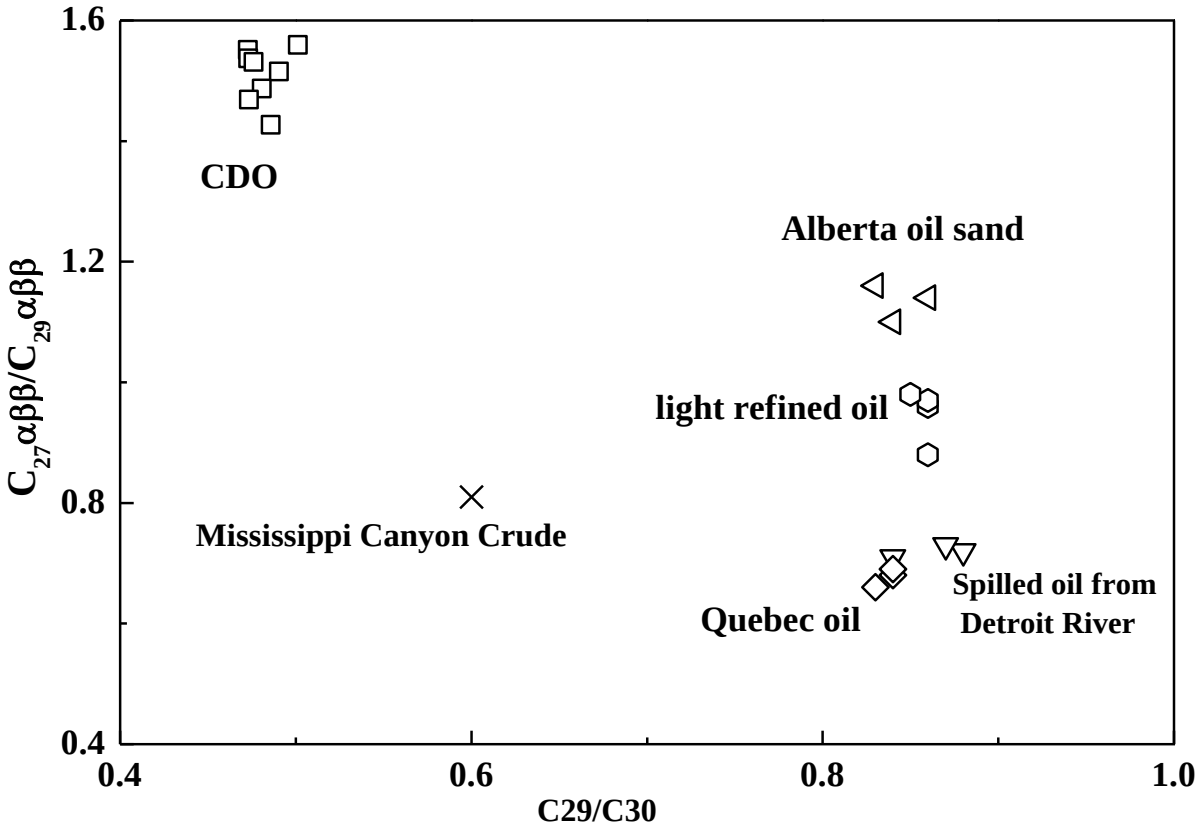


Figure 3.7 Differentiation of CDO from reference oils using the combination of diagnostic ratios of terpanes and steranes (C29/C30 vs. $C_{27}\alpha\beta\beta/C_{29}\alpha\beta\beta$)

significant difference between CDO and WCO using the selected biomarkers. Similarly, double ratios plots between terpanes and steranes could be used to distinguish CDO from other oils to examine any difficulty in oil identification caused by the statistic difference of steranes with variations of days and oil types. Double ratios plots (C29/C30 vs. $C_{27}\alpha\beta\beta/C_{29}\alpha\beta\beta$) shown in Figure 3.7 was employed to differentiate selected oil from other references oils including a light refined oil (Yang et al. 2012), a Mississippi Canyon crude oil (Yang et al. 2013), Spilled oil from Detroit River (Wang et al. 2004), Alberta oil sand (Yang et al. 2011), and an oil sand (Yang et al. 2011). Based on the successful differentiation, most of the stable steranes and all the terpanes in CDO could be used as biomarkers for source identification.

3.4 Summary

In this chapter, fingerprinting of CDO during short-term weathering was evaluated. Through the analysis of the variations of biomarkers and identification of different oils, the recommended diagnostic ratios of biomarkers were ranked.

Diagnostic ratios of sesquiterpanes in CDO and WCO were relatively unstable compared to those in crude oil samples, except the peaks 4:5 and 8:10. When double ratio plots of peaks 4:5 and 8:10 were plotted, CDO could not be identified with a biodegraded Liao River crude oil. Therefore, sesquiterpanes cannot be used as biomarker for CDO fingerprinting in seawater.

Steranes and terpanes were relatively stable in CDO and WCO samples. Based on the double ratio plots of peaks, steranes and terpanes were demonstrated to be applicable as biomarkers to identify CDO. The order of susceptibility of diagnostic ratios of steranes used as biomarkers for CDO identification from the least susceptible to the

most susceptible is DIA27S/27R > C_{27}S/C_{27}R> C_{27}αββR/C_{27}αββS > C_{28}αββR/ C_{28}αββS> C_{29}S/C_{29}R > C_{27}S/C_{27}αββR. The order of susceptibility of selected biomarkers for CDO fingerprinting from the least susceptible to the most susceptible were terpanes > steranes > sesquiterpanes due to the RSD values and the range of the diagnostic ratios in typical oils.

This research for the first time examined the stability and suitability of diagnostic ratios of sesquiterpanes, steranes, and terpanes, for CDO identification during phosichemical weathering in seawater. The output could help fulfill the gaps of CDO fingerprinting using current analysis methods and biomarkers. Future work will be conducted to monitor the performance of biomarkers in more types of oils and under more conditional factors (e g., temperature, volume ratio of dispersants to oil).

CHAPTER 4 ALIPHATIC AND AROMATIC BIOMARKERS FOR FINGERPRINTING OF WEATHERED CHEMICALLY DISPERSED OIL[2]

[2] *The contents of this chapter are based and expanded on the following paper:*

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Role: Xing Song solely worked on this study and acted as the first author of this manuscript under Dr. Baiyu Zhang, Dr. Bing Chen, and Dr. Leonard M. Lye's guidance. Most contents of this paper were written by him and further edited by the other co-authors. Ms. Li helped to conduct parts of the experiments.

4.1 Introduction

The fingerprinting of CDO using existing biomarkers is still a challenge because of limited studies in the field. The appearance of oil-in water emulsion in CDO can dramatically decrease the interface tension, change certain physical properties such as oil viscosity, and further affect the behaviours of CDO (Macnaughton et al. 2003, Swannell and Daniel 1999). Particularly, chemical properties of dispersants can diversify the weathering of CDO and crude oil (Zhuang et al. 2016). In previous studies on the effects of weathering on CDO, a few existing biomarkers for crude oil fingerprinting were directly adopted, such as terpanes and steranes. The previous chapter has discussed the stability of biomarkers for fingerprinting of dispersed oil during a short-term weathering. However, the applicability of existing oil biomarkers for CDO fingerprinting in long-term weathering are not well examined.

4.2 Material and Methods

4.2.1 Experimental materials

Main chemicals were purchased from Sigma Aldrich. Hexanes were of chromatographic grade, DCM and acetone were of analytical grade. The surrogate solution included acenaphthene-d_{10}, phenanthrene-d_{10}, perylene-d_{12}, benzo[*a*]anthracene-*d12*, and terphenyl-d_{14}, which were widely applied for recovery analysis of the targeted analytes (Gallotta and Christensen 2012, Wang et al. 2005, Wang et al. 2006b, Wang et al. 2011). C_{30} 17β(H) 21β(H)-hopane was applied as the internal standard (IS) to calibrate the surrogates (shown in Figure 4.1) as well as to calculate the recovery rate of surrogates for quality

control and quality assurance purpose. Crude oil applied was the Alaska North Slope. Silica gel (200-425 mesh) was activated at 100-110 ºC for least 48 hours before use. Sodium sulfate anhydrous was placed in an oven at 100-110 ºC for 24 hours. Glassware was washed and cleaned using chemical soap, acetone, DCM and hexane at least twice before and after use, respectively.

4.2.2 Weathering and preparation of oil samples

Crude oil samples: Every 0.8 g of crude oil was dissolved in 10 mL of hexane to prepare a crude oil sample. Two μL of internal standard was spiked into 100 μL of oil and then well mixed with hexane until the volume reached 1 mL.

Weathered crude oil (WCO) samples: Fourteen 250 mL Erlenmeyer flasks were used. In each flask, artificial seawater was generated by mixing 120 mL of distilled water and 4.32 g of artificial sea salt (36 ‰ salinity). One hundred μL of crude oil was then added into each flask. WCO samples were generated by shaking flasks using a MaxQ™ 4000 Benchtop Orbital Shaker from Thermo Fisher at a speed of 120 rpm at 30 °C for 1, 10, 20, 30, 40, 50, and 60 days, respectively.

Weathered chemically dispersed oil (CDO) samples: Another fourteen 250 mL Erlenmeyer flasks were used with 120 mL of artificial seawater (36 ‰ salinity) and 100 μL of crude oil filled in each flask. Ten μL of Corexit 9500A dispersant was subsequently added to the surface of oil slick (1:10, $v_{Corexit}/v_{oil}$) in each flask for forming stable oil-in-water emulsion. The flasks

were shaken at a speed of 120 rpm at 30 °C for 1, 10, 20, 30, 40, 50, and 60 days, respectively. Homogenously dispersed oil in the water column was found after weathering. Dispersed oil in the water column was collected near the bottom of the flasks as the CDO samples.

4.2.3 Sample analysis

Organic phase extraction from WCO samples: Fifty μL surrogate solution (contains 3.0-11.2 μg/mL of 5 surrogates) was added into each sample. Oil flumed on the surface of artificial water and adhered to the wall was transferred to a separation funnel using 20 mL DCM for five times. The extraction process was repeated twice using 100 mL of DCM each time. Residual water was thoroughly eliminated by anhydrous Na_2SO_4. DCM was removed using a rotary evaporator. Hexane was then added as a solvent. The organic phase was transferred to a concentrator and concentrated to 10 mL using a gentle nitrogen flow. From the 10 mL sample obtained after concentrating, 1 mL sample was transferred to the concentrator and further concentrated to 0.4 mL.

Organic phase extraction from CDO samples: Twelve mL of each well-dispersed CDO sample (around 1/10 volume of water) was transferred to a 20 mL graduate cylinder with the addition of surrogates. Each CDO sample was then transferred into a separation funnel. Hydrocarbons in each CDO sample were then extracted by 100 mL of DCM three times. Na_2SO_4 anhydrous was used to deplete possibly residual water. Hexane was added to each exact and DCM was removed using a

rotary evaporator. The organic phase was transferred into a 15 mL concentrator and was adjusted to 0.4 mL.

Separation of the saturate fractions and aromatic fractions: Each extracted organic phase was fractionated by a 3 g silica gel column (Wang and Stout 2010, Wang et al. 2006a). The saturate fractions (F1) were first eluted with 12 mL of hexane, and aromatic fractions (F2) were eluted with 15 mL of mixture of hexane: dichloromethane (v/v, 1:1). The F1 and F2 were then concentrated and quantified to 1 mL, respectively, followed by 150 μL of concentrated organic phase spiked into a microvial by a 500 μL syringe. Finally, 2 μL of the internal standard solution (0.1mg/mL) was spiked into each microvial.

Gas chromatography–mass spectrometry (GC-MS) analysis: The aliphatic and aromatic hydrocarbons in crude oil, WCO, and CDO samples were analyzed using a GC-MS (Agilent model 6890). A 30 m DB-5ms capillary GC column was applied. The carrier gas was Helium. The GC oven temperature was set at 50 °C for 2 min, then ramped up 6 °C/min to 300 °C for 20 minutes (Mulabagal et al. 2013, Song et al. 2016). Detailed information for GC-MS analysis was listed in Table 4.1. The SIM mode was used to analyze all the biomarkers. Two types of diagnostic ratios were calculated using peak areas (p). The diagnostic ratio for each pair of individual biomarkers was obtained by calculating the ratio of p of an individual biomarker to that of another individual biomarker in the same sample. In terms of each ratio of an individual biomarker to the set of each type of biomarkers, it was computed using the ratio of p of an individual biomarker to the sum of p of

all identified biomarkers in a selected type of biomarker group. The RSD values (Standard deviation / the average of the diagnostic ratios) were calculated to evaluate the effects of weathering on diagnostic ratios (Daling et al. 2002, Song et al. 2016, Stout et al. 2001, Wang et al. 2013a, Wang et al. 2013b).

4.2.4 Quality assurance and quality control (QA/QC)

The validity and reliability of the experimental results and biomarker analysis were evaluated using a QA/QC protocol. Four crude oil samples were prepared to indicate the original diagnostic ratios of 8 types of biomarkers. Duplicate experiments including sample preparation, weathering and extraction were conducted. Duplicate GC/MS analyses were also adopted to measure F1 and F2 in each 150 μL of organic phase sample. The diagnostic ratio of each pair of biomarkers was displayed by the average (n=8, if biomarkers are detectable) with a corresponding standard deviation. The coefficients of determination (R^2) of calibration of surrogates were > 0.9940 (n=5). The average recoveries (%) of five surrogates, including acenaphthene-d_{10}, phenanthrene-d_{10}, perylene-d_{12}, benzo[*a*]anthracene-d_{12}, and terphenyl-d_{14}, were 62%, 86%, 107%, 95%, and 113%, respectively. The recovery rates were acceptable (50-150%) referred to laboratory QA&QC standards (Dux et al. 1990, EPA 2004, Robbat Jr et al. 1999).

4.3 Results and Discussions

4.3.1 Identification of biomarkers in crude oil samples

The aliphatic biomarkers in the crude oil, including adamantanes, diamantanes,

sesquiterpanes, steranes, and terpanes, were identified in F1 and displayed in Figure 4.1 (a-e). Biomarkers were characterized by their m/z values, retention times, and distribution patterns in the chromatograms of reference oils (Wang et al. 2005, Wang et al. 2006b, Yang et al. 2009). The identified individual peaks with their abbreviations, empirical formula, and target ions are summarized in Table 3. 2 (Song et al. 2016, Wang et al. 2006a, Wang et al. 2005, Wang et al. 2006b). Adamantanes were identified at m/z 136, 135,149, 163, and 177, at F1.

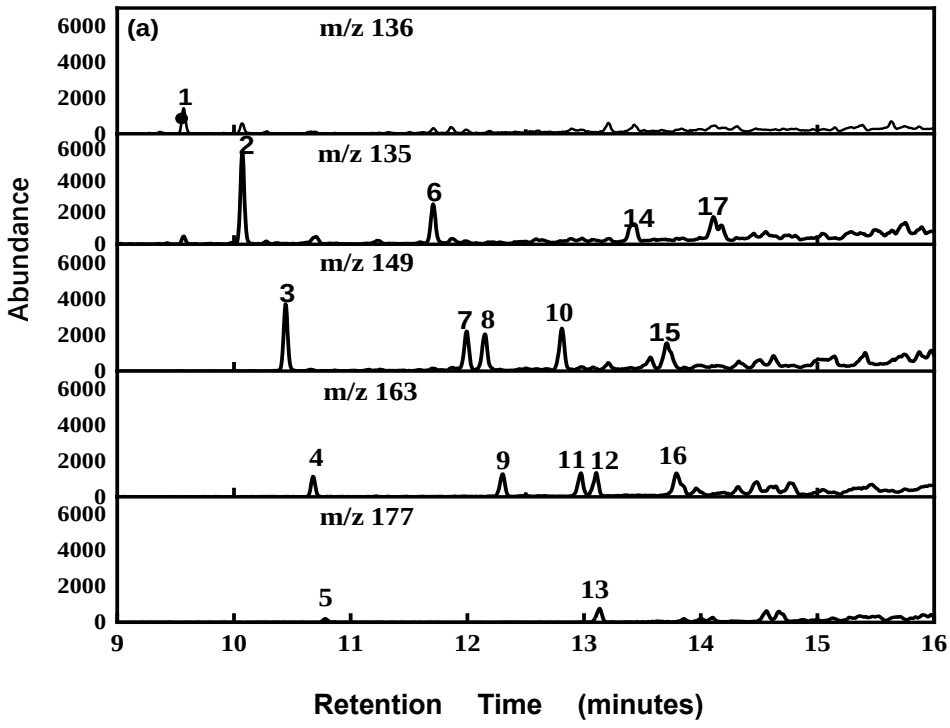
Diamondoids were found at m/z 188, 187, 201, and 215. Sesquiterpanes were identified as C14 to C16 sesquiterpanes at m/z 123, 179, 193, and 207 (Wang et al. 2005, Yang et al. 2009). Identified steranes ranged from C27 to C29 steranes at m/z 217 and 218. Major characteristic steranes were ionic steranes (C27αββR/S-C29αββR/S). All the recognized peaks of terpanes ranged from C23 to C35 at m/z 191.The structural assignments of aromatic biomarkers in crude oil, including MA-steranes, and TA- steranes in F2 were identified in Figure 4.1 (F-G). Alkylated PAHs were identified at their specific m/z (Wang and Stout 2010). Important characteristics of hydrocarbons (Wang et al. 2013a, Wang and Stout 2010, Wang et al. 2006a), such as m/z values, retention time, and chromatograms, were used to further verify the individual peaks of biomarkers. The information of individual peaks of aromatic hydrocarbons is shown in Table 3.2.

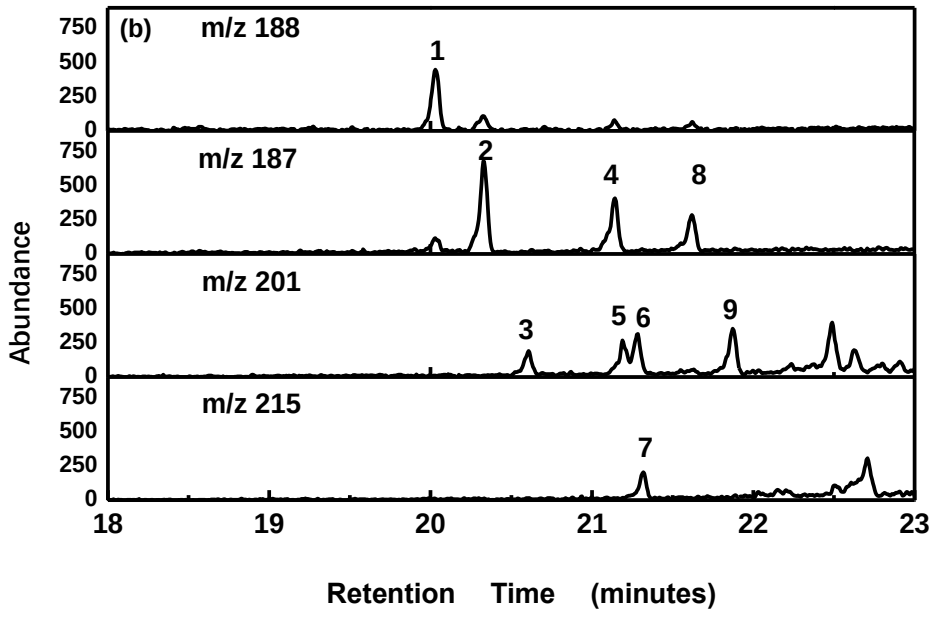
4.3.2 Aliphatic biomarkers in fingerprinting of weathered CDO and WCO samples

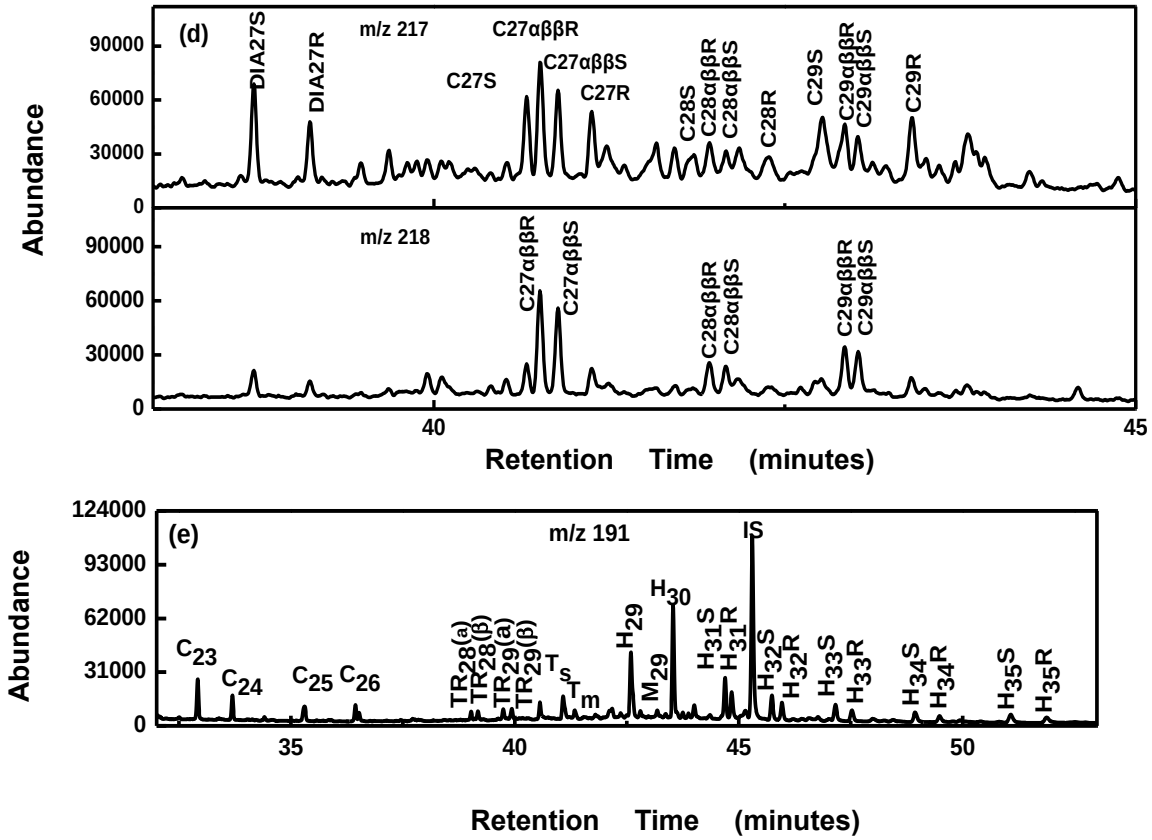
Table 4.1 shows the average diagnostic ratios of two different biomarkers within the group of detectable adamantanes. Observed from the chromatograms, the peak

areas of adamantanes in CDO dramatically decreased during weathering (Figure 4.2). The peaks of adamantanes in CDO samples nearly disappeared from the chromatograms after 40 days of weathering. The weathering noticeably influenced the diagnostic ratios between two biomarkers within the group of adamantanes in the CDO samples as well. The RSD of diagnostic ratios of p7/p8 and p11/p12 in CDO samples changed by 7.1 % and 11.5 %, respectively. These RSD values (around 5%-10%) imply the possible candidates of diagnostic ratios to fingerprint CDO. Nevertheless, the RSD of ratios between other pairs of adamantanes in CDO samples ranged from 16.1 % to 76.7 %. The high RSD values suggest a high impact of weathering on diagnostic ratios between two biomarkers within the group of adamantanes. Therefore, adamantanes are not recommended as indicators to characterize weathered CDO. However, adamantanes could be applicable to fingerprint of crude or slightly weathered oil although they could be degraded. Wang et al. have discovered that the concentration of adamantanes gradually decreased due to evaporation, however, the diagnostic ratios between two different adamantanes did not significantly changed in crude oil samples (Wang et al. 2006b).

Some of the biomarkers within diamantanes, such as p2, and p4-p9, can be found even in CDO samples after 60 days of weathering. However, the peaks of diamantanes in chromatographs after 20 days of weathering noticeably decreased and were hard to be detected. The diamantanes thus are unreliable to fingerprint CDO in a longer-term weathering (>20 days). As such, only the average value, SD,


(a)
m/z 136
m/z 135
m/z 149
m/z 163
m/z 177
Abundance
Retention Time (minutes)


(b)
m/z 188
m/z 187
m/z 201
m/z 215
Abundance
Retention Time (minutes)


(d)
m/z 217
DIA27S
DIA27R
C27S
C27αββR
C27αββS
C27R
C28S
C28αββR
C28αββS
C28R
C29S
C29αββR
C29αββS
C29R
m/z 218
C27αββR
C27αββS
C28αββR
C28αββS
C29αββR
C29αββS
Abundance
90000
60000
30000
0
40
45
Retention Time (minutes)
(e)
m/z 191
IS
C23
C24
C25
C26
TR28(a)
TR28(β)
TR29(a)
TR29(β)
Ts
Tm
H29
M29
H30
H31S
H31R
H32S
H32R
H33S
H33R
H34S
H34R
H35S
H35R
124000
93000
62000
31000
0
35
40
45
50
Abundance
Retention Time (minutes)

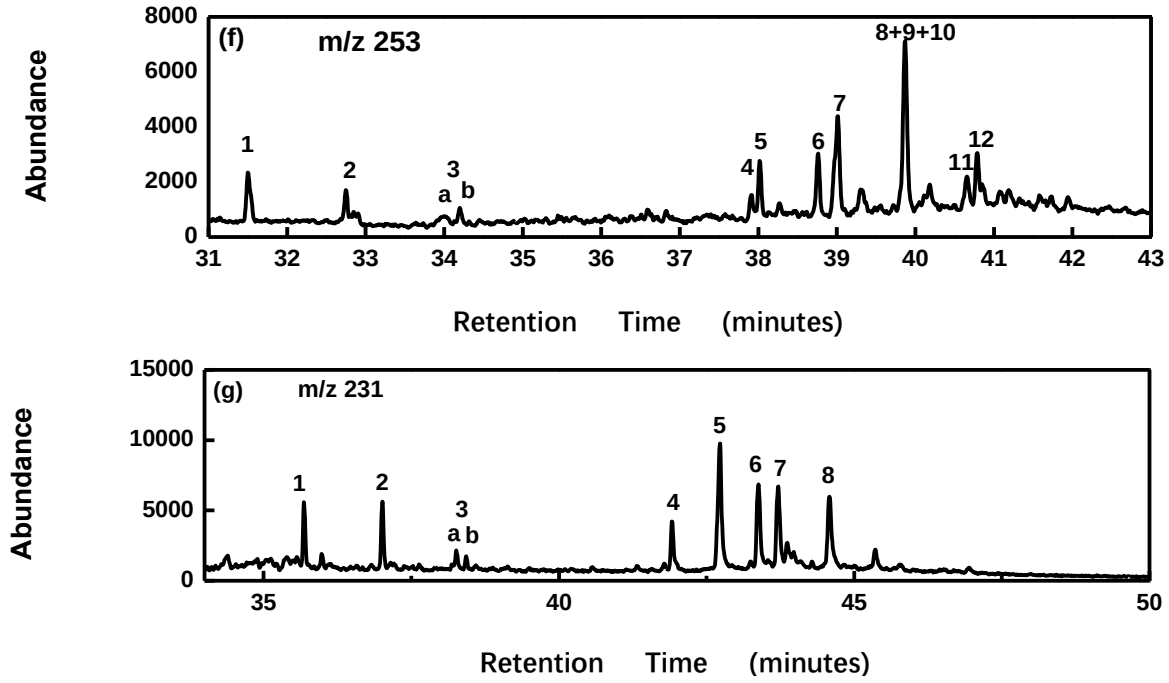


Figure 4.1 Identification of biomarkers in crude oil using GC-MS chromatograms: (a) adamantanes, (b) diamantanes, (c) sesquiterpanes, (d) steranes, (e) terpanes (f) MA-steranes, and (g) TA-steranes

and RSD of diamantanes within 20 days of weathering are available and summarized (Table 4.2) for characterization of shorter-term weathered CDO. Within the first 20 days of weathering, the diagnostic ratio of p5/p6 in CDO was not greatly changed (RSD<5%). Other diagnostic ratios in CDO were only slightly changed (RSD<10%). The ratios above can be used as a supplemental tool for identifying or confirming CDO fingerprinting in a shorter term (< 20 days). The diagnostic ratios of diamantanes in CDO samples were more stable than those in WCO judged by a lower RSD range. The 5.65 % RSD of p4/p3 in WCO samples indicated an insignificant effect by weathering, while other diagnostic ratios of diamantanes in WCO were noticeably affected by weathering (RSD>10%).

The stability of diagnostic ratios of biomarkers within the same group of diamondoids (adamantanes and diamantanes) could be correlated to different weathering conditions. Although diamondoids are not reliable candidates for fingerprinting of weathered oil for an extended weathering time, they can be applied as biomarkers for crude or slightly weathered oil under some circumstances. Wang et al. found that the distribution and concentrations of diamantanes in non-dispersed oil were affected by biodegradation while the degradation extent of diamantanes was relatively lower (Wang et al. 2006b). A similar trend was found in Wei's study (Wei et al. 2007). To our understanding, both of Wang's experiments in the dark and Wei's biodegradation in an oil reservoir did not introduce oil to the water column and did not consider photo-radiation (Wang et al. 2006b, Wei et al. 2007). In our experiments, samples were illuminated by a fluorescent lamp, which provides energy for efficient

photo-oxidation. So, both evaporation and photo-radiation may contribute to the degradation of adamantanes and diamantanes. Evaporation might play the dominant role compared to photo-oxidation due to the high volatile properties of diamondoids. Although the mechanisms and significance of photooxidation are still unclear, it may be attributed to OH- provided in the water column, which may facilitate aerobic oxidation (Wei et al. 2007). The complex composition of oils may affect the weathering of biomarkers in oil. The relative concentrations of compounds in different oils can vary widely (Wang et al. 2006a). Williams et al. (Williams et al. 1986) found that the weathering degree of the same biomarkers in different oils varied.

Our previous study showed the effects of physio-chemical weathering on sesquiterpanes in a relatively short-term (Song et al. 2016). In this study, only parts of the peaks were found in the chromatograms of CDO samples. The peak areas of sesquiterpanes decreased noticeably in a longer weathering duration in CDO samples. The changes of sesquiterpanes may be mainly attributed to evaporation in the early stages of weathering (Bao et al. 2014). The average diagnostic ratios were shown in Table 4.3. The diagnostic ratios changed with the decrease of the peak relative to those of terpanes and steranes based on RSD values. Only the diagnostic ratio of p1/p10 was relatively stable with RSD < 5% when sesquiterpanes are detectable. The diagnostic ratio of p8/p10 was slightly affected by weathering (5% < RSD < 10%) under the same circumstances. It is unclear whether photo-oxidation results in the depletion of the sesquiterpanes. The diagnostic ratios of sesquiterpanes in CDO were more stable than those in WCO, which were consistent with our previous findings. Overall, sesquiterpanes were unstable to fingerprinting long-term weathered CDO.

Table 4.1 Diagnostic ratios of some adamantanes (9 of 17) in CDO and WCO samples

Time	oil	Diagnostic ratios of adamantane (average ± SD)								
		p2/p3	p4/p9	P2/p10	P7/p8	P11/p12	P7/p10	P11/p16	P9/p11	P4/p5
1	CDO	0.96±0.16[a]	0.50±0.08	0.54±0.29	0.94 ±0.02	0.94±0.05	0.67±0.07	0.79±0.14	0.69±0.04	4.70±0.83
10	CDO	1.76[b]	1.16	2.41	0.97	0.85	1.60	0.61	0.85	NA
20	CDO	1.38±0.01[a]	0.70 ±0.02	1.50±0.02	0.87±0.01	0.87±0.03	0.78±0.01	0.56±0.02	0.64±0.00	4.12±0.42
30	CDO	1.51±0.02[a]	1.01±0.01	3.08 ±0.03	1.02±0.00	1.00±0.04	1.06±0.01	0.83±0.01	1.01±0.02	4.91±0.58
40	CDO	1.42±0.04[a]	1.00±0.07	2.61±0.00	1.04±0.06	1.12±0.11	1.08±0.07	0.88±0.09	0.94±0.07	6.10±0.60
50	CDO	NA	NA	NA	NA	NA	NA	NA	NA	NA
60	CDO	NA	NA	NA	NA	NA	NA	NA	NA	NA
Average		1.40	0.87	2.03	0.97	0.95	1.04	0.73	0.83	5.15
RSD		20.63	30.59	49.90	7.09	11.46	34.69	19.08	18.88	16.14
1	WCO	0.80±0.03[a]	0.38±0.01	0.26±0.02	1.01±0.04	0.98±0.01	0.58±0.02	0.47±0.01	0.69±0.01	2.86±0.07
10	WCO	0.72±0.03[c]	1.70±0.02	2.72±0.10	1.01±0.02	0.69 ±0.11	1.24±0.04	0.71±0.04	1.80±0.28	0.94±0.033
20	WCO	1.26±0.04[d]	2.44±2.14	4.54±0.48	0.93±0.06	0.69±0.22	1.12 ±0.12	NA	1.39 ±0.81	NA
30	WCO		NA	NA	NA	NA	NA	NA	NA	NA
40	WCO		NA	NA	NA	NA	NA	NA	NA	NA
50	WCO	1.19±0.02[a]	0.91±0.55	2.32±0.50	0.85±0.08	0.94±0.06	0.83 ±0.32	0.50±0.15	0.75±0.08	0.39±0.15
60	WCO		NA	NA	NA	NA	NA	NA	NA	NA
Average		0.99	1.17	3.00	0.92	0.83	0.93	0.48	1.20	1.62
RSD		27.52	76.20	71.71	5.54	18.65	30.36	3.49	50.01	107.42

[a] n=4; [b] n=1; [c] n=2; [d] n=3;.

Table 4.2 Diagnostic ratios of target diamantanes in CDO and WCO samples

Time	oil	Diagnostic ratios of diamantanes (average ± SD)					
		p1/p2	p2/p3	p5/p6	p4/p3	p9/p7	p4/p8
1	CDO	0.63±0.07[a]	3.22±0.55	0.92±0.16	2.35±0.41	1.75±0.13	1.19±0.16
10	CDO	0.54±0.07 [b]	2.75±0.23	0.84±0.07	2.61±0.91	1.99±0.14	1.27±0.51
20	CDO	0.55 [c]	3.29	0.88	2.10	1.73	1.36
30	CDO	2.12±0.09 [d]	2.39±0.23	0.58±0.01	4.00±0.33	4.78±0.81	0.74±0.08
40	CDO	NA	NA	NA	NA	NA	NA
50	CDO	NA	NA	NA	NA	NA	NA
60	CDO	NA	NA	NA	NA	NA	NA
Average*		0.57	3.09	0.88	2.35	1.82	1.27
RSD*		9.12	9.50	4.55	10.89	7.98	6.69
1	WCO	0.73±0.07[a]	3.62±0.32	1.02±0.04	2.30±0.10	1.72±0.14	1.22±0.10
10	WCO	0.58±0.01[e]	2.86±0.17	0.91±0.08	2.47±0.16	1.87±0.05	1.02±0.01
20	WCO	0.57±0.02[e]	1.34±0.25	0.79±0.03	2.58±0.46	2.71±0.06	0.81±0.04
30	WCO	0.11±0.02[d]	24.98±0.19	0.72±0.02	15.95±2.82	1.91±0.15	4.48±0.51
40	WCO	NA	NA	NA	NA	NA	NA
50	WCO	NA	NA	NA	NA	NA	NA
60	WCO	NA	NA	NA	NA	NA	NA
Average*		0.63	2.61	0.91	2.45	2.10	1.02
RSD*		13.92	44.40	13.04	5.65	25.50	20.34

*Average : from 0-20days, RSD, from 0 to 20 days [a] n=8; [b] n=6; [c] n=1; [d] n=2; [e] n=4.

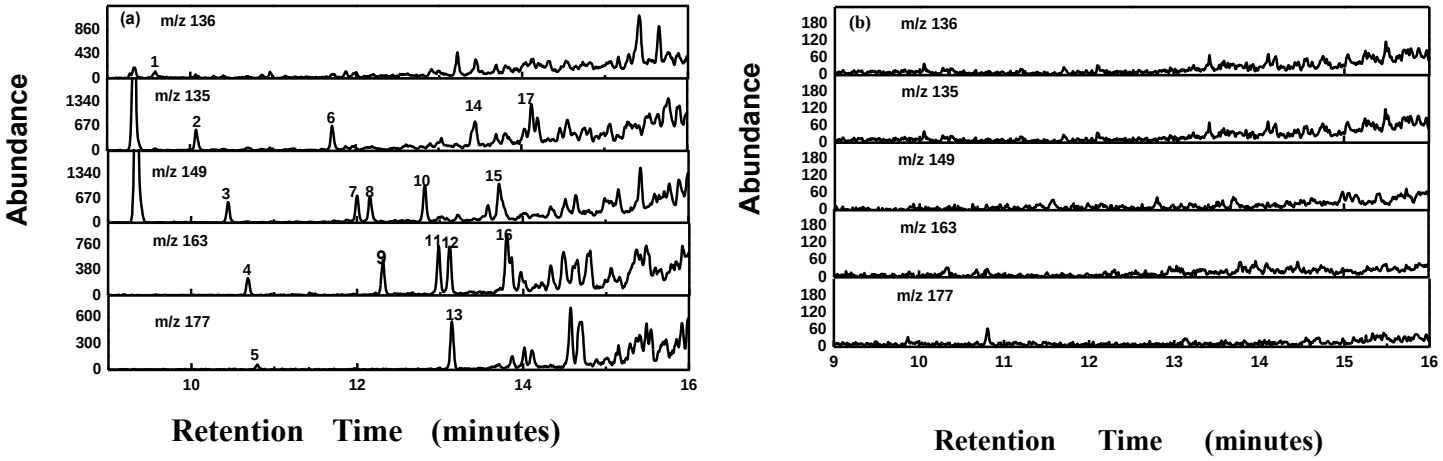


Figure 4.2 Comparison of GC-MS chromatograms of adamantanes (m/z 136, 135, 149, 163, and 177) in CDO samples at the: (a) 1st, and (b) 60th day, respectively

Table 4.3 Diagnostic ratios of target sesquiterpanes in CDO and WCO samples

Time	oil	Diagnostic ratios of sesquiterpanes (average ± SD)							
		p3/p4	p4/p5	p5/p6	p8/p9	p8/p10	p1/p5	p5/p10	p1/p10
1	CDO	1.44±0.15[a]	0.37±0.07	1.85±0.22	2.24±0.86	0.29±0.05	0.29±0.11	0.73±0.01	0.21±0.08
10	CDO	1.26±0.35[b]	0.91±0.33	0.61±0.37	1.89±0.50	0.28±0.04	NA	0.22±0.04	NA
20	CDO	1.09[c]	0.47	1.90	2.58	0.29	0.35	0.57	0.20
30	CDO	1.26[c]	0.69	1.13	1.93	0.32	0.36	0.54	0.20
40	CDO	1.02±0.07[d]	1.16±0.07	1.36±0.07	NA	0.31±0.07	NA	0.49±0.07	NA
50	CDO	NA	NA	NA	NA	NA	NA	NA	NA
60	CDO	NA	NA	NA	NA	NA	NA	NA	NA
Average		1.21	0.72	1.37	2.16	0.30	0.33	0.51	0.20
RSD		13.38	44.86	39.08	14.88	5.48	11.84	36.33	3.10
1	WCO	1.05±0.04[e]	0.41±0.05	1.84±0.29	2.37±0.48	0.30±0.04	NA	0.69±0.12	1.08±0.01
10	WCO	0.66±0.05[b]	0.31±0.04	1.95±0.35	2.16±0.39	0.28±0.00	NA	0.45	0.66
20	WCO		0.39	1.91	3.10	0.30	NA	0.18±0.08	NA
30	WCO	0.98±0.06[c]	0.71±0.08	1.40±0.34	3.58±0.71	0.40±0.03	NA	1.34±0.27	NA
40	WCO	NA	NA	NA	NA	NA	NA	NA	NA
50	WCO	NA	NA	NA	NA	NA	NA	NA	NA
60	WCO	NA	NA	NA	NA	NA	NA	NA	NA
Average		0.90	0.48	1.73	2.70	0.33		0.83	
RSD		22.81	44.13	16.75	28.45	19.01		55.78	

[a] n=6; [b] n=4; [c] n=2; [d] n=1; [e] n=8.

Table 4.4 Diagnostic ratios of target steranes in CDO and WCO samples

Time	oil	Diagnostic ratios of MA-steranes (average ± SD)					
		p1/p2	p1/p8	p8/p10	p10/p11	p7/p11	p7/p8
1	CDO	1.54±0.05	0.62±0.02	1.59±0.03	0.65±0.02	0.50±0.03	0.48±0.03
10	CDO	1.49±0.04	0.59±0.01	1.67±0.06	0.66±0.03	0.48±0.04	0.43±0.02
20	CDO	1.57±0.03	0.61±0.02	1.70±0.03	0.65±0.02	0.48±0.03	0.44±0.01
30	CDO	1. 55±0.03	0.62±0.06	1.64±0.13	0.65±0.01	0.49±0.01	0.46±0.04
40	CDO	1.57±0.04	0.60±0.02	1.72±0.10	0.64±0.04	0.48±0.02	0.44±0.03
50	CDO	1.56±0.05	0.59±0.01	1.64±0.02	0.68±0.02	0.50±0.01	0.45±0.01
60	CDO	1.58±0.08	0.63±0.13	1.72±0.01	0.64±0.03	0.48±0.04	0.44±0.02
Average		1.55	0.61	1.67	0.65	0.49	0.45
RSD		1.92	2.25	2.88	2.10	1.88	3.85
1	WCO	1.51±0.07	0.61±0.04	1.68±0.06	0.68±0.03	0.50±0.03	0.43±0.02
10	WCO	1.56±0.04	0.55±0.01	1.67±0.03	0.64±0.03	0.51±0.04	0.45±0.03
20	WCO	1.55±0.04	0.58±0.03	1.59±0.08	0.68±0.05	0.48±0.03	0.45±0.01
30	WCO	1. 1.55±0.09	0.61±0.05	1.63±0.06	0.66±0.03	0.50±0.02	0.47±0.02
40	WCO	1.55±0.06	0.62±0.04	1.52±0.12	0.68±0.08	0.54±0.05	0.52±0.04
50	WCO	1.54±0.03	0.63±0.05	1.71±0.04	0.68±0.02	0.46±0.01	0.43±0.01
60	WCO	1.55±0.04	0.60±0.02	1.69±0.07	0.64±0.05	0.48±0.02	0.44±0.01
Average		1.55	0.60	1.63	0.66	0.50	0.46
RSD		0.39	5.06	4.21	3.45	5.47	6.95
		p13/p14	p14/p15	p13/p16	p17/p18	p18/p19	p17/p20
1	CDO	0.86±0.04	1.06±0.06	0.89±0.02	0.76±0.02	1.47±0.03	0.92±0.05

10	CDO	0.82±0.02	1.06±0.03	0.89±0.03	0.77±0.04	1.44±0.06	0.96±0.07
20	CDO	0.86±0.02	1.10±0.01	0.97±0.03	0.82±0.04	1.49±0.04	1.04±0.06
30	CDO	0.85±0.03	1.07±0.02	0.97±0.02	0.73±0.02	1.49±0.02	0.95±0.05
40	CDO	0.86±0.02	1.11±0.05	0.96±0.04	0.72±0.03	1.51±0.05	0.94±0.04
50	CDO	0.85±0.02	1.09±0.02	0.95±0.02	0.73±0.02	1.50±0.08	0.93±0.03
60	CDO	0.92±0.03	1.02±0.04	1.00±0.10	0.75±0.03	1.42±0.05	0.95±0.06
Average		0.86	1.07	0.95	0.75	1.47	0.96
RSD		3.25	3.00	4.56	4.52	2.24	4.25
1	WCO	0.89±0.06	1.03±0.05	0.96±0.12	0.75±0.09	1.47±0.02	0.95±0.08
10	WCO	0.94±0.02	0.99±0.01	0.99±0.08	0.75±0.01	1.43±0.02	0.95±0.03
20	WCO	0.96±0.05	1.02±0.02	1.01±0.07	0.74±0.02	1.38±0.09	0.95±0.03
30	WCO	0.90±0.03	0.99±0.03	0.92±0.07	0.76±0.01	1.44±0.04	0.92±0.06
40	WCO	0.91±0.04	1.04±0.04	0.94±0.10	0.80±0.05	1.44±0.03	0.95±0.06
50	WCO	0.87±0.04	1.02±0.04	0.94±0.02	0.75±0.04	1.38±0.07	1.04±0.10
60	WCO	0.88±0.04	1.04±0.04	0.93±0.01	0.74±0.02	1.48±0.06	0.92±0.03
Average		0.91	1.02	0.95	0.76	1.42	0.96
RSD		3.70	2.12	3.80	2.67	2.69	4.81

Table 4.5 Diagnostic ratios of target terpanes in CDO and WCO samples

Time	oil	Diagnostic ratios of MA-steranes (average ± SD)					
		C23/C24	C25/C26	TR28a/b	TR29a/b	Ts/Tm	C29/C30
1	CDO	1.67±0.10	1.42±0.10	0.89±0.13	0.90±0.03	0.60±0.03	0.72±0.04
10	CDO	1.54±0.12	1.29±0.06	0.79±0.04	0.92±0.07	0.65±0.02	0.68±0.01
20	CDO	1.59±0.06	1.37±0.04	0.81±0.02	0.93±0.04	0.65±0.06	0.72±0.05
30	CDO	1. 62±0.05	1.27±0.06	0.80±0.02	0.93±0.02	0.61±0.02	0.74±0.01
40	CDO	1.59±0.06	1.31±0.08	0.78±0.02	1.01±0.05	0.63±0.03	0.73±0.01
50	CDO	1.71±0.08	1.30±0.07	0.79±0.02	0.93±0.03	0.60±0.03	0.72±0.01
60	CDO	1.70±0.06	1.53±0.14	0.91±0.04	0.96±0.03	0.68±0.02	0.77±0.08
Average		1.64	1.36	0.82	0.94	0.63	0.73
RSD		3.75	6.82	6.53	3.76	4.77	3.41
1	WCO	1.66±0.08	1.35±0.11	0.88±0.10	0.89±0.06	0.59±0.04	0.71±0.02
10	WCO	1.61±0.03	1.30±0.06	0.97±0.02	0.93±0.01	0.63±0.01	0.73±0.01
20	WCO	1.69±0.05	1.33±0.03	0.94±0.02	0.90±0.02	0.64±0.01	0.74±0.01
30	WCO	1.65±0.13	1.40±0.07	0.94±0.05	0.94±0.02	0.64±0.03	0.74±0.02
40	WCO	1.67±0.02	1.42±0.07	0.96±0.02	0.96±0.02	0.63±0.01	0.70±0.02
50	WCO	1.73±0.08	1.35±0.07	0.89±0.07	0.91±0.04	0.62±0.03	0.72±0.02
60	WCO	1.67±0.02	1.31±0.04	0.88±0.10	0.92±0.03	0.61±0.03	0.71±0.02
Average		1.67	1.35	0.92	0.92	0.62	0.72
RSD		2.06	3.16	3.93	2.43	3.17	2.37
		H31S/H31R	H32S/H32R	H33S/H33R	H34S/H34R	H35S/H35R	
1		1.38±0.05	1.33±0.10	1.44±0.09	1.61±0.15	1.34±0.11	
10		1.43±0.03	1.32±0.06	1.40±0.12	1.64±0.09	1.44±0.06	

20	1.37±0.05	1.31±0.03	1.55±0.03	1.66±0.11	1.22±0.11
30	1.36±0.02	1.31±0.03	1.45±0.05	1.65±0.07	1.24±0.05
40	1.38±0.03	1.28±0.01	1.52±0.06	1.65±0.03	1.29±0.05
50	1.35±0.01	1.30±0.02	1.48±0.04	1.58±0.03	1.20±0.03
60	1.50±0.01	1.27±0.07	1.52±0.06	1.59±0.09	1.41±0.07
Average	1.39	1.30	1.48	1.62	1.30
RSD	3.82	1.68	3.60	1.87	7.17
1	1.35±0.07	1.32±0.07	1.42±0.09	1.53±0.06	1.22±0.16
10	1.38±0.10	1.27±0.01	1.50±0.03	1.61±0.04	1.33±0.14
20	1.55±0.02	1.26±0.01	1.55±0.01	1.64±0.04	1.34±0.02
30	1.39±0.10	1.24±0.08	1.49±0.05	1.59±0.08	1.27±0.06
40	1.48±0.07	1.24±0.01	1.57±0.03	1.56±0.03	1.47±0.03
50	1.51±0.04	1.28±0.03	1.53±0.09	1.63±0.05	1.41±0.14
60	1.40±0.17	1.29±0.04	1.49±0.04	1.52±0.15	1.36±0.12
Average	1.44	1.27	1.51	1.58	1.34
RSD	5.16	2.18	3.37	3.02	6.21

As reported by Song et al. (2016), almost all the diagnostic ratios between two biomarkers within steranes and those within terpanes were constant with a relatively low or medium extent (Song et al. 2016). The diagnostic ratios between two biomarkers within steranes and those within terpanes within 60 days of weathering were examined, respectively. The diagnostic ratios (average ± standard deviation (SD), n=8) with RSD of steranes (m/z 217) are summarized in Table 4.4, and terpanes (m/z 191) are listed in Table 4.5. RSD values of the majority of diagnostic ratios were lower than 5%, which means that the diagnostic ratios between two biomarkers within steranes and those within terpanes in CDO samples are recalcitrant to the weathering. The diagnostic ratios of targeted biomarkers belonging to steranes and terpanes in WCO were unaffected by weathering as well; that is, steranes and terpanes could be applicable to fingerprinting of both CDO and WCO samples.

4.3.3 Aromatic biomarkers for fingerprinting of weathered CDO and WCO samples

Table 4.6 shows the diagnostic ratios (average ± SD, n = 8), and RSD of TA-steranes in CDO and WCO samples. The RSD of most ratios were less than 5% implying a stable status of ratios during weathering (Figure 4.3). The fingerprinting of CDO using TA-steranes is reliable with weathering and the application of dispersants. The diagnostic ratios (average ± SD, n =8), as well as RSD of MA-steranes in CDO and WCO are given in Table 4.7. The ratios of MA-steranes (Figure 4.3) are stable with low RSD (< 5%). Some isomers of TA- steranes and MA- steranes may be good candidates for oil fingerprinting in CDO due to their similarities although aromatic steranes can be degraded. Both TA-steranes and MA-steranes were grouped based on

the different RSD values of diagnostic ratios between two biomarkers (Figure 4.3). The RSD values of diagnostic ratios between two biomarkers of the same sub-group (e.g. 2.12-5.37 for CDO) were lower than those (6.82-8.42 for CDO) RSD values of diagnostic ratios between two biomarkers belonging to different sub-groups. Therefore, MA-steranes were divided into two subgroups, sub-group 1 (p1-p2) and sub-group 2 (p4-p11). TA-steranes were divided into two subgroups as well, sub-group 1 (p1-p2) and sub-group 2 (p4-p9). This phenomenon is probably due to the small differences in separation efficiencies between two groups of the same aromatic steranes during the fractionation process. Occasional loss of fractions during fractionation can change the proportion of biomarkers between inside and outside the groups of the same type.

The fates of weathering of alkylated-PAHs in aquatic environment were investigated by many researchers (Bacosa et al. 2015, Stout et al. 2016). Alkylated-PAHs with relatively low molecule weights, such as alkylated naphthalene and phenanthrenes, can be affected by evaporation. Alkylated PAHs may also be photo-oxidized (Bacosa et al. 2015). Some alkylated PAHs such as C1-C and C1-F, have relatively high resistance to weathering (Bacosa et al. 2015). However, few studies focused on the changes of diagnostic ratios of alkylated-PAHs in CDO for oil fingerprinting. Table 4.8 shows a summary of the diagnostic ratios of alkylated–PAHs in CDO and WCO. The results indicated that diagnostic ratios of most determined alkylated-PAHs fluctuated during weathering with high RSD values. A few diagnostic ratios, such as C4-N/C1-F, C2-P/C4-P, and C1-C/C2-C, possessed relatively high resistance to weathering (RSD < 10%). Relatively stable diagnostic ratios between two

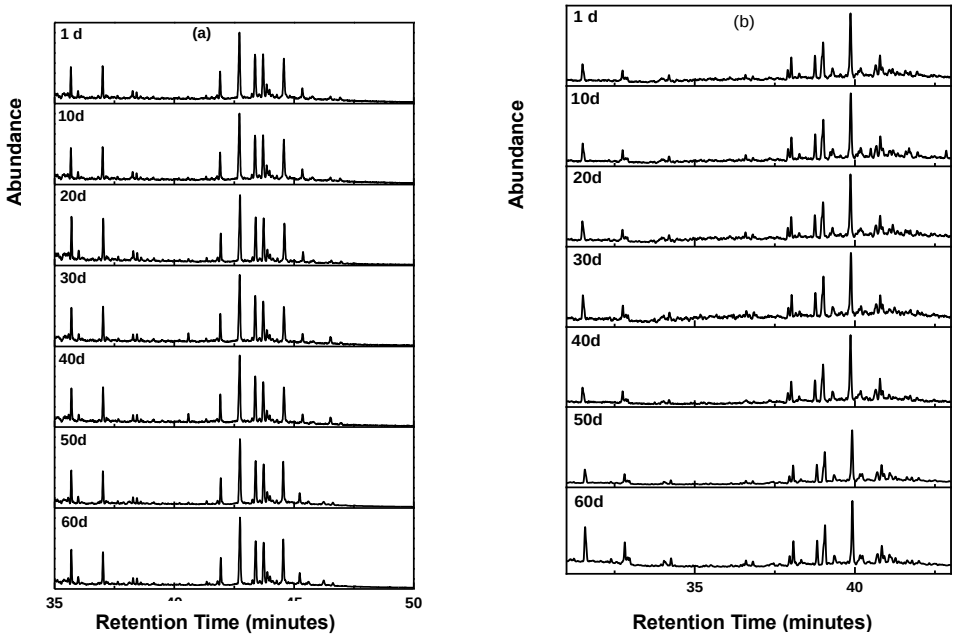


Figure 4.3 Comparison of GC-MS chromatograms of (a) TA-steranes (m/z 231) and (b) MA-steranes (m/z 253) in CDO samples at the 1st, 10th, 20th, 30th, 40th, 50th and 60th day, respectively

Table 4.6 Diagnostic ratios of target TA-steranes in CDO and WCO samples

time	oil	Diagnostic ratios of TA-steranes (average ± SD)					
		p1/p2	3a/3b	p4/p5	p5/p6	p6/p7	p7/p8
1	CDO	0.94±0.06	0.98±0.08	0.28±0.01	1.63±0.05	1.01±0.01	1.00±0.04
10	CDO	0.88±0.02	1.04±0.09	0.29±0.02	1.64±0.04	1.01±0.02	1.05±0.08
20	CDO	0.90±0.05	1.06±0.06	0.29±0.01	1.62±0.05	1.01±0.01	1.06±0.10
30	CDO	0.87±0.01	0.95±0.06	0.29±0.01	1.55±0.14	1.11±0.12	1.09±0.04
40	CDO	0.88±0.02	1.01±0.05	0.29±0.01	1.58±0.07	1.11±0.05	1.04±0.09
50	CDO	0.87±0.02	1.05±0.12	0.29±0.01	1.57±0.03	1.05±0.02	1.04±0.05
60	CDO	0.93±0.06	1.15±0.13	0.31±0.01	1.62±0.04	1.03±0.02	1.04±0.09
Average		0.90	1.03	0.29	1.60	1.05	1.05
RSD		2.96	5.37	2.72	2.12	4.45	2.68
1	WCO	0.93±0.07	0.97±0.06	0.28±0.01	1.64±0.06	0.98±0.06	1.11±0.05
10	WCO	0.90±0.06	0.92±0.04	0.30±0.02	1.59±0.02	1.01±0.01	1.02±0.06
20	WCO	0.92±0.03	0.99±0.04	0.31±0.01	1.59±0.06	1.04±0.05	1.01±0.07
30	WCO	0.94±0.06	1.01±0.10	0.30±0.02	1.60±0.05	1.03±0.01	1.04±0.06
40	WCO	0.95±0.07	0.95±0.03	0.30±0.01	1.62±0.04	0.99±0.02	1.00±0.06
50	WCO	0.95±0.10	0.98±0.04	0.30±0.02	1.62±0.08	1.03±0.02	1.00±0.05
60	WCO	0.92±0.03	0.97±0.03	0.28±0.00	1.58±0.02	1.01±0.02	1.05±0.03
Average		0.93	0.97	0.30	1.61	1.01	1.03
RSD		2.15	3.02	3.31	1.38	2.01	3.70

Table 4.7 Diagnostic ratios of target MA-steranes in CDO and WCO samples

Time	oil	Diagnostic ratios of MA-steranes (average ± SD)						
		p1/p2	p4/p5	p5/p6	p6/p7	p7/p8	p11/p12	p6/p12
1	CDO	1.48±0.09	0.62±0.06	0.74±0.09	0.44±0.02	0.63±0.04	0.91±0.06	1.12±0.06
10	CDO	1.45±0.06	0.63±0.05	0.80±0.08	0.43±0.02	0.66±0.01	0.80±0.06	1.08±0.04
20	CDO	1.48±0.06	0.64±0.07	0.83±0.09	0.43±0.02	0.68±0.03	0.84±0.04	1.14±0.03
30	CDO	1.61±0.20	0.62±0.07	0.83±0.05	0.43±0.01	0.70±0.02	0.79±0.04	1.12±0.04
40	CDO	1.60±0.16	0.58±0.07	0.91±0.03	0.40±0.01	0.69±0.01	0.80±0.07	1.05±0.03
50	CDO	1.57±0.04	0.54±0.02	0.82±0.03	0.44±0.01	0.69±0.01	0.78±0.04	1.15±0.04
60	CDO	1.54±0.08	0.56±0.06	0.86±0.06	0.44±0.01	0.66±0.02	0.82±0.03	1.12±0.09
Average		1.54	0.60	0.83	0.43	0.67	0.82	1.11
RSD		4.37	6.32	6.45	2.87	3.45	5.46	3.07
1	WCO	1.61±0.16	0.54±0.04	0.79±0.06	0.44±0.02	0.67±0.03	0.93±0.03	1.13±0.04
10	WCO	1.62±0.06	0.56±0.05	0.79±0.02	0.43±0.01	0.67±0.02	0.91±0.08	1.10±0.06
20	WCO	1.67±0.05	0.56±0.05	0.81±0.08	0.44±0.01	0.65±0.03	0.93±0.10	1.15±0.02
30	WCO	1.59±0.09	0.52±0.02	0.88±0.09	0.45±0.01	0.68±0.02	0.81±0.09	1.18±0.10
40	WCO	1.61±0.05	0.65±0.09	0.75±0.04	0.42±0.02	0.63±0.02	0.89±0.07	1.13±0.04
50	WCO	1.50±0.03	0.56±0.03	0.80±0.04	0.45±0.02	0.65±0.04	0.82±0.03	1.14±0.14
60	WCO	1.60±0.09	0.53±0.04	0.82±0.06	0.46±0.03	0.66±0.02	0.85±0.02	1.10±0.12
Average		1.60	0.56	0.81	0.44	0.66	0.88	1.13
RSD		3.21	7.63	4.95	2.81	2.33	5.57	2.45

Table 4.8 Diagnostic ratios of alkylated PAHs in CDO and WCO samples

Time	oil	Diagnostic ratios of MA-steranes (average ± SD)					
		C3-N/C4-N	C4-N/C1-F	C4-N/C4-P	C4-N/C1-F	C2-P/C1-C	C1-F/C1-C
1	CDO	3.74±0.23	0.47±0.02	2.48±1.80	1.86±0.06	5.94±0.01	1.70±0.32
10	CDO	2.08±0.67	0.40±0.04	2.27±1.68	1.93±0.05	7.65±0.10	1.46±1.63
20	CDO	0.75±0.23	0.27±0.01	2.69±0.04	1.82±0.10	5.70±0.16	0.75±0.16
30	CDO	0.79±0.06	0.29±0.04	3.34±0.80	1.95±0.19	7.90±0.14	1.25±2.65
40	CDO	1.09±0.53	0.16±0.01	0.89±0.50	1.78±0.08	9.48±0.08	0.79±2.51
50	CDO	0.22±0.09	0.19±0.04	1.39±0.94	1.81±0.18	8.50±0.71	0.95±1.87
60	CDO	0.55±0.32	0.20±0.06	2.71±1.55	1.61±0.04	8.07±0.1.92	1.24±1.92
Average		1.32	0.28	2.25	1.82	7.61	1.16
RSD		92.62	40.31	37.35	6.13	17.82	30.38
1	WCO	3.57±0.27	0.51±0.03	2.79±2.20	1.90±0.07	6.74±2.73	2.00±0.53
10	WCO	2.89±0.56	0.23±0.10	2.65±1.50	1.40±0.15	5.37±0.22	0.96±0.45
20	WCO	1.24±0.02	0.32±0.01	4.24±0.55	1.74±0.03	6.13±1.04	1.28±0.28
30	WCO	4.96	0.05	0.57±0.02	1.71±0.07	5.53±2.56	0.24±0.01
40	WCO	0.54±0.04	0.18±0.04	2.55±0.06	1.46±0.20	6.34±0.70	0.92±0.08
50	WCO	0.24±0.12	0.13±0.04	1.75±0.81	1.75±0.07	8.54±2.81	0.73±0.41
60	WCO	0.92±0.308	0.19±0.12	2.14±1.46	1.78±0.06	6.16±0.35	1.02±0.03
Average		2.05	0.23	2.38	1.68	6.40	1.02
RSD		86.76	65.69	46.75	10.80	16.47	52.48
		C2-P/C4-P	C1-F/C2-F	C1-C/C2-C	C4-P/C1-F	C4-P/C1-C	
1	CDO	9.61±0.07	0.85±0.04	1.04±0.03	1.25±0.12	0.59±0.01	
10	CDO	9.66±0.12	0.75±0.35	1.05±0.05	1.66±0.15	0.61±0.02	
20	CDO	9.63±0.45	0.39±0.26	1.04±0.04	0.67±0.03	0.59±0.04	

30	CDO	11.33±1.55	0.54±0.03	1.17±0.08	0.59±0.09	0.70±0.24
40	CDO	9.44±0.19	0.31±0.04	118±0.07	1.36±1.47	1.25±0.03
50	CDO	9.49±0.67	0.40±0.04	1.13±0.09	2.26±1.31	1.06±0.07
60	CDO	10.91±1.39	0.49±0.09	1.14±0.18	0.57±0.26	0.65±0.02
Average		10.04	0.52	1.11	1.19	0.78
RSD		7.53	33.77	5.41	53.13	34.01
1	WCO	9.50	0.83±0.06	1.01±0.17	0.30±0.41	0.50±1.13
10	WCO	10.35±0.54	0.57±0.18	1.09±0.11	0.64±0.24	0.52±0.03
20	WCO	10.99±0.65	0.66±0.03	1.15±0.13	0.43±0.05	0.56±0.06
30	WCO	9.20±2.39	0.22±0.01	1.21±0.33	2.99±0.21	0.73±0.12
40	WCO	12.15±1.67	1.05±1.21	1.21±0.17	0.59±0.07	0.53±0.02
50	WCO	12.28±2.04	0.31±0.07	1.19±0.11	0.76±0.88	0.76±0.13
60	WCO	10.58±0.30	0.57±0.08	0.98±0.26	0.58±0.02	0.59±0.03
Average		10.72	0.60	1.12	1.07	0.56
RSD		11.11	47.88	8.34	83.80	10.70

biomarkers thus probably imply the same extend of weathering of two biomarkers within the stable ratios or insignificant weathering effect on these biomarkers. The ratios that are only slightly influenced (RSD < 10%) by weathering can be applied to CDO fingerprinting within certain duration.

4.3.4 The stability of diagnostic ratios of the same types of biomarkers in CDO

The resistance of weathering of all the biomarkers is indicated in Figure 4.4. The stability of diagnostic ratios evaluated by RSD values: RSD < 5%, "Unaffected"; 5% < RSD < 10%, "Slightly affected", and RSD > 10%, "Affected" are displayed in Y-axis. The X-axis displays the degree of the resistance of biomarkers to weathering based on the depletion of biomarkers relative to terpanes/steranes and the performance of biomarkers during different weathering processes in the literature (Aeppli et al. 2014, Stout et al. 2016). As some studies on the weathering of biomarkers indicated (Mulabagal et al. 2013, Wang et al. 1998a), steranes and terpanes in weathered oil flume, and oil treated by dispersants, had high-resistance to weathering. The weathering does not affect the diagnostic ratios between two biomarkers within steranes and those within terpanes (RSD < 5%) in weathered CDO. Most of the biomarkers in terpanes and steranes could be well applied to fingerprint weathered CDO. Although TA-steranes have the potential to be photo-oxidized according to Stout's study (Stout et al. 2016), the diagnostic ratios of TA-steranes in CDO did not changed markedly. The same result was observed for the ratios of MA-steranes. Only a few diagnostic ratios of alkylated PAHs in CDO were unaffected or slightly affected by weathering. Other biomarkers, including adamantanes, diamantanes, and sesquiterpanes, were degradable with unstable

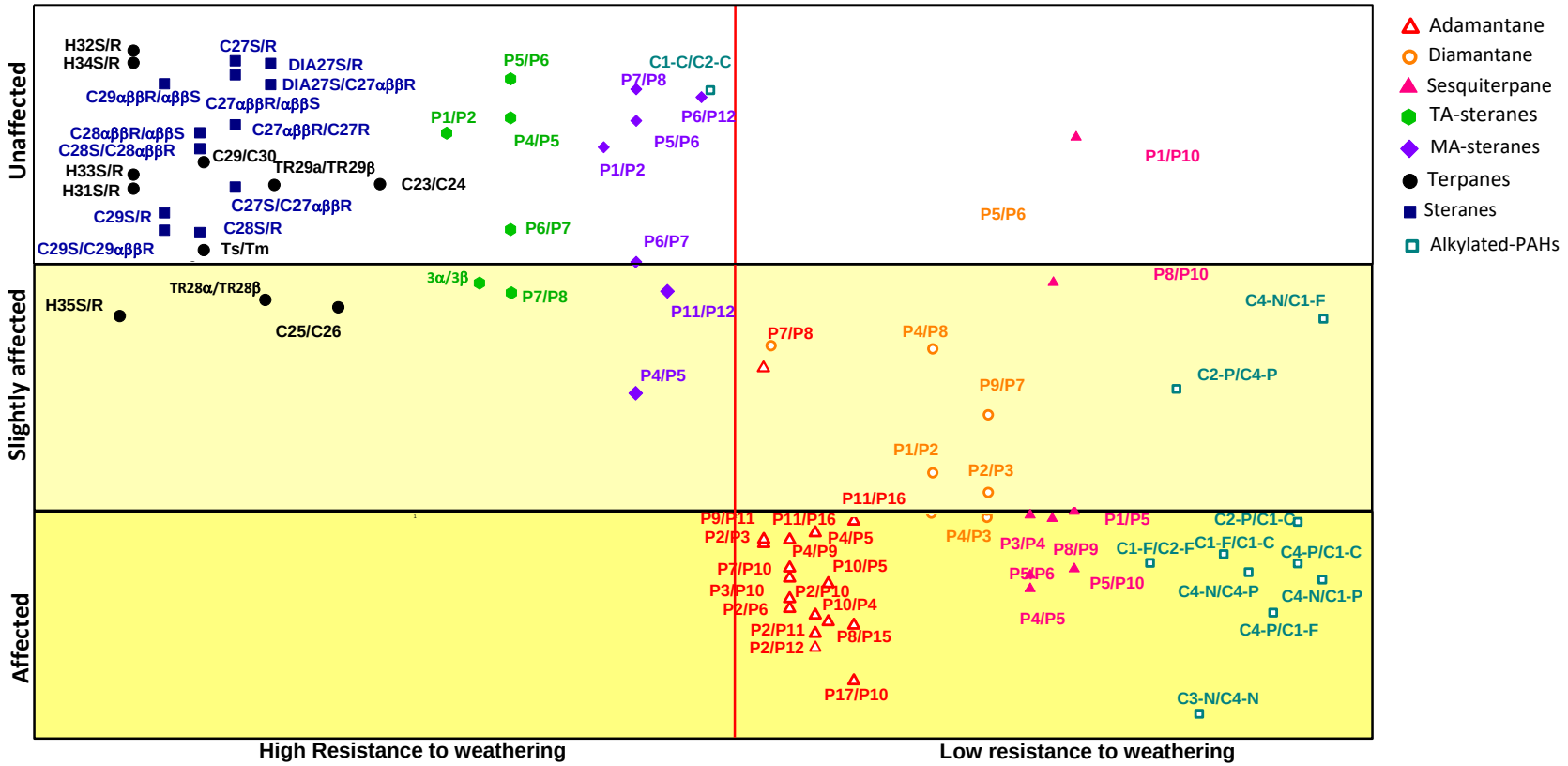


Figure 4.4 Stability of diagnostic ratios between biomarkers in CDO

diagnostic ratios (RSD > 10%). The diagnostic ratios of biomarkers in the column of "degradable" were more stable compared with those in WCO (Tables 4.1-4.8).

4.3.5 The stability of diagnostic ratios of two types of biomarkers in CDO

The most commonly applied diagnostic ratios are established through the same types of biomarkers (Bayona et al. 2015, Radović et al. 2014, Wang et al. 2006a). Few studies involved two or more types of biomarkers in diagnostic relationships. The intricate relationships between two types of biomarkers are mainly steranes and terpanes. Among more than 40 biomarkers, only 2-3 ratios from 8-9 biomarkers are auxiliarily used to identify oil. These biomarkers included C27αββR(S), C28αββR(S), and C29αββR(S), which are steranes, as well as Ts or Tm, and C29 or C30, which are terpanes (Aeppli et al. 2014). The full names of the biomarkers could be found in Table 3.2. Few studies differentiated oils involving the ratios in other types of chemicals, such as adamantanes and diamantanes (Wang et al. 2006b). Therefore, the authors screened a few possibly valid diagnostic relationships from all the selected biomarkers in CDO samples based on the stability of diagnostic ratios of biomarkers during weathering.

Two groups of the ratios are established containing terpanes/steranes (Group 1) and TA-steranes/MA-steranes (Group 2). The ratios are only calculated by using the abundances from the same chromatograms. The RSD values of diagnostic ratios of both Group 1 and Group 2 displayed in Figure 4.5 showed some applicable combinations and a few susceptible ones. For Group 1, the

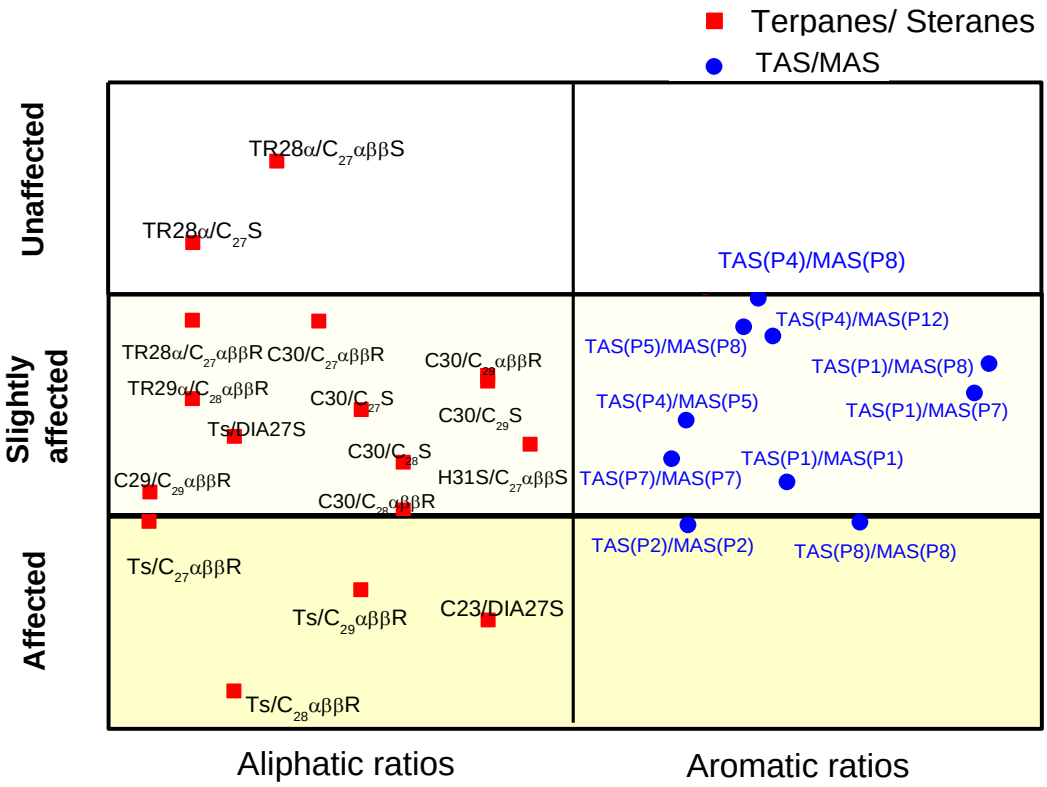


Figure 4.5 Stability of diagnostic ratios of two biomarkers within the group of aliphatics and within the group of aromatics

results indicated that the diagnostic ratios between two different biomarkers within the group of steranes and those within the group of terpanes were relatively stable since low RSD values (i.e., RSD < 5%) of the diagnostic ratios were observed. However, much higher RSD values of diagnostic ratios between two different biomarkers (one from the group of terpanes and another from the group of steranes) were detected, leading to the result that the associated diagnostic ratios were not stable enough to be recommended for dispersed oil fingerprinting. The RSD values of only 2 of the selected 18 ratios were lower than 5% probably induced by weathering with systematic errors. Systematic errors were caused during sampling, extraction, elution, and concentration processes, resulting from different recovery rates of surrogates. The stable diagnostic ratios are $TR_{28}(\alpha)$/ $C_{27}\alpha\beta\beta R(S)$ and $TR_{28}(\alpha)$/ $C_{27}S$. $TR_{28}(\alpha)$ may be a good candidate of terpanes to fingerprint CDO combined with steranes, because the RSD values of diagnostic ratios containing $TR_{28}(\alpha)$ were relatively low. Contrarily, the diagnostic ratios related to C30, a widely applied terpanes for fingerprinting of crude oil, were slightly affected by weathering and systematic errors due to their RSD values (5%<RSD<10%). So, the ratios of $TR_{28}(\alpha)$ to the total abundance of steranes ($T_{steranes}$) were examined and compared with C30/ $T_{steranes}$ and $T_{terpanes}$ /$T_{steranes}$ (Figure 4.6 (A)). $TR_{28}(\alpha)$ was confirmed as a reasonable biomarker bridging to steranes, because the RSD of $TR_{28}(\alpha)$/ $T_{steranes}$ (0.038-0.042) were only 3.54 from 1 to 60 days of weathering. The relative abundance of C30 to steranes was also slightly affected by weathering and systematic errors (5.92% of RSD). The ratios of C30 to steranes may still be valid if the high accuracy of oil identification is not required. All the ratios

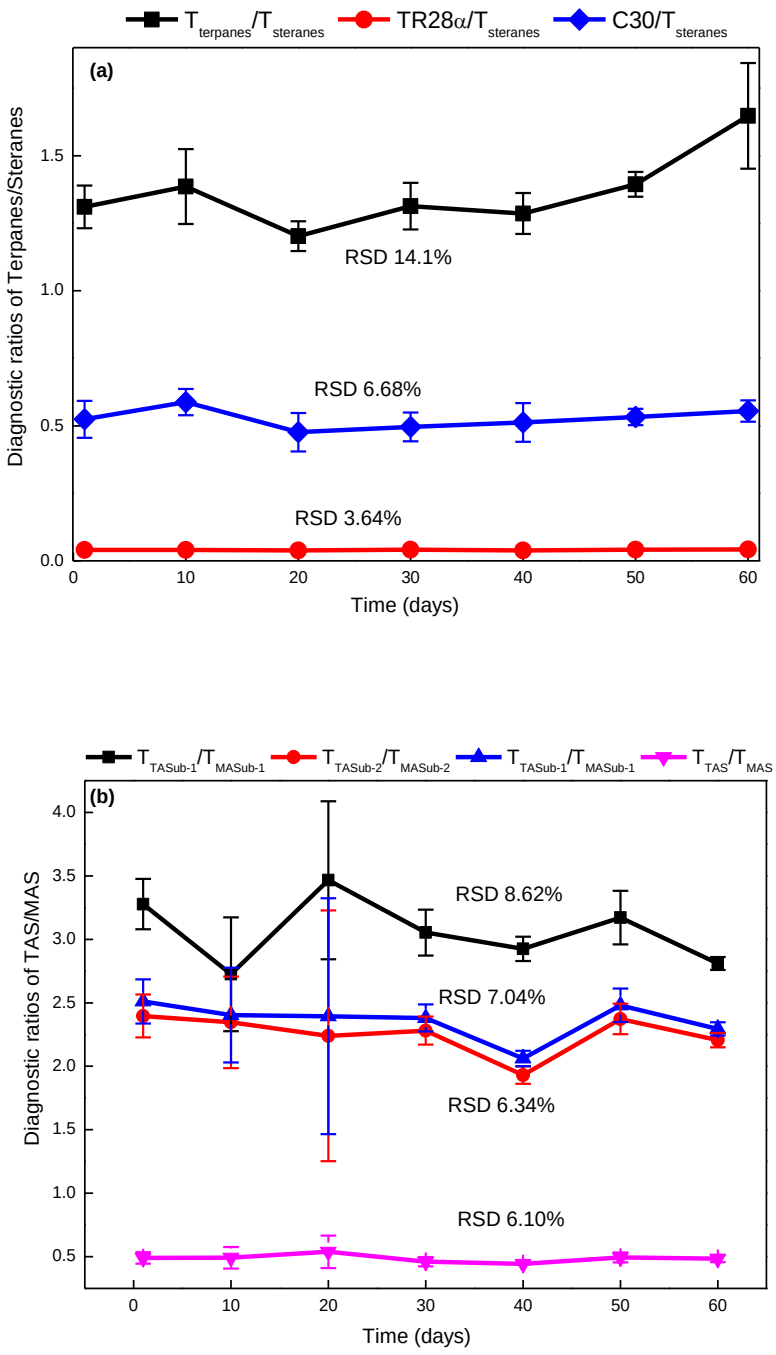


Figure 4.6 The stability of diagnostic ratios of biomarkers (average+ SD, n=8) in: (a) terpanes/steranes, (b) TA-steranes/MA-steranes

regarding T_s, another well-applied terpanes in fingerprinting crude oils, were not constant with a varying RSD from 5.58 to 18.2%. T_s is thus not recommended to be combined with steranes for identifying weathered chemically dispersed oil. The stability of the same ratios in WCO was quite different. C30 was the most stable one when associated with steranes. In WCO samples, RSD of the diagnostic ratios of C30 to $C_{27}\alpha\beta\beta R(S)$, C_{28} S, and C_{29} S are 2.85, 4.98, and 3.73 %, respectively. The ratio of C30 to the total of steranes was 1.72%. The RSD of $TR_{28}(\alpha)$ ranged from 5.52 to 7.65% with 6.65% of the RSD of $TR_{28}(\alpha)$/total steranes.

For Group 2, the RSD of the ratios between a biomarker within TA-steranes (TAS) and another biomarker within MA-steranes (MAS) was from 5% to 10%, indicating lower stability compared with the diagnostic ratios between two biomarkers of the same types of aromatic steranes. These diagnostic ratios may be considered as secondary tools for fingerprinting of CDO as well as terpanes/steranes located at the same RSD range (5%-10%).

The ratios of total abundance of TAS and MAS were also examined in Figure 4.6(B). The total abundance of classified sub-groups of TA-steranes and MA-steranes (defined as T_{TAsub1} and T_{TAsub2}, T_{MAsub1} and T_{MAsub2} in section 4.3) were also involved in the diagnostic relationships, respectively, given the possible significant effects of systematic errors mentioned above (in **3.3**). Overall, the diagnostic ratios of two types of aromatic steranes were steadier than those in two types of aliphatic biomarkers, because most of the RSD of selected ratios of aromatic steranes were less than 10% (Figure 4.6). Meanwhile,

the RSD of total TA/MA steranes (T_{TAS}/T_{MAS}) was 6.10%, which indicated a potential group of valid diagnostic ratios. The RSD of detected ratios regarding the total abundance of sub-groups in TAS and MAS ranged from 6.34 to 8.62%. The RSD of T_{TAsub1} / T_{MAsub2} were higher than that of others. From individual ratios, the calculated ratios belonging to T_{TAsub1} / T_{MAsub2}, such as TAS(p1)/MAS(p8) and TAS(p1)/MAS(p7), were not the highest. But no RSD of any ratio was less than 5%. The ratios in Group 2 and those related to C30 and other steranes can be at the same priority level.

The experimental results showed that the diagnostic ratios of different types of biomarkers were less stable than those of the same types of biomarkers. This was probably because of the effects of the diverse degree of weathering as well as the discrepancies of efficacies of elution for different types of biomarkers. From Figure 4.4, half of the isomers of aromatic steranes were slightly changed during the weathering (RSD: 5%-10%). The ratios of diagnostic ratios containing the variable peaks would be affected. The unstable ratios generated from biomarkers in different sub-groups were found in some samples even within the same weathering days. The RSD of $T_{TAsub-1}$/ $T_{MAsub-1}$ was larger than those of other groups. In addition, the ranges of chromatography signals plus carbon numbers may not affect the stability of selected biomarkers. The RSD values of $TR_{28}(\alpha)$/ $T_{steranes}$ were the lowest, whereas the abundance of total steranes (C27-C29) was 25 times larger than that of $C_{27}(\alpha)$ (C27).

4.4. Summary

This chapter systematically examined the diagnostic ratios of 8 types of biomarkers for CDO fingerprinting. Three types of aliphatic biomarkers, including adamantanes, diamantanes, and sesquiterpanes, were not recommended to characterize weathered dispersed oil with a long-term weathering. Some diagnostic ratios between two biomarkers within adamantanes, those within diamantanes, and those within sesquiterpanes might be applicable as secondary tools to fingerprinting CDO within a shorter-term weathering. Most of the diagnostic ratios based on steranes, terpanes, and aromatic-steranes (TA-steranes, and MA-steranes) in CDO were recalcitrant during the experiments. Therefore, these biomarkers could be applied for CDO fingerprinting. Parts of the diagnostic ratios of alkylated-PAHs can be applied for CDO identification in some cases although they can be more easily degraded than other biomarkers, such as terpanes and steranes. Some potential applicable diagnostic ratios between two biomarkers of different types were also screened. The screened stable biomarkers and corresponding diagnostic relations help fulfil the gaps of CDO fingerprinting. Future work will be focused on the evaluation of fingerprinting of CDO under more specific weathering status (e g., photo-oxidation and biodegradation) and with formal statistical analysis methods, such as principal component analysis.

CHAPTER 5 DIFFERENTIATION OF WEATHERED CHEMICALLY DISPERSED OIL FROM WEATHERED CRUDE OIL[3]

[3] *The contents of this chapter are based and expanded on the following paper:*

Song, X., Lye, L. M., Chen, B., & Zhang, B. (2019). Differentiation of weathered chemically dispersed oil from weathered crude oil. Environmental monitoring and assessment, 191(5), 270, DOI: 10.1007/s10661-019-7392-5
Role: Xing Song solely worked on this study and acted as the first author of this manuscript under Dr. Baiyu Zhang, Dr. Bing Chen, and Dr. Leonard M. Lye's guidance. Most contents of this paper were written by him and further edited by the other co-authors.

5.1 Introduction

Possible candidate biomarkers for fingerprinting of different CDO have been investigated through some experiments (Olson et al. 2017, Song et al. 2016, Song et al. 2018). However, whether the application of dispersants can affect the weathering of biomarkers is unknown. As such, multivariate analysis methodologies, such as PCA, will play an important role in objectively differentiating chemically dispersed oil (CDO) from weathered crude oil (WCO) or non-dispersed oil. To our knowledge, fingerprinting of CDO using PCA has not been reported yet. This chapter mainly aims to differentiate CDO from WCO using multiple PCA algorithms based on the diagnostic ratios of 7 types of biomarkers, including adamantanes, diamantanes, sesquiterpanes, steranes, terpanes, TA-steranes, and MA-steranes.

5.2 Materials and Methods

5.2.1 Oil-weathering experiments and data collection

Based on our previous results from a long-term (1, 10, 20, 30, 40, 50 ,and 60 days of weathering) general weathering of dispersed oil and crude oil (Song et al. 2018), 8 types of biomarkers were selected to differentiate CDO from WCO. Briefly, the experiments could be summarized as follows. Three types of oil samples were prepared: 1) crude oil samples: crude oil samples were prepared by dissolving crude oil in hexane, 2) CDO: aliquot 100 μL crude oil was pipetted to artificial seawater with following addition of 10 μL dispersant (Corexit 9500A); and 3) WCO; aliquot 100 μL crude oil without dispersant was pipetted into artificial seawater. CDO and WCO were shaken at 120 rpm for certain days to simulate oil weathering.

CDO and WCO samples were extracted for sample analysis when the weathering

process has completed (Song et al. 2016, Song et al. 2018). The extraction into the organic phase was accomplished using DCM. The extracts were cleaned and eluted using a chromatographic column filled with silica gel. The organic phase was concentrated and analyzed using a GC-MS (Agilent model 6890) equipped with a DB-5ms capillary column (30 m) (Song et al. 2016, Song et al. 2018). The validity and reliability of the experiment were evaluated using QA/QC programs. All the weathering simulations, sample pre-treatments, and sample analyses were conducted in duplicate. Each detectable biomarker thus has 8 data of peak areas using GC-MS analysis. The undetectable biomarkers (especially light-molecular ones) have less than 8 data points. Calibrated surrogates were introduced to sample preparation to ensure the validity of sample treatment. Internal standards were applied to monitoring the stability of GC-MS system.

Eight types of biomarkers, containing adamantanes, diamantanes, sesquiterpanes, terpanes, steranes, TA-steranes, MA-steranes, and alkylated PAHs, were selected. The peak areas of identified biomarkers in each sample (crude oil, CDO, and WCO samples) were calculated. More than 100 diagnostic ratios were calculated based on their peak areas shown in Table 3.2. The diagnostic ratios included the ratios from the same types of biomarkers (e.g. Ts/Tm, C29/C30: terpanes/terpanes) and the ratios from two types of biomarkers (e.g. Ts/C27S, TR28a/C29αββR: terpanes/steranes). The average values of diagnostic ratios of two individual biomarkers were obtained through the ratios of peak areas. The average values of diagnostic ratios were set as variables to evaluate the effects of the application of dispersants and weathering duration on selected biomarkers, respectively. Weathering days (1-60 days) of CDO were abbreviated as C1-C60, and W1-W60 were used to represent weathering days

(1-60 days) of WCO samples. The abbreviations of the diagnostic ratios are shown in Table 5.1.

5.2.2 Principal component analysis

PCA is a widely recognized multivariate analysis technique that uses orthogonal transformation to convert the variables of original data into uncorrelated variables. PCA extracts eigenvalues and eigenvectors from the covariance of original correlated variables to a new smaller set of independent uncorrelated variables (principal components) (Jeffers 1967, Singh et al. 2004, Tipping and Bishop 1999, Wold et al. 1987). The principal components, z_i's are weighted by the combinations of original variables with eigenvectors as shown in Equation (1):

$$\begin{pmatrix} z_1 = \alpha'_{11}x_1 + \alpha'_{12}x_2 + \cdots + \alpha'_{1j}x_j \\ z_2 = \alpha'_{21}x_1 + \alpha'_{22}x_2 + \cdots + \alpha'_{2j}x_j \\ \cdots \\ z_i = \alpha'_{i1}x_1 + \alpha'_{i2}x_2 + \cdots + \alpha'_{ij}x_j \end{pmatrix} \quad (1)$$

Where, α_i is the i th vector representing components loading, j donates the number of variables, and x denotes the variables.

Covariance was firstly employed to the data sets for measurement of linear correlation between 2 variables. Pearson correlation was then applied to exam the correlation of scaled variables derived from the original data. Other non-parametric correlation methods based on ranks of observations could also describe non-linear but monotonic correlation to obtain eigenvalues and eigenvectors (Alberto et al. 2001, Ma et al. 2010). Two types of non-parametric correlation, Spearman ρ and Kendall τ, are thus employed in the data sets in case of non-linear but monotonic association between 2 ordinal variables. They may be helpful with the variables with different and

Table 5.1 Abbreviation of the peaks of diagnostic ratios

Type of diagnostic ratios	Abbreviation	Peak No.	Type of diagnostic ratios	Abbreviation	Peak No.
Adamantane/adamantanes	Ad1	p2/p3	Diamantanes/diamantanes	Dia1	p1/p2
Adamantane/adamantanes	Ad2	p2/p6	Diamantanes/diamantanes	Dia2	p2/p3
Adamantane/adamantanes	Ad3	p3/p10	Diamantanes/diamantanes	Dia3	p5/p6
Adamantane/adamantanes	Ad4	p4/p9	Diamantanes/diamantanes	Dia4	p4/p3
Adamantane/adamantanes	Ad5	p2/p10	Diamantanes/diamantanes	Dia5	p9/p7
Adamantane/adamantanes	Ad6	p2/p11	Diamantanes/diamantanes	Dia6	P4/P8
Adamantane/adamantanes	Ad7	p2/p12	Sesquiterpanes/Sesquiterpanes	Ses1	p3/p4
Adamantane/adamantanes	Ad8	p17/p10	Sesquiterpanes/Sesquiterpanes	Ses2	p4/p5
Adamantane/adamantanes	Ad9	p10/p4	Sesquiterpanes/Sesquiterpanes	Ses3	p5/p6
Adamantane/adamantanes	Ad10	p10/p5	Sesquiterpanes/Sesquiterpanes	Ses4	p8/p9
Adamantane/adamantanes	Ad11	p7/p8	Sesquiterpanes/Sesquiterpanes	Ses5	p8/p10
Adamantane/adamantanes	Ad12	p11/p12	Sesquiterpanes/Sesquiterpanes	Ses6	p1/p5
Adamantane/adamantanes	Ad13	p7/p10	Sesquiterpanes/Sesquiterpanes	Ses7	p5/p10
Adamantane/adamantanes	Ad14	p11/p16	Sesquiterpanes/Sesquiterpanes	Ses8	p1/p10
Adamantane/adamantanes	Ad15	p9/p11	Sesquiterpanes/Sesquiterpanes	Ses9	P3/P6
Adamantane/adamantanes	Ad16	p4/p5	Sesquiterpanes/Sesquiterpanes	Ses10	P6/P8
Adamantane/adamantanes	Ad17	p8/p15			
Steranes/steranes	St1	p1/p2	Steranes/steranes	St7	p13/p14
Steranes/steranes	St2	p1/p8	Steranes/steranes	St8	p14/p15
Steranes/steranes	St3	p8/p10	Steranes/steranes	St9	p13/p16
Steranes/steranes	St4	p10/p11	Steranes/steranes	St10	p17/p18
Steranes/steranes	St5	p7/p11	Steranes/steranes	St11	p18/p19
Steranes/steranes	St6	p7/p8	Steranes/steranes	St12	p17/p20

incomparable means in the same data set, such as variables containing the diagnostic ratios of terpanes and TA-steranes.

The PCA results were applied to assess the effects of the application of dispersants as well as the weathering duration on diagnostic ratios of biomarkers. Principal components (PCs) were set to cover at least 80% of variances using covariance, Pearson correlation, and non-parametric methods (Spearman and Kendall), respectively. The PCAs were performed using both Minitab 17 (Minitab Inc. 2017) and XSLTAT software, an Excel based software. Both software showed consistent results.

Pearson's correlation coefficient can be calculated using Equation 2:

$$\rho_{X,Y} = \frac{\mathrm{cov}(x,y)}{\sigma_X \sigma_Y} = \frac{\mathrm{E}[(X-\mu X)(Y-\mu Y)]}{\sigma_X \sigma_Y} \quad (2)$$

Where cov(X,Y) is the covariance of X and Y, σ_X is the standard deviation of X, σ_Y is the standard deviation of Y.

Spearman correlation (r_s) is approximately the Pearson correlation coefficient between ranked variables. If Spearman correlation is used then X and Y are changed to the rank of X, and the rank of Y.

$$r_s = \rho_{X,Y} = \frac{\mathrm{cov}(r_x, r_y)}{\sigma_{r_x} \sigma_{r_y}} \quad (3)$$

Where cov (r_x, r_y) is covariance of the ranked variables x and y, σ donates the standard deviations of the ranked variables.

Kendall τ is a reasonable coefficient to evaluate the concordance of ranked variables (Kendall 1948). If there are two sets of ranked variables (A and B), one of the two

ranks will be naturally re-ordered. The pair of ranked numbers in any two variables $\binom{n}{2}$ will be scored as right order (+1) or inverse order (-1) based on the natural sequence. The scores in both ranks then are multiplied to reach a score, as concordance (positive scores, as C) or discordance (negative scores, as D).

$$\tau = \frac{C-D-Q}{\frac{1}{2}n(n-1)} \quad (4)$$

5.3 Results and Discussion

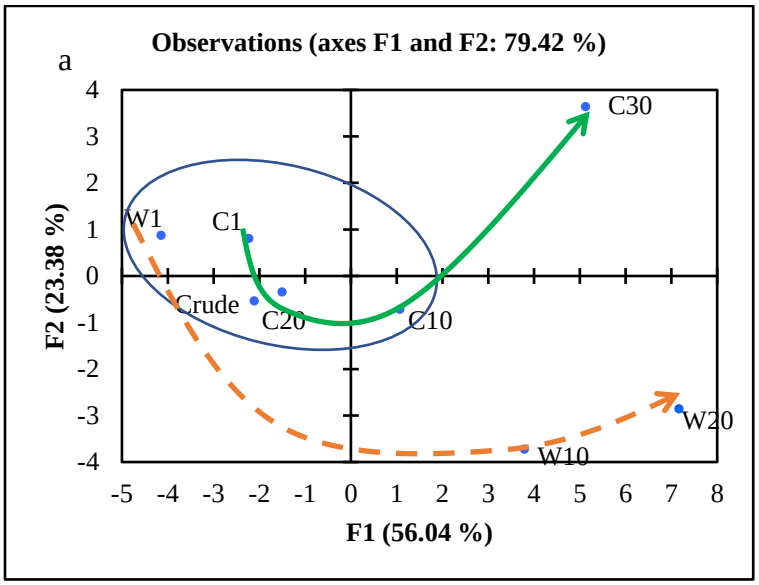
5.3.1 The effects of dispersants and weathering on low-molecular biomarkers

PCA was firstly applied to differentiate CDO from WCO using both the diagnostic ratios of adamantanes and the diagnostic ratios of diamantanes. PCA was conducted using the average values of the same diagnostic ratios selected on the same samples. Table 5.2 shows the Pearson matrix as an example of the correlation matrix. Tables 5.3 and 5.4 show the eigenvectors and factor scores of Pearson matrix, respectively. The scores plots using the three PCA methods are displayed in Figure 5.1 (a-c). Raw data are listed in Table 5.5. Slightly weathered CDO (1-20 days) are grouped with crude oil, and slightly weathered crude oil (1 day) according to experimental conditions associated with hierarchical cluster analysis (CA) shown in Figure 5.2. Other WCO (10-20 days weathering) are clearly differentiated from the slight weathered CDO as well as CDO with a relatively longer weathering duration. This implied that the addition of dispersants may attribute to the variations of degree and fate of weathering of diamondoids (C30 versus W10 and W20) besides weathering duration (C1 versus C30). Although few studies tracked the weathering degrees of biomarkers, especially the same types of biomarkers, after applying dispersants using statistical methods,

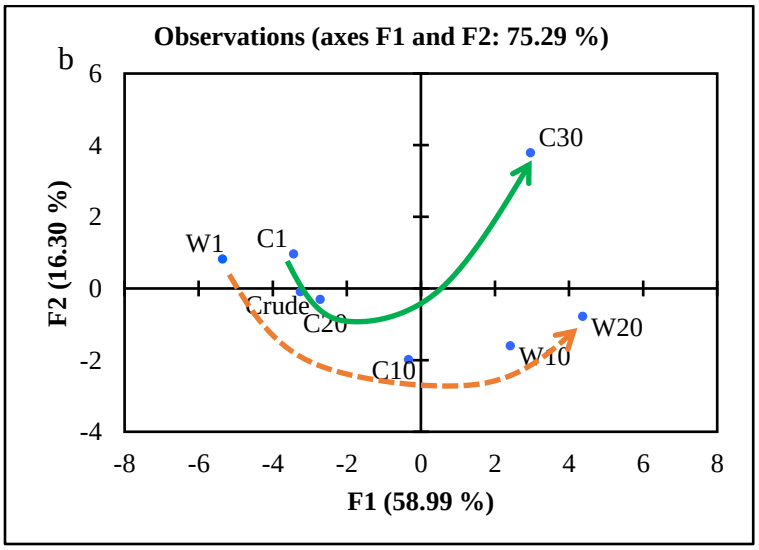
some hydrocarbons in chemically dispersed oil have diverse resistances to weathering processes compared to those in non-dispersed (naturally-dispersed) oil sharing the same weathering conditions (Bacosa et al. 2015, Prince et al. 2013). Even in dispersed oil, hydrocarbon weathering highly linked to the size of oil droplets (Brakstad et al. 2015). Biomarkers in dispersed oil could perform variable and discordant degradation rate as well. The first component (PC1) explained 56-59% of total variances. The second component (PC2) presented 14-23% of total variances. The third component (PC3) presented 5-10% of total variances. The combination of PC1 to PC3 is sufficient to interpret the influence of weathering duration and the application of dispersants on the variations of diagnostic ratios. The diagnostic ratios of diamantanes and adamantanes can be applied to differentiate CDO, crude oil, and, WCO as shown in Figure 5.3. For example, in Pearson methods, the diagnostic ratios of Ad1, Dia1, Dia 4, and Dia 5, are weighted on relatively heavily weathered CDO (C30). Crude and relatively slightly weathered CDO and WCO are related to some diagnostic ratios, such as Dia 2 and 3, Dia 6, and Ad 9. The diagnostic ratios (Ad 2-6, Ad 13, and Ad 15) located near the corresponding oil are probably correlated to WCO. Meanwhile, some specific diagnostic ratios are always linked with unique oil samples reflecting the impacts of use of dispersants and weathering duration. For example, crude oil appeared in three PCA biplots are always corelated with Ad1, Dia 2 and Dia 3. Dia 4 and Dia 5 can trace CDO (C30 for Pearson and Spearman PCA, and C10 for Kendall PCA). WCO can always be differentiated using Ad3-6, Ad13, and Ad15.

Besides, if data points from CDO are connected using a curve following the general order of weathering days from 1 day to 60 days, the curve direction goes counterclockwise in Pearson PCA (green line in Figure 5.1 a). The direction for WCO

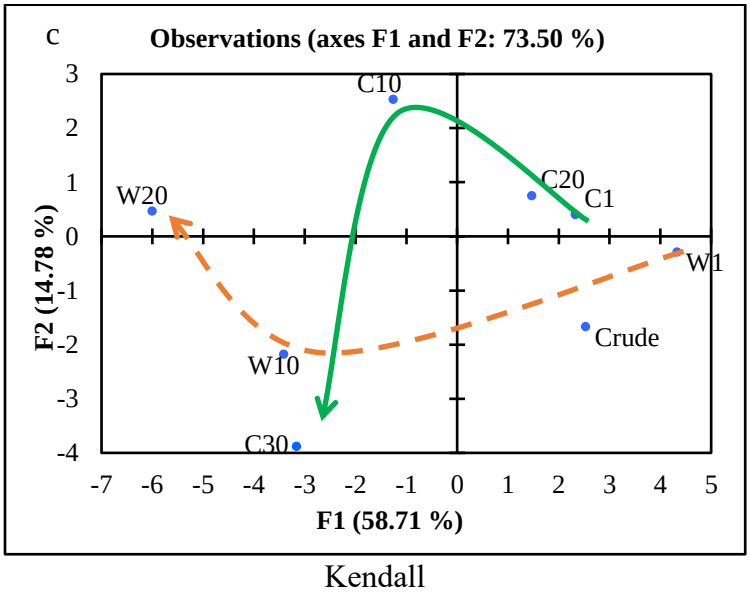
is counterclockwise as well when the curve is drawn as the same sequence (orange line in Figure 5.1 a). The direction of data of CDO is the same direction as the direction of



Pearson



Spearman



Kendall

Figure 5.1 PCA results using the diagnostic ratios of adamantanes and those of diamantanes using: a) Pearson b) Spearman, and c) Kendall PCA

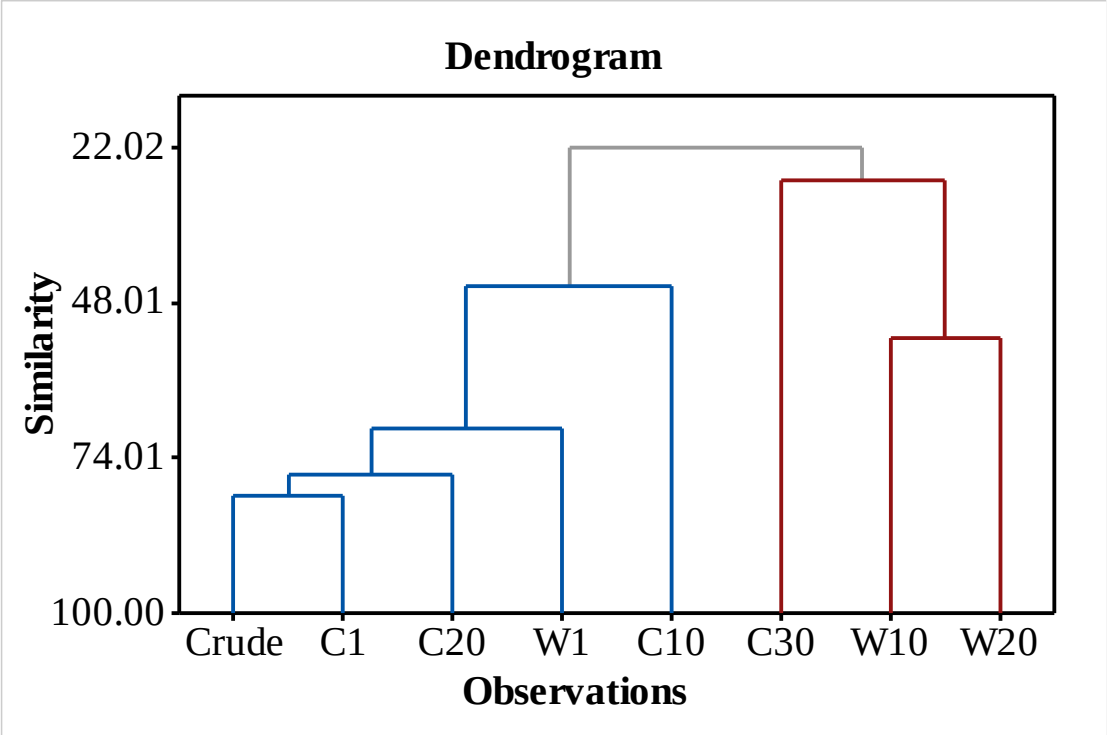
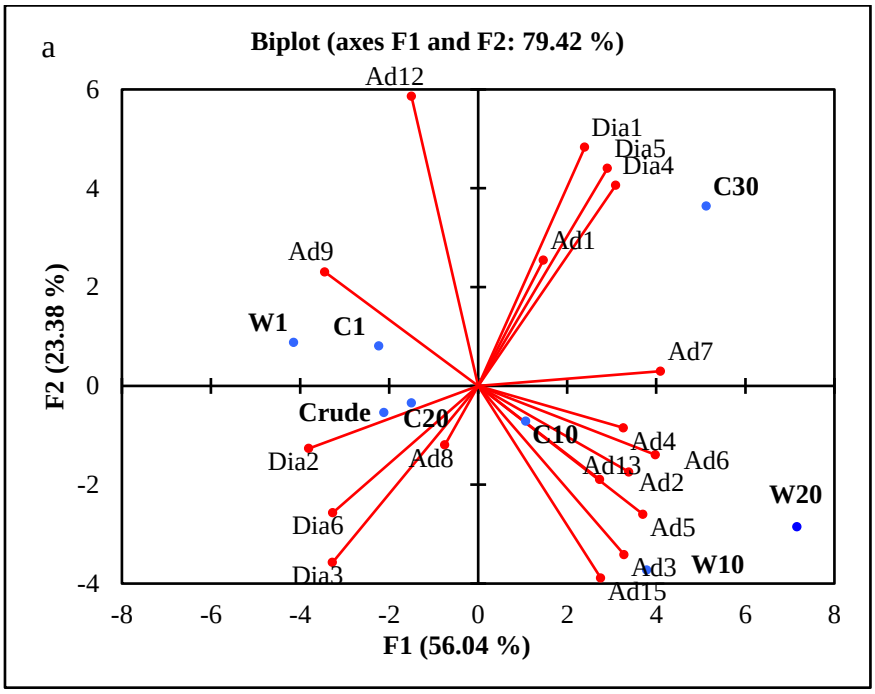
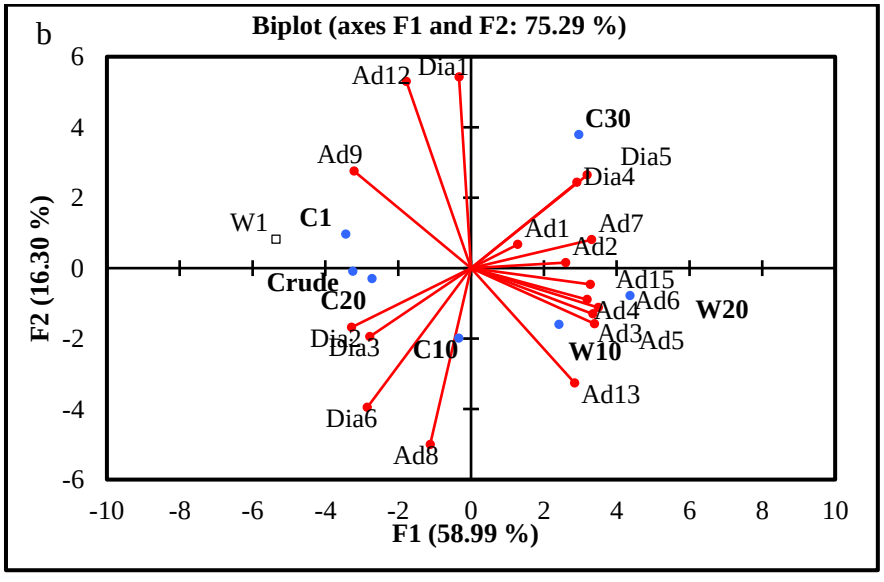


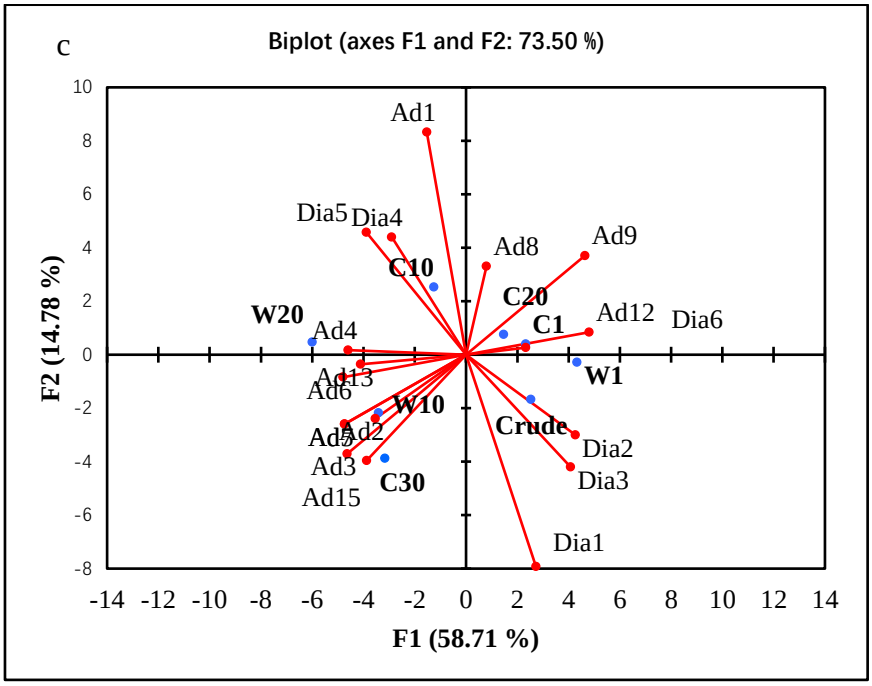
Figure 5.2 Dendrogram showing the clustering of CDO and WCO samples using adamantanes and diamantanes



Pearson



Spearman



Kendall

Figure 5.3 PCA biplot using the diagnostic ratios of adamantanes and those of diamantanes using: a) Pearson b) Spearman, and c) Kendall PCA

Table 5.2 Pearson correlation matrix of diagnostic ratios of adamantanes and diamantanes

Variables	Ad1	Ad2	Ad3	Ad4	Ad5	Ad6	Ad7	Ad8	Ad9	Ad12	Ad13	Ad15	Dia1	Dia2	Dia3	Dia4	Dia5	Dia6
Ad1	**1**	0.043	-0.065	0.319	0.172	0.220	0.357	0.376	-0.333	0.019	0.567	-0.252	0.261	-0.455	-0.676	0.448	0.446	-0.154
Ad2	0.043	**1**	0.927	0.609	0.927	0.912	0.852	-0.560	-0.744	-0.696	0.344	0.839	0.198	-0.720	-0.526	0.362	0.443	-0.743
Ad3	-0.065	0.927	**1**	0.694	0.965	0.933	0.805	-0.414	-0.833	-0.830	0.512	0.941	0.010	-0.714	-0.361	0.227	0.274	-0.662
Ad4	0.319	0.609	0.694	**1**	0.710	0.839	0.597	-0.263	-0.697	-0.826	0.502	0.462	-0.051	-0.933	-0.454	0.197	0.319	-0.605
Ad5	0.172	0.927	0.965	0.710	**1**	0.964	0.890	-0.279	-0.871	-0.781	0.645	0.891	0.095	-0.790	-0.517	0.359	0.387	-0.700
Ad6	0.220	0.912	0.933	0.839	0.964	**1**	0.895	-0.383	-0.838	-0.787	0.573	0.798	0.180	-0.908	-0.606	0.434	0.502	-0.810
Ad7	0.357	0.852	0.805	0.597	0.890	0.895	**1**	-0.327	-0.827	-0.460	0.620	0.696	0.521	-0.771	-0.822	0.732	0.745	-0.842
Ad8	0.376	-0.560	-0.414	-0.263	-0.279	-0.383	-0.327	**1**	0.177	0.218	0.417	-0.322	-0.310	0.308	0.257	-0.206	-0.348	0.561
Ad9	-0.333	-0.744	-0.833	-0.697	-0.871	-0.838	-0.827	0.177	**1**	0.684	-0.795	-0.689	-0.084	0.705	0.561	-0.334	-0.353	0.501
Ad12	0.019	-0.696	-0.830	-0.826	-0.781	-0.787	-0.460	0.218	0.684	**1**	-0.444	-0.714	0.436	0.703	0.061	0.201	0.101	0.350
Ad13	0.567	0.344	0.512	0.502	0.645	0.573	0.620	0.417	-0.795	-0.444	**1**	0.449	-0.004	-0.529	-0.446	0.313	0.224	-0.247
Ad15	-0.252	0.839	0.941	0.462	0.891	0.798	0.696	-0.322	-0.689	-0.714	0.449	**1**	-0.052	-0.507	-0.172	0.148	0.133	-0.561
Dia1	0.261	0.198	0.010	-0.051	0.095	0.180	0.521	-0.310	-0.084	0.436	-0.004	-0.052	**1**	-0.187	-0.763	0.931	0.921	-0.623
Dia2	-0.455	-0.720	-0.714	-0.933	-0.790	-0.908	-0.771	0.308	0.705	0.703	-0.529	-0.507	-0.187	**1**	0.679	-0.454	-0.550	0.789
Dia3	-0.676	-0.526	-0.361	-0.454	-0.517	-0.606	-0.822	0.257	0.561	0.061	-0.446	-0.172	-0.763	0.679	**1**	-0.895	-0.929	0.752
Dia4	0.448	0.362	0.227	0.197	0.359	0.434	0.732	-0.206	-0.334	0.201	0.313	0.148	0.931	-0.454	-0.895	**1**	0.970	-0.764
Dia5	0.446	0.443	0.274	0.319	0.387	0.502	0.745	-0.348	-0.353	0.101	0.224	0.133	0.921	-0.550	-0.929	0.970	**1**	-0.818
Dia6	-0.154	-0.743	-0.662	-0.605	-0.700	-0.810	-0.842	0.561	0.501	0.350	-0.247	-0.561	-0.623	0.789	0.752	-0.764	-0.818	**1**

Table 5.3 Eigenvectors

	F1	F2	F3	F4	F5	F6	F7
Ad1	0.099	0.225	0.510	0.231	-0.309	0.419	-0.172
Ad2	0.279	-0.103	-0.185	-0.073	-0.277	0.443	0.212
Ad3	0.275	-0.212	-0.108	-0.153	-0.010	-0.029	0.122
Ad4	0.243	-0.136	0.120	0.492	0.211	-0.332	0.203
Ad5	0.293	-0.144	0.014	-0.163	-0.004	0.219	0.027
Ad6	0.305	-0.095	-0.014	0.059	0.098	0.076	0.073
Ad7	0.301	0.089	0.006	-0.194	-0.084	0.051	0.058
Ad8	-0.121	-0.017	0.549	-0.279	0.469	0.234	0.389
Ad9	-0.267	0.111	-0.173	0.154	0.440	0.489	-0.027
Ad12	-0.204	0.364	-0.042	-0.219	-0.057	-0.023	-0.371
Ad13	0.188	-0.058	0.476	-0.300	0.070	-0.323	-0.273
Ad15	0.230	-0.242	-0.160	-0.390	0.180	0.126	-0.277
Dia1	0.111	0.450	-0.161	-0.144	0.052	-0.156	0.359
Dia2	-0.279	0.019	-0.097	-0.394	-0.201	-0.076	0.272
Dia3	-0.233	-0.319	-0.098	-0.080	0.252	-0.061	0.026
Dia4	0.186	0.397	-0.016	-0.151	0.196	-0.078	-0.088
Dia5	0.202	0.381	-0.069	0.032	0.088	-0.053	0.292
Dia6	-0.268	-0.151	0.210	-0.100	-0.399	-0.053	0.350

Table 5.4 Factor loadings

	F1	F2	F3	F4	F5	F6	F7
Ad1	0.316	0.441	0.756	0.233	-0.189	0.206	-0.046
Ad2	0.894	-0.202	-0.274	-0.074	-0.170	0.217	0.057
Ad3	0.881	-0.416	-0.160	-0.155	-0.006	-0.014	0.033
Ad4	0.778	-0.266	0.178	0.496	0.129	-0.163	0.055
Ad5	0.939	-0.282	0.021	-0.164	-0.002	0.108	0.007
Ad6	0.978	-0.186	-0.021	0.059	0.060	0.037	0.020
Ad7	0.963	0.175	0.010	-0.196	-0.052	0.025	0.016
Ad8	-0.388	-0.034	0.814	-0.281	0.288	0.115	0.105
Ad9	-0.856	0.218	-0.256	0.156	0.270	0.240	-0.007
Ad12	-0.652	0.714	-0.062	-0.221	-0.035	-0.011	-0.100
Ad13	0.603	-0.114	0.707	-0.303	0.043	-0.159	-0.074
Ad15	0.737	-0.474	-0.238	-0.393	0.110	0.062	-0.075
Dia1	0.355	0.883	-0.238	-0.145	0.032	-0.076	0.097
Dia2	-0.893	0.036	-0.144	-0.397	-0.123	-0.037	0.073
Dia3	-0.746	-0.626	-0.146	-0.080	0.155	-0.030	0.007
Dia4	0.595	0.778	-0.024	-0.153	0.120	-0.038	-0.024
Dia5	0.648	0.748	-0.102	0.033	0.054	-0.026	0.079
Dia6	-0.858	-0.297	0.311	-0.100	-0.244	-0.026	0.094

Table 5.5 Diagnostic ratios of adamantanes and those of diamantanes for PCA

	Ad1	Ad2	Ad3	Ad4	Ad5	Ad6	Ad7	Ad8	Ad9	Ad12	Ad13	Ad15	Dia1	Dia2	Dia3	Dia4	Dia5	Dia6
Crude	0.64	0.84	1.33	0.8	0.84	1.32	1.19	0.75	2.45	0.9	0.82	0.82	0.73	3.59	0.98	2.22	1.7	1.28
C1	1.10	1.01	0.70	0.56	0.79	1.14	1.13	0.68	3.58	0.98	0.73	0.73	0.63	3.22	0.92	2.35	1.75	1.19
C10	1.76	0.86	1.37	1.16	2.41	3.02	2.56	2.42	1.27	0.85	1.60	0.85	0.54	2.75	0.84	2.61	1.99	1.27
C20	1.38	1.74	1.09	0.70	1.50	1.80	1.58	1.06	2.70	0.87	0.78	0.64	0.55	3.29	0.88	2.10	1.73	1.36
C30	1.51	2.49	2.05	1.01	3.08	4.96	4.94	0.55	1.57	1.00	1.06	1.01	2.12	2.39	0.58	4.00	4.78	0.74
W1	0.80	0.51	0.32	0.38	0.26	0.37	0.37	1.52	5.45	0.98	0.58	0.69	0.73	3.62	1.02	2.30	1.72	1.22
W10	0.72	3.33	3.97	0.93	4.88	5.86	4.07	0.82	0.66	0.69	1.20	1.96	0.58	2.86	0.91	2.47	1.87	1.02
W20	1.26	3.11	3.61	2.44	4.54	7.73	3.90	0.41	0.68	0.54	1.12	1.39	0.57	1.34	0.79	2.58	2.71	0.81

WCO (both counterclockwise and clockwise). This trend indicates the effects of weathering duration on the variation of biomarkers for CDO and WCO.

Meanwhile, the curves of CDO and WCO located in different areas clearly implied the impacts of the use of dispersants in variations of biomarkers. However, the trends were not always found if all the data plots included, such as C20 in Pearson PCA (in Figure 5.1 a). One data plot in CDO or WCO sequence (1-60 days of weathering) at most is omitted to obtain a clearer trend. The data plot in the middle of the weathering duration is primarily selected to be omitted, to clarify the effects of weathering duration. The same directions of the curves were found in Spearman PCA (Figure 5.1 b), but not in Kendall PCA (Figure 5.1 c). The different directions of curves may result from different PCA methods. Different ranking methods may result in diverse information loss related to the effects of weathering duration on the values of diagnostic ratios.

Meanwhile, PCA successfully differentiated CDO from WCO using the diagnostic ratios of adamantanes. From the loading plots, Crude, C1, and C20 are located in a similar zone. CDO with longer weathering duration (C30-C40) is clearly differentiated from WCO (W10-20). The PCA results from adamantanes may represent the application of dispersants as well as the effects of weathering days. Two PCs were selected, explaining 80% of the variance. The scores plots showed in Figure 5.4 illustrated the isolation of W20 and C30. The data are listed in Table 5.6. The trend is concordant with identified clusters using CA (Figure 5.5). Crude oil is grouped with W1 and C1 and C20, and C10 is grouped with C30 and C40 from CA results. W10 and W20 are differentiated from CA. Both CA and PCA could clarify the difference of

diagnostic ratios between CDO and WCO as well as weathering duration. Contrast with the results using adamantanes as variables, W10 is classified as the group with slight weathered oil. The higher resistance to evaporation of diamantanes may lead to a lower variation of the diagnostic ratios of diamantanes compared with adamantanes (Wang et al. 2006b). It might be confirmed that the selected PCA methods could apparently differentiate weathered CDO from WCO using adamantanes. Many studies (Bao et al. 2014, Daling et al. 2014) showed that the major weight loss of oil is caused by evaporation. Compared with other PCA results, oil with different weathering degrees can be easily differentiated when degraded components are selected as variances (Ismail et al. 2016). It is still unclear whether photo-oxidation contributed to the differences of the first stage (0-10 days of weathering) and later stages of weathering (longer than 2- days). The results obtained from the PCA implies that adamantanes and diamantanes may be degraded in two ways in different rates. The PCA results using only diamantanes could also obtain similar results (Figure 5.6) with values of diagnostic ratios (Table 5.7). Oil samples with longer weathering days are differentiated from other samples with relatively shorter weathering duration (0-20 days). Weathered non-dispersed oil are separated from weathered dispersed oil in all PCA methodologies. The diagnostic ratios of ad1 and ad7 (the detailed ratios could be found in Table 5.2) are always correlated with CDO, while Ad3, Ad5, Ad6, and Ad15 were associated with WCO, compared with PCA results using both adamantanes and diamantanes. These indicators probably are key indicators for differentiation CDO from WCO using adamantanes.

Two principal components should be sufficient for fingerprinting using diagnostic ratios of sesquiterpanes as observations (Figure 5.7). The diagnostic ratios are listed in

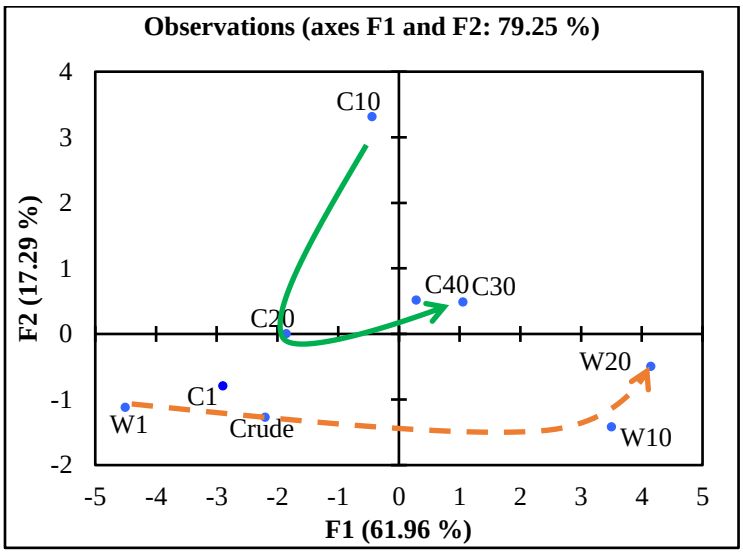


Figure 5.4 Differentiation of CDO from WCO and crude oil using adamantanes

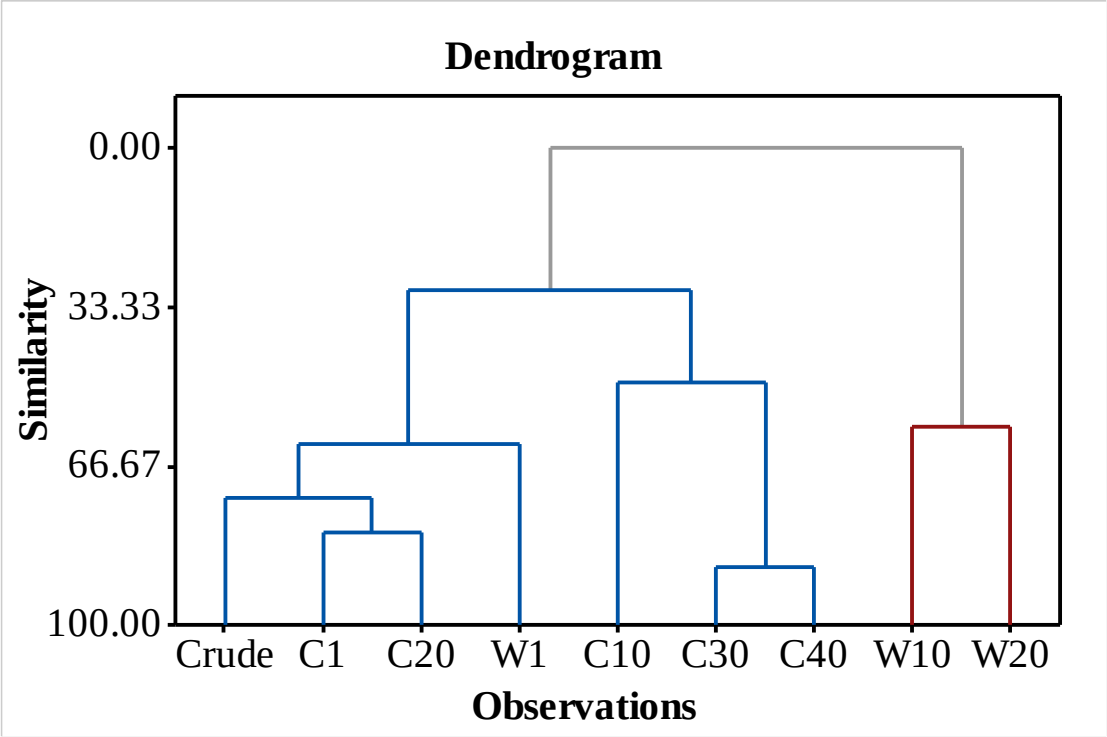


Figure 5.5 Dendrogram showing the clustering of CDO and WCO samples using adamantanes

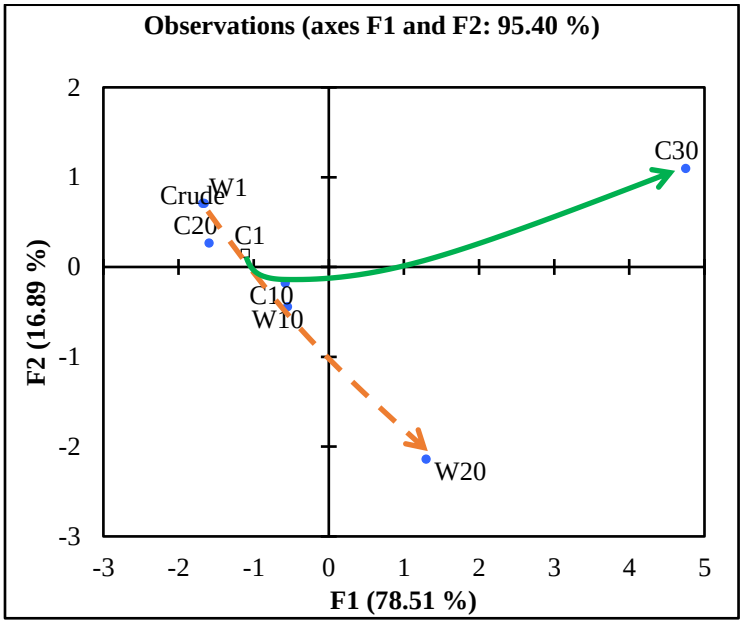


Figure 5.6 Differentiation of CDO from WCO and crude oil using diamantanes

Table 5.6 Diagnostic ratios of adamantanes

	Ad1	Ad2	Ad3	Ad4	Ad5	Ad6	Ad7	Ad8	Ad9	Ad12	Ad13	Ad15
Crude	0.64	0.84	1.33	0.8	0.84	1.32	1.19	0.75	2.45	0.90	0.82	0.82
C1	1.10	1.01	0.70	0.56	0.79	1.14	1.13	0.68	3.58	0.98	0.73	0.73
C10	1.76	0.86	1.37	1.16	2.41	3.02	2.56	2.42	1.27	0.85	1.60	0.85
C20	1.38	1.74	1.09	0.70	1.50	1.80	1.58	1.06	2.70	0.87	0.78	0.64
C30	1.51	2.49	2.05	1.01	3.08	4.96	4.94	0.55	1.57	1.00	1.06	1.01
C40	1.42	2.23	1.85	1.00	2.61	3.88	4.32	0.56	1.59	1.12	1.08	0.94
W1	0.80	0.51	0.32	0.38	0.26	0.37	0.37	1.52	5.45	0.98	0.58	0.69
W10	0.72	3.33	3.97	0.93	4.88	5.86	4.07	0.82	0.66	0.69	1.20	1.96
W20	1.26	3.11	3.61	2.44	4.54	7.73	3.90	0.41	0.68	0.54	1.12	1.39

Table 5.7 Diagnostic ratios of diamantanes

	Dia1	Dia2	Dia3	Dia4	Dia5	Dia6
Crude	0.73	3.59	0.98	2.22	1.70	1.28
C1	0.63	3.22	0.92	2.35	1.75	1.19
C10	0.54	2.75	0.84	2.61	1.99	1.27
C20	0.55	3.29	0.88	2.10	1.73	1.36
C30	2.12	2.39	0.58	4.00	4.78	0.74
W1	0.73	3.62	1.02	2.30	1.72	1.22
W10	0.58	2.86	0.91	2.47	1.87	1.02
W20	0.57	1.34	0.79	2.58	2.71	0.81

Table 5.8 Diagnostic ratios of sesquiterpanes

	p3/p4	p4/p5	p5/p6	p8/p10	p5/p10	p3/p6	p6/p8
crude	1.08	0.50	2.05	0.27	0.81	1.12	1.48
C1	1.44	0.37	1.85	0.29	0.73	0.96	1.39
C10	1.26	0.91	0.61	0.28	0.22	0.48	1.59
C20	1.09	0.47	1.90	0.29	0.57	0.99	1.02
C30	1.26	0.69	1.13	0.32	0.54	1.27	1.52
C40	1.02	1.16	1.36	0.31	0.49	1.62	1.16
w1	1.05	0.41	1.84	0.30	0.69	0.79	1.26
W10	0.66	0.31	1.95	0.28	0.45	0.39	0.83
W30	0.98	0.71	1.40	0.40	1.34	0.96	2.45

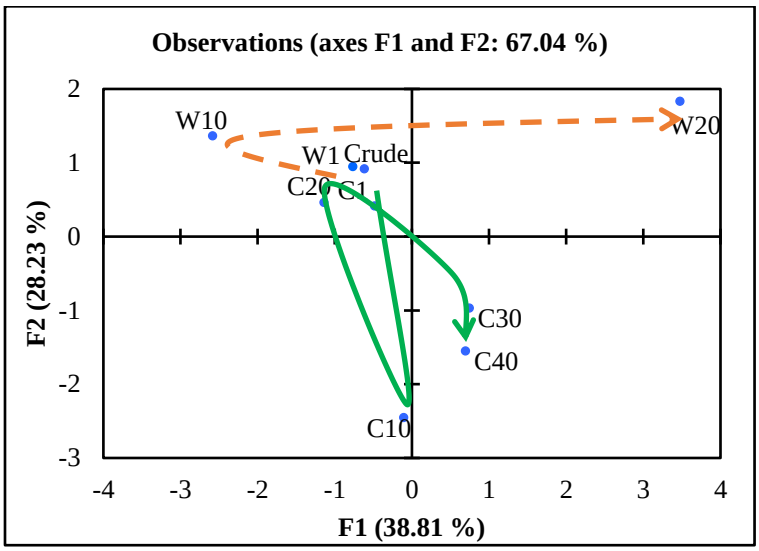


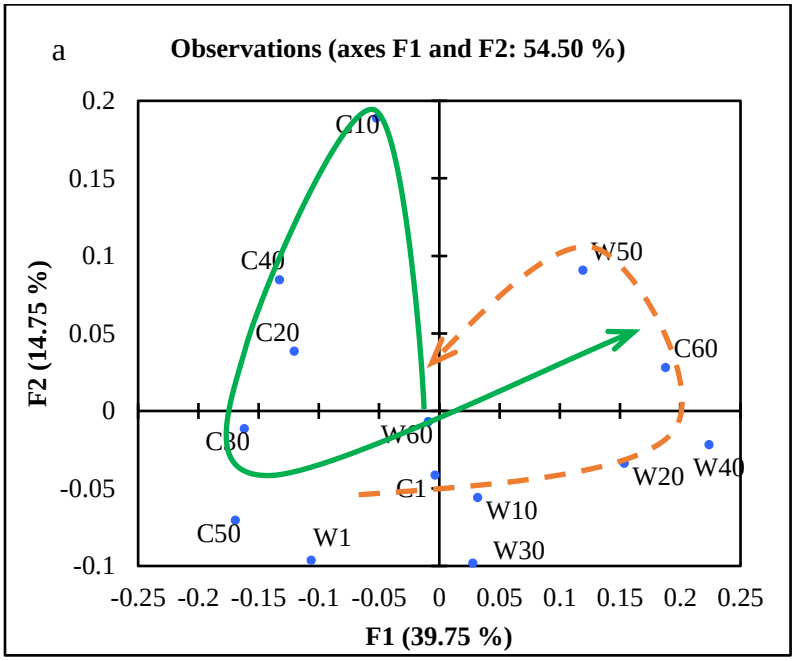
Figure 5.7 Differentiation of CDO from WCO and crude oil using sesquiterpanes

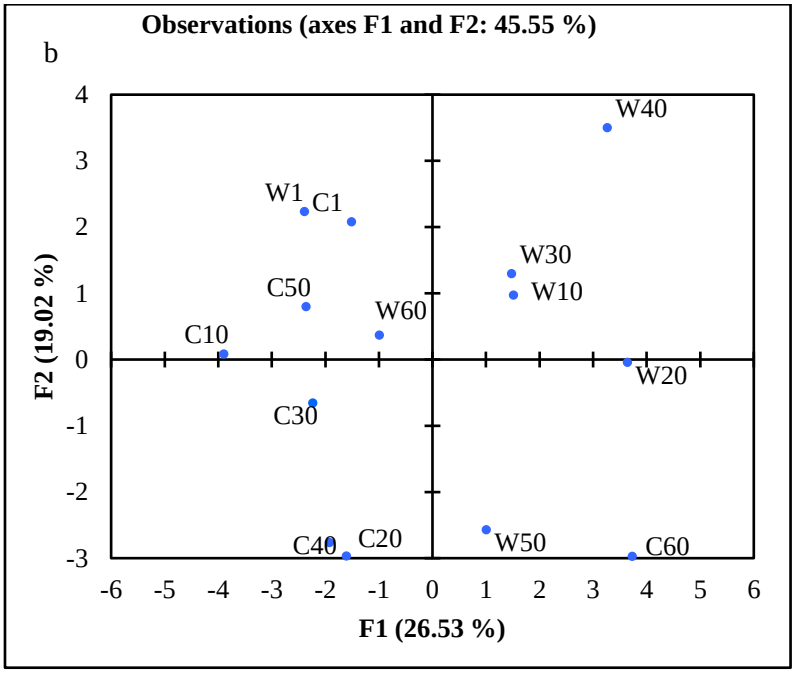
Table 5.9. PC2 involves longer weathering days (C40, W30 as well as C10). The assessment is similar to diamantanes. PCA clearly indicates the differences between long-term weathering and short-term weathering. Additionally, short-term weathering (less than 10 days) is identified using the diagnostic ratios of sesquiterpanes of CDO and WCO. The changes of the values of p3/p4, and p4/p5 may indicate the degree of weathering of CDO, while the degree of weathering of WCO is related to p3/p6 and p5/p10.

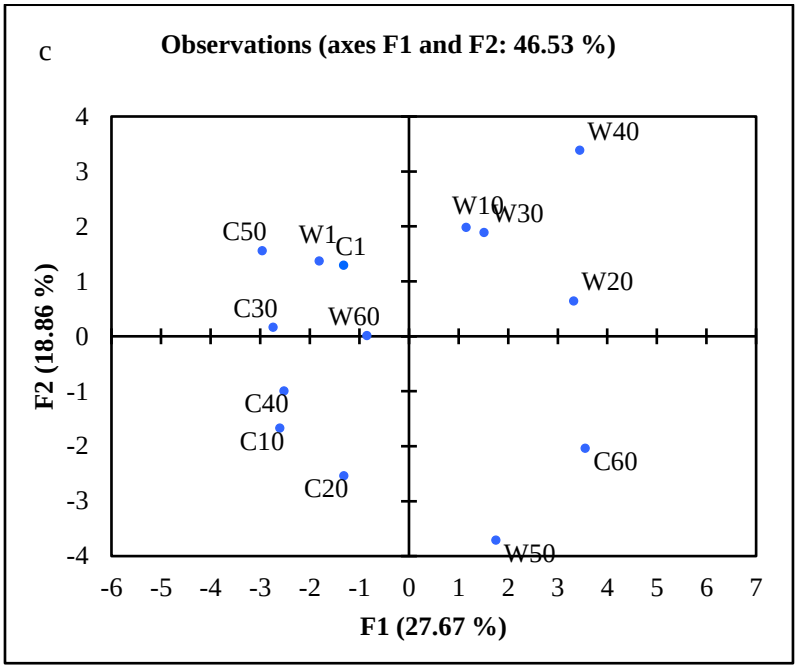
The curve connecting data plots of adamantanes with the order of weathering duration are similar in pattern to those displayed in Figure 5.1. The directions of the curves for both CDO (green line) and WCO (orange line) are counterclockwise (Figure 5.4). The same trend is observed in Figure 5.6. The rotation of line from both CDO and WCO were clockwise using sesquiterpanes.

5.3.2 The effects of dispersants and weathering on high-molecular biomarkers

Only one principal component was obtained during PCA using the diagnostic ratios of steranes, terpanes, TA-steranes, and MA-steranes alone, respectively. Eighty percentage of the diagnostic ratios of these biomarkers have a relatively low RSD values (<5%) based on our previous study (Song et al. 2018). The high recalcitrance of the biomarkers to weathering probably is the main reason of low variances. The slight difference of resistance to weathering of different types of biomarkers may be important to identify CDO from WCO. PCA is then conducted using the diagnostic ratios of steranes and terpanes using 4 PCA methodologies shown in Figure 5.8 (a-d). The diagnostic ratios are given in Table 5.9. The PCA basically separated CDO (left zone) and WCO (right zone) into two zones. The duration of weathering of CDO and


a
Observations (axes F1 and F2: 54.50 %)
F2 (14.75 %)
F1 (39.75 %)
C10
C40
C20
C30
C50
W1
W60
C1
W50
C60
W20
W40
W10
W30


b
Observations (axes F1 and F2: 45.55 %)
F2 (19.02 %)
F1 (26.53 %)
W40
W1
C1
W30
W10
C50
W60
C10
W20
C30
C40
C20
W50
C60



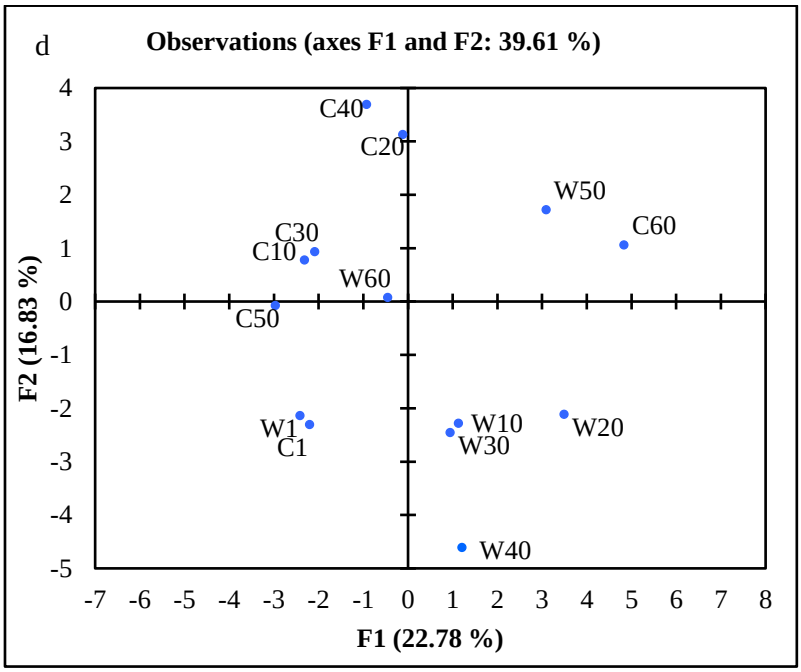
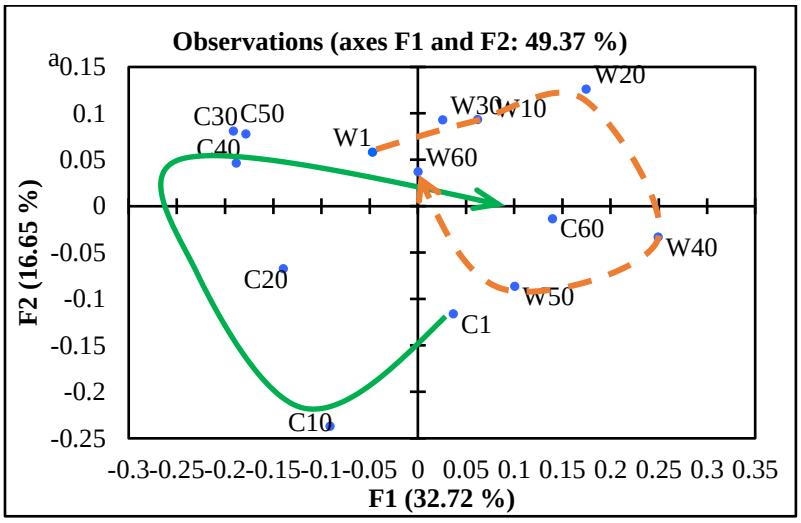
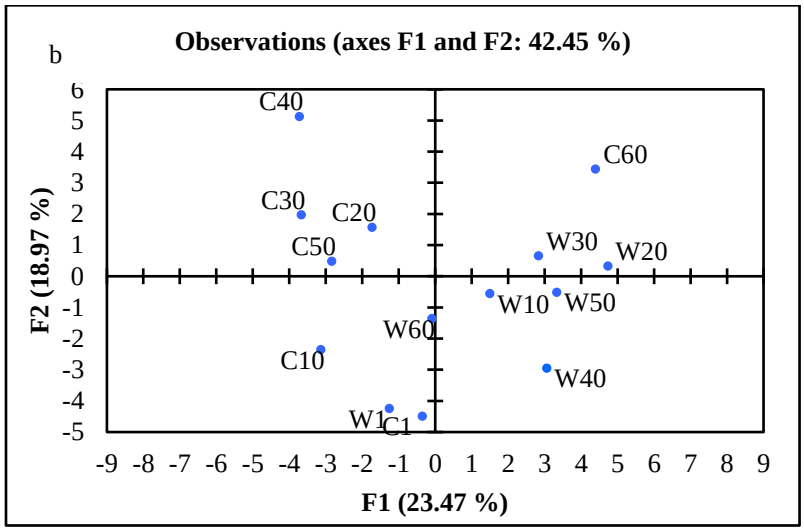
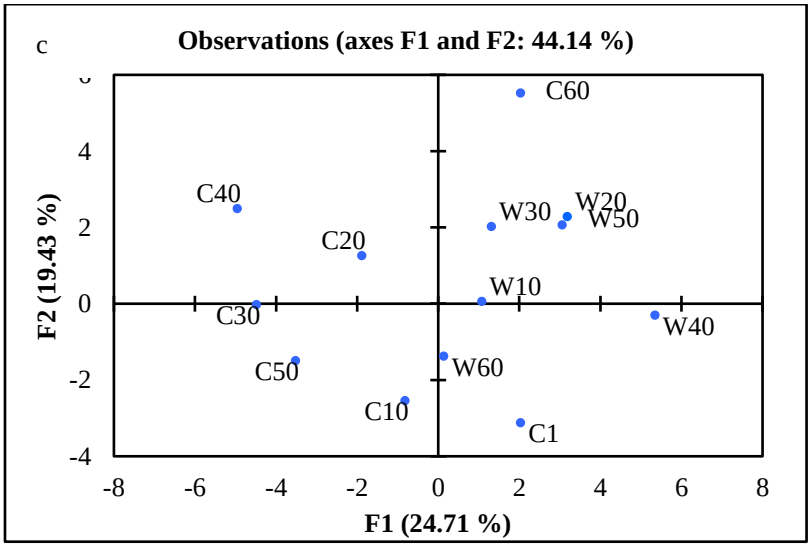


Figure 5.8 Differentiation of CDO from WCO by the diagnostic ratios of the combination of steranes and terpanes using: a) Covariance, b) Pearson c) Spearman, and d) Kendall PCA


a
Observations (axes F1 and F2: 49.37 %)
F2 (16.65 %)
F1 (32.72 %)
C30
C50
C40
W1
W30
W10
W20
W60
C60
W40
C20
W50
C1
C10


b
Observations (axes F1 and F2: 42.45 %)
F2 (18.97 %)
F1 (23.47 %)
C40
C60
C30
C20
C50
W30
W20
W10
W50
W60
C10
W40
W1
C1


c
Observations (axes F1 and F2: 44.14 %)
F2 (19.43 %)
F1 (24.71 %)
C60
C40
W30
W20
W50
C20
W10
C30
W40
C50
W60
C10
C1

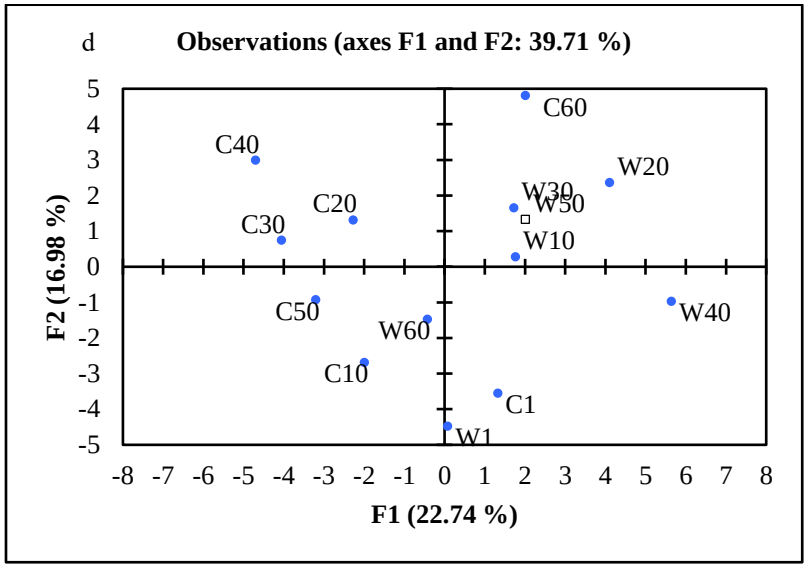


Figure 5.9 Differentiation of CDO from WCO by the diagnostic ratios of the combination of high-molecular aliphatic and aromatic biomarkers using: a) Covariance, b) Pearson c) Spearman, and d) Kendall PCA

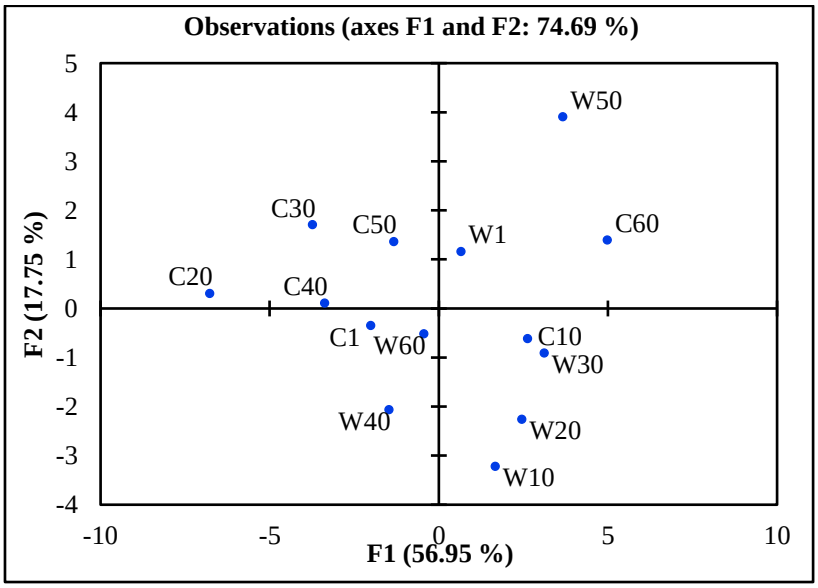


Figure 5. 10 Differentiation of CDO from WCO using diagnostic ratios of two types of biomarkers (terpanes/steranes)

Table 5.9 Diagnostic ratios of steranes and terpanes

	c23/c24	c25/C26	C27a/b	C28a/b	Ts/Tm	C29/C30	H31S/R	H32S/R	H33S/R	H34S/R	H35S/R
C1	1.67	1.42	0.89	0.90	0.60	0.72	1.38	1.33	1.44	1.61	1.34
C10	1.54	1.29	0.79	0.92	0.65	0.68	1.43	1.32	1.40	1.64	1.44
C20	1.62	1.37	0.81	0.93	0.65	0.72	1.37	1.31	1.55	1.66	1.22
C30	1.66	1.27	0.80	0.93	0.61	0.74	1.36	1.31	1.45	1.65	1.24
C40	1.59	1.31	0.78	1.01	0.63	0.73	1.38	1.28	1.52	1.65	1.29
C50	1.71	1.30	0.79	0.93	0.60	0.72	1.35	1.30	1.48	1.58	1.20
C60	1.70	1.53	0.91	0.96	0.68	0.77	1.50	1.27	1.52	1.59	1.41
W1	1.66	1.35	0.88	0.89	0.59	0.71	1.35	1.32	1.42	1.53	1.22
W10	1.61	1.30	0.97	0.93	0.63	0.73	1.38	1.27	1.50	1.61	1.33
W20	1.69	1.33	0.94	0.90	0.64	0.74	1.55	1.26	1.55	1.64	1.34
W30	1.65	1.40	0.94	0.94	0.64	0.74	1.39	1.24	1.49	1.59	1.27
W40	1.67	1.42	0.96	0.96	0.63	0.70	1.48	1.24	1.57	1.56	1.47
W50	1.73	1.35	0.89	0.91	0.62	0.72	1.51	1.28	1.53	1.63	1.41
W60	1.67	1.31	0.88	0.92	0.61	0.71	1.40	1.29	1.49	1.52	1.36
st1	st2	st3	st4	st5	st6	st7	st8	st9	st10	st11	st12
1.54	0.62	1.59	0.65	0.50	0.48	0.86	1.06	0.89	0.76	1.47	0.92
1.49	0.59	1.67	0.66	0.48	0.43	0.82	1.06	0.89	0.77	1.44	0.96
1.57	0.61	1.70	0.65	0.48	0.44	0.86	1.10	0.97	0.82	1.49	1.04
1.55	0.62	1.64	0.65	0.49	0.46	0.85	1.07	0.97	0.73	1.49	0.95
1.57	0.60	1.72	0.64	0.48	0.44	0.86	1.11	0.96	0.72	1.51	0.94
1.56	0.59	1.64	0.68	0.50	0.45	0.85	1.09	0.95	0.73	1.50	0.93

1.58	0.63	1.72	0.64	0.48	0.44	0.92	1.02	1.00	0.75	1.42	0.95
1.51	0.61	1.68	0.68	0.50	0.43	0.89	1.03	0.96	0.75	1.47	0.95
1.56	0.55	1.67	0.67	0.51	0.45	0.94	0.99	0.99	0.75	1.43	0.95
1.55	0.58	1.59	0.68	0.48	0.45	0.96	1.02	1.01	0.74	1.38	0.95
1.55	0.61	1.63	0.66	0.50	0.47	0.90	0.99	0.92	0.76	1.44	0.92
1.55	0.62	1.52	0.68	0.54	0.52	0.91	1.04	0.94	0.80	1.44	0.95
1.54	0.63	1.71	0.62	0.46	0.43	0.87	1.02	0.94	0.75	1.38	1.04
1.55	0.60	1.69	0.64	0.48	0.44	0.88	1.04	0.93	0.74	1.48	0.92

Table 5.10 Diagnostic ratios of combination of high-molecular biomarkers

	1	2	3	4	5	6	7	8	9	10	11	12	13
	c23/c24	c25/C26	C27a/b	C28a/b	Ts/Tm	C29/C30	H31S/R	H32S/R	H33S/R	H34S/R	H35S/R	st1	st2
C1	1.67	1.42	0.89	0.90	0.60	0.72	1.38	1.33	1.44	1.61	1.34	1.54	0.62
C10	1.54	1.29	0.79	0.92	0.65	0.68	1.43	1.32	1.40	1.64	1.44	1.49	0.59
C20	1.62	1.37	0.81	0.93	0.65	0.72	1.37	1.31	1.55	1.66	1.22	1.57	0.61
C30	1.66	1.27	0.80	0.93	0.61	0.74	1.36	1.31	1.45	1.65	1.24	1.55	0.62
C40	1.59	1.31	0.78	1.01	0.63	0.73	1.38	1.28	1.52	1.65	1.29	1.57	0.60
C50	1.71	1.30	0.79	0.93	0.60	0.72	1.35	1.30	1.48	1.58	1.20	1.56	0.59
C60	1.70	1.53	0.91	0.96	0.68	0.77	1.50	1.27	1.52	1.59	1.41	1.58	0.63
W1	1.66	1.35	0.88	0.89	0.59	0.71	1.35	1.32	1.42	1.53	1.22	1.51	0.61
W10	1.61	1.30	0.97	0.93	0.63	0.73	1.38	1.27	1.50	1.61	1.33	1.56	0.55
W20	1.69	1.33	0.94	0.90	0.64	0.74	1.55	1.26	1.55	1.64	1.34	1.55	0.58
W30	1.65	1.40	0.94	0.94	0.64	0.74	1.39	1.24	1.49	1.59	1.27	1.55	0.61
W40	1.67	1.42	0.96	0.96	0.63	0.70	1.48	1.24	1.57	1.56	1.47	1.55	0.62
W50	1.73	1.35	0.89	0.91	0.62	0.72	1.51	1.28	1.53	1.63	1.41	1.54	0.63
W60	1.67	1.31	0.88	0.92	0.61	0.71	1.40	1.29	1.49	1.52	1.36	1.55	0.60

	14	15	16	17	18	19	20	21	22	23	24	25
	st3	st4	st5	st6	st7	st8	st9	st10	st11	st12	TAS1	TAS2
C1	1.59	0.65	0.50	0.48	0.86	1.06	0.89	0.76	1.47	0.92	0.94	0.98
C10	1.67	0.66	0.48	0.43	0.82	1.06	0.89	0.77	1.44	0.96	0.88	1.04
C20	1.70	0.65	0.48	0.44	0.86	1.10	0.97	0.82	1.49	1.04	0.90	1.06
C30	1.64	0.65	0.49	0.46	0.85	1.07	0.97	0.73	1.49	0.95	0.87	0.95
C40	1.72	0.64	0.48	0.44	0.86	1.11	0.96	0.72	1.51	0.94	0.88	1.01
C50	1.64	0.68	0.50	0.45	0.85	1.09	0.95	0.73	1.50	0.93	0.87	1.05

C60	1.72	0.64	0.48	0.44	0.92	1.02	1.00	0.75	1.42	0.95	0.93	1.12
W1	1.68	0.68	0.50	0.43	0.89	1.03	0.96	0.75	1.47	0.95	0.93	0.97
W10	1.67	0.67	0.51	0.45	0.94	0.99	0.99	0.75	1.43	0.95	0.90	0.92
W20	1.59	0.68	0.48	0.45	0.96	1.02	1.01	0.74	1.38	0.95	0.92	0.99
W30	1.63	0.66	0.50	0.47	0.90	0.99	0.92	0.76	1.44	0.92	0.94	1.01
W40	1.52	0.68	0.54	0.52	0.91	1.04	0.94	0.80	1.44	0.95	0.95	0.95
W50	1.71	0.62	0.46	0.43	0.87	1.02	0.94	0.75	1.38	1.04	0.95	0.98
W60	1.69	0.64	0.48	0.44	0.88	1.04	0.93	0.74	1.48	0.92	0.92	0.97

	26	27	28	29	30	31	32	33	34	35	36	37
	TAS3	TAS4	TAS5	TAS6	TAS7	MAS1	MAS2	MAS3	MAS4	MAS5	MAS6	MAS7
C1	0.28	1.63	1.01	1.00	0.31	1.48	0.62	0.74	0.44	0.63	0.91	1.12
C10	0.29	1.64	1.01	1.05	0.30	1.45	0.63	0.80	0.43	0.66	0.80	1.08
C20	0.29	1.62	1.01	1.06	0.35	1.48	0.64	0.83	0.43	0.68	0.84	1.14
C30	0.29	1.55	1.11	1.09	0.32	1.61	0.62	0.83	0.43	0.70	0.79	1.12
C40	0.29	1.58	1.11	1.04	0.31	1.60	0.58	0.91	0.40	0.69	0.80	1.05
C50	0.29	1.57	1.05	1.04	0.30	1.57	0.54	0.82	0.44	0.69	0.78	1.15
C60	0.31	1.62	1.03	1.04	0.35	1.58	0.56	0.86	0.44	0.66	0.82	1.12
W1	0.28	1.64	0.98	1.11	0.27	1.61	0.54	0.79	0.44	0.67	0.93	1.13
W10	0.30	1.59	1.01	1.02	0.31	1.62	0.56	0.79	0.43	0.67	0.91	1.10
W20	0.31	1.59	1.04	1.01	0.33	1.67	0.56	0.81	0.44	0.65	0.93	1.15
W30	0.30	1.60	1.03	1.04	0.40	1.59	0.52	0.88	0.45	0.68	0.81	1.18
W40	0.30	1.62	0.99	1.00	0.30	1.61	0.65	0.75	0.42	0.63	0.89	1.13
W50	0.30	1.62	1.03	1.00	0.31	1.50	0.56	0.80	0.45	0.65	0.82	1.14
W60	0.28	1.58	1.01	1.05	0.30	1.60	0.53	0.82	0.46	0.66	0.85	1.10

Table 5.11 Diagnostic ratios of two types of biomarkers (terpanes/steranes)

	TS/1	c23/1	C30/7	c30/8	h31s/10	c27a/7	c27a/8	C30/13	C30/14
C1	1.12	2.57	8.47	4.58	2.63	0.65	0.36	9.76	8.12
C10	1.34	2.48	10.80	4.67	3.05	0.75	0.32	11.07	9.12
C20	1.10	2.21	8.75	3.81	2.42	0.70	0.31	8.25	7.10
C30	1.19	2.48	8.76	4.01	2.50	0.74	0.34	8.74	7.46
C40	1.18	2.37	8.95	4.09	2.84	0.68	0.31	9.22	7.77
C50	1.25	2.85	9.54	4.27	2.81	0.75	0.33	9.48	8.14
C60	1.37	3.38	9.63	4.20	2.85	0.73	0.32	10.47	9.59
W1	1.25	2.75	10.73	4.64	3.06	0.79	0.34	9.96	8.90
W10	1.51	2.46	10.23	4.65	3.30	0.67	0.30	9.79	9.19
W20	1.50	2.82	10.07	4.51	3.02	0.69	0.31	9.85	9.42
W30	1.40	2.92	9.77	4.56	3.05	0.69	0.32	10.65	9.55
W40	1.35	2.53	9.12	4.81	2.75	0.65	0.34	9.26	8.36
W50	1.32	3.40	10.15	4.39	2.79	0.82	0.35	10.65	9.30
W60	1.28	2.57	10.51	4.60	2.88	0.74	0.32	9.86	8.38
	c27a/10	28a/14	C30/17	C30/18	TS/8	TS/14	TS/18	c29/18	t1/t2
C1	0.53	0.75	8.55	6.87	0.76	1.36	1.15	5.04	1.31
C10	0.54	0.90	9.34	7.19	0.79	1.55	1.22	5.24	1.39
C20	0.52	0.80	7.26	5.91	0.66	1.24	1.03	4.20	1.20
C30	0.55	0.86	8.35	6.10	0.73	1.36	1.11	4.49	1.31
C40	0.54	0.90	8.40	6.15	0.70	1.32	1.05	4.50	1.29
C50	0.55	0.90	8.65	6.26	0.75	1.43	1.10	4.54	1.39
C60	0.55	0.97	9.47	7.07	0.88	2.01	1.49	5.42	1.65

W1	0.57	0.87	9.02	6.74	0.75	1.44	1.09	4.78	1.40
W10	0.51	0.85	9.02	6.75	0.84	1.66	1.21	4.97	1.34
W20	0.49	0.87	9.13	6.76	0.86	1.81	1.30	5.03	1.42
W30	0.52	0.93	9.13	6.89	0.85	1.80	1.30	5.07	1.48
W40	0.51	0.78	8.22	6.62	0.84	1.45	1.15	4.67	1.28
W50	0.61	0.97	9.20	6.92	0.83	1.75	1.30	5.01	1.61
W60	0.53	0.83	9.03	6.65	0.76	1.38	1.10	4.73	1.35

WCO is identified (anticlockwise) with only a discordance of data point using the covariance method. The weathering of different types of biomarkers may gradually be affected by the application of dispersant, but insignificantly influenced by weathering duration. The diagnostic ratios of steranes terpanes, TA-steranes, and MA-steranes are combined to operate PCA using four methods (Figure 5.9) with diagnostic ratios in Table 5.10. Four PCA methods accomplished the differentiation of CDO (left zone) from WCO (right zone). The PCA results of the diagnostic ratios of different types of biomarkers also could differentiate CDO from WCO as shown in Figure 5.10 with data in Table 5.11. The duration of weathering of CDO and WCO is identified (anticlockwise) by the covariance method. Since the diagnostic ratios of the same types of biomarkers were stable, the difference between diagnostic ratios in CDO and WCO implied the influence of use of dispersant on weathering process of different types of biomarkers.

In addition, when data plots are linked using a curve, the counterclockwise trend is suitable for CDO and WCO using terpanes and steranes (Figure 5.8 a). But the line cannot be drawn using other PCA methods. The available trend of the plots may be narrowed down to Pearson PCA. In terms of the combination of high-molecular aliphatic and aromatic biomarkers (Figure 5.9 a), the direction is clockwise when Pearson PCA was applied. The order become subtle when using other non-parametric methods. The omitted information may correlate to the effects of weathering duration on the variations of diagnostic ratios. Some secondary information is omitted during the ranking process. The impacts of weathering duration on diagnostic ratios is of secondary importance compared to the effects of application of dispersants.

5.4 Summary

CDO samples were differentiated from WCO samples using all the low-molecular biomarkers or combinations of high molecular biomarkers by multiple PCA methods. The application of dispersants can affect the weathering fate of biomarkers to differentiate the weathering process of CDO from WCO. The differences of CDO and WCO samples were induced by the effects of weathering duration as well. The overall trend of weathering duration can be displayed in scores plots from PCA analyzes. Involved biomarkers play a paramount role for CDO differentiation. The results implied the diverse degrees of weathering of different types of biomarkers and reflected the importance and possibility of application of biomarkers to trace the behaviors of weathered dispersed oil. More indices including diagnostic ratios and isotopic index will be used in further studies to better trace the weathering of oils, and application of countermeasures of oil spill using fingerprinting.

CHAPTER 6 BIOMARKER TRACED BIODEGRADATION OF OIL TREATED BY A GREEN DISPERSANT[4]

[4] *The contents of this chapter are based and expanded on the following paper:*

Song, X., Zhang, B., Chen, B., Liu, B., Lye, L. M., & Zhang, K.D. (2019). Biomarker traced biodegradation of oil treated by a green dispersant. Ready for submission.

Role: Xing Song solely worked on this study and acted as the first author of this manuscript under Dr. Baiyu Zhang, Dr. Bing Chen, and Dr. Leonard M. Lye's guidance. Most contents of this paper were written by him and further edited by the other co-authors. Dr. Bo Liu helped seawater sampling, respirometer setup, and microtox setup. Mr. Kedong Zhang generated surfactants (before modification) with me and helped to conduct biodegradation (before modification).

6.1 Introduction

The increasing demand for high biodegradability of spilled oil promoted the development of green dispersants in recent years. Currently, limit studies evaluated the biodegradability of oil treated by new dispersants. Besides, the effects of dispersants on fingerprinting and the biodegradation degree of dispersed oil, especially of oil treated by new dispersants, are imperative but less tackled. In this chapter, a commercial dispersant and a new green dispersant generated in the NRPOP laboratory at Memorial University were adopted. Modification of the green dispersant was conducted through improved filtration. The aims of this chapter are to better understand the fingerprinting of biodegraded oil dispersed by the two dispersants, and to further evaluate the biodegradability of dispersed oil through developing an oil tracing methodology based on oil fingerprinting. The changes of the diagnostic ratios from eight types of biomarkers were examined and categorized to diverse functions including source identification, oil quantification, biodegradation status evaluation, and differentiation of the effects of dispersants. The research outputs would give a better understanding of CDO fingerprinting and biodegradation extents of oil treated by different dispersants, especially by new dispersants. The findings would also help to promote the development, improvement, and applications of green dispersants.

6.2 Methods and Materials

6.2.1 Chemicals and materials

Alaska North Slope (ANS) and No.6 marine fuel oil (MF #6) were selected as targeting

oils. ANS crude oil was a typical medium-graded oil widely used in references for the fate and behaviors of spilled oil. Marine fuels were playing a leading role in numbers and amounts of oil spills during shipping (Huijer 2005). Surrogates associated with internal standard, C_{30} 17β(H) 21β(H)-hopane, obtained from Sigma Canada were applied for quality assurance and quality control. All the organic solvents, including hexane, dichloromethane (DCM), and methanol were analytical or chromatogram grade. Alcalase enzyme was purchased from Sigma Canada. Seawater near offshore Newfoundland was sampled in winter and stored at 4 ºC in the fridge before usage. Filter paper (1.5 μm) was purchased from Fisher Scientific Canada. The supplies for acute toxicity determination, including diluent, osmotic adjusting solution (OAS), and reconstitution solution for the mixture of a bacterium, were bought from Modern Water Company.

6.2.2 Improved production of a green dispersant

Shrimp waste was prepared and the associated dispersant production was modified based on a previous study (Zhang et al. 2018a). Briefly, shrimp waste was collected from purchased northern pink shrimp (Pandalus borealis) from the local fish market in Newfoundland, Canada. The shrimp waste was grounded in a food processor (Black & Decker Model FP2700SC) and then stored at -20 ºC. The frozen shrimp waste was thawed at room temperature for approximately an hour. Shrimp waste was homogeneously mixed with the distilled water (1:1, w/v) in colonial flasks. The mixture was heated in a 90 ºC water bath for 15 min to inhibit the indigenous hydrolysis. Aliquots of Alcalase enzyme were added into flasks as

optimized proportions (2.25:100 v/v) when the mixture was cooled to room temperature. The solution was hydrolyzed through a shaker following the optimized conditions: shaking rate 120 rpm, temperature 56 ºC, for one hour. The Alcalase enzyme was then inactivated using a 90 ºC water bath for 15 min. The flasks were then cooled to room temperature and subsequently centrifuged at 10,000 rpm for 12 min. The supernatant was collected and filtered using 1.5 um filter paper in a separation apparatus for four times. The residue solid debris and shrimp oil were excluded through filtration to obtain a clear water solution (Figure 6.1). Dry powders were generated using a freeze dryer and stored in a desiccator. Aquatic samples were generated using dried 500 mg of powder mixed with 100 mL of distilled water. The powder was dissolved into water column shortly and appeared orange. The performance of filtered dispersants in oil dispersion was not affected by the filtration and was compatible to chemical dispersant (Figure 6.2). The acute toxicity of the modified dispersants was measured as the comparison to the observations from the previous study (Zhang et al. 2018a). The Microtox, a widely applied toxicity measurement tool, was used to assess the acute toxicity (Cook and Wells 1996). Turbidity and color correction were considered in this experiment (Campisi et al. 2005). Duplicate experiments were employed to ensure the reproducibility of the toxicity test.

6.2.3 Biodegradation of oil treated by the green dispersant

Aerobic respirometers were applied to investigate the biodegradation of oil treated by the modified shrimp waste-based dispersant. Each respirometer flask

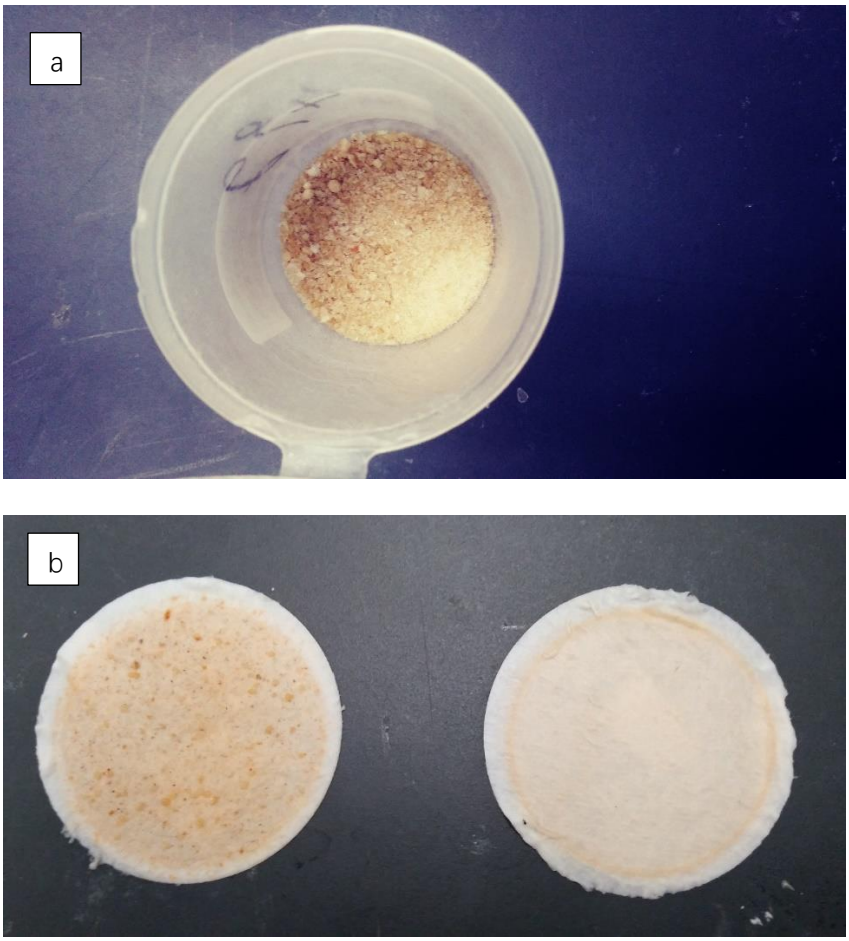


Figure 6.1 Modified dispersants

Figure 6.1 Modified dispersants: (a) Green dispersant (Powder) after filtration

(b) Insoluble compounds of dispersant in filter paper

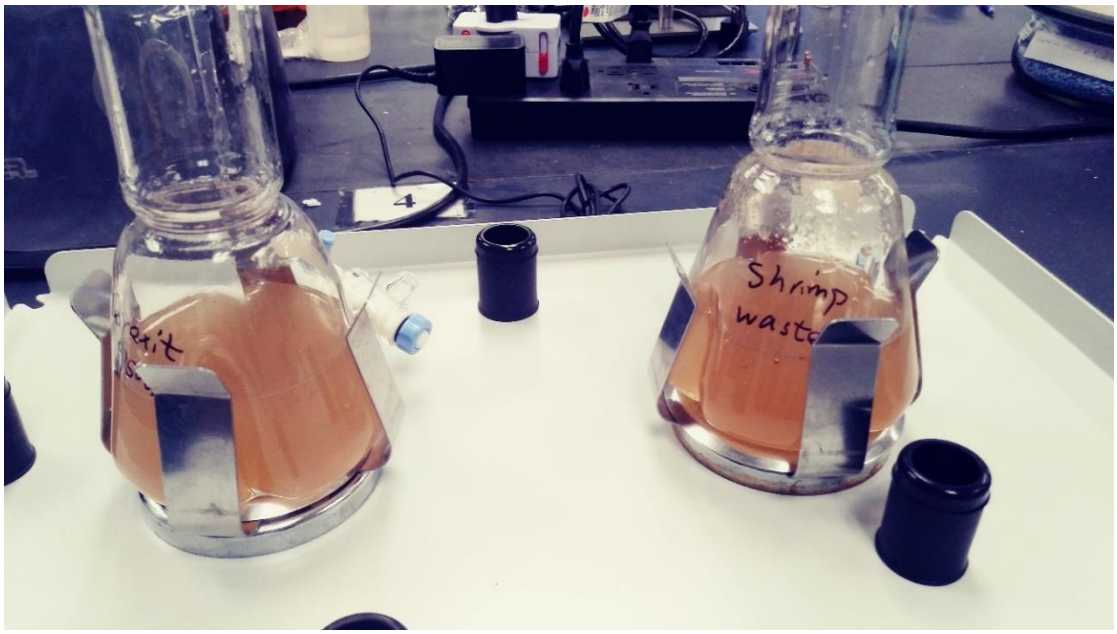


Figure 6.2 Effectiveness determination: Corexit 9500A (left) and Shrimp waste (right)

was filled by 500mL of seawater with a magnetic stir. Approximately 7.5 ug of crude oil (two types: ANS or MF #6) was added into 500ml seawater to generate a concentration of 15 ppm of oil (Prince et al. 2013). Shrimp waste powder (50mg, 0.1 CMC) was added into the flask. The amounts were adjusted to effectively dispersed 15ppm oil based on the effectiveness experiments. Extra nitrogen and phosphorus sources were added into the flasks as the nutrients to support the biodegradation. Oxygen flow was continuously added from the top of the respirometer to provide an aerobic environment. Each flask was completely covered thoroughly by an aluminum paper to create a dark environment. The weathering duration of ANS were 0, 15, and 30 days. The weathering duration of MF #6 were 0, 30, and 45 days. The flasks were sealed and weathered in duplicate.

Two comparison groups are simultaneously conducted to delve into the fingerprinting of biodegraded oil treated by dispersants as well as the biodegradation itself. Comparison experiments include the addition of Corexit 9500A (0.4 μL) or without the addition of dispersants into each flask. Three formations of oils were thus generated: oil dispersed by shrimp waste (SW), chemically dispersed oil (CDO), and non-dispersed oil as blank (B). The biodegradation conditions of the three groups are the same.

6.2.4 Characterization of biomarkers and diagnostic index

All oil components in the whole flasks were extracted when biodegradation was finished. Oil and residues attached to the wall of bottles were carefully thoroughly

washed using 50 ml DCM with glass rob for at least three times. Each water sample was extracted by 100ml DCM three times. DCM was removed using rotatory evaporation. The solvent was then transferred to hexane and concentrated to approximate 0.4 mL. The different fractions of hydrocarbons were cleaned and fractionated using a silica gel-based chromatogram column. The aliphatic hydrocarbons were eluted by 3g silica gel using 12 mL hexane. Aromatic hydrocarbons were eluted by 15ml the mixture of hexane and DCM. The two fractions were both concentrated to 1 mL.

The samples were then analyzed through GC-MS analysis. The GC-MS system and operation conditions followed our previous method for fingerprinting of biomarkers in chemically dispersed oil (Song et al. 2018) and shown in Table S1. Selected Ion mode (SIM) and full scan mode were used to identify and quantify primary hydrocarbons and biomarkers in oil. Each diagnostic ratio was calculated by the ratio of the peak areas of biomarkers.

6.2.5 Fingerprinting of oil treated by dispersants during biodegradation

The changes of diagnostic ratios during biodegradation could be applied to achieve different oil tracing functions, including oil source identification, biodegradation rate calculation, biodegradation status estimation, and the effects of dispersants on biodegradation. The procedure to integrate these functions was proposed in Figure 6.3 based on previous standard protocols and integration with oil fingerprinting. The relative standard deviation (standard deviation/average *100%) values were calculated to examine the stability of diagnostic ratios (Stout et al. 2001). The

diagnostic ratios with less than 5% of RSD values indicated that the targeting ratios are unaffected by biodegradation. The diagnostic ratios were slightly affected by weathering when RSD values from 5% to 10% of RSD value. When RSD value was larger than 10%, biodegradation was recognized as a significant contributor to the variations of diagnostic ratios. The biomarkers with high resistance to biodegradation, such as hopanes, could be applied as IS for the calculation of the rate and concentration of oil biodegradation (Prince et al. 1994, Prince et al. 2013). The changes of other biomarkers to these recalcitrant biomarkers could be used to achieve quantification functions for oil identification. In the case of readily degradable biomarkers, if calculated diagnostic ratios were stable during biodegradation, they could be feasible to identify the source of biodegraded oil (Daling et al. 2002). Unstable biomarkers could be applied to trace biodegradation degrees and the effects of dispersants through the statistical comparison of diagnostic ratios from of oil treated by SW, oil treated by Corexit 9500A, and non-dispersed oil during biodegradation (Arey et al. 2007b). Statistical analysis, such as Principal component analysis (PCA), was recommended to differentiate biodegraded oils, which reflect the effects of dispersants and other factors (Ismail et al. 2016, McGregor et al. 2012).

6.2.6 Quality control and quality assurance

The experimental procedures and data analysis were strictly managed by QA/QC protocol. All the experiments were conducted in duplicate. Two aliquots from each 150μL of extracted organic phase (aliphatic and aromatic fractions) were measured

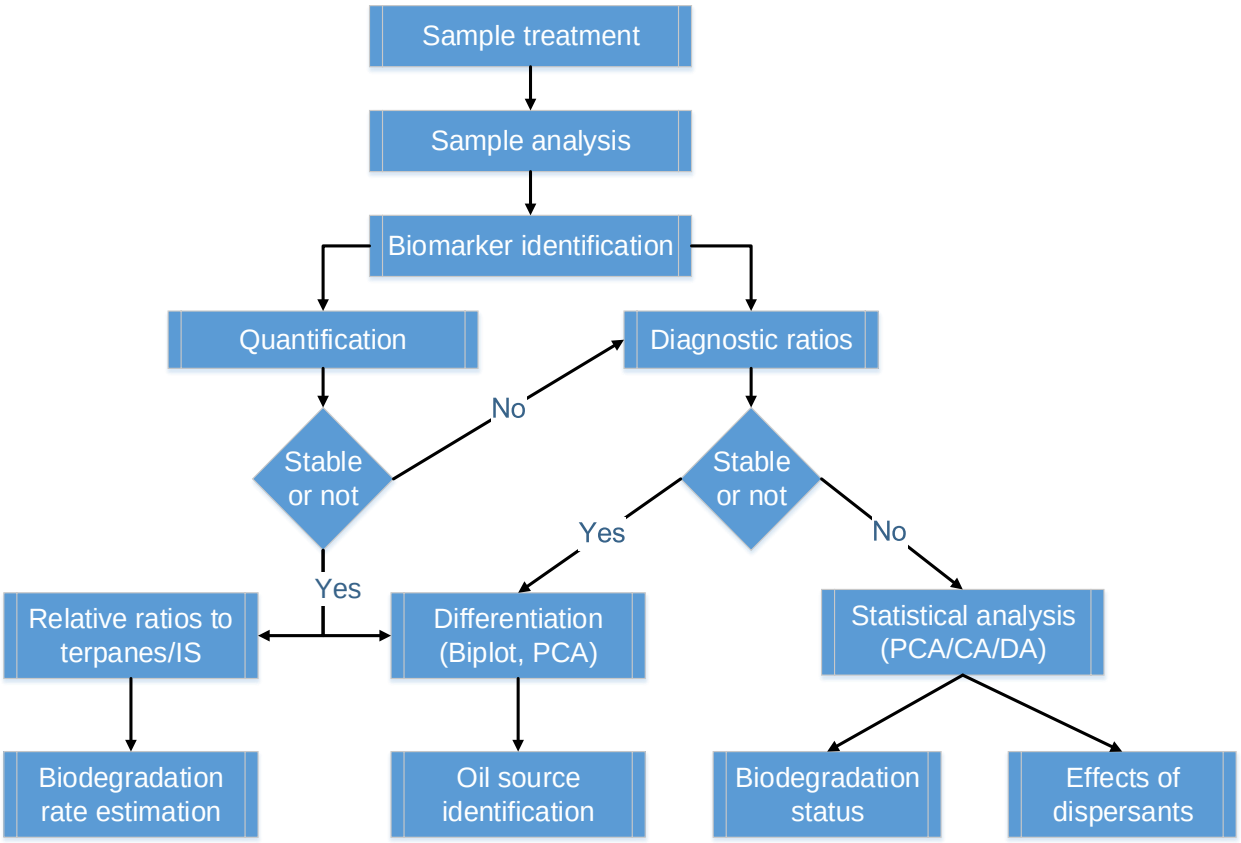


Figure 6.3 Biodegradation tracing for oil treated by dispersants

by GC-MS for duplicate analyses. The coefficients of determination (R^2) of calibration of surrogates were > 0.9940 (n=5). The average recoveries (%) of surrogates was from 90% to 110%, including acenaphthalene-d_{10}, phenanthrene-d_{10}, perylene-d_{12}, benz[a]anthracene-d_{12}, and terphenyl-d_{14}. The coefficients of determination of the calibration for quantification of n-alkanes and PAHs determination were >0.99 (n=5).

6.3 Results and Discussion

6.3.1 Identification of stable biomarkers as internal standards for oil characterization

Seven types of biomarkers and alkylated-PAHs were identified in crude oil, biodegraded crude oil, and biodegraded oil treated by dispersants for both MF and ANS oil samples. The identification information of aliphatic and aromatic biomarkers was summarized in Table 3.2. The relative peaks areas of other biomarkers to terpanes in ANS were shown in Figure 6.4. The order of selected biomarkers in ANS resistance to biodegradation was terpanes = TA-steranes = MA-steranes > steranes > diamantanes > sesquiterpanes > adamantanes. The relative peaks areas of biomarkers to terpanes in MF was calculated as well. The order for MF was terpanes = TA-steranes = steranes > diamantanes > sesquiterpanes > adamantanes. Not surprisingly, high-molecular steroids had a higher resistance to biodegradation. As a group of recalcitrant biomarkers, aromatic steranes were not changed significantly compared to hopanes. The observations showed similar resistance to biodegradation of aromatic steranes compared to terpanes. The depletion of aromatic steranes indicated that the biodegradation of oil could affect fingerprinting results using some

aromatic biomarkers, while these biomarkers were not significantly affected by physiochemical weathering. Regular steranes degraded approximately 20% relative to terpanes. The destruction of steranes indicated that the degree of oil biodegradation was changing “medium” level to “heavy” level (Seifert and Moldowan 1979). The preference of steroids biodegradation in this study was consistent with the resistance order of biomarkers biodegradation observed in experiments and biodegraded oil in field samples (Seifert and Moldowan 1979, Wardroper et al. 1984).

For diamondoids, the diamantanes degraded slower than adamantanes, consistent with previous studies related to weathering of diamondoids of crude oils (Wang et al. 2006b). Diamantanes had relatively high resistance to biodegradation compared to adamantanes, they were affected by microbial depletion as well. This biodegradation preference was the same as the results regarding the biodegradability of diamondoids in non-dispersed oil. The abundance of all the adamantanes decreased to a low level approximately near to the detection limit.

The RSD of each diagnostic ratio was calculated to evaluate the stability of biomarkers. The analytical results showed in Figure 6.5. Biomarkers located in the middle (zone A) indicating low RSD values (<5%) for both MF and ANS oil during biodegradation. Diagnostic ratios in zone B had stable values (RSD<5%) in biodegraded oil treated by SW for ANS but unstable values for MF (RSD>5%). Diagnostic ratios in zone C had stable values for MF but unstable values for ANS. Diagnostic ratios in zone D were unstable in biodegraded oil treated by SW for both ANS and MF. Families of steranes, terpanes, and TA-steranes indicated relatively stable diagnostic index with lower RSD values. MA-steranes had high stability to

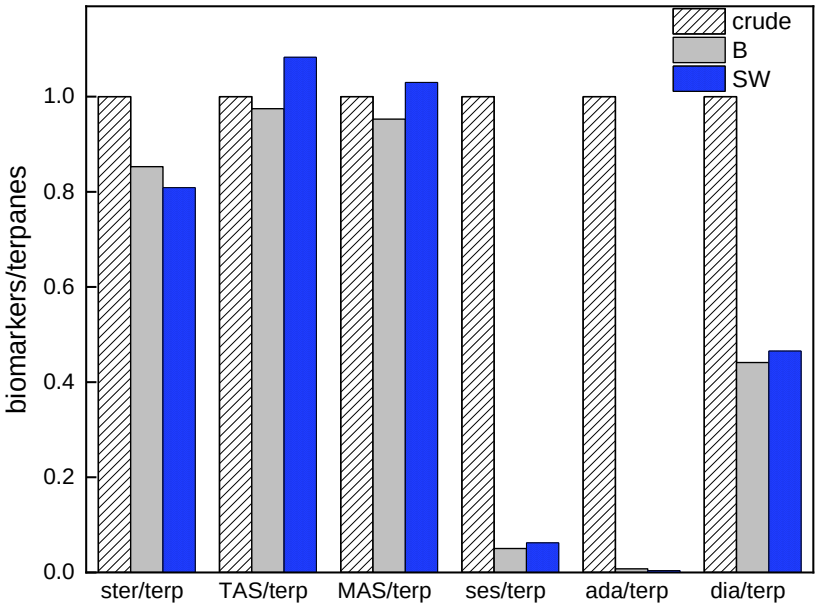


Figure 6.4 The ratios of peak areas of other biomarkers to terpanes in ANS (Ts+Tm+C29+C30)

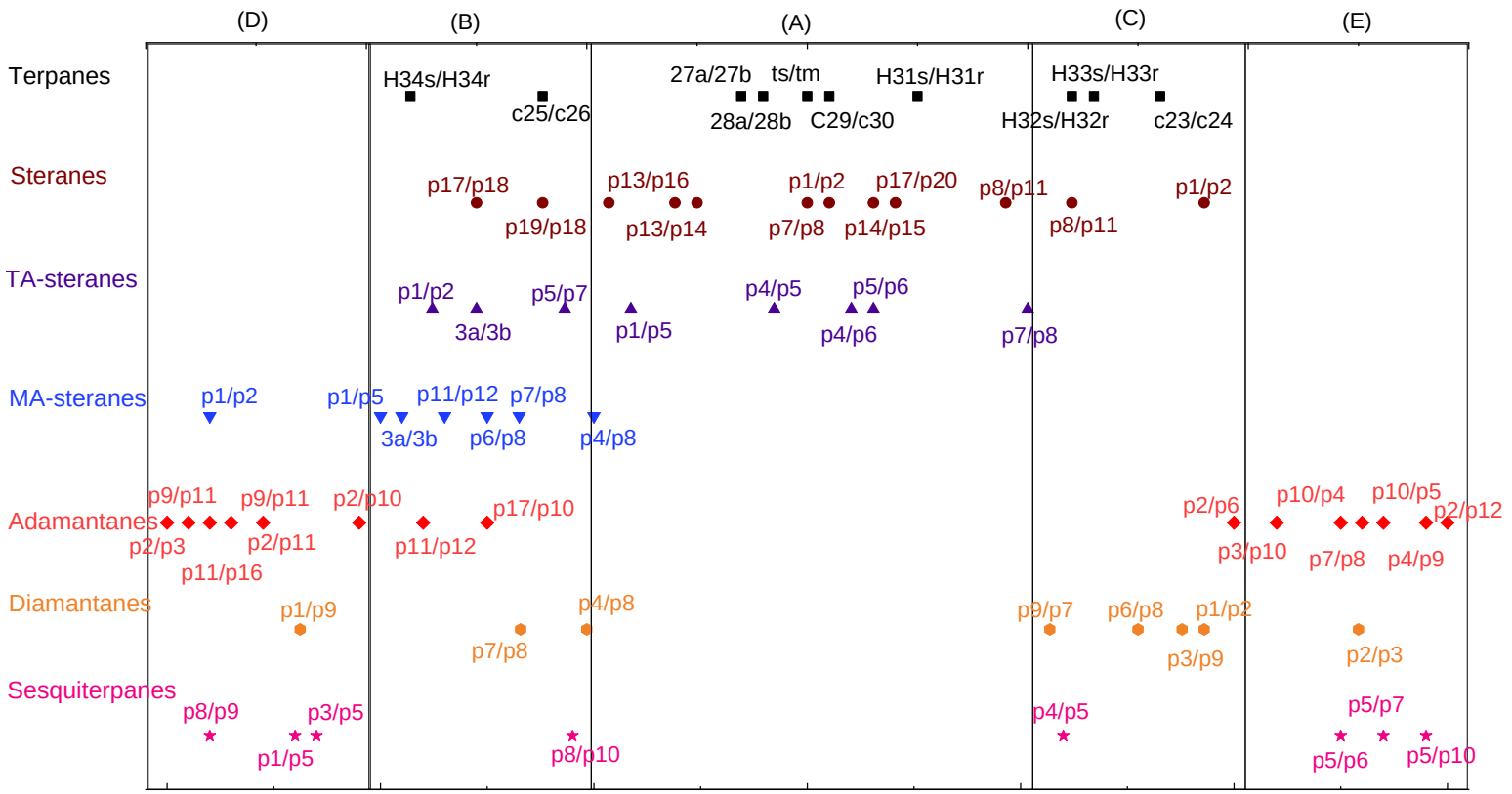


Figure 6.5 Stability of biomarkers for fingerprinting of biodegraded oil treated by shrimp-waste based dispersants: (a) stable diagnostic ratios for both MF and ANS; (b) relatively more stable in ANS, (c) relatively more stable in MF, (d-e) affected by weathering

biodegradation (RSD< 5%). Other biomarkers with low molecular weight were degraded during biodegradation with unstable diagnostic index, consistent with the physio-chemical weathering of dispersed oil (Song et al. 2018). Ninety percentage of the diagnostic ratios were affected by biodegradation. Overall, the diagnostic ratios of diamantanes were changed. The experiment flasks were avoiding light and well-sealed, the main degradation pathway of light molecular biomarkers could be considered as biodegradation with limited evaporation. Although diagnostic ratios of adamantanes were significantly affected by evaporation, the internal ratios of diamantanes have high stability with the effects of evaporation (Li et al. 2014). The diagnostic variations of diamantanes were mainly contributed to biodegradation. The differences of their diagnostic ratios could reflect the biodegradation process. It might be difficult to absolutely exclude the effects of evaporation. The sesquiterpanes were degraded with few stable diagnostic indexes. The RSD values of sesquiterpanes in biodegradation were more stable than those in physio-chemical weathering from our previous findings (Song et al. 2018), this trend probably reflected the different influences between physio-chemical weathering and biodegradation. The stability of biomarkers was determined corresponding to 100% depletion of the n-alkanes ($<C_{30}$) and 70% depletion of alkanes (n>30). The stable biomarkers were still can be applied for source identification even when most of n-alkenes are highly and even absolutely biodegraded.

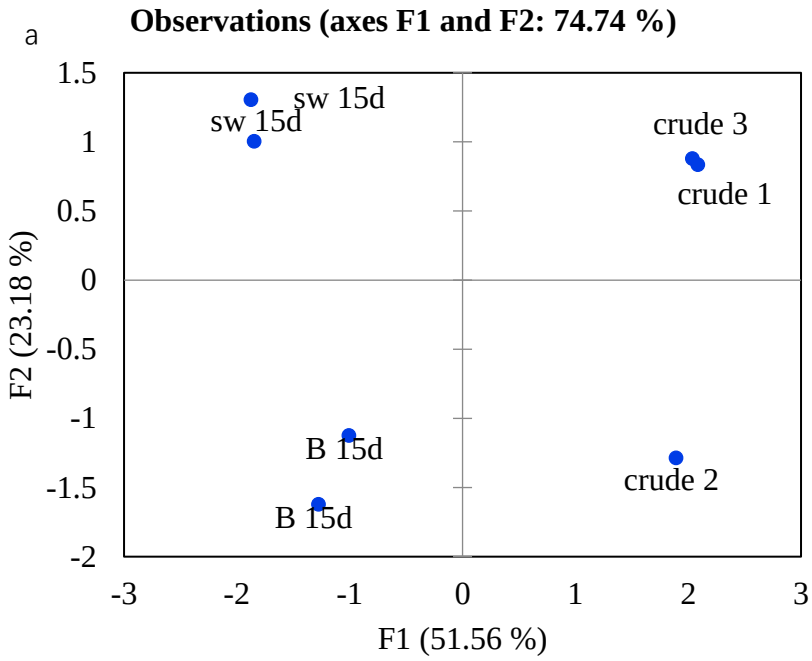
6.3.2 Screening of biomarkers for biodegradation tracing and differentiation of dispersed oil

As a useful tool for oil fingerprinting and maturity, diamondoids in oil could not exist stably in water column during weathering. Although aromatic steranes were stable, the

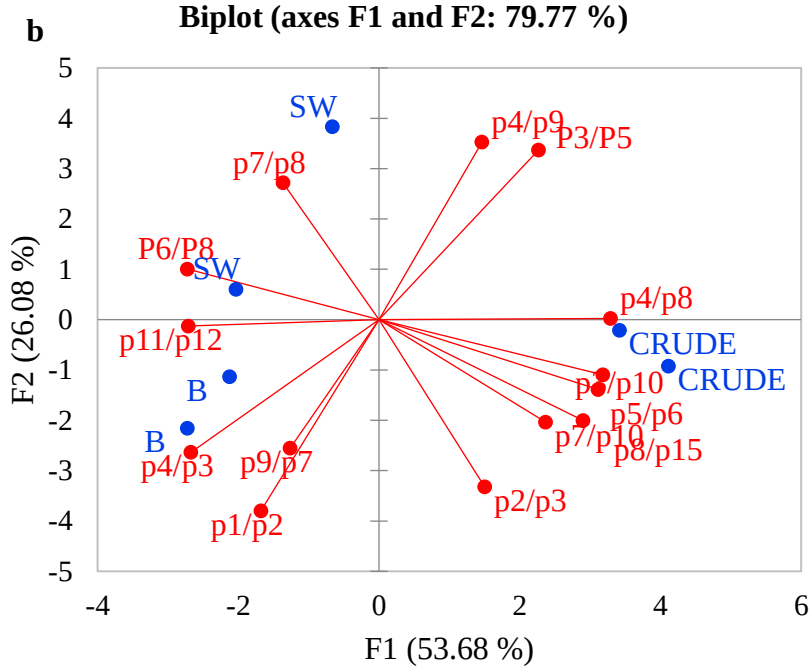
complicated distributions of MAS in #6 MF were adequate but hard to be identified. The application of types of biomarkers depended on different oil types. It was difficult to identify the difference among SW, CDO, and B through peak areas. The stable diagnostic ratios were not suitable options for differentiation of different oils. Biomarkers with slightly and hardly varied diagnostic ratios were selected to identify different oils using PCA. The analytical results using adamantanes were shown in Figure 6.6 (a). Two main components covered 80% of the total variance. Crude oil was clearly differentiated dispersed oil from non-dispersed oil by PC1. The oils dispersed by shrimp waste were in different areas as well using PC2. The selected biomarkers could achieve fingerprinting functions. Adamantanes coupled with diamantanes could differentiate dispersed and non-dispersed oil as well. The differentiation could be realized using adamantanes coupled with diamantanes as shown in Figure 6.6 (b). The diagnostic ratios tracing different oils were displayed as well. Sesquiterpanes could be applied to differentiate biodegraded oil from crude oil (Figure 6.6 c). However, sesquiterpanes might not become good candidates to differentiate oil dispersed by diverse dispersants. The analytical results from diamondoids and sesquiterpanes implied the specific selection of biodegradation of hydrocarbons applied by different dispersants.

As the main compounds in MF oil, alkylated PAHs played important roles in the characterization and monitoring of MF due to their high contents. The variations of the diagnostic ratios of alkylated-PAHs in biodegraded MF were examined in Figure 6.7. RSD was applied to evaluate the differences in diagnostic ratios. Four types of functions of the diagnostic ratios were defined based on the variations. The diagnostic ratios with low RSD (<5%) and the insignificant difference could be considered as

a


Observations (axes F1 and F2: 74.74 %)
sw 15d
sw 15d
crude 3
crude 1
B 15d
B 15d
crude 2
F2 (23.18 %)
F1 (51.56 %)
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-3
-2
-1
0
1
2
3

b


Biplot (axes F1 and F2: 79.77 %)
SW
p4/p9
P3/P5
p7/p8
P6/P8
SW
p4/p8
CRUDE
p11/p12
B
p8/p10
CRUDE
p5/p6
B
p7/p10
p8/p15
p4/p3
p9/p7
p2/p3
p1/p2
F2 (26.08 %)
F1 (53.68 %)
5
4
3
2
1
0
-1
-2
-3
-4
-5
-4
-2
0
2
4
6

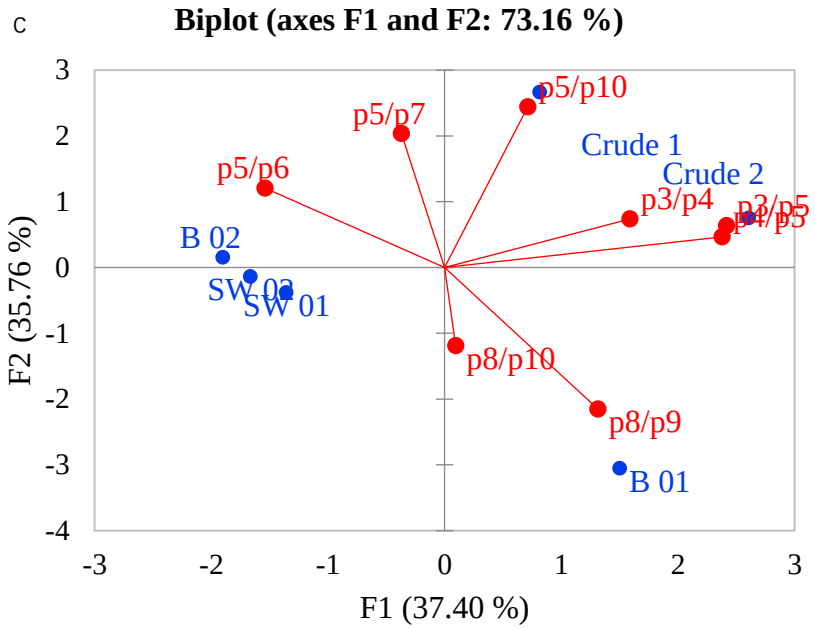


Figure 6.6 Differentiation of different dispersed oil using: (a) adamantanes, (b), adamantanes and diamantanes, and (c) sesquiterpanes

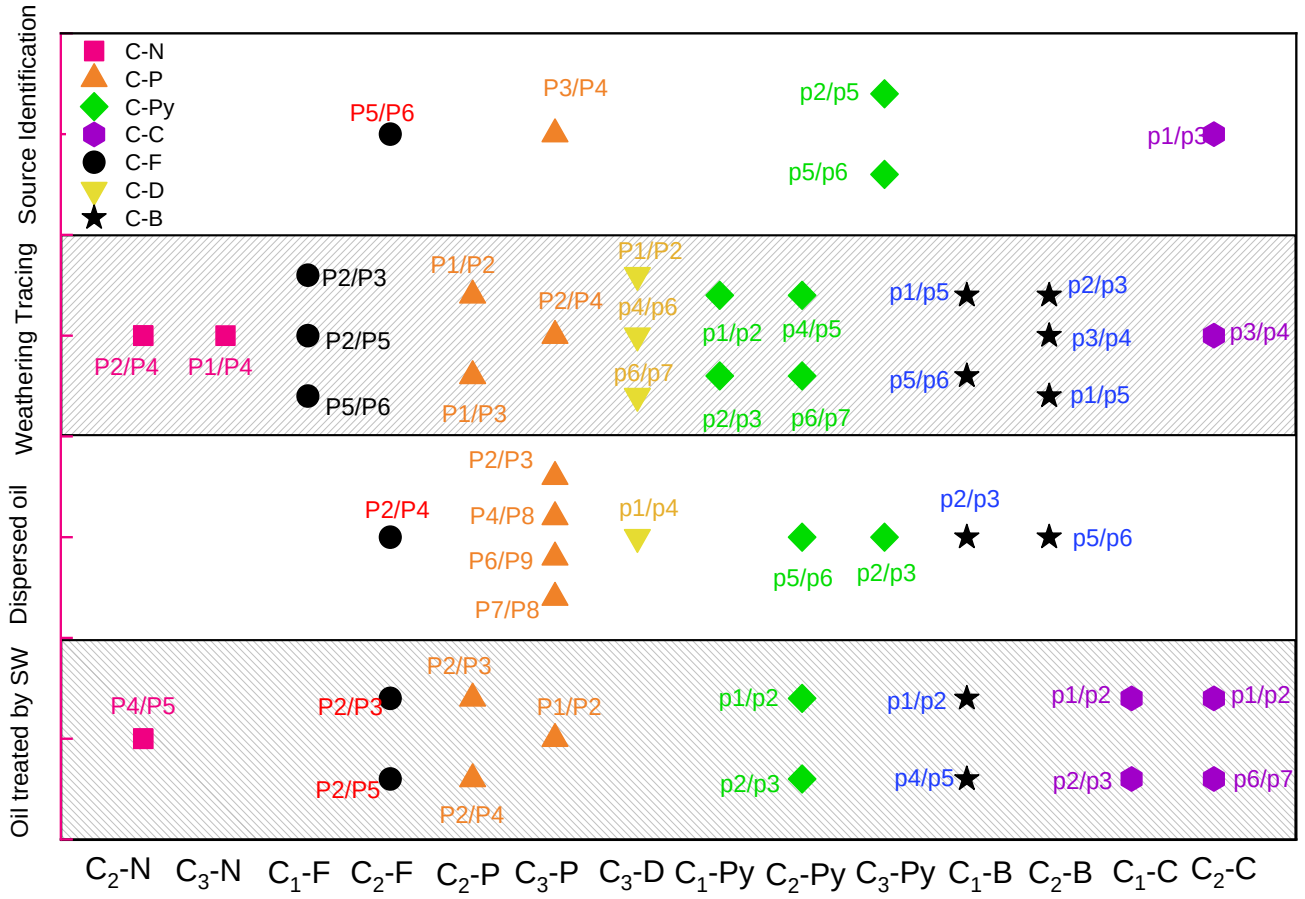
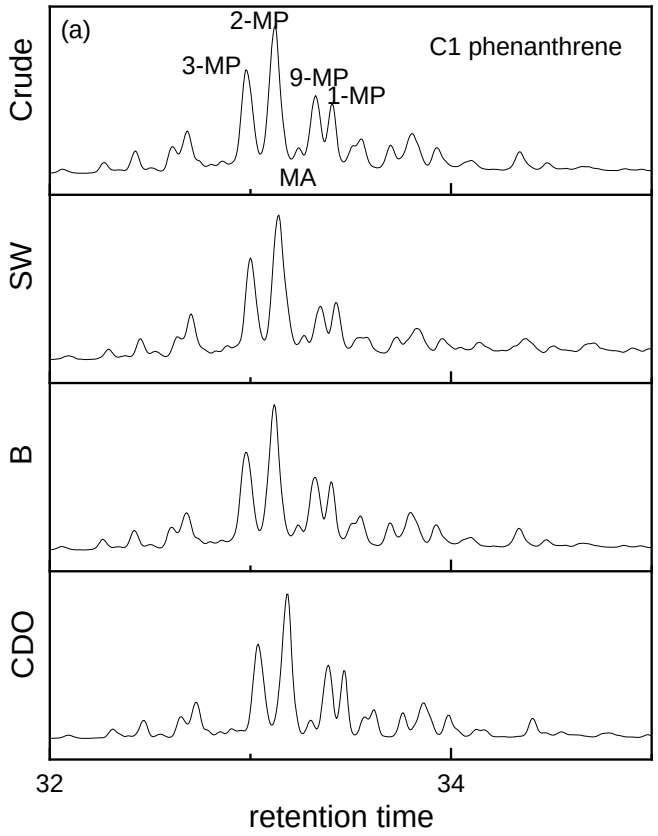


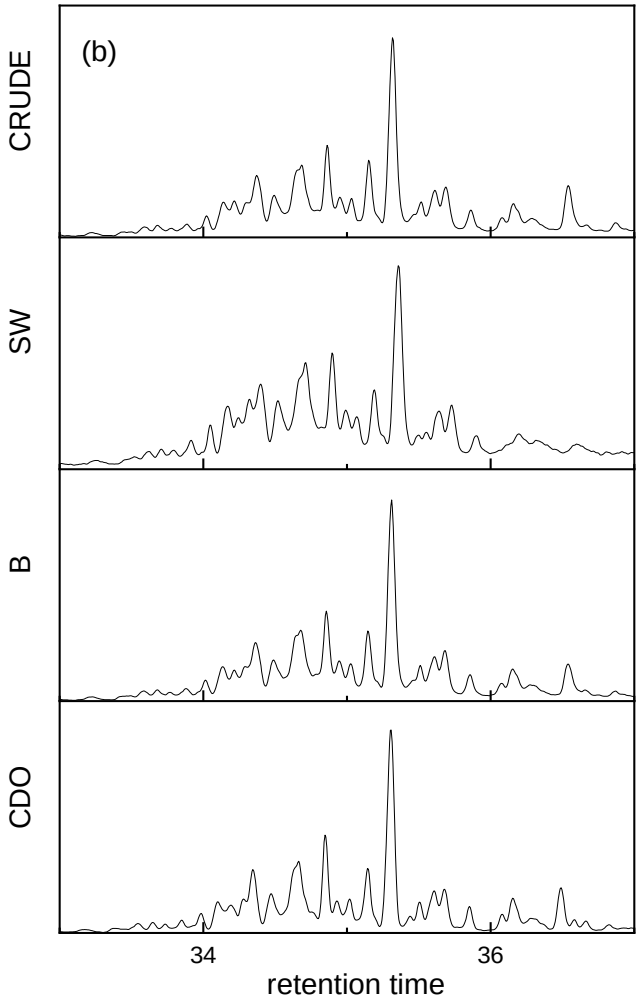
Figure 6.7 Functions of alkylated-PAHs for fingerprinting of biodegraded MF oil

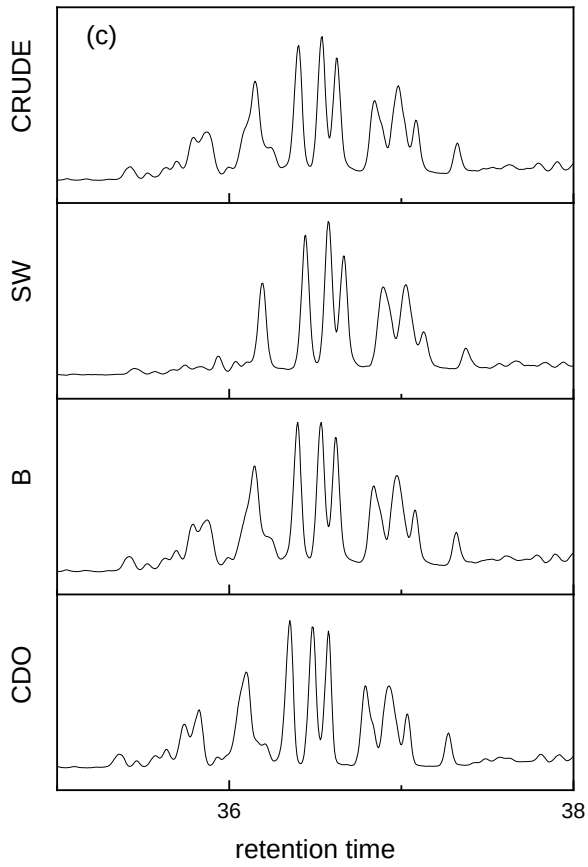
stable ones for source identification even though alkylated-PAHs are partially degraded. If the diagnostic ratios of biodegraded oils were closer to each other compared with initial ratios, the ratios could be applied to trace general biodegradation (weathering process).Similarly, if diagnostic ratios in dispersed oil differed from initial ratios as well as biodegraded crude oil, the diagnostic ratios could differentiate the application of dispersants. Some specific biomarkers with only significant vibrations in oil treated by SW were applicable to differentiate oil treated by SW and may further trace the effects of different types of dispersants. The diagnostic ratios of alkylated-PAHs from the same types were feasible to trace biodegradation of dispersed oil observed form the results (Figure 6.7). Four functions can be satisfied by diverse groups of diagnostic ratios. Some diagnostic ratios with diverse functions were shown in Figure 6.8-6.10 to clearly illustrate the differences from the chromatograph. These diagnostic ratios could be used to differentiate different oils and examine the weathering status of biodegraded oil coupled with other statistical analyses.

6.3.3 Improvement of shrimp-waste based dispersant (SWD) and the associated impact on oil biodegradation

The 5-minutes acute toxicity of the modified SWD with various concentrations ranged from 18.5 to 28.2 g/L. The 15-minutes acute toxicity was 10.5-52.5 g/L. The SWD concentration applied was 5g/L in baffled flask tests. The toxicity of the modified SWD after filtration was similar to that of SWD before filtration. The toxicity of the SWD before or after filtration was low compared to Corexit 9500A. In real world cases, the SWD concentration in marine environments could be lower than that in laboratory-scale experiments owing to dilution after


(a)
2-MP
C1 phenanthrene
3-MP
9-MP
1-MP
MA
Crude
SW
B
CDO
32
34
retention time


(b)
CRUDE
SW
B
CDO
34
36
retention time


(c)
CRUDE
SW
B
CDO
36
38
retention time

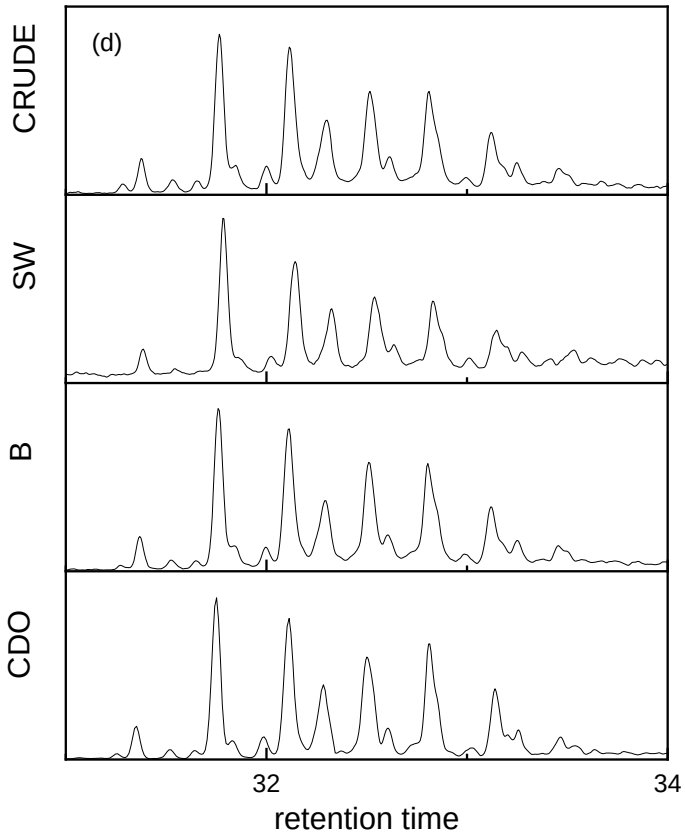
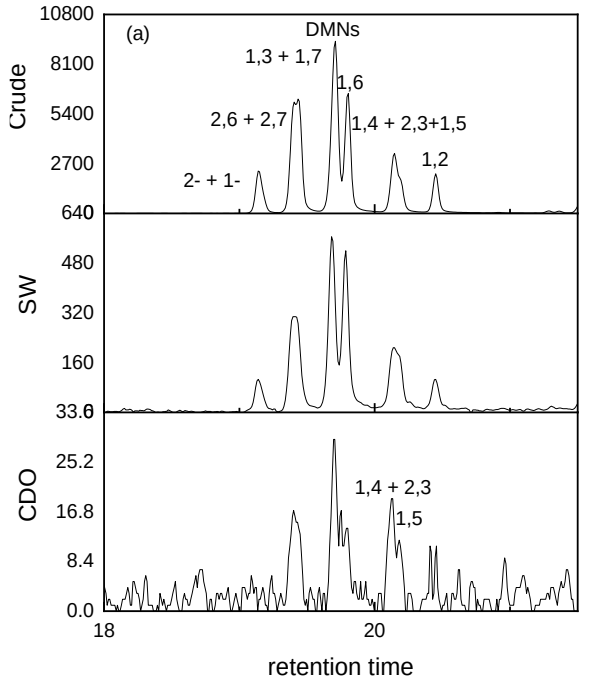


Figure 6.8 Chromatography of relatively stable alkylated PAHs during biodegradation: (a) C_1-P, (b) C_4-P, (c) C_3-Py (d) C_3-D



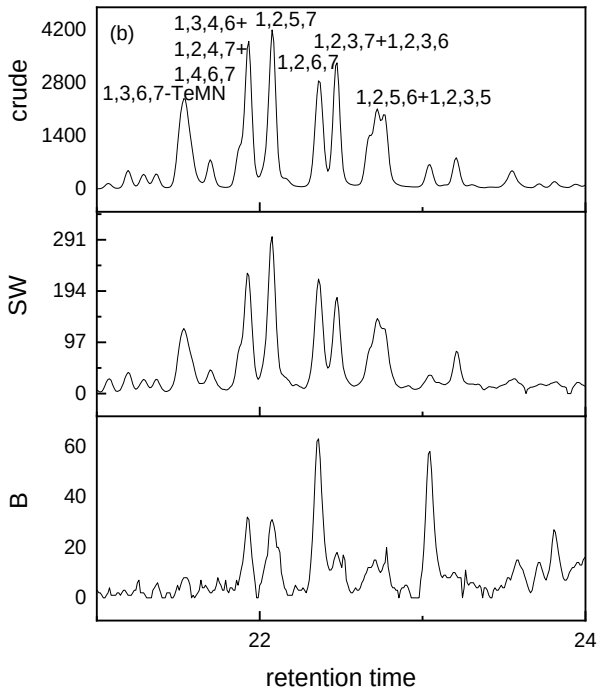


Figure 6.9 Special selection of biodegradation of homologous alkylated-PAHs for (a) C2-Naphthalenes and (b) C4-Naphthalenes

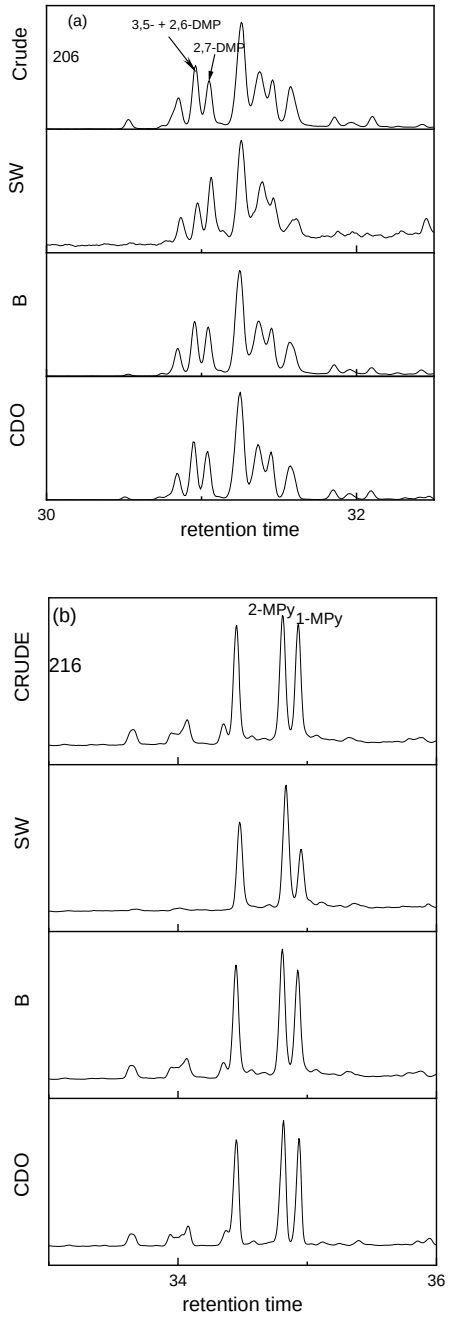


Figure 6.10 Special differentiation of biodegradation of dispersed oil using homologous alkylated-PAHs (a) C2-Phenanthrenes and (b) C2-Pyrenes

application. The biodegradation of oil treated by unmodified and modified SWD were compared to evaluate the role of SWD improvement in facilitating biodegradation. The ratios of the concentration of n-alkanes to that of blank were used to evaluate the promotion or inhibition of biodegradation by the SWD improvement. The results illustrated in Figure 6.11 indicated a better acceleration of biodegradation after filtration. The ratios of the concentration of n-alkanes in oil treated by unmodified SWD to the blank (non-dispersed oil) were 4-15, implying an inhibition of biodegradation. The inhibition might contribute to the aggregates of organic compounds of surfactants with oil. The ratios of the concentrations of oil applied by modified SWD to non-dispersed oil were much lower than those in oil treated by unmodified SWD. The range of the ratios decreased below to 1 for most of the ratios.

6.3.4 Applications of stable biomarkers for characterization of crude oil and primary biodegradation of oil treated by modified SWD

The biodegradation of n-alkanes was evaluated in Figure 6.12. Alkanes were identified at m/z 63, 70, and 85, and further confirmed by n-alkanes standards and references. The selected stable biomarkers were used to quantify the biodegradation rates of n-alkanes. The peak areas of n-alkanes to three terpanes (C29, C30, and H31S) was applied to evaluate the biodegradation rate. The peak areas of individual n-alkanes were compared with those of terpanes, TA-steranes, and MA-steranes, respectively. The degradation rates in MF and ANS ranged from 30% to 100%. The degradation rate generally decreased with the increase of chain length and the numbers of branch chain. Most of the alkanes were depleted with carbon numbers less than 30. The biodegradation of

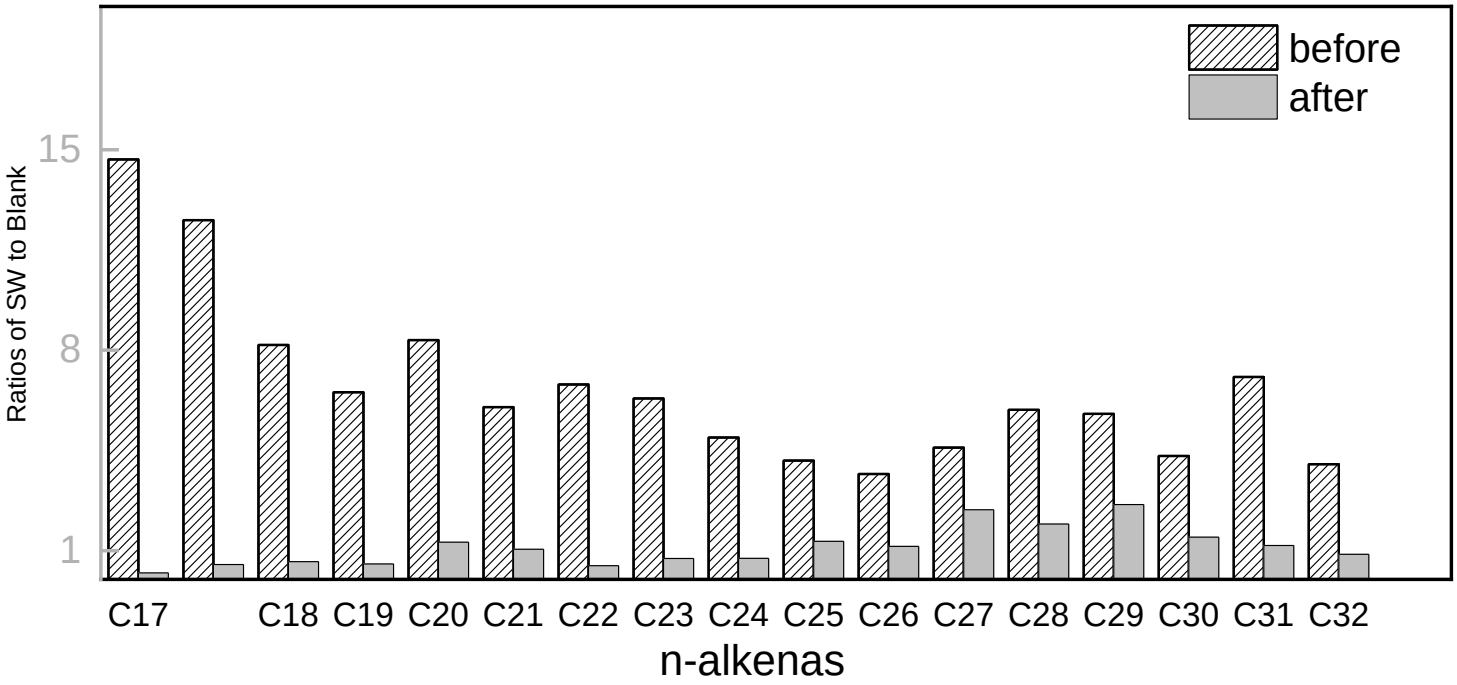


Figure 6.11 Acceleration of biodegradation of oil treated by SW after modification through filtration (red color: unmodified SW, green color: modified SW)

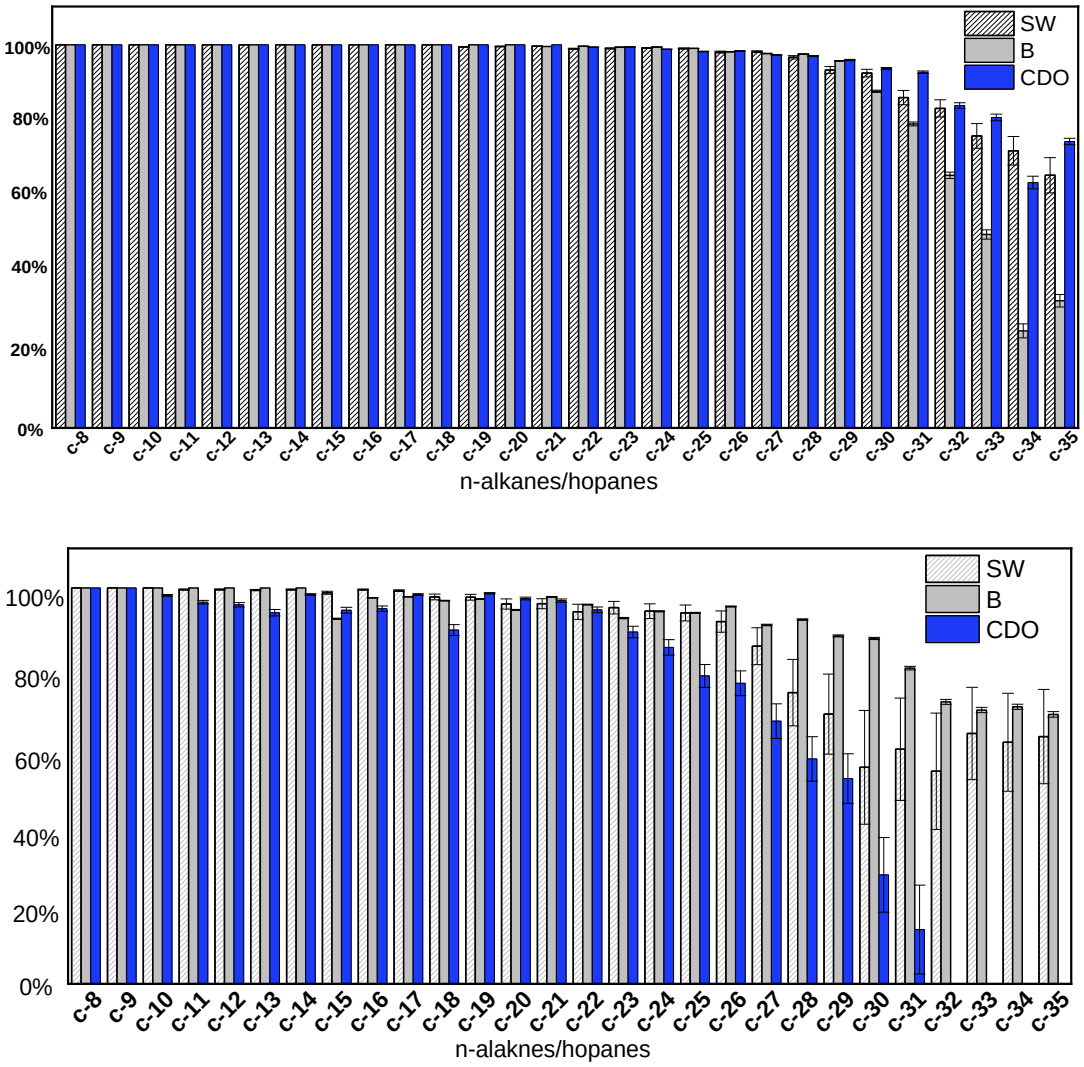


Figure 6.12 Comparison of biodegradation of n-alkanes in oil treated by SW (SW), crude oil (B), and Corexit 9500A (CDO) for (a) MF, biodegradation duration: 30days; and (b) ANS, biodegradation duration:15 days.

n-alkanes of SWD was similar to B and CDO from C8-C31 for MF oil. For n-alkanes with more carbon numbers (n>32), SWD had lower capacity to degradation compared to crude oil.

In the case of ANS oil biodegradation, the biodegradation rate of n-alkanes with carbon numbers from 8-26 in SW were compatible to those in B. The degradation of C_{27}-C_{32} alkanes of SW was 30% lower than those in B samples. The biodegradation levels of higher molecular alkanes (C_{33}-C_{35}) in SW and B were compatible. The selectivity of alkanes in different oils implied the effects of oil composition on biodegradation rates. Compared with chemical dispersant, the biodegradation of oil treated by SW was promoted for both MF and ANS oils. Supplementation of the dispersants obtained similar levels of presenting compounds compared to those in non-dispersed oil. The results indicated that the addition of SWD generally did not inhibit biodegradation and somehow facilitated the biodegradation of n-alkanes.

The mass losses of 7 types of alkylated-PAHs during biodegradation were evaluated through the comparison of peaks of individual PAHs with those in TA-steranes. TA-steranes were selected as the internal index for the following reasons. They have steady peaks relative to hopanes during biodegradation indicating their high resistance to biodegradation showed in Figure 6.4. TA-steranes located in the same fractions with PAHs after elution process, and they were clearly identified from both crude ANS and MF. Many PAHs, including C-N, C-F, C-P, and C-B, were depleted below to the detection limit of GC-MS for ANS oil with the disappearance of diagnostic ratios. MF oil had a

higher concentration of alkylated PAHs, implying a valuable pathway to monitoring the effects of dispersants on PAHs degradation (Figure 6.13). The alkylated homologues of naphthene (2-rings) in SW and CDO were the most susceptible to biodegradation followed by alky-fluorene, alky-phenanthrene, alky-dibenzothiophene, and alky-pyrenes. The mass losses of PAHs of dispersed oil with less aromatic rings were much remarkably higher than PAHs with more aromatic rings in oil treated by SWD.

The observation was consistent with the reported correlation between biodegradation resistance and the numbers of aromatic rings (Cerniglia and Heitkamp 1989, Wang et al. 1994b). The biodegradation rates of naphthalene, fluorene, phenanthrene, and dibenzothiophene in SWD were faster than those in CDO and B samples, indicating the stimulation of biodegradation of PAHs with the presence of SWD. The stimulation of biodegradation could be explained by the formation of micelles caused by the addition of surfactants (Volkering et al. 1995) and low toxicity of purified dispersant. For 4-rings PAHs, the degradation rate in SWD was not stimulated compared with other samples due to limited degradation. The high resistance to biodegradation was probably attributed to the lack of initial oxygenation caused by photooxidation (Bacosa et al. 2015). This phenomenon might highlight the significant influence of photooxidation on alkylated-PAHs with more numbers of aromatic rings. The biodegradation reactivities of alkylated-PAHs in SWD could be estimated according to the loss of PAHs (biodegradation rate) as well. It was clearly displayed that the biodegradable reactivity of alky-naphthalene, alky-fluorene, alky-phenanthrene, alky-dibenzothiophene, and alky-pyrene reduced with the increasing alkylated

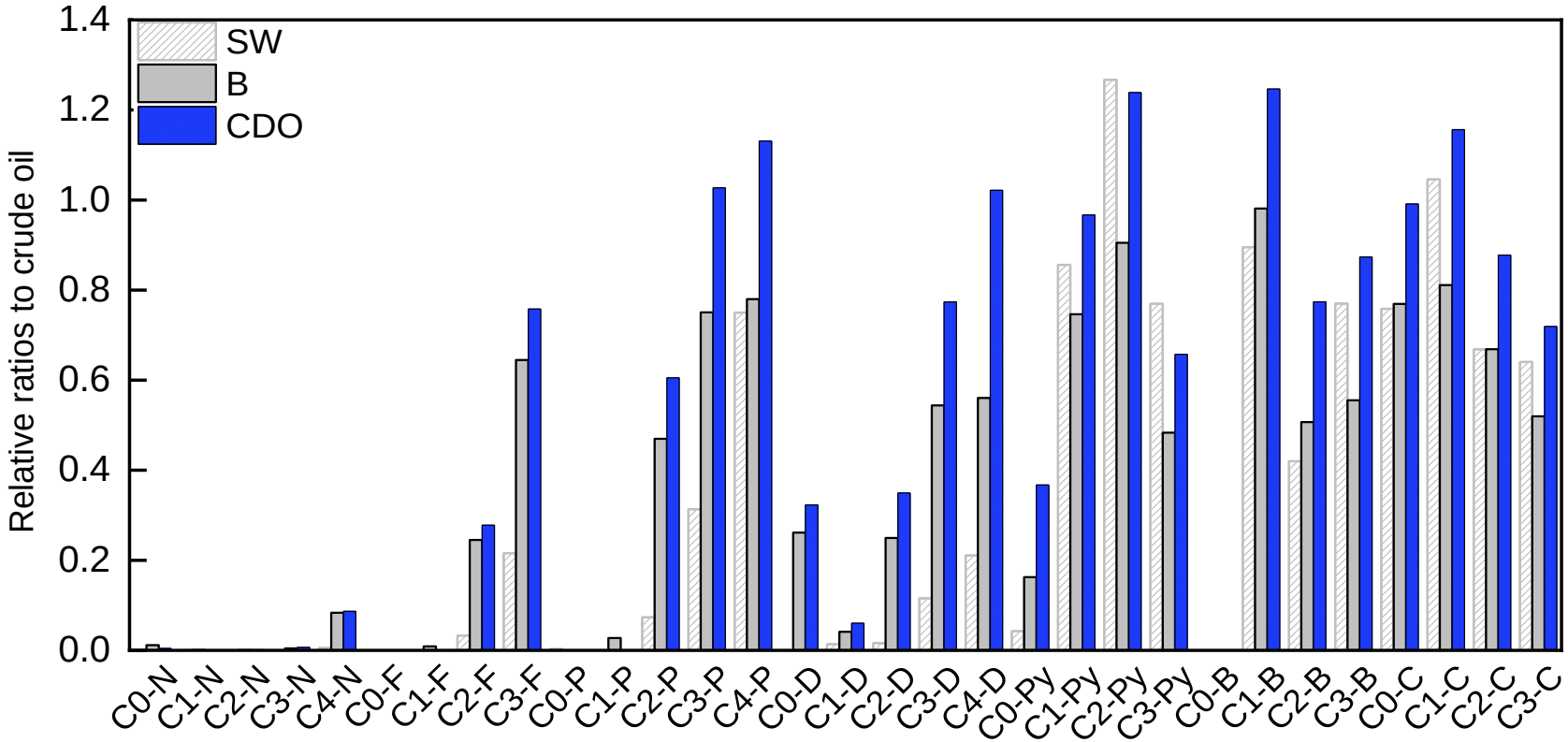


Figure 6.13 Comparison of biodegradation of PAHs in oil treated by SW (SW), crude oil (B), and Corexit 9500A (CDO) for MF (30 days)

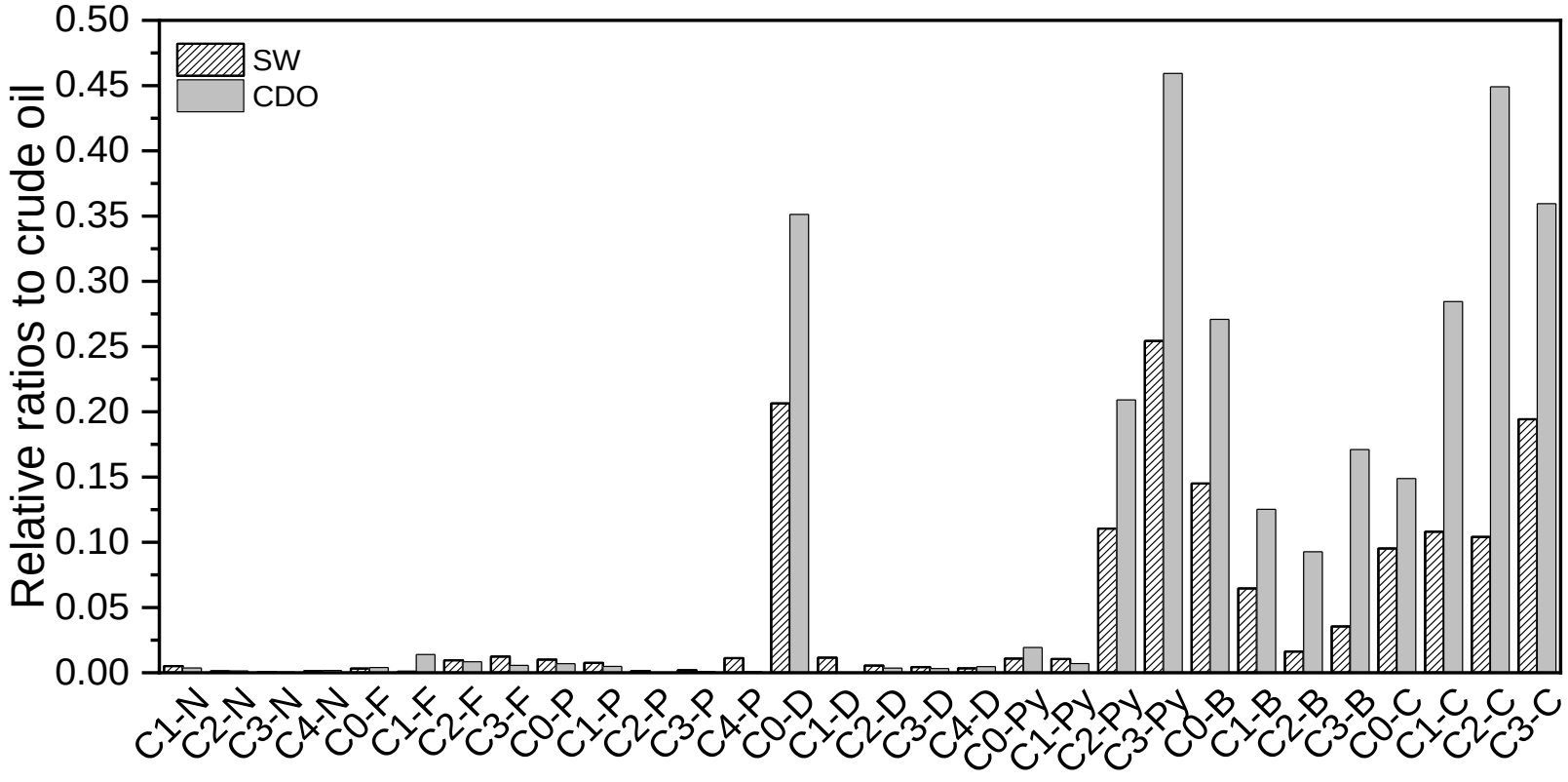


Figure 6.14 Comparison of biodegradation of PAHs in oil treated by SW (SW) and Corexit 9500A (CDO) for MF (45 days)

numbers (C1>C2>C3>C4) in 30-day biodegradation. After 45 days of biodegradation, resistant alkylated-PAHs were degraded by 50-90% (Figure 6.14). The resistance of alkylated chrysenes to biodegradation increased with the alkylated numbers.

6.4 Summary

Biodegradation experiments were performed to evaluate the biodegradability of oil treated by a modified green SWD through developing an oil tracing methodology. The developed methodology was adopted to classify the different functions of the diagnostic ratios of biomarkers based on their stability during biodegradation. Common biomarkers and homologues of alkylated-PAHs were involved. The developed methodology would help to better understand the accurate oil tracing functions for the biodegradation of oil treated by new dispersants.

Results indicated that biodegradation of SWD was remarkably promoted after the modification of SWD production. The biodegradation rates of n-alkanes (n<26) treated by modified SWD was compatible compared n-alkanes in oil without the addition of dispersants. The degradation rate of n-alkanes (n>26) varied with the types of oils, implying the lower capacity of SWD for further biodegradation. The application of SWD enhanced the degradation rates of alkylated-naphthalene, fluorene, phenanthrene, and dibenzothiophene. Alkylated-PAHs with more benzene rings were not degraded in the experiments. Results also indicated that the modified SWD promoted the biodegradation of some alkylate-PAHS.

CHAPTER 7 IMPACTS OF ENVIRONMENTAL FACTORS ON WEATHERING OF BIOMARKERS IN DISPERSED OIL [5]

[5] *The contents of this chapter are based and expanded on the following paper:*

Song, X., Zhang, B., Chen, B., and Lye, L. M. (2019). Impacts of environmental factors on weathering of biomarkers in dispersed oil. Ready for submission.

Role: Xing Song solely worked on this study and acted as the first author of this manuscript under Dr. Baiyu Zhang, Dr. Bing Chen, and Dr. Leonard M. Lye's guidance. Most contents of this paper were written by him and further edited by the other co-authors.

7.1 Introduction

Biomarkers had diverse but clear responses to different weathering processes in marine environments, mainly induced by evaporation, photooxidation, and biodegradation. The variations of weathering conditions, such as temperature, oil concentration, and salinity, may make the fate and behaviors of the biomarkers more complicated and distinguishable (Bacosa et al. 2015, Prince et al. 2003, Radović et al. 2014). When chemical dispersants were involved, CDO could generate diverse impacts on the degradation rate and weathering preference of hydrocarbons, including biomarkers, in marine environments (Joo et al. 2013, Yamada et al. 2003). The changes of biomarkers thus may be utilized to trace the dominant weathering processes and conditions affecting the fate and behaviors of CDO, though few studies evaluated its applicability from the perspective of fingerprinting. This chapter considered some important weathering conditions as variables, including temperature (2 and 30 °C), the salinity of seawater (5 and 35 psu), the concentration of CDO (70 and 700 ppm), seawater composition (natural seawater and artificial seawater), and weathering duration (30 days and 60 days). The CDO fingerprinting were analyzed through GC-MS coupled with multiple statistical analyses. The influences of environmental factors on weathering of biomarkers were discussed.

7.2 Materials and Methods

7.2.1 Materials

Organic solvents, including hexane mixture and dichloromethane, were of chromatographic grade purchased from Sigma Aldrich Canada. Well recognized

surrogate candidates for oil fingerprinting were selected, including acenaphthalene-d_{10}, phenanthrene-d_{10}, benz[a]anthracene-d_{12}, perylene- d_{12}, and terphenyl-d_{14}. C_{30} 17β(H) 21β(H)-hopane was selected as the internal standard. Alaska North slope crude oil was sealed and stored in 4 ºC without illumination. Silica gel was activated in approximate 110 ºC for at least 48 hours before use. Glassware was washed and cleaned using both DCM and chemical soap at least twice before and after experiments, respectively. Seawater was collected nearby a harbor of St. John's Newfoundland.

7.2.2 Weathering experiments

Crude oil samples: Approximately 0.8g of ANS crude oil was dissolved in hexane and concentrated to 1mL.

Higher concentration (700 ppm) of CDO samples: Aliquot 100 μL of crude oil was added into artificial seawater No.1 (36 ‰ salinity), artificial seawater No.2 (5 ‰ salinity) and real seawater, respectively. Nine μL of Corexit 9500A dispersant was pipetted to oil slick following the oil-dispersant ratios as 10:1 to generate 700 ppm of chemically dispersed oil.

Lower concentration (70 ppm) of CDO samples: Aliquot 10 μL of crude oil was added into artificial seawater No.1 (36 ‰ salinity), artificial seawater No.2 (5 ‰ salinity) and real seawater, respectively. Corexit 9500A dispersant was pipetted to oil slick following the oil-dispersant ratios as 10:1 to generate 70 ppm of chemically dispersed oil.

Oil weathering: Aliquot CDO samples were placed into two shakers with different temperature control: 30 ºC and 2 ºC, respectively. The flasks were shaked at the speed

of 120 rpm for 30 or 60 days, separately (Figure 7.1). Oil was observed homogenously dispersed in the water column when weathering experiments ended.

7.2.3 Sample analysis

Fifty μL of the surrogate mixture was syringed into weathered samples. For the low concentration of CDO, Oil fractions were extracted using 10 mL of DCM in a 250mL of a separatory funnel for at least four times. In case of the higher concentration of CDO, oil fractions were extracted using 20mL of DCM for at least four times and transferred into a 250 mL of Erlenmeyer flask. Water in extracts was removed by sodium sulfate (anhydrous). The organic phase was then transferred into a rotary evaporator. Another 5 mL of DCM was added into the Erlenmeyer flask to flush residue oil. Dried extracts from CDO were dissolved by hexane and finally concentrated to 0.4 mL or less.

Crude oil samples and CDO samples were fractionated using a self-generated silica gel column. Briefly, the aliphatic biomarkers were firstly eluted by 12 mL hexane, and the aromatic biomarkers were separated by 15 mL of the mixture of hexane: DCM (v:v, 1:1). The eluted fractions were concentrated to 1 mL for GC-MS determination.

The biomarkers were analyzed using a GC-MS coupled with a 30 m DB-5ms capillary column based on Song et al. (2018). SIM mode was used to characterize and analyze biomarkers. The peak areas of each biomarker were calculated. The relative standard deviations (RSD) values (standard deviations/ the average *100%), referred to as the Coefficient of Variation (CV), were used to evaluate the effects of different factors on the variations of biomarkers.

7.2.4 Principal component analysis

Principal component analysis (PCA), a powerful multivariate technique, is used to analyze a data matrix containing several intercorrelated variables. The most important information was extracted by reducing the dimensions of the variables. (Jeffers 1967, Singh et al. 2004, Tipping and Bishop 1999, Wold et al. 1987). In this chapter, covariance correlation was employed to the data sets composed by biomarkers with relative higher molecular weight, because they have successfully differentiated the chemically dispersed oil (CDO) from weathered crude oil (WCO). The PCA results were helpful for the analysis of the effects of different factors involved in this study on the changes in the diagnostic ratios of biomarkers. Two or three principal components (PCs) were set to cover at much as possible (80%) of variances. The PCAs were performed using XLSTAT software, an Excel-based software by Addinsoft.

7.2.5 Multiple correspondence analysis

Multiple correspondence analysis (MCA) is a statistical technique to analyze the correlation of several categorical dependent variables in the data sets. It could be seen as conceptually similar to PCA, because MCA applied to categorical variables, whereas PCA worked on quantitative variables (Abdi and Valentin 2007). MCA converted the targeting data matrix composed by the categorical variables to nominal variables by ranking different levels for variables. MCA is rarely applied in oil fingerprinting as a critical analytical approach. When biomarkers were degraded to a undetectable level after weathering, they are rarely selected for the quantitative analysis (Christensen et al. 2005, Miao et al. 2015). MCA would have advantages in fingerprinting when some of the targeting petroleum hydrocarbons were found absolutely depleted or under the detection limit involving multiple affecting factors in

the study. Indeed, the severely degraded biomarkers indicated a significant contribution to the weathering, implying their correlation to different oil weathering processes and conditions. Therefore, this chapter introduced MCA to analyze the effects of the environmental factors on biomarkers (adamantanes, diamantanes, and sesquiterpanes) of dispersed oil using biomarkers that are significantly degraded to an undetectable level. The MCAs were conducted using XLSTAT software as well.

7.2.6 QA/QC

The validity and reliability of the experimental results and biomarker analysis were evaluated using a QA/QC protocol. Four crude oil samples were prepared to indicate the original diagnostic ratios of biomarkers and alkylated-PAHs. Duplicate experiments, including sample preparation, weathering, and extraction were conducted. Duplicate GC/MS analyses were also adopted to measure F1 and F2 in each 150μL of organic phase sample. The diagnostic ratios of each biomarker were displayed by the average (n=8, if biomarkers are detectable) with a corresponding standard deviation. The coefficients of determination (R^2) of calibration of surrogates were > 0.9940 (n=5). The average recoveries (%) of five surrogates, including acenaphthalene-d_{10}, phenanthrene-d_{10}, perylene-d_{12}, benz[a]anthracene-d_{12}, and terphenyl-d_{14}, ranged from 80% to 100%.

7.3 Results and Discussion

7.3.1 Source identification of dispersed oil

Biomarkers and alkylated-PAHs were identified in dispersed oil samples. Briefly, the peak areas of each individual biomarkers were integrated from the SIM mode of chromatograms in GC-MS analysis software.

The stability of each diagnostic ratio was measured by the average values, SD and RSD values of the diagnostic ratios under all experimental conditions. The stability of eight groups of biomarkers was summarized in Figure 7.1. The low-molecular-weight biomarkers, involving adamantanes, diamantanes and sesquiterpanes, were substantially declined and somewhat decreased to an undetectable level in most of the samples. The 95% of the diagnostic ratios from these biomarkers were remarkably changed. Many of them can not be recorded, because the corresponding biomarkers were barely examined or roughly shaped from GC chromatograph. Since there were theoretically few marine microorganisms in artificial seawater, the loss of the biomarkers reflected their low resistance to physiochemical weathering, involving evaporation and photooxidation (Song et al. 2018).

While, the refractory biomarkers (terpanes, steranes, TA-, and MA-steranes) were not significantly against different weathering conditions. Forty-three percent of these diagnostic ratios were evaluated as stable indicators for oil source identification of dispersed oil based on the low RSD values (<5%). The RSD values of 46% of the diagnostic ratios ranged from 5 to 10%. The observations implied a slight effect of the variations of environmental factors on

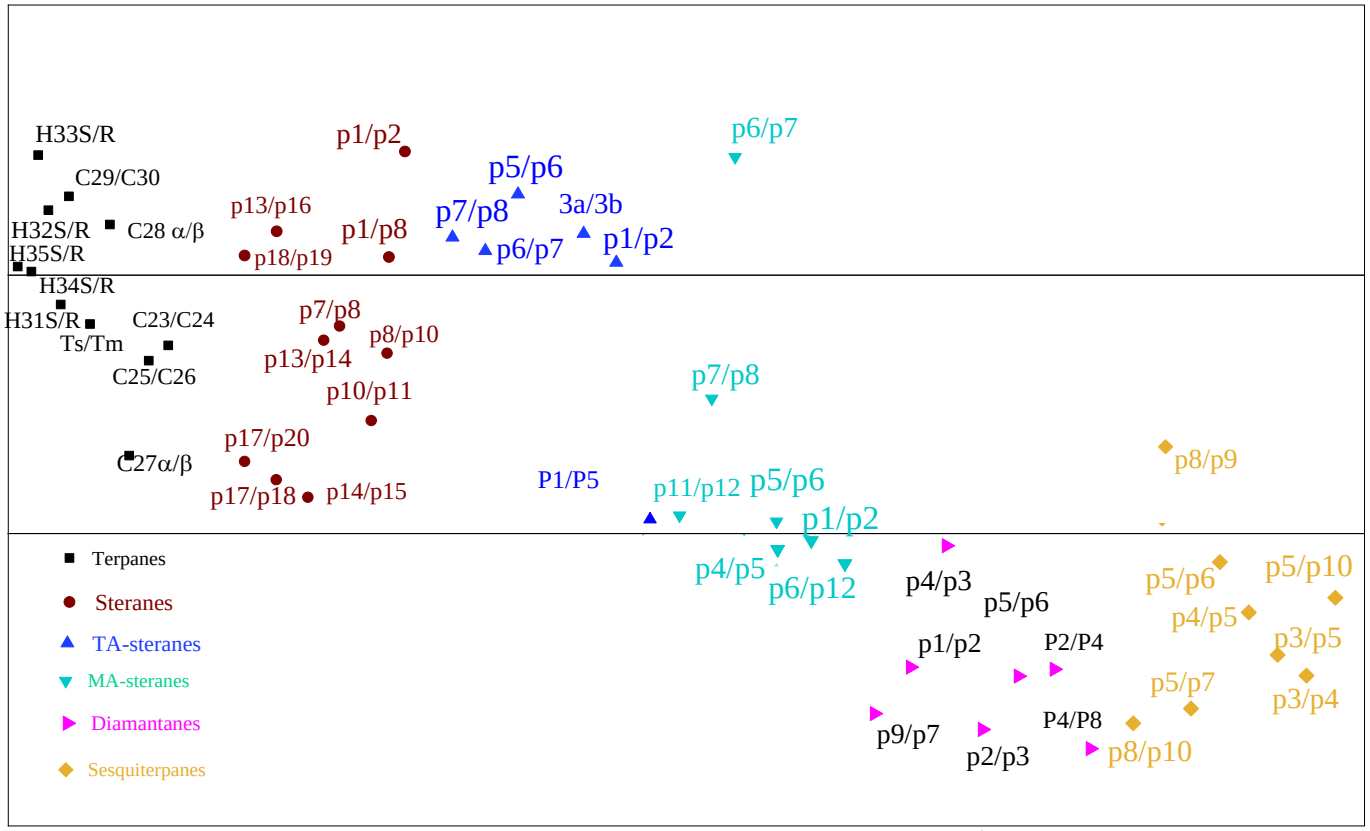


Figure 7.1 Source identification of dispersed oil using biomarkers under different environmental conditions

oil source identification using these biomarkers. Most of the diagnostic ratios composed by individual alkylated PAHs varied and affected by different scenarios and the application of dispersants. A few diagnostic ratios in CDO had different ratios with those in WCO after 60-days weathering, including C3-naphthalenes (P2/P3 and P4/P5), C4-naphthalenes (P1/P2/P3), C1-fluorenes (P2/P3), and C4-phenanthrenes (P4/P5). Only a few of the diagnostic ratios were still have a lower RSD value. The diagnostic ratios of alkylated-PAHs in CDO were affected by the selected environmental factors.

7.3.2 Impacts of weathering scenarios on the behaviors of aliphatic biomarkers

Impacts of different weathering conditions and processes on degradation of aliphatic biomarkers were evaluated through statistical analysis (MCA or PCA). The massive loss of low-molecular weight biomarkers generated great difficulties in recognizing and quantifying of the corresponding diagnostic ratios. The variations of the data could be categorized as rankings based on two conditions: (1) diagnostic ratios could be calculated due to the apparent resolution of biomarkers in chromatograms, and (2) diagnostic ratios could not be recognized due to the remarkable loss of biomarkers. MCA was applied to analyze the data sets if diagnostic ratios can not be clearly recognized after weathering. PCA was utilized to analyze the data set with clearly identified biomarkers in GC chromatograph.

The analytical results of diamantanes using MCA (PC1 and PC2) were shown in Figure 7.2. Three principal components (PC1, PC2, and PC3) were extracted, explaining the 48.6, 28.2, and 12.8 % of the total variance, respectively. The

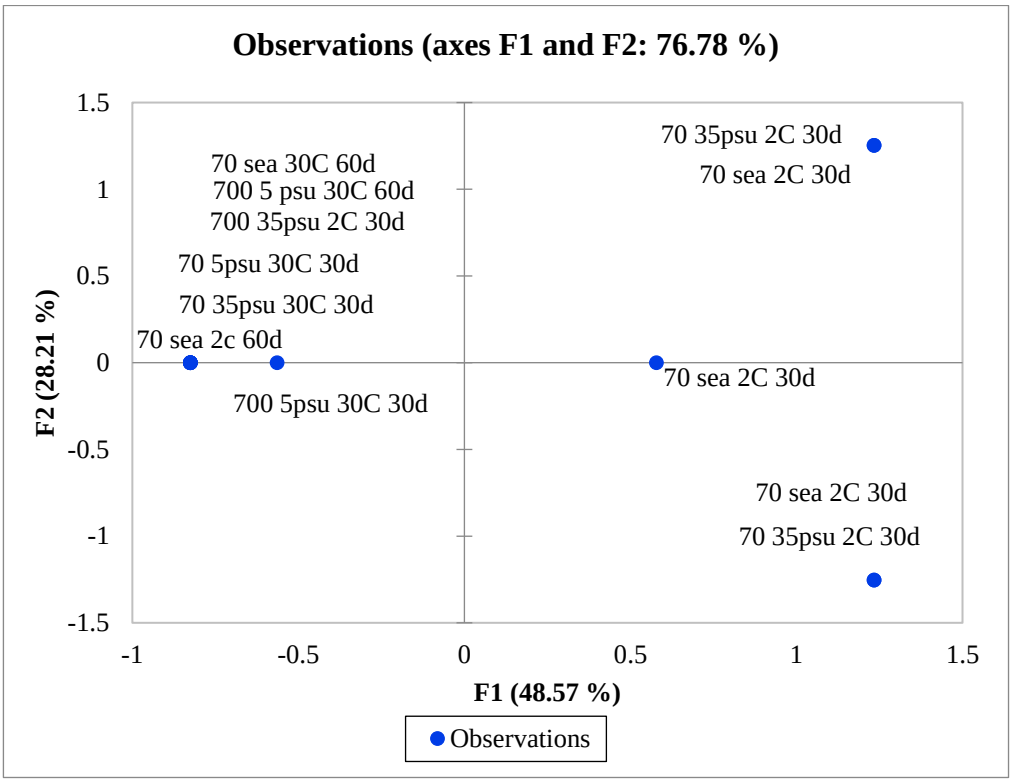


Figure 7.2 MCA results of dispersed oil using diamantanes

samples with higher temperature and higher concentration levels, were differentiated with other samples. The analytical results indicated the high correlation between the changes of diamondoids and the variation of temperature and dilution. Evaporation of petroleum hydrocarbons was temperature related (Fingas 1997). Nevertheless, the influence of photooxidation in aliphatic biomarkers may be limited, because the higher resistance of aliphatic hydrocarbons compared to aromatic hydrocarbons probably implied a similar weathering degree for aliphatic biomarkers (Garrett et al. 1998). Meanwhile, the photooxidation excited by singlet oxygen mechanism (one of the main mechanisms) was temperature-dependent (Anderson and Johns 1986, Shankar et al. 2015, Vergeynst et al. 2019). The main pathways of the depletion of diamondoids were thus evaporation and dilution. Impacts of photooxidation may not be absolutely ignored, because this study did not accurately vary the long-term effects of the intensity of the illustration. The biomarkers (e.g., p1/p2, p4/p3, p5/p6, and p4/p8) applied for differentiation could be used to track the occurrence of evaporation in dispersed oil.

MCA analysis regarding the sesquiterpanes was displayed in Figure 7.3. The first two principal components were sufficient to describe the variability of biomarkers in CDO with different weathering conditions, involving 85% of the total variance. Plots of samples with shorter duration and lower temperature stand closer, which indicated the significant impact of temperature and weathering duration. The correlation of two variables (weathering duration and

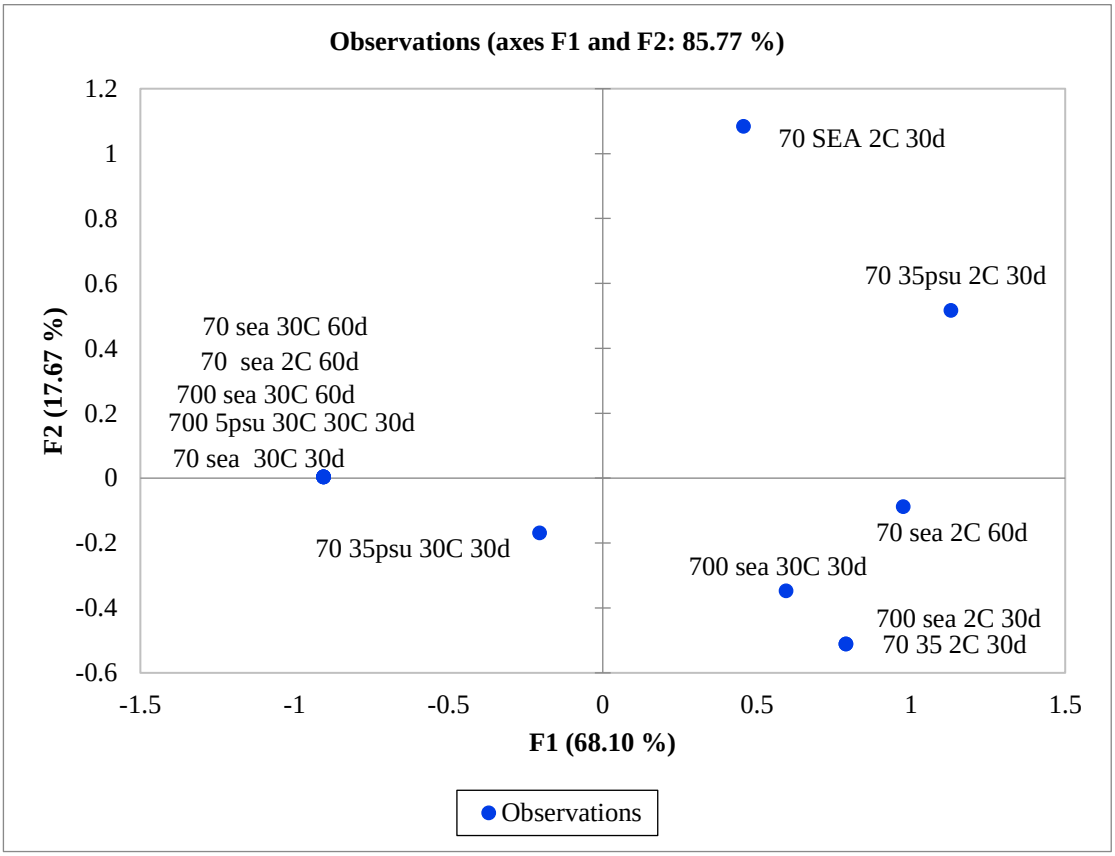


Figure 7.3 MCA results of dispersed oil using sesquiterpanes

temperature) and the degradation of sesquiterpanes were established based on the observations on the right side of the MCA results. This observation still built up a link of the degradation of low-molecular-weight biomarkers to the impact of evaporation. The minor effect of photo-oxidation on aliphatic hydrocarbons in weathered crude oils from previous studies was another indirect evidence of the roles of evaporation (Bacosa et al. 2015, Prince et al. 2003, Vergeynst et al. 2019).

The correlation between high-molecular-weight biomarkers, such as terpanes and TA-steranes, were analyzed using PCA, as all the diagnostic ratios could be calculated. In terms of terpanes, four principal components were screened contributing 80% percentage of the variations (Figure 7.4 (a)). As the RSD values of terpanes ranged from 0-10%, the effects of environmental conditions, containing type of seawater, dissolution, temperature, and salinity, on CDO fingerprinting were limited. The samples marked with “700 rpm” have similar contributions to the first two PCs, could be differentiated from other samples. These components contributed to the effects of oil concentration. Samples with a lower concentration and shorter weathering duration could be correlated, marked in the red circle, indicating the effects of oil concentration and weathering duration. The overlap probably implied the interactions of these factors.

Similarly, samples with higher salinity (35psu) could be categorized and circled, which was a subset of the red circle. The differentiation implied a minor effect of the salinity on the variations of diagnostic ratios of terpanes. Figure 7.4 (b)

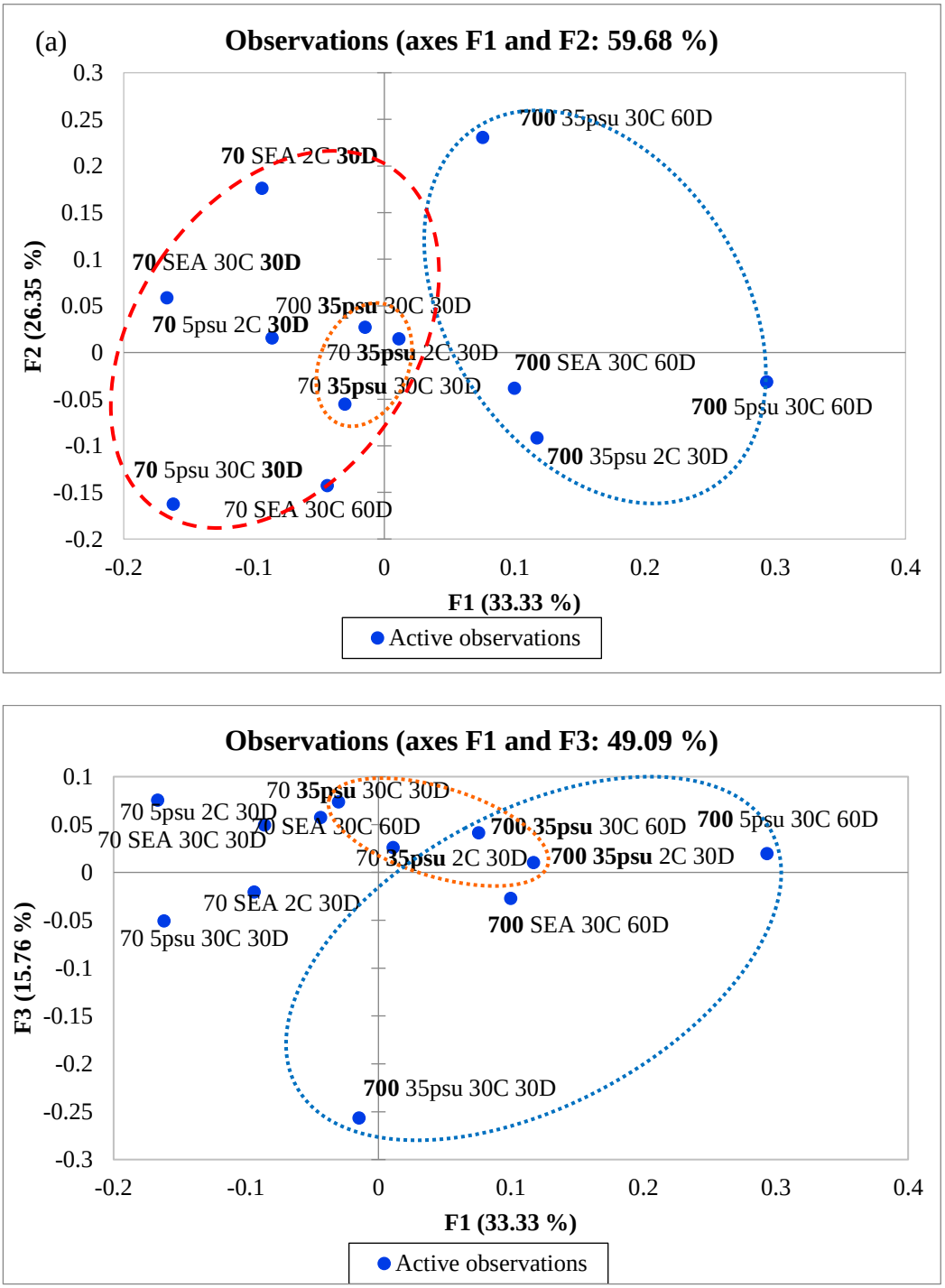


Figure 7.4 PCA score plot of CDO samples using the diagnostic ratios of terpanes: (a) the correlation between PC1 and PC2, and (b) the correlation between PC1 and PC3.(Blue circle: higher concentration; Red circle: lower concentration and lower weathering duration; Orange circle: higher salinity)

displayed the correlation of samples using PC1 and PC3. The similar differentiation result could be observed, implying the order of the importance of selected effects: oil concentration/ weathering duration>salinity. The effects of seawater could be recognized in figure as well. Meanwhile, the interactions of different factors may contribute to the variations of diagnostic ratios as well, though the interactions were not thoroughly investigated in this thesis. Since the high correlation between these important factors and biodegradation and high resistance of hopanes to photooxidation, the probably dominate weathering is biodegradation for hopanes (Lee et al. 2013, Stout et al. 2016). The diagnostic ratios tracking the important factors, involving C27α/β, C28α/β, and C23/C24, reflected their higher sensitivity to biodegradation, which is consistent with literature (Bost et al. 2001, Zhao and Machel 2011).

7.3.3 Impacts of weathering scenarios on the behaviors of aromatic biomarkers

PCA results of oil samples using TA-steranes were shown in Figure 7.5. The first three PCs contributed to 80% of the total variance. Differed from the differentiation results using terpanes, oil concentration became a minor distributor in the score plots (marked as a blue circle). Dispersed oil samples with different temperatures could be differentiated from those of other plots (red circle). Temperature probably could be a potentially important factor affecting the subsequent degradation if the weathering continued. Since the negligible evaporation of steranes, temperature was a significant factor related to the oxidation driven by free-radical chain reaction (Shankar et al. 2015). The differentiation might imply the different preference and the potential pathway of photooxidation of TA-steranes. Photooxidation may not significantly affect the

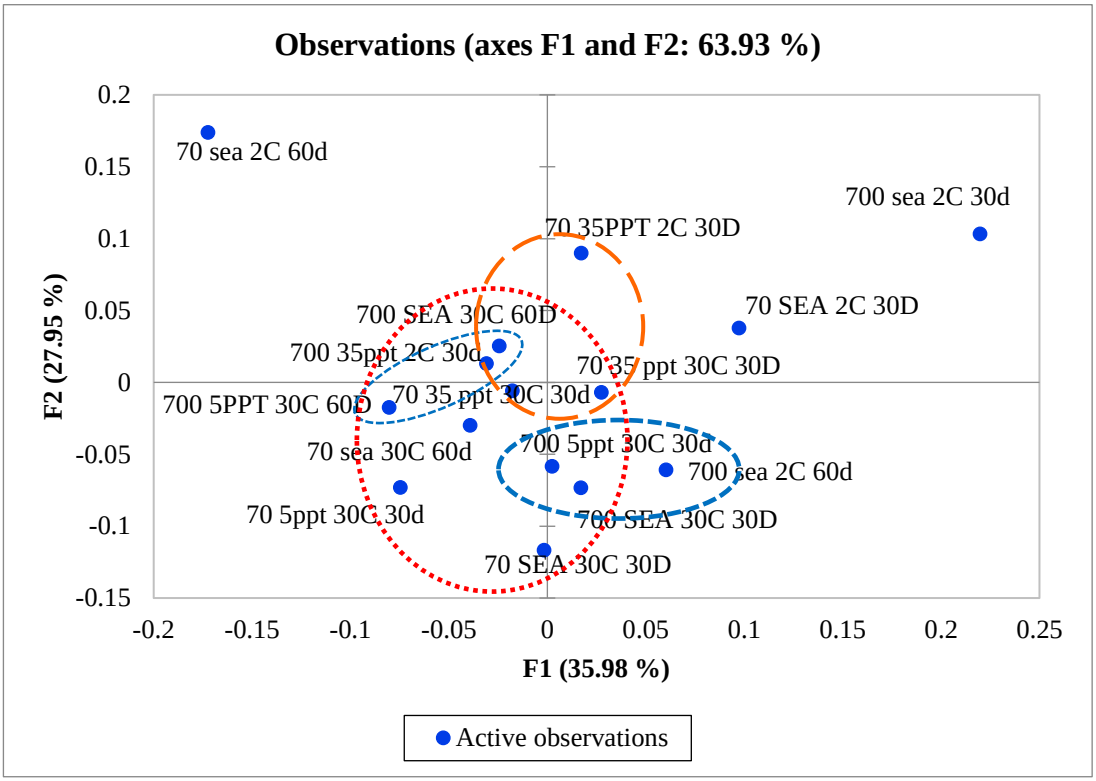
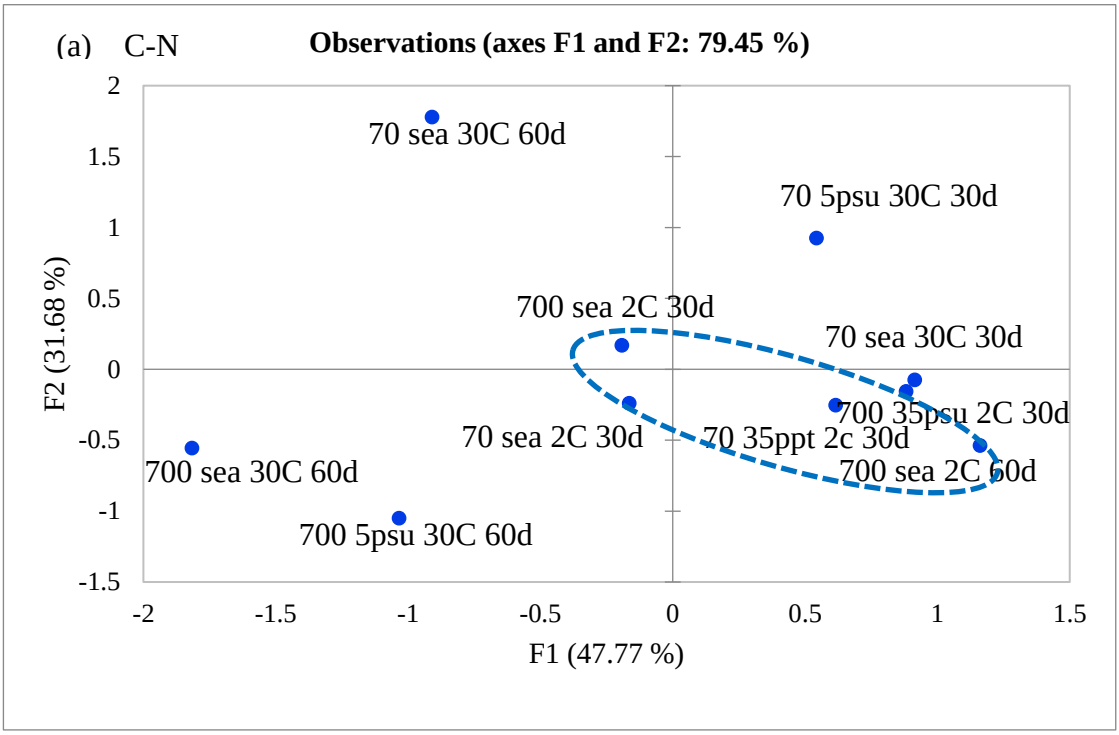


Figure 7.5 PCA score plot of CDO samples using the diagnostic ratios of TA-steranes

(Blue circle (Squire dot): higher concentration; Red circle (Round dot): lower concentration and lower weathering duration; Orange circle (long dash): higher salinity)



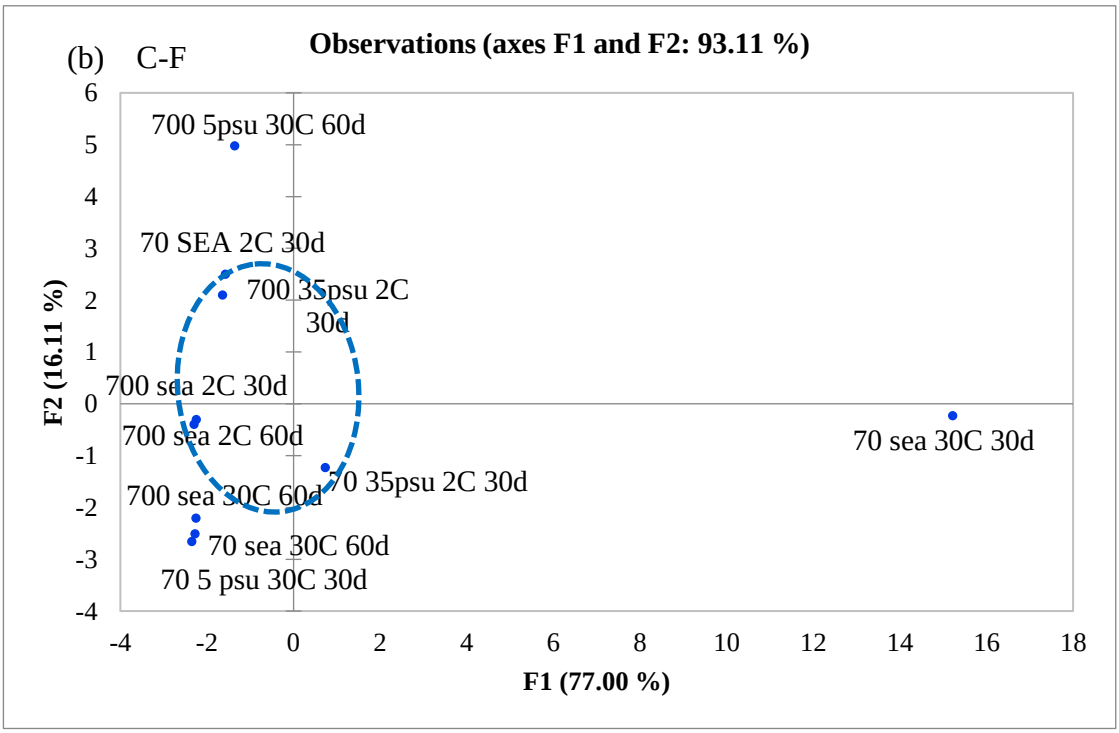


Figure 7.6 PCA score plot of CDO samples using the diagnostic ratios: (1) alkylated-naphthalene (C-N) and (2) alkylated-fluorene (C-F)

(Blue circle (Squire dot): lower temperature)

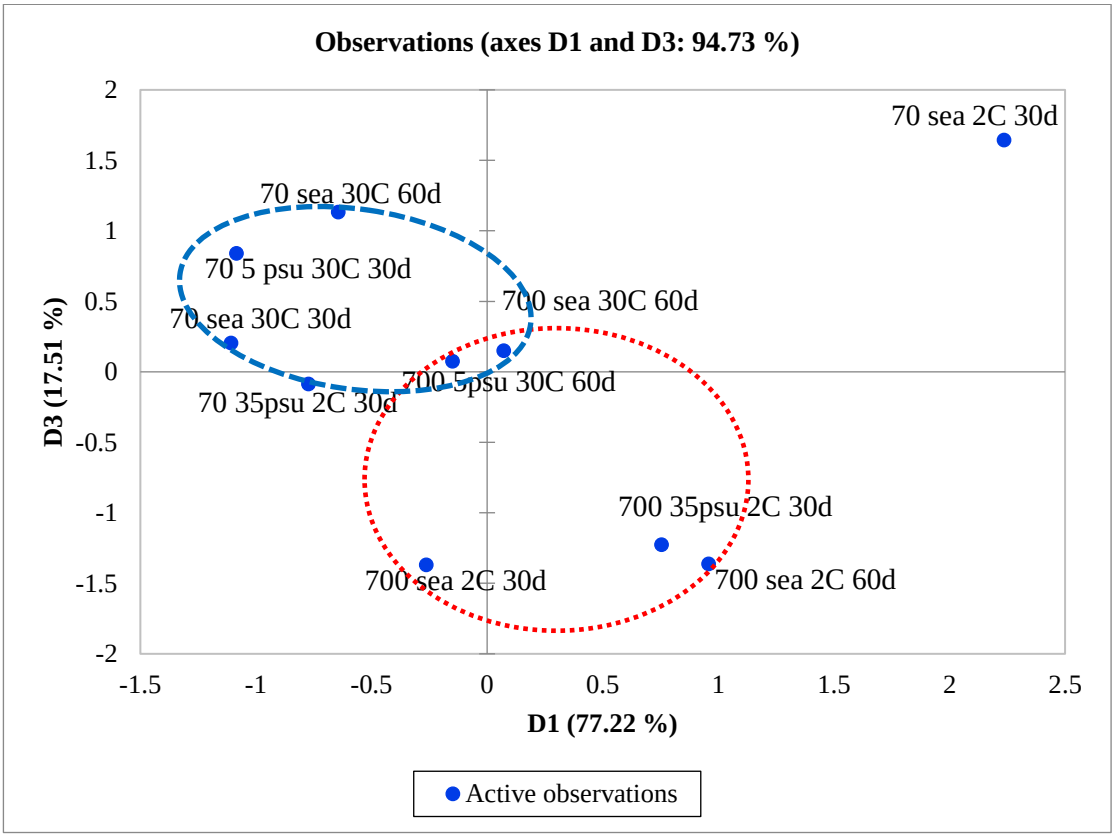


Figure 7.7 PCA score plot of CDO samples using the diagnostic ratios of alkylated-phenanthrene (C-P)

(Blue circle (Squire dot): lower temperature; Red circle (round dot): higher oil concentration)

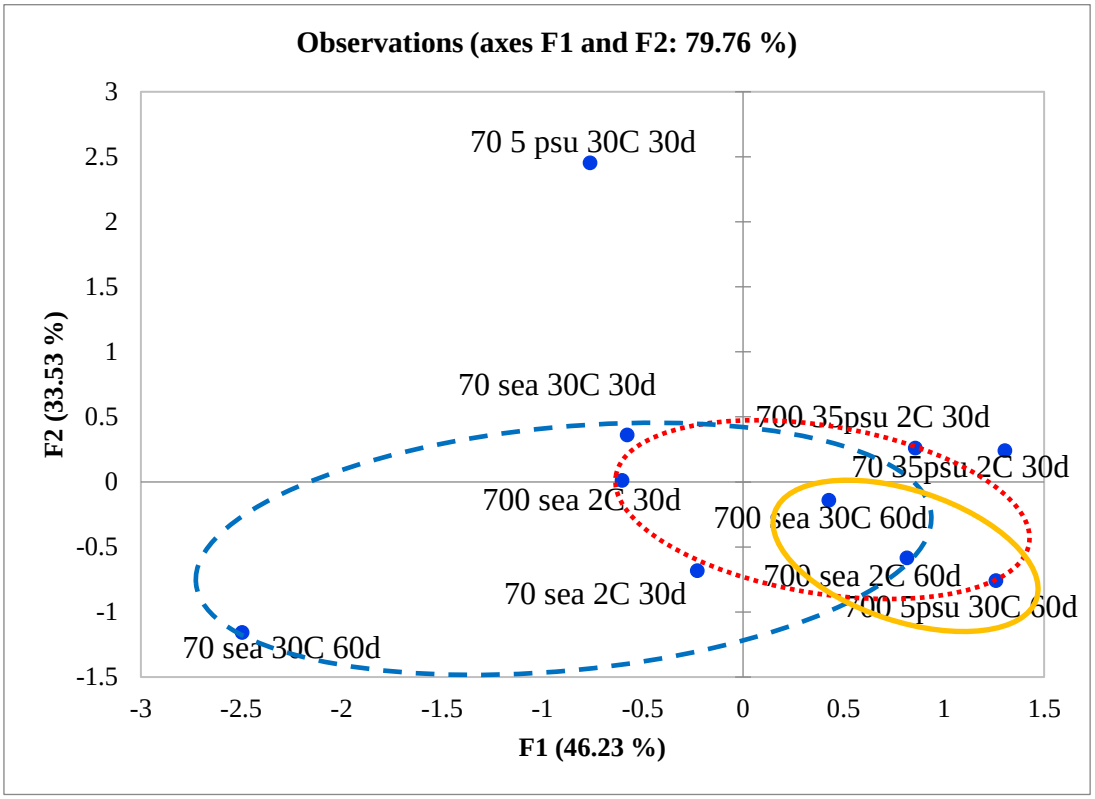


Figure 7.8 PCA score plot of CDO samples using the diagnostic ratios of alkylated-benzothiophene (C-B)

(Blue circle (Squire dot): seawater; Red circle (round dot): higher oil concentration; orange (solid): longer weathering duration)

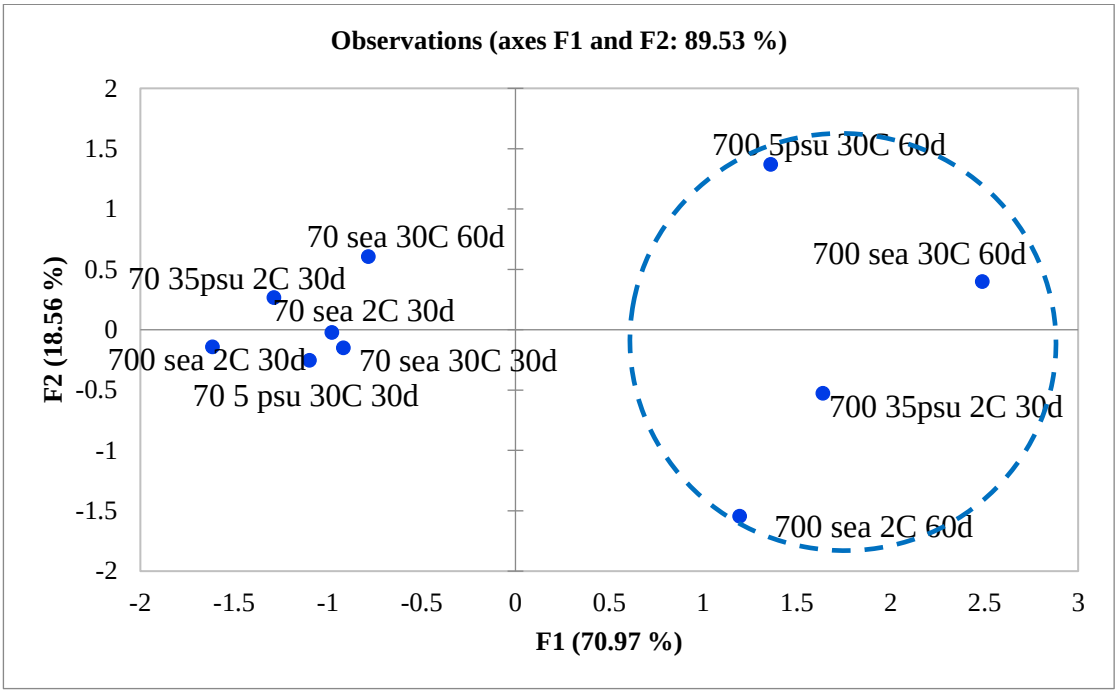


Figure 7.9 PCA score plot of CDO samples using the diagnostic ratios of alkylated-dibenzothiophene (C-D)

(Blue circle (Squire dot): higher oil concentration)

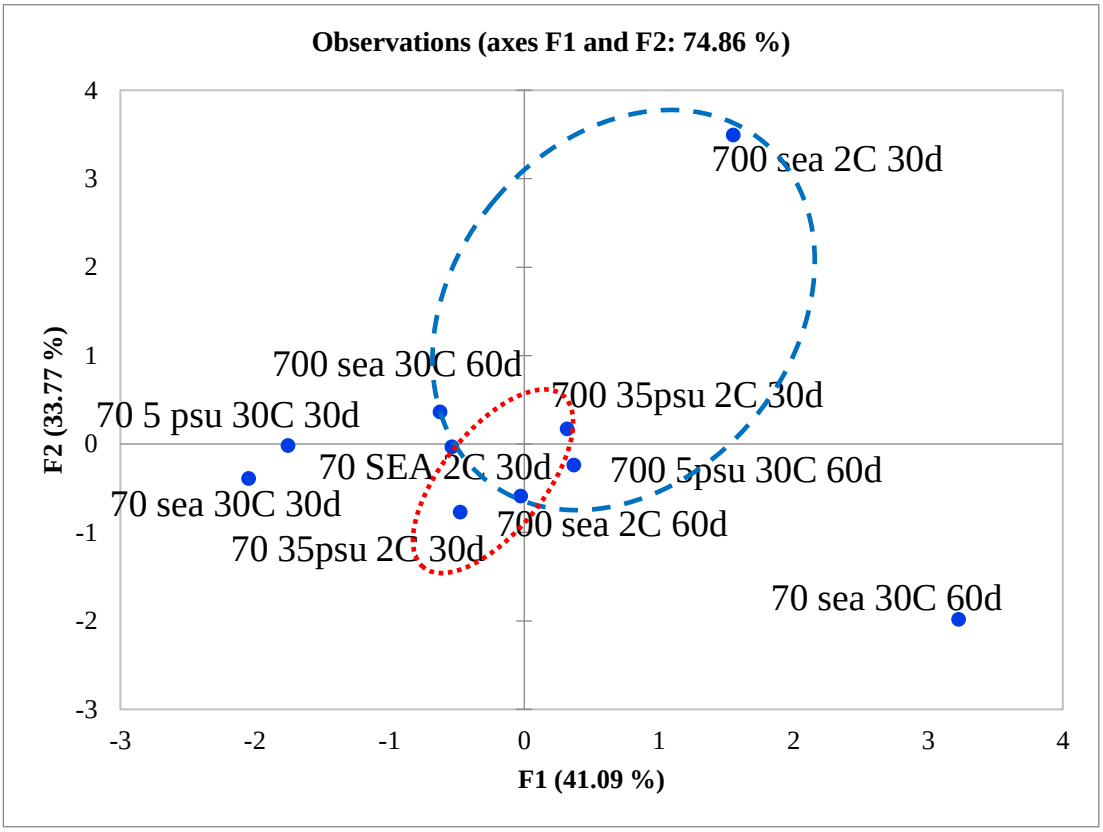


Figure 7.10 PCA score plot of CDO samples using the diagnostic ratios of alkylated-pyrene (C-Py)

(Blue circle (Dash): higher oil concentration; red circle (round dash): (lower temperature))

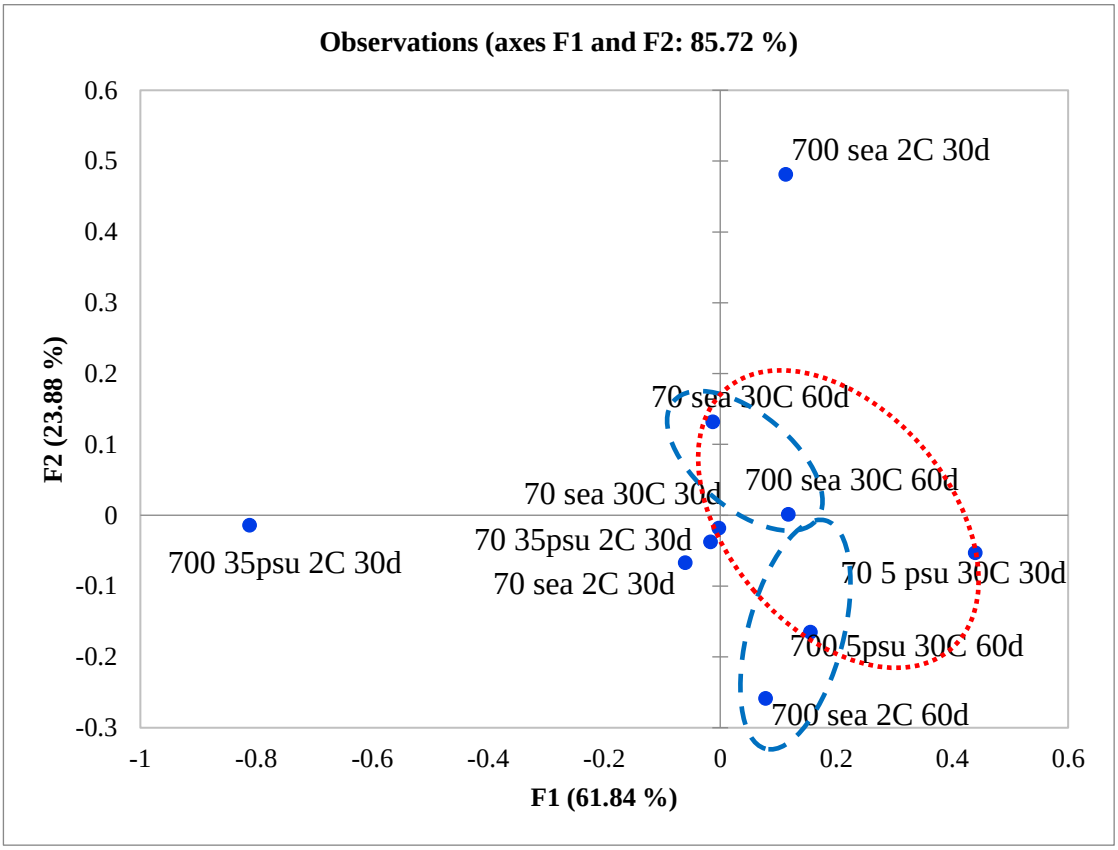


Figure 7.11 PCA score plot of CDO samples using the diagnostic ratios of alkylated-chrysene (C-Chrysene)

(Blue circle (Dash): longer weathering duration; red circle (round dash): (higher temperature

diagnostic ratios, though the significant roles of photooxidation on aromatic hydrocarbons were observed (Bacosa et al. 2015, Garrett et al. 1998, Vergeynst et al. 2019).

PCA was performed to evaluate the impacts of 5 environmental factors in using 7 types of alkylated-PAHs, involving C-N, C-F, C-P, C-Py, C-B, C-D, and C-C, respectively. The PCA results were displayed in Fig 7.6-7.11. Generally, 2-3 PCs were extracted, revealing at least 80% of the total variance. Environmental factors had distinguishable impacts on different alkylated-PAHs. For example, the temperature is correlated with the weathering of C-N, C-F, and C-P, but rarely linked to the weathering of other alkylated PAHs. Oil concentration was the main factor in differentiating the weathering of 6 types of alkylated-PAHs. The composition of seawater only had remarkable effects on the weathering of C-B. The overlap of the circles in figures, such as Figure 7.8, 7.10, and 7.11 implied the impact of interactions of multiple environmental factors. Although the quantitative analysis of the interaction effects using PCA is severe, the presence of the interaction effects demonstrated the complicated impact of environmental factors on the weathering of biomarkers in CDO.

Since alkylated PAHs were susceptible to physio-chemical weathering, especially photooxidation, the changes of the diagnostic ratios contributed to physiochemical weathering and biodegradation. PAHs with lower molecular weight, such as naphthalene and fluorenes, were readily affected by evaporation, photooxidation, and biodegradation. PAHs with higher molecular weight and higher ring numbers had a higher resistance to evaporation, but their

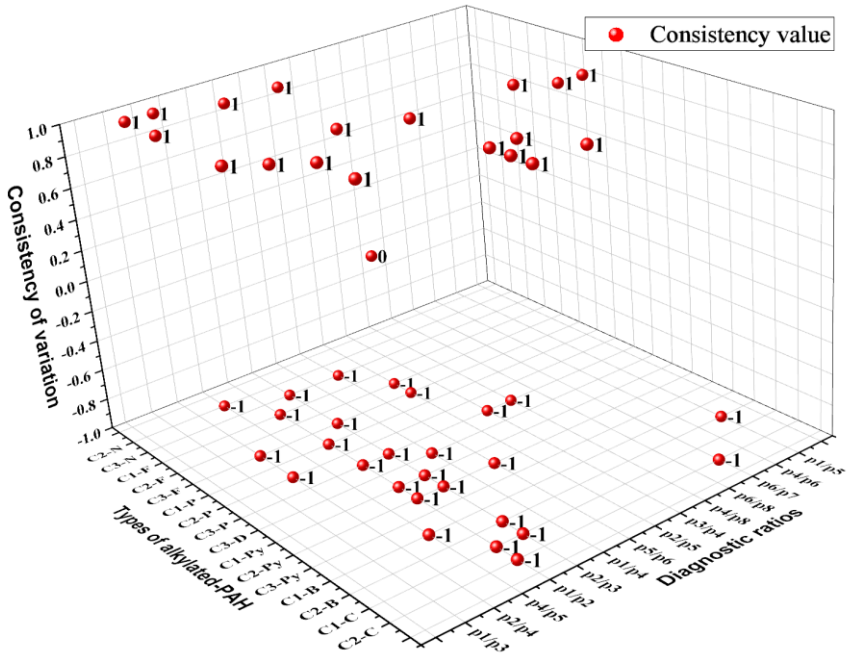


Figure 7.12 The consistency of the variations of the diagnostic ratios in biodegradation and natural weathering experiments

degradation was dominated by photooxidation associated with biodegradation according to the previous research (Fayad and Overton 1995). The changes of the diagnostic ratios of alkylated-PAHs may bring difficulties in tracing different weathering status because multiple weathering processes could simultaneously alter the fate and behaviors of alkylated-PAHs.

In this chapter, the function of tracking the fate and behaviors of CDO using alkylated-PAHs were evaluated by the variations of diagnostic ratios based on weathering experiments in this chapter and the data from the biodegradation experiment (Chapter 6). The changes in the patterns of the biomarkers were expressed by the increase (+1), the decreasing trend (-1), or barely any change (0) of alkylated-PAHs. If the changes of diagnostic ratios in physiochemical and biodegradation were different, the diagnostic ratios were marked as (-1). If the changes in two weathering experiments had the same trend of decrease or increase, the diagnostic ratios were marked as (+1). The scores were applied to determine the consistency of the variations of the diagnostic index for differentiation biodegradation and natural weathering. The results of the consistency of the variation of patterns were evaluated shown in Figure 7.12. Approximate 50% of the diagnostic ratios from individual alkylated-PAHs did not have a constant decrease or increase with different weathering process (with a consistency value of -1). The results indicated the different preferences of biodegradation and physiochemical weathering. Some diagnostic ratios of alkylated-PAHs illustrating different weathering processes were screened.

7.4 Summary

Fingerprinting is a vital technology to identify the source and trace the fate and behaviors of CDO. The variation of biomarkers can be attributed to oil weathering processes with environmental changes. Those complicated conditions could affect the weathering degrees of biomarkers in CDO. This chapter conducted weathering experiments under various weathering conditions. The variations of biomarkers were analyzed using multiple analytical methods, including PCA, MCA, and the consistency of variations trend. The important weathering conditions to CDO fingerprinting were analyzed, involving low temperature, low concentration of dispersed oil, weathering duration, and salinity. The main weathering pathways of different biomarkers were discussed. Research outputs could provide a better scientific understanding on the variations of biomarkers and necessary knowledge CDO fingerprinting.

CHAPTER 8 CONCLUSIONS AND RECOMMENDATIONS

8.1 Summary

Fingerprinting is a crucial technology to trace the sources and behaviors of spilled oil in marine environments. Biomarkers, as complex hydrocarbons in oil from formally living organisms, have been widely used for offshore oil spill fingerprinting. The use of dispersants enhances the stay of dispersed oil in a water column and changes the crucial properties of spilled oil. The existence and concentrations of some biomarkers in chemically dispersed oil (CDO) may differ from those in crude oil and weathered oil. Such differences could affect the diagnostic ratios among different biomarkers. Dispersants thus may affect the suitability of existing biomarkers in oil source identification and the evaluation of oil weathering status during an offshore spill.

Research gaps have been identified in the thesis. Firstly, studies are extremely limited regarding the applicability of existing biomarkers in fingerprinting CDO under various weathering conditions. Secondly, if these biomarkers were affected by physiochemical weathering and biodegradation, research efforts need to be placed on how the biomarkers behave and how to trace the CDO weathering processes, and whether the addition of dispersants leads to significant the variations of biomarkers. Thirdly, oil weathering processes and conditions are crucial to better understand and monitor the fates and behaviors of CDO, but there is lack of detailed and reliable research in the field.

To help fill the gaps and improve the current fingerprinting technique for tackling dispersed oil, this thesis work is aimed at 1) evaluating the stability of biomarkers in CDO through the laboratory simulation of different weathering processes; 2) unraveling the statistical difference between CDO and WCO; 3) understanding the

effects of biodegradation on fingerprinting of CDO and evaluating the biodegradation of CDO treated by a modified SWD; and 4) seeking the effects of different weathering processes and conditions on the variations of biomarkers.

To assess the applicability of biomarkers through physiochemical weathering, multiple weathering experiments, including short-term and long-term weathering, were conducted (Chapter 3 and Chapter 4). In Chapter 3, fingerprinting of chemically dispersed oil during a short-term weathering was evaluated. Through the analysis of the variations of biomarkers and identification of different oils, the recommended diagnostic ratios of biomarkers were ranked. Diagnostic ratios of sesquiterpanes in CDO and WCO were relatively unstable compared to those in crude oil samples, except the peaks 4:5 and 8:10. When double ratio plots of peaks 4:5 and 8:10 were plotted, CDO could not be identified with a biodegraded Liao River crude oil. Therefore, sesquiterpanes could not be used as biomarker for CDO fingerprinting in seawater. Steranes and terpanes were relatively stable in CDO and WCO samples. Based on the double ratio plots of peaks, steranes and terpanes were demonstrated to be applicable as biomarkers to identify CDO. The order of susceptibility of diagnostic ratios of steranes used as biomarkers for CDO identification from the least susceptible to the most susceptible was DIA27S/27R > C_{27}S/C_{27}R> C_{27}αββR/C_{27}αββS > C_{28}αββR/ C_{28}αββS> C_{29}S/C_{29}R > C_{27}S/C_{27}αββR. The order of susceptibility of selected biomarkers for CDO fingerprinting from the least susceptible to the most susceptible were terpanes > steranes > sesquiterpanes due to the RSD values and the range of the diagnostic ratios in typical oils. This research for the first time examined the stability and suitability of diagnostic ratios of sesquiterpanes, steranes, and terpanes, for CDO identification during phosichemical

weathering in seawater. The output could help fulfill the gaps of CDO fingerprinting using current analytical methods and biomarkers. Future work will be conducted to monitor the performance of biomarkers using more types of oils and with more conditional factors (e g., temperature, volume ratio of dispersants to oil) taken into consideration.

Chapter 4 systematically examined the diagnostic ratios of 8 types of biomarkers for CDO fingerprinting. Three types of aliphatic biomarkers, including adamantanes, diamantanes, and sesquiterpanes, are not recommended for characterization of weathered dispersed oil with a long-term weathering. Some diagnostic ratios between two biomarkers within adamantanes, those within diamantanes, and those within sesquiterpanes might be applicable as secondary tools to fingerprinting CDO within shorter-term weathering. Most of the diagnostic ratios based on steranes, terpanes, and aromatic-steranes (TA-steranes, and MA-steranes) in CDO were recalcitrant during the experiments. Therefore, these biomarkers could be applied for CDO fingerprinting. Parts of the diagnostic ratios of alkylated-PAHs can be applied for CDO identification in some cases although they can be more easily degraded than other biomarkers, such as terpanes and steranes. Some potential applicable diagnostic ratios between two biomarkers of different types were also screened. The screened stable biomarkers and corresponding diagnostic relations help fulfill the gaps of CDO fingerprinting. Future work will be focused on the evaluation of fingerprinting of CDO under more specific weathering conditions (e g., photo-oxidation and biodegradation) and with formal statistical analysis methods being used, such as principal component analysis.

To elucidate whether there are statistical differences between weathered dispersed oil and weathered crude oil, several principal component analyses (PCA) were applied to differentiate weathered chemically dispersed oil from weathered crude (non-dispersed) oil using 103 diagnostic ratios of the same type of biomarkers and those of two types of biomarkers as input data. CDO samples were differentiated from WCO samples using all the low-molecular biomarkers or combinations of high molecular biomarkers by multiple PCA methods. The application of dispersants can affect the weathering fates of biomarkers to differentiate the weathering process of CDO from WCO. The differences in CDO and WCO samples were attributed to the effects of weathering duration as well. The overall trend of weathering duration can be displayed in scores plots from PCA analyzes. Involved biomarkers play a paramount role for CDO differentiation. The results implied the diverse degrees of weathering of different types of biomarkers and reflected the importance and possibility of application of biomarkers to trace the behaviors of weathered dispersed oil. More indices including diagnostic ratios and isotopic index will be used in further studies to better trace the weathering of oils, and application of countermeasures of oil spill using fingerprinting.

To examine the influence of biodegradation in the variations of biomarkers, and further evaluate biodegradation rate of CDO treated by a modified SWD, biodegradation experiments were performed to evaluate the biodegradability of oil treated by a modified green SWD through developing an oil tracing methodology. The developed methodology was adopted to classify the different functions of the diagnostic ratios of biomarkers based on their stability during biodegradation. Common biomarkers and homologues of alkylated-PAHs were involved. The

developed methodology would help to better understand the accurate oil tracing functions for the biodegradation of oil treated by new dispersants. Results indicated that biodegradation of SWD was strongly promoted after the modification of SWD production. The biodegradation rates of n-alkanes (n<26) treated by modified SWD was compatible compared n-alkanes in oil without the addition of dispersants. The degradation rate of n-alkanes (n>26) varied with the types of oils, implying the lower capacity of SWD for further biodegradation. The application of SWD enhanced the degradation rates of alkylated-naphthalene, fluorene, phenanthrene, and dibenzothiophene. Alkylated-PAHs with more benzene rings were not degraded in the experiments. Results also indicated that the modified SWD promoted the biodegradation of some alkylate-PAHS.

To understand the correlation between different weathering processes and the variations of biomarkers, multiple weathering experiments, involving the variations weathering processes and conditions, were conducted. The variations of the selected diagnostic ratios were evaluated using MCA and PCA. Although some biomarkers, containing some steranes, terpanes, TA-, and MA-steranes show high resistance to weathering, the concentration of dispersed oil, temperature and salinity dominates the variations of biomarkers in different dispersed oils. The variations of the diagnostic ratios composed by diamondoids and sesquiterpanes in dispersed oil were mainly linked by evaporation. The slight variations of terpanes and steranes were related to biodegradation. The diagnostic ratios of alkylated-PAHs could be affected by multiple weathering conditions. The consistent trend of the variations was applied to evaluate the importance of photo-oxidation and biodegradation. Stable terpanes steranes may be linked to biodegradation, and the

degradation of aromatic steranes may be attributed to photooxidation.

8.2 Research Contributions

The major research contributions of this work can be summarized in the following aspects:

1) Knowledge on the fingerprinting of dispersed oil

The comprehensive study regarding fingerprinting of dispersed oil was firstly comprehensively evaluated. The stability of eight types of biomarkers was examined in several weathering experiments, including physiochemical weathering, biodegradation, and natural weathering. The results filled the knowledge gaps through obtaining applicable biomarkers for fingerprinting of dispersed crude oil. Different weathering degrees of biomarkers indicated the impact of dispersants on stability of biomarkers and the possibility of application of degraded biomarkers to trace behaviors of weathered CDO. The results could provide solid legal liability for the responsibility of oil spills involving the application of dispersants.

2) Provide an advanced fingerprinting strategy for dispersed oil fingerprinting

An advanced fingerprinting strategy for fingerprinting dispersed oil (Figure 8.1) was proposed and verified using 5 chapters in this thesis. In this strategy, biomarkers could be categorized based on their stability. Four essential functions, including source identification, oil differentiation, weathering tracking, and oil monitoring could be realized through diverse proper methodologies using biomarkers. The strategy has been verified using over 35,000 original data during experiments. The strategy can

provide reliable approaches and technical support to the development and modification of standard methods regarding oil monitoring, semi-quantitative characterization, and fingerprinting of oil treated by chemical agents, or oil contaminated samples.

3) Scientific significance of weathering tracking through fingerprinting technology

The variations of biomarkers induced by different weathering processes were analyzed through multiple weathering experiments coupled with diverse analytical methods. The effects of evaporation, photooxidation, and biodegradation were traced using diverse biomarkers. The analytical results would give a better understanding of the fate and behaviors of biomarkers in spilled CDO in marine environments and have scientific value for further research. The developed oil tracing methodology, including applying MCA on oil fingerprinting and assessing consistency trend of alkylated PAHs, could provide new thoughts and direction for environmental forensic.

4) Knowledge on biodegradation of a new green dispersant evaluated using biomarkers

Production of a newly generated shrimp waste-based dispersant was further improved using a modified protocol. Biodegradability of oil treated by the new dispersant was for the first time evaluated using identified biomarkers. The results would help to promote the development, improvement, and application of more new dispersants in the future.

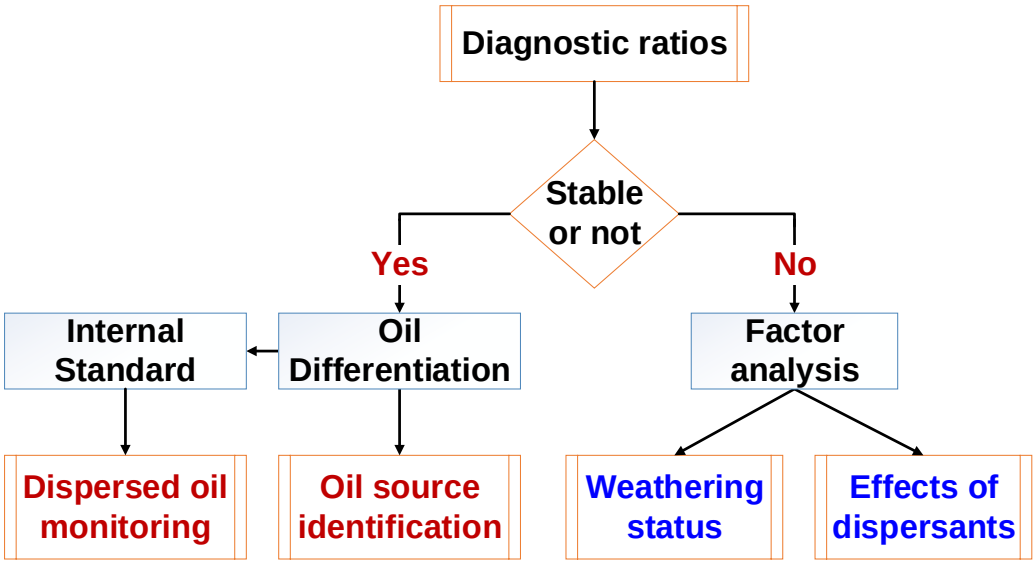


Figure 8.1 An advanced fingerprinting strategy for dispersed oil fingerprinting

8.3 Recommendations for Future Work

1) Photo-oxidation had significant and diverse effects on the fates of dispersed oils in the water column. Impact of photo-oxidation, especially sunlight, on dispersed oil weathering under different environmental conditions need to be further conducted.

2) Applied statistical analytical methods, including principal component analysis and multiple correspondence analysis, need to be further improved to attain accurate recognition of the differentiation and significant factors contributing to the main PCs. More analytical methods should also be introduced to fingerprinting analysis for better reservation of information during dimension reduction.

3) Some low-molecular weight aliphatic biomarkers could be easily degraded and depleted during weathering. Aliphatic and aromatic components of spilled oil however could be converted to polar products, such as resins. Therefore, the behaviors of more polar hydrocarbons, such as naphthenic acid, should be further investigated.

4) Since the marine environments are complicated and critical for oil degradation, more factors and real conditions should be considered, such as the presence of ice and variations of mixing energy. Moreover, scale-up weathering experiments are also desired.

5) Cost-benefit analysis regarding the effects of dispersed oil on marine environments using fingerprinting strategy is recommended to be investigated.

8.4 Selected Publications

Refereed Journal Articles

Song, X., Zhang, B., Chen, B. and Cai, Q. (2016) Use of sesquiterpanes, steranes, and terpanes for forensic fingerprinting of chemically dispersed oil. *Water, Air, & Soil Pollution* 227(8), 281.

Song, X., Zhang, B., Chen, B., Lye, L. and Li, X. (2018) Aliphatic and aromatic biomarkers for fingerprinting of weathered chemically dispersed oil. *Environmental Science and Pollution Research*, 1-13.

Song, X., Lye, L. M., Chen, B., & Zhang, B. (2019). Differentiation of weathered chemically dispersed oil from weathered crude oil. *Environmental monitoring and assessment*, 191(5), 270.

Song, X, Jing, L., Chen, B., Zhu, Z., Cai, Q., Ye, X., Zheng, X., Zhang, B., and Hill, S. (2020). Droplet size distribution of pre-dispersed oil under simulated deep-water conditions. Ready for submission.

Song, X., Chen, B., Zhang, B., Zhang, K., Lye, L. M., and Liu, B.. (2020). Effectiveness of the dispersants generated from the hydrolysate of shrimp waste in frazil ice-covered water. Ready for submission.

Song, X., Zhang, B., Chen, B., Liu, B., Lye, L. M., & Zhang, K.D. (2020). Biomarker traced biodegradation of oil treated by a green dispersant. Ready for submission.

Song, X., Zhang, B., Chen, B., and Lye, L. M. (2020). Impacts of environmental factors on weathering of biomarkers in dispersed oil. Ready for submission.

Zhang, K., Zhang, B., **Song, X.**, Liu, B., Jing, L., & Chen, B. (2018). Generation of shrimp waste-based dispersant for oil spill response. Environmental Science and Pollution Research, 25(10), 9443-9453.

Cai, Q., Zhang, B., Chen, B., **Song, X.**, Zhu, Z., & Cao, T. (2015). Screening of biosurfactant-producing bacteria from offshore oil and gas platforms in North Atlantic Canada. *Environmental monitoring and assessment*, 187(5), 284.

Conference Proceedings and activities

Song, X., Chen, B., Zhang, B.Y. and Li, P. (2014) The modelling of ecological effects of chemically dispersed oil in typical marine species in offshore Newfoundland. Proceedings of the International Society for Environmental Information Sciences (ISEIS) 2014 Annual Conference, August 6-8, 2014, St. John's, Canada. OCR1339: 1-12

Cai, Q., Zhang, B., Chen, B., Li, P., **Song, X.**, & Zhu, Z. (2014, October). Behavior of Corexit dispersants in the Gulf of Mexico after the Deepwater Horizon oil spill. In Proceedings of the International Conference on Marine and Freshwater Environments (iMFE 2014)-Our Water, Our Future (Vol. 1, No. 1).

Song, X., Zhang, B., Chen, B., and Cai Q. (2014, 08). Identification of typical biomarkers in dispersed oil in baffled flask test. IMFE 2014. St. John’s, Canada.

Song, X., Chen, B., Zhu, Z., Jing L., Cai, Q., Ye, X., Zhang, B., and Zheng X. (2016, 06). A Preliminary Study on Droplet Size Distribution of Chemically Dispersed Crude Oil under High Pressure Conditions. 39th AMOP, Halifax, Canada.

Song, X., Chen, B., Zhang, B., Zhang, K., Liu, B., and Lye, L.M. (2017, 10). Effectiveness of bio-dispersants generated from the hydrolysate of shrimp waste in frazil ice-covered water. 40th AMOP, Calgary, Canada.

Song, X., Zhang, B., Chen, B., Lye, L.M., and Lee K. (2017, 10). Utility of Eight Types of Biomarkers in Differentiating Weathered Dispersed Oil from Weathered Crude Oil. PEOPLE 2017, St. John's, Canada.

Song, X., B., Chen, Cai, Q., Zhang, B., Zhang, K., , and Stoyanov S.R. (2018, 10). Weathering of dispersed oil in ice-infested water. 41th AMOP, Victoria, Canada.

Song, X., Zhang, B., Chen, B., Ye, X., Liu B., and Lye, L.M (2019, 10). Fingerprinting of chemically dispersed oils using aliphatic and aromatic biomarkers. PEOPLE 2019 Symposium, St. John's, Canada.

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