

ELUCIDATION OF THE PHOTOCATALYTIC REMOVAL PATHWAYS OF
HUMIC SUBSTANCES: PROGRESS TOWARDS MECHANISTIC EXPLANATIONS

by

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Dedicated to my family...

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ABSTRACT

The objective of this research was to characterize the spectroscopic properties of humic and fulvic acids of different origins as model compounds to represent the natural organic matter in aquatic systems. The photocatalytic oxidation of model humic and fulvic acids was carried out using TiO₂ Degussa P-25 as the photocatalyst. The degradation kinetics was assessed based on pseudo first order and Langmuir Hinshelwood (L-H) kinetic models. The related data for aquatic and terrestrial humic substances were comparatively presented in terms of UV-vis parameters such as Color₄₃₆, Color₄₀₀, UV₃₆₅, UV₃₀₀, UV₂₈₀, UV₂₅₄ and total organic carbon (TOC) removal. Furthermore, the molecular and structural characteristics of the humic acid molecule relative to changes during photocatalytic oxidation were monitored by spectroscopic techniques.

On the basis of their diverse chemical and physical properties such as molecular weight, molecular size, elemental composition and source of origin, substantial differences were observed in photocatalytic removal efficiencies of humic and fulvic acids. For all of the humic substances, higher removal rates were achieved in terms of UV₂₅₄ values compared to that of Color₄₃₆. Moreover, humic acids exhibited higher pseudo first order removal rates with respect to that for fulvic acid. The declining trend of the specified UV-vis parameters, the related changes in the fluorescence spectra (initial increase of the fluorescence intensities, formation of new fluorophores, shift of the spectra to lower wavelength region and decline of intensity after long photocatalytic irradiation times), decrease of TOC content during degradation, the spectral changes in FTIR and NMR indicated the oxidative degradation of humic substances.

Considering the complexity and polydispersity of the humic macromolecules, they were fractionated into well defined subcomponents of known molecular sizes using ultrafiltration through membranes in the range of 100-1 kDa. The effect of photocatalytic oxidation on the molecular size fractions of humic substances were also evaluated on a comparative basis by UV-vis and fluorescence spectroscopy.

As confirmed by the spectroscopic evaluation of the molecular size distribution data, photocatalytic degradation of humic acid leads to the formation of lower molecular size (small fractions) and higher UV-absorbing compounds. For fractions less than 10 kDa, UV₂₅₄ absorbing moieties in treated humic acid samples become higher than that of raw humic substances designating the generation of new species during photocatalysis.

Based on the comparison between the synchronous scan fluorescence spectra of the molecular size fractions of raw and oxidized humic substances, it could be concluded that oxidative cleavage of the molecule leads to a blue shift of the spectra which is indicative to the formation of new fluorophores in each size fraction. It is assumed that oxidative degradation of the macromolecule occurs through an unselective pathway, by the reaction of •OH radicals with the various moieties within each fraction.

ÖZET

Bu çalışmanın amacı su kaynaklarında bulunan doğal organik maddeleri temsilen kullanılan farklı kökenli hümik ve fulvik asitlerin spektroskopik olarak karakterizasyonunu yapmaktır. Model hümik ve fulvik asitlerin fotokatalitik oksidasyonu TiO_2 Degussa P-25 fotokatalizör kullanılarak yapılmıştır. Bozunma kinetiği Langmuir Hinshelwood ve birinci dereceden reaksiyon kinetik modelleri üzerinden değerlendirilmiştir. Hümik maddelerin ilgili verileri Renk_{436} , Renk_{400} , UV_{365} , UV_{300} , UV_{280} ve UV_{254} UV-vis parametreleri ve toplam organik karbon (TOK) giderimi üzerinden karşılaştırmalı olarak verilmiştir. Ayrıca, hümik asitlerin fotokatalitik oksidasyona bağımlı olarak değişen moleküler ve yapısal özellikleri spektroskopik yöntemlerle izlenmiştir.

Hümik ve fulvik asitlerin fotokatalitik giderimlerinde molekül ağırlığı, molekül büyüklüğü, elementel kompozisyonu ve kökenleri gibi bir takım fiziksel ve kimyasal özelliklerine bağlı olarak farklılıklar görülmüştür. Bütün hümik maddeler için, Renk_{436} giderimine kıyasla UV_{254} değerleri cinsinden daha yüksek giderim hızına ulaşılmıştır. Ayrıca, hümik asitler fulvik asitlere göre daha yüksek birinci derece giderim hızları göstermektedir. Belirtilen UV-vis parametrelerindeki azalma eğilimi, floresan spektralarındaki ilgili değişiklikler (floresan yoğunluğundaki ilk artış, yeni floroforların oluşumu, spektranın daha düşük dalga boylu bölgeye kayması ve uzun fotokatalitik bozunma sürecinden sonra floresan yoğunluğunun düşmesi), parçalanma ile TOK miktarındaki azalma ve FTIR ve NMR spektralarındaki değişiklikler hümik maddelerin oksidatif olarak parçalandığını göstermektedir.

Hümik makromolekülünün karmaşıklığı ve polidispers yapısı düşünülerek, ultrafiltrasyonla 100-1 kDa aralığında membranlar kullanılarak tanımlanmış molekül büyüklüklerindeki komponentlere ayrılmıştır. Fotokatalitik oksidasyonun hümik maddelerin molekül fraksiyonlarına olan etkisi UV-vis ve floresan spektroskopisiyle değerlendirilmiştir.

Moleküler büyüklük dağılımı verilerinin spektroskopik olarak değerlendirilmesiyle onaylandığı gibi, hümik asitlerin fotokatalitik olarak parçalanması düşük moleküler

ağırlıklı (küçük fraksiyonlar) ve yüksek UV-absorbe eden bileşiklerin oluşumuna neden olmaktadır. 10 kDa'dan küçük fraksiyonlar için, oksidasyona tabii tutulmuş hümik asitlerdeki UV₂₅₄ absorbe eden yapıların, ham hümik asitlerdeki benzer fraksiyonlardan daha yüksek değerlerde olması fotokataliz sırasında yeni yapıların oluştuğunu göstermektedir.

Ham ve okside olmuş hümik asitlerin moleküler büyüklük dağılımlarının senkronize taramalı floresan spektrumları karşılaştırıldığında, molekülün oksidatif parçalanması ile spektrumun mavi öteleme göstermesinin her bir hümik asit fraksiyonunda yeni floroforların oluşumuna sebep olduğu söylenebilir. Makromolekülün oksidatif parçalanmasının, seçici olmayan •OH radikallerinin her bir fraksiyondaki farklı yapılarla tepkimesiyle oluştuğu düşünülmektedir.

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LIST OF ABBREVIATIONS

Symbol	Explanation
$\Delta\lambda$	Bandwidth
A	Related UV-vis parameter (m^{-1}) or TOC content of HS (mg L^{-1})
AHA	Aldrich humic acid
AOP	Advanced oxidation processes
BLF	Black Light Fluorescent Lamp
Color ₄₀₀	Absorbance at 400 nm
Color ₄₃₆	Absorbance at 436 nm
CZE	Capillary zone electrophoresis
Da	Dalton
DBP	Disinfection byproduct
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
F.I.	Fluorescence index
FA	Fulvic acid
FTIR	Fourier transformation infrared
HA	Humic acid
HOMO	Highest occupied molecular orbital
HPLC	High pressure liquid chromatography
HS	Humic substances
IHSS FA	International humic substance society fulvic acid
IHSS HA	International humic substance society humic acid
IHSS SHA	International humic substance society soil humic acid
k	Pseudo first order reaction rate constant (min^{-1})
K	Pseudo first order reaction rate constant, (min^{-1})
K_{LH}	Adsorption coefficient (m)
k_{LH}	Reaction rate constant ($\text{m}^{-1} \text{min}^{-1}$)
L-H	Langmuir Hinshelwood

LUMO	Lowest unoccupied molecular orbital
NHA	Nordic humic acid
NMR	Nuclear magnetic resonance spectroscopy
NMWCO	Nominal molecular weight cut off
NOM	Natural organic matter
PSS	Polystyrene sulfonate
Py-GC-MS	Pyrolysis-gas chromatography-mass spectroscopy
QS	Quinone sulfate
R	Pseudo first order rate
RHA	Roth humic acid
R _{LH}	Rate of the reaction ($\text{m}^{-1} \text{min}^{-1}$)
ROS	Refractory organic substances
RPE	Relative photonic efficiency
SCOA	Specific color absorbance
SCOA ₄₃₆	Specific color absorbance at 436 nm
SOM	Soil organic matter
SUVA	Specific UV absorbance
SUVA ₂₅₄	Specific UV absorbance at 254 nm
SUVA ₃₆₅	Specific UV absorbance at 365 nm
t	Irradiation time (min)
t _{1/2}	Half life (min)
THM	Trihalomethane
THMFP	Trihalomethane formation potential
UV ₂₅₀	Absorbance at 250 nm
UV ₂₅₄	Absorbance at 254 nm
UV ₂₈₀	Absorbance at 280 nm
UV ₃₀₀	Absorbance at 300 nm
UV ₃₆₅	Absorbance at 365 nm

1. INTRODUCTION

Although there is a lot of information about the properties of humic substances, the level of knowledge concerning their physical-chemical characterization is still ambiguous. In the last years, humic substances increasingly became an object of environmental research. They are typically abundant in drinking water supplies because of substantial inputs of dissolved and particulate organic material from the surrounding watershed or because of autochthonous production of humic substances by degradation of algal biomass.

Humic substances (HS) play an important role in transport, binding, analysis and remediation of pollutants in natural environments due to their ubiquitous occurrence and chemical reactivity. Moreover, they act as precursors for the formation of carcinogenic disinfection by-products (DBPs) during the chlorination process in water treatment systems. Humic materials are still among the least understood and characterized components in the environment. Hence, continued development of new analytical methods and approaches is required for their analysis.

The objective of this research is to characterize the spectroscopic properties of humic substances from different origins as model compounds to represent natural organic matter in aquatic systems and to observe the alterations of spectroscopic properties during photocatalytic oxidation process. Model humic and fulvic acid compounds of terrestrial and aquatic origin, namely, Aldrich humic acid (AHA), Roth humic acid (RHA), International Humic Substance Society (IHSS) humic acid standard (IHSS HA), IHSS fulvic acid standard (IHSS FA), Nordic humic acid reference (NHA), and IHSS soil humic acid standard (IHSS SHA) were used in bench scale experiments to mimic natural organic matter in drinking water supplies.

In the first part, the studied humic substances were characterized with respect to their UV-vis and fluorescence spectroscopic properties. Considering the complexity of the humic macromolecules, they were fractionated into well defined subcomponents of defined molecular sizes using ultrafiltration through membranes in the range of 100-1 kDa. Moreover, the spectroscopic properties of each size fraction were characterized and

compared by UV-vis spectroscopy and fluorescence spectroscopy in emission and synchronous scan modes.

In the second part, photocatalytic oxidation of model humic and fulvic acids was carried out using TiO₂ Degussa P-25 as the photocatalyst. The related data of humic substances were presented in terms of the specified UV-vis parameters as Color₄₃₆, Color₄₀₀, UV₃₆₅, UV₃₀₀, UV₂₈₀ and UV₂₅₄. The degradation kinetics were comparatively assessed based on pseudo first order and Langmuir Hinshelwood (L-H) kinetic models. Furthermore, the molecular and structural characteristics of the humic acid molecule relative to changes during photocatalytic oxidation were monitored by fluorescence spectroscopy in emission and synchronous scan excitation modes, Fourier transform infrared (FTIR) and nuclear magnetic resonance spectroscopy (NMR) utilizing liquid state ¹H NMR and ¹³C NMR.

In the last section, the molecular size distribution profiles of the partially oxidized humic substances were comparatively presented by both the specified UV-vis properties and synchronous scan fluorescence spectra.

The structural and conformational identification of humic matter is extremely challenging because of their highly heterogeneous nature in relation to their photophysical, photochemical, and photobiological roles in natural waters. Hence, it is intended to fulfill the lack of knowledge about the spectral changes of humic substances during oxidation processes and elucidate the effects of the molecular size distribution of raw and treated humic samples.

2. THEORY

2.1. Humic Substances

The main contributors to the organic carbon, globally distributed in aquatic and terrestrial environments, are humic substances, which are products of the decomposition of plant and animal residues. They are chemically complex, high molecular weight polydisperse polyelectrolytes, comprising organic acids with both aromatic and aliphatic features. Humic substances (HS) comprise a general class of biogenic, refractory, and yellow to black organic substances (Suffet and MacCarthy, 1989).

Table 2.1. Some important functional groups of humic substances

Functional group	Structure
Acidic groups	
Carboxyl	$\text{R}-\text{C}=\text{O}(-\text{OH})$
Enol	$\text{R}-\text{CH}=\text{CH}-\text{OH}$
Phenolic OH	$\text{Ar}-\text{OH}$
Quinone	$\text{Ar}=\text{O}$
Neutral groups	
Alcoholic OH	$\text{R}-\text{CH}_2-\text{OH}$
Ether	$\text{R}-\text{CH}_2-\text{O}-\text{CH}_2-\text{R}$
Ketone	$\text{R}-\text{C}=\text{O}(-\text{R})$
Aldehyde	$\text{R}-\text{C}=\text{O}(-\text{H})$
Ester	$\text{R}-\text{C}=\text{O}(-\text{OR})$
Basic groups	
Amine	$\text{R}-\text{CH}_2-\text{NH}_2$
Amide	$\text{R}-\text{C}=\text{O}(-\text{NH}-\text{R})$

R: represents an aliphatic backbone of straight, branched, saturated or unsaturated carbon

Ar: is an aromatic ring

As presented in Table 2.1, they are known to contain functional groups i.e. $-\text{C}=\text{C}$, $-\text{COOH}$, $-\text{OH}$, $-\text{OCH}_3$, $-\text{C}=\text{O}$, $-\text{NH}$ or $-\text{NH}_2$, arrangements such as redox quinone-semiquinone-hydroquinone, charge-transfer planar complexes. Both their dark colour and paramagnetism most likely result from the electronic structure of their core; aromatic

subunits, an extended (p-electron) system in the semiquinone moieties, and the electron donor-acceptor nature (Suffet and MacCarthy, 1989).

Due to their structural versatility, humic substances are involved in many environmental processes, especially in binding, transport, and deposition of inorganic and organic pollutants e.g. heavy metal ions, pesticides (Suffet and MacCarthy, 1989). Imparting undesirable color and acting as precursors for undesirable trihalomethane formation during the chlorination process, their presence in drinking water supplies causes problems, the consequences of which may lead to the deterioration of human health (Reckhow et al., 1990; Rook, 1977).

The complex polymeric nature and interaction between component chains of humic material make structural analysis difficult; however, compositional information can be obtained from elemental and functional group analysis as presented in Table 2.2.

Table 2.2. Elemental analysis of humic substances (Schnitzer and Khan, 1972)

Element	Humic acid (wt %)	Fulvic acid (wt %)
C	53.6-58.7	40.7-50.6
H	3.2-6.2	3.8-7.0
N	0.8-5.5	0.9-3.3
O	32.8-38.3	39.7-49.8
S	0.1-1.5	0.1-3.6

The elemental analysis of humic and fulvic acids from a range of environmental sources show that the atomic H/C ratio is quite low, and is lower for humic acid than fulvic acid, which is consistent with a higher aromatic content for humic acid. The atomic O/C ratio is also lower for humic acid than fulvic acid, reflecting the higher content of polar groups in fulvic acid.

The greater water solubility of fulvic acids compared to humic acids can be attributed to the higher content of polar groups, particularly carboxyl groups. Among the other functional groups present in smaller quantities are ether, aldehyde and amine.

Oxygen is the major heteroatom in humic substances and occurs predominantly in the following functional groups: -COOH, phenolic and alcoholic -OH, ketonic and quinoid -C=O, and -OCH₃ (ether and ester). The estimated abundances of these groups in humic and fulvic acids are given in Table 2.3.

Table 2.3. Estimated abundance of functional groups (mequiv g⁻¹)* in humic and fulvic acids (Schnitzer and Khan, 1972)

Functional group	Humic acid	Fulvic acid
Total acidic groups	5.6-8.9	6.4-14.2
Carboxyl, COOH	1.5-5.7	5.2-11.2
Phenolic, OH	2.1-5.7	0.3-5.7
Alcoholic, OH	0.2-4.9	2.6-9.5
Quinoid/keto, C=O	0.1-5.6	0.3-3.1
Methoxy, OCH ₃	0.3-0.8	0.3-1.2

*mequiv g⁻¹ equivalent to mmol of each group per g of humic substances

Different descriptions of HS are used according to specific environments of origin and research interests. As a consequence, a wide variety of terms such as dissolved organic matter (DOM), natural organic matter (NOM), soil organic matter (SOM), and refractory organic substances (ROS) are commonly used in literature. Often they are related to operational definitions based on fractionation procedures. The term “humic substances” is used as a generic name to describe coloured material or its fractions obtained on the basis of solubility characteristics. The fraction called humic acids is not soluble in water basics under acidic conditions (pH < 2) but is soluble at higher pH values. They can be extracted from soil by various reagents. Humic acids are the major extractable component of soil humic substances. They are dark brown to black in colour. The fraction called fulvic acids is soluble in water under all pH conditions. They remain in solution after removal of humic acid by acidification. Fulvic acids are light yellow to yellow-brown in colour. Finally the fraction called humins is not soluble in water at any pH value and even under alkaline medium. Humins are basically black in colour. Usually the carbon content of material from a given source increases in the series such as FA < HA < Humin.

Differences between humic acids and fulvic acids can be explained by variations in molecular weight, the number of functional groups (carboxyl and phenolic OH) and the extent of polymerization. The postulated relationships are depicted in Figure 2.1, in which it can be seen that colour, carbon and oxygen contents, acidity and degree of polymerization all change systematically with increasing molecular weight. The low molecular weight fulvic acids have higher oxygen but lower carbon contents than the high molecular weight humic acids. The total acidities of fulvic acids (900-1400 meq/100 g) are considerably higher than that of humic acids (400-870 meq/100 g) (Tipping, 2002).

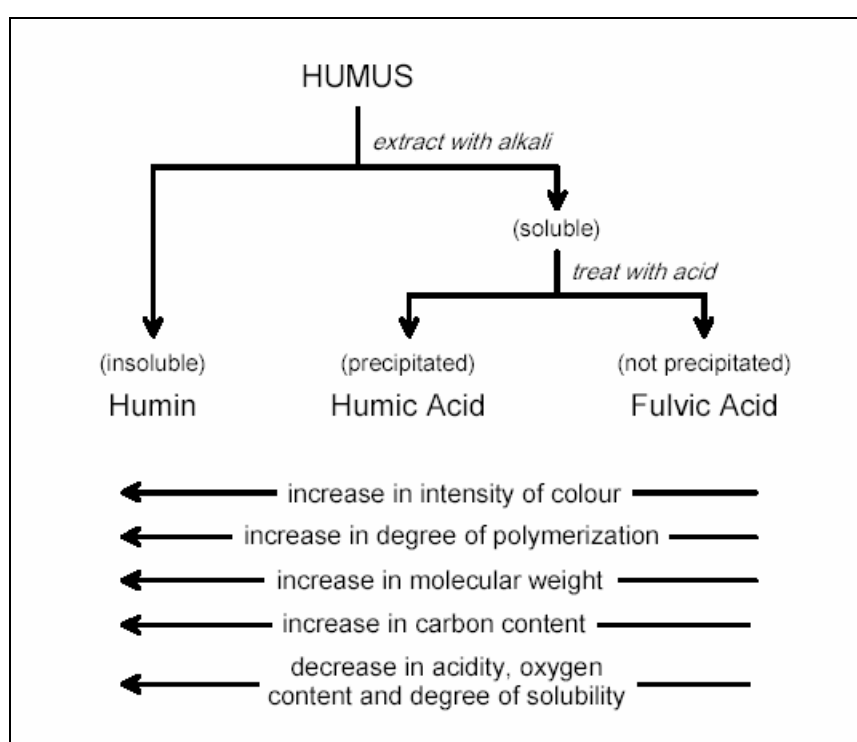


Figure 2.1. Postulated relationships between humic substances.

Humic materials have a wide range of molecular weights and sizes ranging from a few hundred to as much as several hundred thousand atomic mass units. In general, fulvic acids have lower molecular weights than humic acids. It is also known that soil derived humic materials are larger than aquatic humic substances (Gaffney et al., 1996).

Humic acids are thought to be complex aromatic macromolecules with amino acids, amino sugars, peptides, aliphatic compounds involved in linkages between the aromatic groups. The hypothetical structure for humic acid, shown in Figure 2.2 contains free and

bound phenolic OH groups, quinone structures, nitrogen and oxygen as bridge units and COOH groups variously placed on aromatic rings.

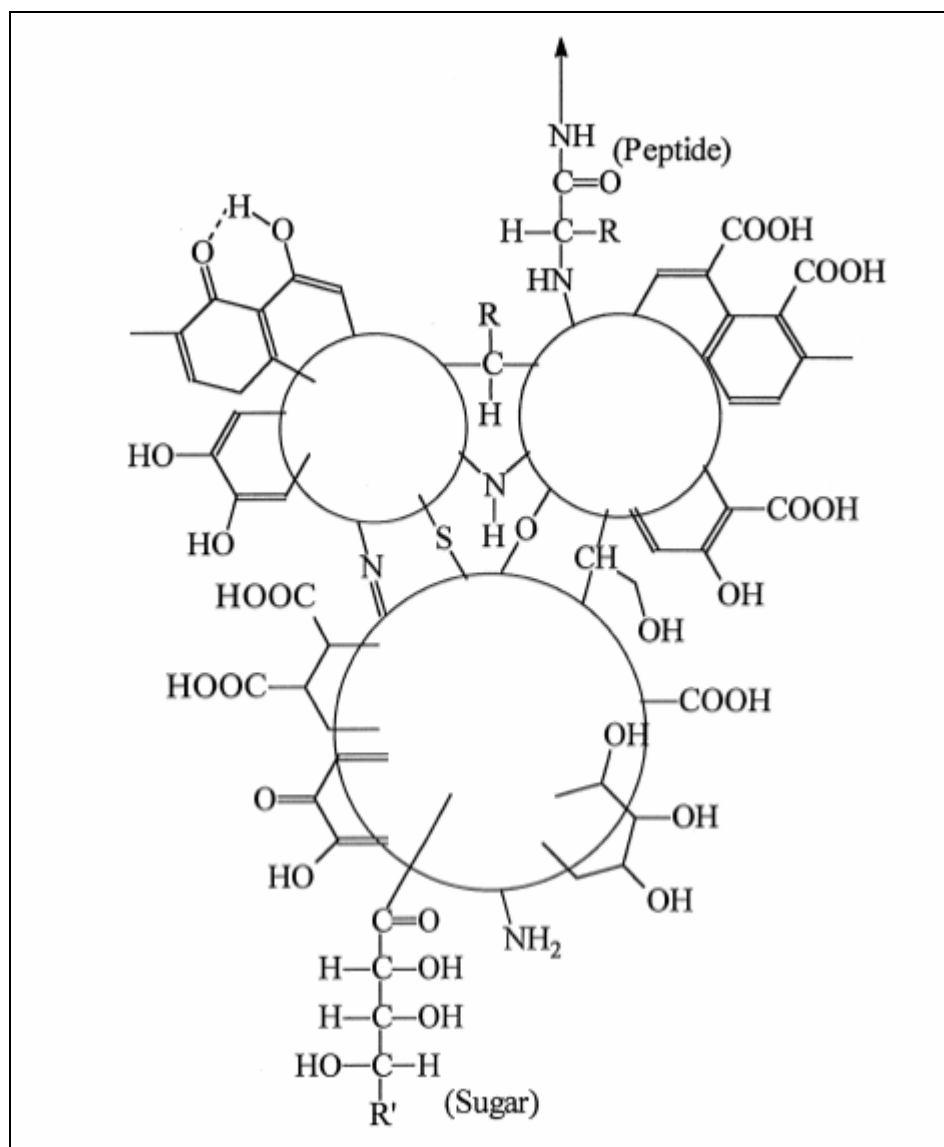


Figure 2.2. Hypothetical structure of model humic acid (Fetsch et al., 1998).

The hypothetical model structure of fulvic acid (Buffle's model) contains both aromatic and aliphatic structures, both extensively substituted with oxygen-containing functional groups (Figure 2.3). However, the structures of fulvic acid are more aliphatic and less aromatic than humic acids. The reason for their high solubility in water at all pH values is mainly due to the presence of carboxylic acid, phenolic and ketonic groups in appreciable amounts.

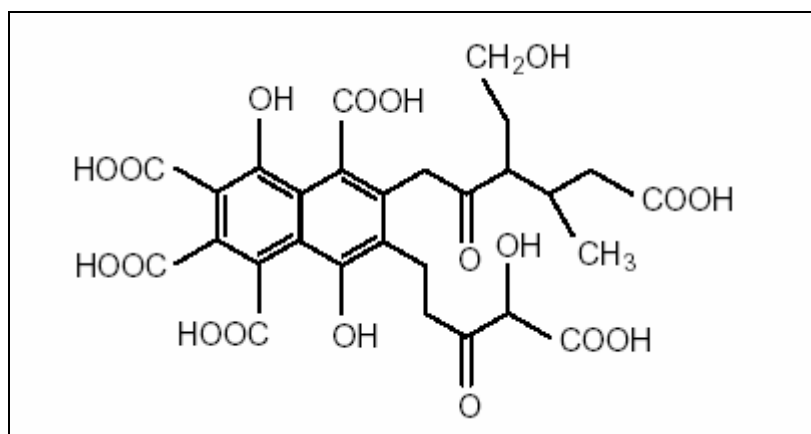


Figure 2.3. Structure of fulvic acid (Buffle et al., 1977).

Depending on the pH of the solution functional groups in humic molecule are dissociated or protonated. Dissociated functional groups carry negative charges. Electrostatic repulsions between neighboring negatively charged sites causes stretching of the molecule. Furthermore, the electrostatic forces are influenced by ionic strength, by the presence of cationic species etc. (Ghosh and Schnitzer, 1980). It was reported that humic molecules can change from a large, flexible and linear shape at high pH, low ionic strength and low humic concentration, to a small, rigid and spherocolloidal conformation at low pH, high ionic strength and high humic concentration (Ghosh and Schnitzer, 1980).

Humic substances are mixtures not defined by a single chemical characteristic; hence, their concentrations are difficult to measure. The most useful humic related determinant that provides a useful indication is the dissolved organic carbon (DOC). Concentrations of DOC in freshwater streams, rivers and lakes which are known to be due to the presence of humic substances vary from less than 0.5 mg L^{-1} to 100 mg L^{-1} depending on the nature of the catchment, including the climate, the trophic status of the water body, and pollutant inputs.

Chemical and physical studies of humic substances and their interactions with environmental contaminants are essential in order to explain their impact on the environmental fate, bioavailability and toxicity of organic and metal contaminants in natural water systems. However, elucidation of the important role of humic substances is complicated by their highly heterogeneous nature.

A number of physical and chemical measurements have been employed to characterize humic substances, including elemental analysis, ultrafiltration, UV-vis spectroscopy, fluorescence spectroscopy, Fourier transform infrared (FTIR) spectroscopy, pyrolysis-gas chromatography-mass (Py-GC-MS) spectroscopy, ^1H and ^{13}C -NMR spectroscopy (Senesi et al., 1991; Westerhoff et al., 1999; Shin et al., 1999; Schulten and Gleixner, 1999; Kalbitz et al., 2000).

Nuclear magnetic resonance spectroscopy (NMR) has been widely used to provide information about humic substances and their chemistry. Applying ^{13}C -NMR spectroscopy appears to be well suited for obtaining an inventory of the chemical composition of humic materials and for revealing differences in functional group content between samples. Spectra can be acquired in solution-state and solid-state NMR-spectroscopy. Information about the presence of functional groups, aromatic systems or branched alkyl groups can be obtained (Pommes et al., 2000).

High pressure liquid chromatography (HPLC) using gels have proved useful in combination with UV-vis, fluorescence, light scattering and sensitive dissolved organic carbon detection techniques, yielding information on molecular absorbance, size distribution, molar mass and reactivity. Using both chemical and spectroscopic methods, various studies have been conducted for the characterization of humic substances of different origin (Frimmel, 1998; Shin et al., 1999; Aguer and Richard, 1996).

Despite of the application of powerful analytical methods like Fourier transform infrared spectroscopy, pyrolysis mass spectrometry (Py-MS) and solid phase nuclear magnetic resonance e.g.; ^{13}C -NMR, molecular structure identification in the classical sense has not been successful (Hayes, 1998). Humic materials are still among the least understood and characterized components in the environment. Hence, continued development of new analytical methods and approaches is required for the characterization and analysis of humic substances.

2.2. Spectroscopic Characterization of Humic Substances

2.2.1. UV-vis Spectroscopy

Ultraviolet-visible spectroscopy (UV=200-400 nm, visible=400-800 nm) corresponds to electronic excitations between the energy levels that correspond to the molecular orbitals of the systems. For a molecule, this is a process where electrons are promoted from their electronic ground state to an excited state. As a rule, energetically favored electron promotion will be from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO).

The absorbance of UV light by a molecule depends on the electronic structure of the molecule. The UV spectrum, therefore, indicates the presence of specific bonding arrangements in the molecule. A diagram showing the various kinds of electronic excitation that may occur in organic molecules is illustrated in Figure 2.4.

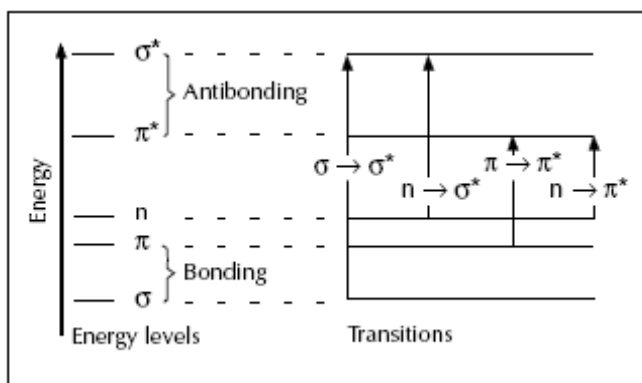


Figure 2.4. Electronic molecular energy levels (Skoog and Leary, 1992).

Valence electrons are found in three types of electron orbitals: single, or σ , bonding orbitals; double or triple (π bonding orbitals); and non-bonding orbitals (lone pair electrons). Sigma (σ) bonding orbitals tend to be lower in energy than π bonding orbitals, which in turn are lower in energy than non-bonding orbitals. When electromagnetic radiation of the correct frequency is absorbed a transition occurs from one of these orbitals to an empty orbital, usually an antibonding orbital σ^* or π^* . Most of the transitions from

bonding orbitals have too high frequency, so most of the absorptions involve only $\pi \rightarrow \pi^*$, $n \rightarrow \sigma^*$ and $n \rightarrow \pi^*$ transitions.

UV-vis spectra of humic substances are featureless with absorption increasing at lower wavelengths and contain no discrete absorption bands due to the overlapping of the broad absorption bands of the chromophores. The absorption in the UV region is mainly caused by the excitation of electron lone pair, usually oxygen ($n \rightarrow \pi^*$) and by conjugated C=C double bonds ($\pi \rightarrow \pi^*$). The absorption in the visible region is caused by lone pair electrons and charge-transfer systems. The absorbance decreases as the wavelength increases, as typical for HS (Schnitzer and Khan, 1972; Stevenson, 1982). A slight maximum could be indicated at approximately 275 nm, which is probably due to quinone structure (Schnitzer and Khan, 1972). The spectral absorption exhibits a dependence on pH values (Chen et al., 1977; Langhals et al., 2000) with decreasing absorbance as solution pH decreases. This dependence reflects the acid-base forms of the chromophores within the molecules or, as suggested by Chen et al. (1977), an increase in particle size due to macromolecular associations is expected.

Absorbances at 254 nm (UV_{254}) and 436 nm ($Color_{436}$) are generally used for the quantification of humic substances. UV_{254} is interchangeably measured with total organic carbon (TOC) as a surrogate parameter to represent the natural organic matter content in surface waters (Najm et al., 1994). The UV absorptivity at 280 nm was also introduced to represent total aromaticity, because, π - π^* electron transition occurs in this UV region (ca. 270-280 nm) for phenolic arenes, benzoic acids, aniline derivatives, polyenes and polycyclic aromatic hydrocarbons with two or more rings (Chin et al., 1994; Traina et al., 1990). Normalization of absorbance to DOC (or TOC) defined as specific absorbance value (SUVA) is very useful for comparing different samples (Frimmel et al., 2002).

It was reported by Abbt-Braun et al., (2004) that a plot of the specific absorption in the visible range ($Color_{436}/DOC$) against the specific absorption in the UV range (UV_{254}/DOC) exhibit higher values for HA fractions for both the UV and visible range than that of FA fractions. This result indicates that the double bond density is related to the hydrophobic character.

Various absorption wavelengths have been cited in literature for the spectral differentiation of humic substances (Chen et al., 1977; De Haan et al., 1982; Stevenson, 1982; Buffle et al., 1982; Hayes et al., 1989; Traina et al., 1990; Wang et al., 1990; Chin et al., 1994; Peuravuori and Pihlaja, 1997; Chen et al., 2002; Abbt-Braun and Frimmel, 2002). However, they usually served as additional indexes characterizing humic materials (Choudhry, 1981). A summary of related data compiled from literature is presented in Table 2.4. Absorption spectra of humic acids in the visible region seem to have been largely ignored because of their featureless character. But this very featurelessness is induced by the brown color of HAs, which is a part of their definition. Thus it seems reasonable to look for some structural information on humic acids hidden in their apparently featureless spectra. Presence of various organic moieties in the structure of humic acids should be reflected in their absorption spectra.

Table 2.4. UV-vis characterization of aquatic humic matter (adapted from Hautala et al., 2000)

Wavelength	Correlative characteristics	References
250, 330, 350 nm	DOC, TOC	De Haan et al., 1982
285 nm	DOC	Buffle et al., 1982
254 nm	DOC, TOC, COD, BOD	Mrkva, 1983; Reynolds and Ahmad, 1997
272, 280 nm	Aromaticity, molecular weight	Traina et al., 1990; Chin et al., 1994; Li et al., 1998
250 nm/365 nm	Aromaticity, molecular weight	Peuravuori and Pihlaja, 1997
465 nm/665 nm	Humification, molecular weight, condensation of aromatic carbon	Bloom and Leenheer, 1989; Stevenson, 1982; Chen et al., 1977

In general, the data showed that UV absorption is a good indicator of the unsaturated C content of samples and that it can be used as a fast, simple and sensitive method for molecular characterization. Therefore, the absorption spectra with reference to the selective wavelengths exhibit structural information as well as DOC content.

2.2.2. Fluorescence Spectroscopy

Absorption of UV radiation by a molecule excites it from a vibrational level in the electronic ground state to one of the many vibrational levels in the electronic excited state. This excited state is usually the first excited singlet state. A molecule in a high vibrational level of the excited state will quickly fall to the lowest vibrational level of this state by losing energy to other molecules through collision. The molecule will also partition the excess energy to other possible modes of vibration and rotation. Fluorescence occurs when the molecule returns to the electronic ground state, from the excited singlet state, by emission of a photon. If a molecule which absorbs UV radiation does not fluoresce it means that it must have lost its energy some other way. These processes are called radiationless transfer of energy. A schematic presentation of the fluorescence is illustrated in Figure 2.5 below.

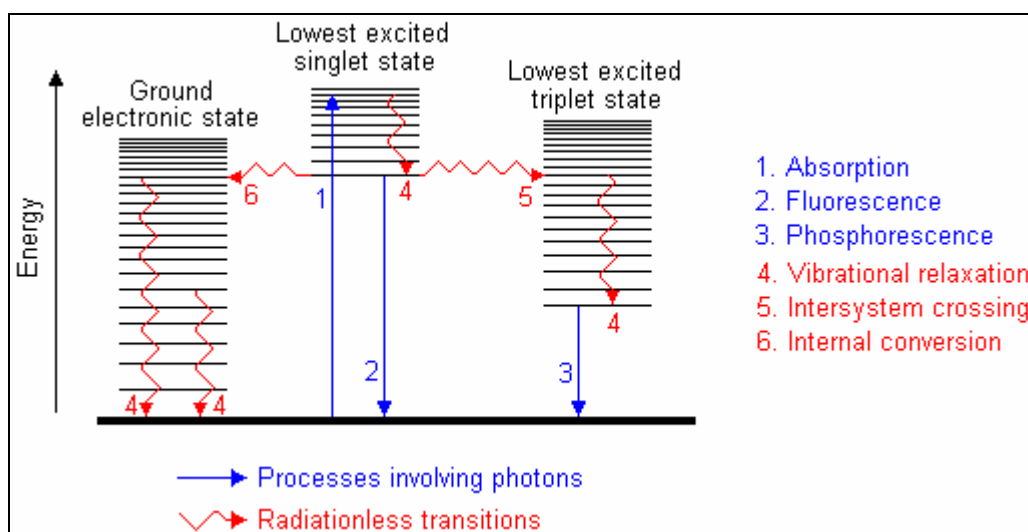


Figure 2.5. Possible physical process following absorption of a photon by a molecule (Skoog and Leary, 1992).

Fluorescence occurs when a molecule absorbs light photons from the UV-visible light spectrum, known as excitation, and then rapidly emits light photons as it returns to its ground state. Fluorescence characterizes the relationship between absorbed and emitted photons at specified wavelengths. It is a precise quantitative analytical technique which is inexpensive and easily mastered.

Absorption (excitation) can result in a range of transitions to various vibrational sublevels of excited singlet states. This is the reason that molecular absorption spectra are often seen to consist of broad peaks. Excitation is then followed by nonradiative relaxation to the lowest sublevel of the S_1 state, via vibrational relaxation and internal conversion (Figure 8). Internal conversion, singlet–triplet intersystem crossing and fluorescence then compete for relaxation to the ground state (S_0). The wavelength of the fluorescence emission is determined by the difference in energy between S_1 and S_0 states. The greater the conjugation in the molecule, the lesser the difference in energy, resulting in a longer wavelength of fluorescence.

All chemical compounds absorb energy which causes excitation of electrons bound in the molecule, such as increased vibrational energy or, under appropriate conditions, transitions between discrete electronic energy states. For a transition to occur, the absorbed energy must be equivalent to the difference between the initial electronic state and a high-energy state. This value is constant and characteristic of the molecular structure. This is termed the excitation wavelength. If conditions permit, an excited molecule will return to ground state by emission of energy through heat and/or emission of energy quanta such as photons. The emission energy or wavelength of these quanta are also equivalent to the difference between two discrete energy states and are characteristic of the molecular structure. Fluorescence occurs when a molecule absorbs photons from the UV-vis light spectrum (200-900 nm), causing transition to a high-energy electronic state and then emits photons as it returns to its initial state, in less than 10^{-9} sec. Some energy, within the molecule, is lost through heat or vibration so that emitted energy is less than the exciting energy; i.e., the emission wavelength is always longer than the excitation wavelength. The difference between the excitation and emission wavelengths is called the Stokes shift.

The sensitivity and nondestructive nature of fluorescence techniques have been emphasized in literature to be well suited for studies of natural organic matter (Senesi et al., 1991). Despite this observation, although the fluorescent structures constitute minor components of the humic macromolecule, their variety and the dependence of their properties on parameters such as molecular weight, concentration and pH render their investigation particularly useful. Therefore, humic acids could also be characterized by fluorescence spectroscopy.

The intrinsic fluorescence of humic substances contains information relating to structure, functional groups, conformation, and heterogeneity as well as dynamic properties related to their intramolecular and intermolecular interactions (Senesi et al., 1991; Mobed et al., 1996).

Fluorescence spectroscopy is generally acquired in emission, excitation and synchronous scan excitation modes. The emission spectrum is recorded by measuring the relative intensity of radiation emitted as a function of wavelength for a fixed excitation wavelength. The excitation spectrum is recorded by measuring the emission intensity at a fixed wavelength while varying the excitation wavelength. On the other hand, synchronous scan excitation spectra are obtained by measuring the fluorescence intensity while simultaneously scanning over both the excitation and emission wavelengths and keeping a constant between them as $\Delta\lambda = \lambda_{em} - \lambda_{exc}$ (Senesi, 1990).

Excitation and emission spectra have provided limited discrimination among various humic fractions, identifying only broad classes of humic and fulvic acids. Synchronous fluorescence spectroscopy has been used in attempts to resolve the broad, significantly overlapping peaks in the excitation and emission spectra.

Table 2.5 presents the effects of functional group substitution on aromatic rings on fluorescence. While UV absorbance of aromatic rings is less sensitive, both the location and intensity of fluorescence peaks are quite sensitive to functionality.

In general, electron donating functional groups (-OH, -NH₂) increase the fluorescence intensity while electron accepting functional groups (-COOH) decrease the fluorescence intensity. The presence of carbonyl containing substituents such as hydroxyl, alkoxyl and amino groups causes a shift of fluorescence to longer wavelengths (Senesi, 1990).

HAs display a red shift in the fluorescence maxima relative to FAs from similar sources. This has been attributed to the presence of high molecular weight fractions, electron withdrawing substituents and a higher degree of conjugation in the HAs. On the

other hand, the presence of electron withdrawing substituents in FA structure contributes to the relatively short wavelengths of the fluorescence maxima (Mobed et al., 1996).

Table 2.5. Substituent effects on aromatic rings (Compiled from Senesi, 1990; Miano and Senesi, 1992)

Substituent	λ_{em}	Intensity
Alkyl	None	Slight increase or decrease
OH, OCH ₃ , OC ₂ H ₅	Increase	Increase
COOH, COH, COR, COOR	Increase	Large decrease
NH ₂ , NHR, NR ₂	Increase	Increase
NO ₂ , NO	Large increase	Large decrease
SH	Increase	Decrease

Previous work, in the field of fluorescence spectroscopy for characterisation of organic matter has established that humic substances typically fluoresce in the excitation range of 300–400 nm and the emission range of 400–500 nm (Goslan et al., 2004).

2.2.3. Fourier Transform Infrared (FTIR) Spectroscopy

The technique works on the fact that bonds and groups of bonds vibrate at characteristic frequencies. A molecule that is exposed to infrared (IR) absorbs infrared energy at frequencies which are characteristic to that molecule. During FTIR analysis, a spot on the specimen is subjected to a modulated infrared beam. The specimen's transmittance and reflectance of the infrared rays at different frequencies is translated into an infrared absorption plot consisting of reverse peaks. The resulting FTIR spectral pattern is then analyzed and matched with known signatures of identified materials.

An IR spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms, no two compounds produce the same infrared spectrum.

More information on the functionality of HS can be obtained from Infrared (IR) spectra. There are studies in literature which use IR for the changes in the structural characteristics of humic substances during oxidation processes (Fukushima et al., 2001, Gül, 2002). IR can be used for the determination of functional groups. However, the characterization is mainly qualitative (Bloom and Leenheer, 1989) and, in general, only some specific bands can be clearly assigned (Table 2.6).

Table 2.6. Infrared absorption bands of humic acids (Gaffney et al., 1996)

Frequency (cm ⁻¹)	Assignment
3400	H bonded OH
3230 (sharp)	Aromatic C-H stretch
2970 (sharp)	Aliphatic C-H stretch
1740 (sharp)	COOH stretch
1585	COO ⁻¹ asymmetric stretch
1415	COO ⁻¹ symmetric stretch
1100-1045	C-C or C-OH stretch

The broad bands around 3400 cm⁻¹ are due to O-H stretching bonds of phenolic structures and alcohols and N-H stretching band of amines and amides. The absorption band at 1709 cm⁻¹ is very likely caused by the C=O stretch vibration of acids, esters, aldehydes and ketones. The second band at 1622 cm⁻¹ is typical for FA, which is due to C=C stretching vibration of unsaturated (conjugated ketones) and aromatic structures (conjugated phenyl group). The C=O stretching frequency (around 1620 nm) is characteristic in amides or quinoid structures of FA. The C-O stretching bands of polysaccharide structures appear around 1070 cm⁻¹.

2.2.4. Nuclear Magnetic Resonance (NMR) Spectroscopy

Recently, nuclear magnetic resonance spectroscopy has become one of the most important spectroscopic methods to obtain information about humic substances and their chemistry. Various NMR techniques are used to provide information about the chemical bonding modes of H, C, N and P. The NMR phenomenon is based on the fact that nuclei of

atoms have magnetic properties that can be utilized to yield chemical information. Quantum mechanically subatomic particles (protons, neutrons and electrons) have spin. In some atoms (eg ^{12}C , ^{16}O , ^{32}S) these spins are paired and cancel each other out so that the nucleus of the atom has no overall spin. However, in many atoms (^1H , ^{13}C , ^{31}P , ^{15}N , ^{19}F etc.) the nucleus does possess an overall spin.

In NMR, the sample is placed in a strong magnetic field, pulses of RF energy excite the nuclei, the 'resonance frequency' is measured as the nuclei return to their ground state (relax). Different environments around the nuclei will give different frequencies and these can be quantified. The absorption of energy during this transition forms the basis of the NMR method.

Quantitative ^{13}C NMR spectra have been employed to reveal differences in functional group content between samples (Pommes et al., 2000) whereas NMR diffusion measurements has provided information about differences in average size or the size distribution for the polydisperse humic substances (Dixon and Larive, 1997; Morris et al., 1999).

The heterogeneous nature of HS results in many overlapping signals that complicate interpretation. However, progress has been made by introducing techniques such as dipolar decoupling (DD), cross polarization magic-angle spinning (CPMAS), DEPT (distortionless enhancement by polarization transfer), INEPT (insensitive nuclei enhanced by polarization transfer) and others. In addition to ^1H and ^{13}C heteroatoms, ^{15}N and ^{31}P (Newman and Tate, 1980) were investigated. Several reviews have been published describing the application of NMR to structural investigations (Hayes et al., 1989; Hayes, 1998).

One dimensional ^1H NMR spectra of humic substances can be split into three main regions such as 0.8-3 ppm, attributable to aliphatic protons; 3-5.5 ppm representing a wide range of protons associated with oxygen containing functional groups the protons of which can be exchangeable or nonexchangeable and the 6-8.5 ppm region attributed to aromatic and amide protons (Hayes, 1998).

A representative table showing the chemical shifts of ^{13}C -NMR bands of humic acids is given below (Table 2.7).

Table 2.7. Chemical shifts of ^{13}C -NMR bands of humic acids (Abbt-Braun et al., 2004)

Chemical shift, ppm	Assignment of structural groups
0-50	Aliphatic structures; sp^3 carbons in chains and rings, methyl groups, primary aliphatic amines, some α -carbons of amino acids
50-60	Overlap region; methoxyl groups (56 ppm), tertiary and quaternary sp^3 carbons, aliphatic ethers, α -carbons of amino acids, secondary aliphatic amines
60-100	Oxygen substituted alkyl carbons: sp^3 carbons with single or double O/N-substitution
100-160	Aromatic structures:
90-110	-anomeric carbons of carbohydrates
100-140	-C substituted and unsubstituted sp^2 carbons
140-160	-O/N substituted sp^2 carbons
160-190	Carboxyl groups; acids, esters, amides
190-230	Carbonyl groups; ketones, aldehydes

^{13}C NMR has several advantages over ^1H NMR in terms of its power to elucidate organic and biochemical structures, including the ability to provide information about the backbone of molecules rather than the periphery. Also, the chemical shift for ^{13}C in most organic compounds is about 200 ppm compared with 10–15 ppm for the proton, and as a consequence, there are less overlaps of peaks. When studying humic substances, solid state ^{13}C NMR has been preferentially used over solution ^{13}C NMR. In solution ^{13}C NMR, the conformational rigidity of aliphatic carbons in poorly dissolved humic hydrophobic domains prevents the fast spinlattice relaxation of these carbons, thereby reducing spectral signals in the aliphatic range (McDonald et al., 2004).

2.3. Advanced Oxidation Processes

Light can have a dramatic effect on a molecule or solid, because, when it absorbs light, its ability to lose or gain electrons is often altered. This electronically excited state is both a better oxidizing and a better reducing agent than its counterpart in the ground. Hence, electron transfer processes involving excited-state electrons can generate highly reactive species like hydroxide ($\cdot\text{OH}$) and superoxide ($\text{O}_2^{\cdot-}$) radicals which can be used to chemically decompose a pollutant into harmless end-products (Table 2.8). Hydroxyl radical exhibits quite high oxidation potential as 2.80 V with reference to the other oxidizing agents.

Table 2.8. Oxidation potentials of common substances and agents for pollution abatement

Oxidizing agent	Oxidation potential, V
Fluorine	3.06
Hydroxide radical ($\cdot\text{OH}$)	2.80
Ozone	2.07
Hydrogen peroxide	1.77
Chlorine dioxide	1.57
Chlorine gas	1.36
Oxygen	1.23
Hypochlorite	0.94
Iodine	0.54
Superoxide radical ($\text{O}_2^{\cdot-}$)	-0.33

Alternatively, light can be used directly to break up pollutant molecule bonds photolytically. In general, oxidation processes, which rely on the generation of reactive species such as hydroxyl radicals are called Advanced Oxidation Processes (AOPs). Many oxidation practices, such as TiO_2/UV , $\text{H}_2\text{O}_2/\text{UV}$, Photo-Fenton and ozone processes (O_3 , O_3/UV , $\text{O}_3/\text{H}_2\text{O}_2$) are currently employed for this purpose. All AOPs have in common, that the formation of hydroxyl radicals is the rate-limiting step.

2.3.1. Photocatalytic Oxidation (TiO₂/UV Process)

Among the catalytic approaches to environmental remediation, that of heterogeneous photoassisted catalysis, known as photocatalysis is the sole example involving utilization of light along with a catalytic solid. It is well known that as the photons with energies greater than the band gap are adsorbed by semiconductors such as ZnO, CdS, and TiO₂, electron hole pairs are generated and then transferred to the surface where they are trapped and react with surface adsorbed molecules.

Heterogeneous photocatalysis has come to describe the field of study and the technology in which irradiated semiconductor particles generate charge carriers (e⁻ and h⁺) that are ultimately poised at the particle surface. These carriers undergo various processes, the most important of which are photoreductions (e.g. metal ions of Au, Pt, Ag, Rh, Hg, Pb, and others) and photooxidations of a large variety of organic substances to their complete mineralization.

The process has several advantages when compared to biological and traditional chemical oxidation processes. First, the photocatalytic reaction is not specific and therefore capable of destroying a spectrum of organic chemicals like hydrocarbon fuels, surfactants, pesticides. Second, the process is very powerful often achieving a complete mineralization of organics. Third, the process is immune to organic toxicity, which makes the photocatalytic process particularly attractive for the degradation of recalcitrant and toxic xenobiotic compounds. Fourth, the process can be applied equally well to liquid (e.g. wastewater, groundwater) and gaseous streams (e.g. VOC emission). Finally there is a potential to utilize sunlight instead of an artificial light as a UV source thereby reducing the energy cost for the process.

The semiconductor photocatalytic process is based on aqueous phase hydroxyl radical chemistry and couples low energy UV-A light with semiconductors acting as photocatalyst. The overall process has been proved to destroy many organic compounds completely to carbon dioxide. The overall process can be summarized as follows:



TiO₂ based photocatalysis has been the focus of numerous investigations in recent years, particularly owing to its application for the complete mineralization of undesirable organic contaminants to CO₂, H₂O and inorganic constituents (Ollis et al., 1991).

TiO₂ in particular has beneficial characteristics, such as its chemical and physical stability, as well as the strong oxidizing power of the photogenerated holes, with which most organic compounds can be oxidized to carbon dioxide at ambient temperature and pressure.

Degussa P-25 has become a research standard as a TiO₂ source because it has reasonably well defined nature (nonporous, BET surface area= 55±15 m² g⁻¹, a 70:30 anatase:rutile mixture, average particle size 30 nm) and a substantially higher photocatalytic activity than most other readily available samples of TiO₂.

The complex primary events affecting band-gap irradiation of TiO₂ particles have been studied with detailed laser flash photolysis measurements (Bahnemann et al., 1997). The relevant reactions at the TiO₂ surface which yield •OH radicals and cause the degradation of the organic compounds could be outlined by equations (2)-(9).

Charge separation



Bulk recombination



Surface trapping



Surface recombination



Interfacial charge transfer



The fundamental parameters such as the influence of oxygen, pH, TiO_2 concentration, TiO_2 type and light intensity on the photocatalytic oxidation have been studied in detail (Bahnemann et al., 1997). Our recent research efforts in this area have focused on assessing the factors that underlie the parameters related to the photocatalytic removal of humic substances (Bekbölet et al., 1998; Bekbölet et al., 2002; Uyguner and Bekbölet, 2004a).

2.3.2. Humic Acid Treatment by Advanced Oxidation Processes

In the past few years, several methods for the removal of natural organic matter from natural source waters have been intensively researched, including enhanced coagulation, activated carbon adsorption and membrane separation, (Crozes et al., 1995; Schafer et al., 1998; Graham, 1999). However, due to the refractory character of humic substances or the formation of by-products that are not further oxidized, a more through oxidation named advanced oxidation processes have been the alternative method to be investigated for the complete mineralization of natural organic matter. All advanced oxidation processes are characterized by a common chemical feature: the capability of exploiting the high reactivity of OH radicals in driving oxidation processes which are suitable for achieving the complete abatement and mineralization of even less reactive pollutants. The OH radicals are reactive species characterized by their nonselectivity to attack the most of the organic molecules with rate constants in the order of 10^6 to $10^9 \text{ M}^{-1} \text{ s}^{-1}$. The advanced oxidation processes offer various ways for the production of OH radicals, hence allow a better compliance with the specific treatment requirements.

Starting with Arai et al. (1986) oxidative removal of humic substances in natural waters with the application of advanced oxidation methods such as UV, UV and H_2O_2 , O_3 , radiation and O_3 has been a topic of various researches (Gilbert, 1988; Backlund, 1992; Kulovaara, 1996; Kulovaara et al., 1996; Wang et al., 2000).

Backlund (1992) studied the degradation of aquatic humic material by UV light in the presence of H_2O_2 and reported that the formation of the same acidic degradation products which accounted for 80 % of the dissolved organic matter was enhanced. UV_{254} and DOC value were found to be decreasing after 30 minutes of irradiation.

To address the aquatic carbon cycling with the contribution of humic substances to the organic photochemistry of lakes and rivers; De Haan (1993) studied the in situ photodegradation of incubated humic lake water samples in natural sunlight.

Later on, Dahlen et al. (1996) revised the UV-induced degradation of organic matter focusing on the production of low molecular weight organic acids such as oxalic, malonic, formic and acetic acids. Capillary zone electrophoresis (CZE) method was used for the identification and quantification of these organic acids.

Arai et al. (1986) investigated the combined effect of radiation and ozone on degradation of humic acid in water. Humic acid solution (5 mg L^{-1}) was irradiated with C_0 γ -rays at room temperature in the presence of ozone at different concentrations. The total organic carbon (TOC) reduction rate was found to increase both with the ozone concentration and the dose rate.

The ozonation of humic substances was reported to lead to a quick decolorization (Gilbert, 1988) and a decrease in UV-absorbance with the evidence of the formation of low molecular weight compounds (Anderson et al., 1986; Backlund, 1992).

With the employment of combined systems such as $\text{O}_3 + \text{H}_2\text{O}_2$, $\text{UV} + \text{H}_2\text{O}_2$, $\text{UV} + \text{O}_3$, etc. more effective results were achieved (Camel and Bermond, 1998; Wang et al., 2000; Kosaka et al., 2001; Liao et al., 2001).

2.3.3. Photocatalytic Oxidation of Humic Substances

Recently, the photocatalytic degradation of humic acids using TiO_2/UV process has been extensively studied by Bekbölet and coworkers (Bekbölet 1996; Bekbölet and Balcioğlu, 1996; Bekbölet and Özkösem, 1996; Bekbölet et al., 1998; Bekbölet et al.,

2002; Uyguner and Bekbölet, 2004a). Spectrophotometric examination of the data revealed almost 50 % decrease in terms of Color₄₃₆ (which correlates to color) and UV₂₅₄ (which correlates to total organic carbon, TOC) parameters.

Eggins et al. (1997) have investigated the semiconductor photocatalysis for the treatment of humic substances in drinking water by measuring color in Hazen units, 254 nm/400 nm absorbance ratio, fluorescence and size exclusion high performance chromatography.

Later on, with the study of Cloethe et al. (1999) covering measurements of TOC parameters and THM (trihalomethane) formation potentials, the removal of humic substances from the water samples via photocatalytic oxidation has been confirmed.

The research efforts in this area are mainly based on assessing the factors that describe the kinetics and mechanistic pathways of photocatalytic removal of humic acids. The inhibition and competition effects on the TiO₂/UV system in the presence of humic acid, different TiO₂ brands, various metal ions (Zn, Cu, Cr, Mn, Ca and Mg), some common anions (chloride, nitrate, sulfate and phosphate), oxyanions (hypochlorite, chlorite, chlorate) and oxidizing species (hydrogen peroxide and bicarbonate) have also been studied to understand the limitations of the photocatalytic system (Bekbölet and Balcıoğlu, 1996; Gönenç and Bekbölet, 2001; Li et al., 2002; Bekbölet et al., 2002; Wiszniowski et al., 2003; Uyguner and Bekbölet, 2004a). Recently, the degradation kinetics of a sequential oxidation system that is the combination of preozonation followed by photocatalysis has also been studied (Kerç et al., 2003). Moreover, the properties of photocatalytically oxidized humic acids were investigated focusing on their spectroscopic characterization by UV-vis and fluorescence spectra (Uyguner and Bekbölet, 2005b; Uyguner and Bekbölet, 2005c).

A few other papers have been published that cover the photocatalytic decomposition of humic acids on TiO₂ (Minero et al., 1999; Wiszniowski et al., 2002; Palmer et al., 2002). Furthermore, photocatalytic degradation of humic acid in artificial sea water was examined in detail (Al-Rasheed and Cardin, 2003a; Al-Rasheed and Cardin, 2003b).

In addition to UV/TiO₂ mediated degradation of humic substances, the sensitized degradation of humic acids on TiO₂ under visible light irradiation has also been investigated (Cho and Choi, 2002; Epling and Lin, 2002).

However, up to date, the photocatalytic oxidation mechanism of humic acids has not been fully understood. That is a result of the lack of knowledge about the structural components of humic substances. Although certain gross structural characteristics are known, there is still lack of structural knowledge relative to changes in the molecular and structural characteristics of humic acid during these processes.

3. MATERIALS AND METHODS

3.1. Materials

All the chemicals used in this study are analytical grade reagents.

3.1.1. Humic Substances

Humic substances of different origin (terrestrial and aquatic) were used in bench scale experiments. The Suwannee River Fulvic Acid (IHSS FA) and Suwannee River Humic Acid (IHSS HA) standard materials, isolated from the Suwannee River, Georgia, as well as soil humic acid (IHSS SHA) (Lot No: 1S102H) as a terrestrial source and Nordic Humic Acid (NHA) were purchased from the International Humic Substance Society. Commercial humic acid samples were supplied from Aldrich (humic acid sodium salt) (AHA) and Roth (humic acid) (RHA). Brand names were used throughout the text for the purpose of differentiating between humic substances. There is no commercial meaning other than maintaining relevancy to the literature.

A stock solution of 1000 mg L^{-1} was prepared by dissolving the humic or fulvic acid in distilled deionized water and filtering through filter paper except for RHA which was prepared according to Urano et al., (1983). Stock solutions of the humic substances were stored in amber glass bottles and were protected from light to prevent decomposition. Appropriate dilutions of humic substances were made weekly from the stock solution using distilled deionized water that has conductivity less than $10 \mu \text{ S cm}^{-1}$. The pH of the humic substances were adjusted to a pH of 6.0 ± 0.5 by adding standard HCl (0.1 N) or NaOH (0.1 N).

3.1.2. Titanium Dioxide

Commercial Degussa P-25 TiO_2 powder was used as the photocatalyst for the degradation of humic substances. The phase composition of P-25 is 70 % anatase and 30 % rutile. The BET surface area is $50 \pm 15 \text{ m}^2 \text{ g}^{-1}$ and the average particle size is 30 nm.

3.2. Methods

3.2.1. Experimental Set-Up

A cylindrical pyrex reaction vessel with a diameter of 7.5 cm, a height of 3.5 cm was used as the photoreactor. A 125 W Black Light Fluorescent Lamp (BLF) with an output spectrum of 320-440 nm was used as the light source. The lamp exhibited a maximum emission at 365 nm and no emission below 300 nm and above 500 nm. The intensity of incident light as measured by potassium ferrioxalate actinometer (Hatchard and Parker, 1956) was 2.85×10^{16} quanta sec^{-1} . In all of the experiments, the distance between the lamp and the surface of the suspension in the reactor was kept constant at 16.5 cm (Bekbölet, 1996). The photoreactor was enclosed by a mirror casing to focus the light in the center of the photoreactor. The whole system was placed in a box the inner walls of which were covered with Al-foil. Volume of the reaction mixture was 50 mL. Continuous stirring of the suspension was provided by means of a Framo-Geraetechnik M21/1 magnetic stirrer throughout the duration of the photocatalytic oxidation experiments

3.2.2. Experimental Procedure

3.2.2.1. Photocatalytic Oxidation. An appropriate concentration of humic acid or fulvic acid solution was prepared by dilution from the stock solution. TiO_2 loadings used in the experiments were 0.10, 0.25 and 1.00 mg mL^{-1} . 50 mL of humic or fulvic acid solution was added directly to the reaction vessel containing the required amount of TiO_2 . The slurry was sonicated for one minute in Ultrasonic LC30 water bath to ensure a homogeneous mixture and then placed on a magnetic stirrer to be irradiated for certain reaction periods ranging from the start of the experiment to 120 minutes. The procedure was repeated with a new sample for each specified irradiation time for all of the runs. Since the photocatalytic oxidations were conducted in a glass reactor open to the atmosphere, the volume loss due to the evaporation of water at longer irradiation periods such as 60 minutes was unavoidable. Therefore, after each experiment, volume corrections were made with distilled deionized water to compensate for the evaporation loss. All of the glassware used were calibrated.

For the removal of TiO_2 , centrifugation was applied for 10 minutes at a rate of 5000 rpm using a Hettich EBA 8S centrifuge. It was followed by filtration through a $0.45\ \mu\text{m}$ Millipore Millex-HA cellulose based membrane filter that is attached to a 10 mL syringe. Time dependent changes of filtered samples after photocatalysis were characterized by UV-vis and fluorescence spectroscopy.

3.2.2.2. Molecular Size Fractionation via Ultrafiltration. Molecular size fractionation of the raw and photocatalytically treated humic substances (50 % degradation with respect to Color_{436}) was performed using a 10 mL Amicon Model 8010 ultrafiltration stirred cell. A schematic diagram of the stirred cell system is presented in Figure 3.1.

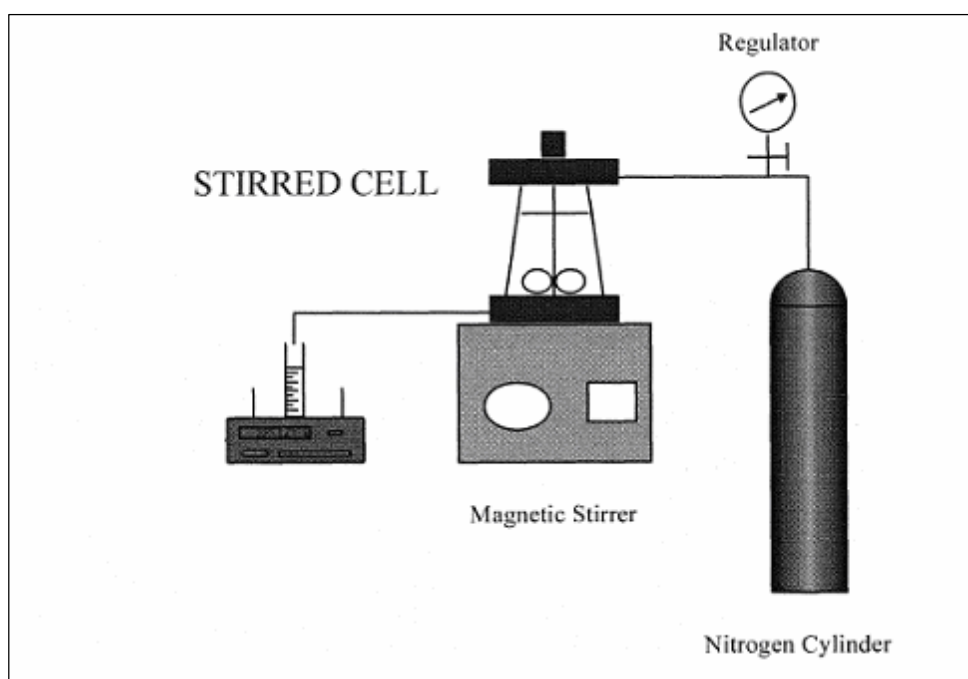


Figure 3.1. Schematic diagram of stirred cell system.

Initially, the samples were filtered through $0.45\ \mu\text{m}$ (approximately 450 kDa) Millipore cellulose acetate membrane filters. Later on, filtration was repeated in a series mode using cellulose membranes with nominal molecular weight cutoffs ranging from 1 kDa to 100 kDa with a sequence of decreasing pore size. Millipore YM series cellulose membrane filters with 25 mm diameter were used within the stirred cell. The specifications of the membranes as stated by the manufacturer are tabulated in Table 3.1.

The cell was operated on a magnetic stirrer. A nitrogen gas tube equipped with a pressure control valve was attached to the stirred reactor to maintain the required pressure inside the cell. At the beginning of each run the membranes were rinsed with deionized water. After use prior to storage YM membranes were washed with 0.1 M NaOH solution and stored in 10 % ethanol/water solution according to manufacturer's recommendations.

After each batch of ultrafiltration, the fractions of humic acid samples were characterized by UV-vis and fluorescence spectra as specified previously.

Ultrafiltration yields fractions containing a mixture of molecules with a variety of sizes that are larger and smaller than the particular membrane pore size. Therefore, fractionation can be seen as a gradual process where basically the composition of the original sample is changed such that from high to low nominal molecular weight cut off (NMWCO) of the membranes, the sample is consistently being depleted in large molecules (larger than the actual NMWCO of the actual membrane).

Table 3.1. Specifications of the cellulose membranes used in ultrafiltration

Membrane type	Nominal molecular weight cutoff (kDa)	Operating pressure (kg cm ⁻²)
YM 100	100	1.0
YM 30	30	3.5
YM 10	10	3.5
YM 3	3	3.5
YM 1	1	3.5

Calibration of fractionation methods with suitable standards has been suggested by Müller and Frimmel, (2002). Hence, it becomes possible to minimize non specific interactions. Samples are subsequently fractionated according to the experimentally defined standardized fractions rather than arbitrarily choosing fraction limits. Fractions are less prone to artifact formation and therefore are a better basis for subsequent analysis.

Due to their similarity to humic substances with respect to chemical and hydrodynamic properties, polystyrene sulfonate (PSS) standards have been used in literature as substitute standards for NOM components (Müller and Frimmel, 2002). For the calibration of the ultrafiltration membranes, PSS standards of 30 kDa and 10 kDa at a concentration of 5 mg L⁻¹ each (as TOC) were dissolved in demineralized water. The electrical conductivity of the solution was adjusted to 1 mS cm⁻¹ with NaCl. 20 mL of PSS was filtered through the membrane, and PSS solutions in the original solution, the concentrate and the permeate were determined by UV absorbance at 224 nm. In order to check the validity of the ultrafiltration system, a mass balance across the membrane based on UV data to determine PSS rejection was calculated to be < 5 %.

3.2.3. Analytical Methods

3.2.3.1. Total Organic Carbon (TOC) Analysis. Total organic carbon (TOC, mg L⁻¹) measurements of humic substances were performed on a Shimadzu TOC-V CSH Total Organic Carbon Analyzer. Calibration of the instrument was done using potassium hydrogen phthalate in the concentration range of 5-25 mg L⁻¹.

3.2.3.2. UV-vis Measurements. UV-vis absorption spectra were recorded on a Shimadzu UV-160A double beam spectrophotometer employing Hellma quartz cuvettes of 1.0 cm optical path length. Humic and fulvic acids were characterized by UV-vis spectroscopy. Absorbance values at 436 nm (Color₄₃₆), 400 nm (Color₄₀₀), 365 nm (UV₃₆₅), 300 nm (UV₃₀₀), 280 nm (UV₂₈₀), 254 nm (UV₂₅₄) and 250 nm (UV₂₅₀) were recorded for the evaluation of UV-vis parameters as absorbance ratios such as E₂₅₀/E₃₆₅ (UV₂₅₀/UV₃₆₅), E₂₅₄/E₃₆₅ (UV₂₅₄/UV₃₆₅), E₂₈₀/E₃₆₅ (UV₂₈₀/UV₃₆₅), E₃₀₀/E₄₀₀ (UV₃₀₀/Color₄₃₆), E₂₅₀/E₄₃₆ (UV₂₅₀/Color₄₃₆), E₂₅₄/E₄₃₆ (UV₂₅₄/Color₄₃₆), E₂₈₀/E₄₃₆ (UV₂₈₀/Color₄₃₆), and E₃₀₀/E₄₃₆ (UV₃₀₀/UV₃₆₅). Specific UV absorbance (SUVA₂₅₄, cm⁻¹ mg⁻¹ L) was used to represent TOC normalized aromatic moieties (UV₂₅₄) whereas specific color absorbance (SCOA₄₃₆, cm⁻¹ mg⁻¹ L) was defined as Color₄₃₆/TOC to signify organic carbon normalized color forming moieties. SUVA₃₆₅ was also calculated in a similar fashion as the ratio of the UV₃₆₅ absorbing species to TOC.

3.2.3.3. Fluorescence Measurements. Fluorescence spectra in the emission and synchronous scan modes were recorded on a Perkin Elmer LS 55 Luminescence Spectrometer equipped with a 150W Xenon arc lamp and a red sensitive photomultiplier tube. A 1-cm pathlength quartz cell was used. The method of measuring fluorescence is that the cuvette holder excites the sample over the entire path length and reads the emitted light at right angles. Both the excitation and the emission slits of the instrument were 10 nm. A scan speed of 400 nm min⁻¹ was used with a slit width opening of 10 nm. Opening the slit wider allows more light energy from the xenon light source to excite the molecules in the sample. A larger slit width applies more excitation light energy to a sample, and a less-well defined spectral purity of the excitation and emission bands (e.g., broader emission bands rather than sharper bands) results (Westerhoff et al., 1999). Synchronous scan spectra were recorded in the excitation wavelength range of 300-650 nm excitation wavelength range using the bandwidth of $\Delta\lambda=18$ nm between the excitation and emission monochromators (Senesi, 1990). The scan speed was 400 nm min⁻¹. The emission spectra were scanned over the range of 400-600 nm at a constant excitation wavelength of 350 nm (Senesi, 1990; Hautala et al., 2000). Since all of the spectra were recorded on the same instrument using the same experimental parameters, a comparative discussion of the spectra is acceptable although, no corrections for fluctuation of instrumental factors and for scattering effects (e.g. primary and secondary inner filter effects) were applied to the data (Senesi, 1990; Peuravuori et al., 2002).

Quinone sulfate (QS) solution (1 $\mu\text{g L}^{-1}$ QS in 0.1 N H₂SO₄) was used during the study as a standard solution to monitor the relative energy emitted by the xenon lamp in the fluorimeter (Seitz, 1981; Scully and Lean, 1994). During this study no change in quinone sulfate fluorescence was observed, and consequently the standard was used as a sensitivity check rather than as an absolute calibration tool.

3.2.3.4. Fourier Transform Infrared (FTIR) Analysis. FTIR analysis was performed at TUBITAK Marmara Research Center (Material and Chemical Technologies Research Institute) using a Perkin Elmer Spectrum One FTIR after mixing 0.5 mg of the freeze dried (lyophilized) humic substance with 150 mg KBr. The lyophilization process was also performed at TUBITAK (Food Science and Technology Research Center) by freezing the humic samples at -70 °C and drying them under vacuum. The FTIR spectra were scanned

over the frequency range from 4000 cm^{-1} to 400 cm^{-1} at a resolution of 2 cm^{-1} . The sample preparation for the FTIR analysis of oxidized humic substances is a long procedure. Humic substances must first be photocatalytically oxidized according to the experimental procedure outlined in section 3.2.2.1 and then lyophilized. However, to recover the appropriate amount of sample from the lyophilization process, the experimental procedure has to be repeated many times. Due to the problems encountered during sample preparation such as the requirement for high sample volume and lyophilization problems, the FTIR measurements could only be performed on raw and selected samples of oxidized Aldrich humic acid and IHSS fulvic acid.

3.2.3.5. Nuclear Magnetic Resonance (NMR) Analysis. The solution ^1H NMR and proton decoupled ^{13}C NMR spectra of raw and oxidized humic acid samples were recorded on a Varian Mercury-VX 400 MHz BB spectrometer after lyophilizing the samples and dissolving them in D_2O . For ^{13}C NMR the spectral width was set to 25 kHz, typically 60 000 FIDs were collected. For ^1H NMR the spectral width was set to 6 kHz and 25 000 FIDs were collected. The experiments were performed in Boğaziçi University Advanced Technologies Research and Development Center. NMR analysis of humic substances is a costly and long time consuming procedure. Acquiring the spectra for humic substances requires more than a week. Moreover, the samples have to be lyophilized prior to analysis. Additionally, there are problems encountered during sample preparation such as the need for high concentration of samples which necessitates repeating the experimental procedure many times. Therefore, the NMR spectra were acquired only for raw and selected samples of the photocatalytically oxidized Aldrich humic acid. The ^1H and ^{13}C NMR spectra were also tested for Roth humic acid however due to solubility problems in D_2O , the analysis could not be performed.

3.2.3.6. Actinometry. Potassium ferrioxalate actinometer that was developed by Hatchard and Parker (1956) was used for the determination of light intensity of the lamp of the photocatalytic reactor. The measurements were followed according to a procedure outlined by Mayo and Shizuka (1976).

3.2.3.7. Spectrophotometric Determination of Phenol. The concentration of phenol in the photocatalytic oxidation experiments was determined by the direct photometric method according to Standard Methods (1999).

3.2.3.8. Other Measurements. The concentrations of the inorganic species formed during photocatalytic degradation of humic substances were measured by Dionex LC20 Ion Chromatography coupled with Dionex CD25 Conductivity Detector.

The photocatalytic mineralization of humic substances was checked by conductivity measurements of the humic substances using a WTW LF 320 conductivity meter (Standard Methods, 1999).

4. RESULTS AND DISCUSSION

In this study, model compounds of terrestrial and aquatic origin, namely, Aldrich humic acid, Roth humic acid, Nordic humic acid, IHSS fulvic acid, IHSS humic acid, and IHSS soil humic acid were used to represent natural organic matter in drinking water supplies. Aldrich humic acid is well known and widely used in literature. On the other hand, Roth humic acid has been extensively studied by Bekbölet and coworkers. Therefore, those humic acids were introduced as the first two in all of the tables and figures. Subsequently, other humic substances obtained from IHSS were presented irrespective of their origins.

In the first part, the studied humic substances were characterized with respect to their UV-vis and fluorescence spectroscopic properties. Considering the complexity of the humic macromolecules, they were fractionated into well defined subcomponents of defined molecular sizes using ultrafiltration through membranes in the range of 100-1 kDa. Moreover, the spectroscopic properties of each size fraction were characterized and compared by UV-vis spectroscopy and fluorescence spectroscopy in emission and synchronous scan modes.

In the second part, photocatalytic oxidation of model humic and fulvic acids was carried out using TiO₂ Degussa P-25 as the photocatalyst. The related data were presented in terms of the UV-vis properties of humic substances and the degradation kinetics were comparatively assessed based on pseudo first order and Langmuir Hinshelwood kinetic models. Furthermore, the molecular and structural characteristics of the humic acid molecule relative to changes during photocatalytic oxidation were monitored by fluorescence spectroscopy (emission and synchronous scan), FTIR and NMR (¹H NMR, and ¹³C NMR).

In the last section, the molecular size distribution profiles of the partially oxidized humic substances were comparatively presented by both the specified UV-vis properties and synchronous scan fluorescence spectra.

4.1. Spectroscopic Analysis of Humic Substances

In this section, the studied humic substances namely, Aldrich humic acid, Roth humic acid, IHSS soil humic acid, Nordic humic acid, IHSS humic acid and IHSS fulvic acid and their molecular size fractions in the range of 0.45 μ m to 1 kDa were characterized by UV-vis spectroscopy and fluorescence spectroscopy in emission and synchronous scan modes.

4.1.1. UV-vis Spectroscopic Properties of Humic Substances

UV-vis absorption is commonly accepted as conventional and versatile for characterization of dissolved organic matter in natural waters. Absorption of light in the UV range by humic substances is caused by π -electrons and reflects aromatic and carboxylic electron systems as well as their conjugates. On the other hand, functional groups with quinoide structures and keto-enol-systems are more responsible for the absorption in the visible range (Frimmel et al., 2002).

The UV-vis spectra of humic substances are commonly broad; they do not exhibit any obvious features and monotonously decrease with increasing wavelength (Schnitzer and Khan, 1972; Traina et al., 1990; Chen et al., 2002; Uyguner and Bekbölet, 2005a). Accordingly, the UV-vis spectra of the studied humic substances show a gradually decreasing absorptivity (Figure 4.1).

IHSS fulvic acid has lower absorbance values compared to those of other humic acids indicating very low double bond character. On the other hand, Roth humic acid has the highest absorbance values in the range of 240-440 nm region. The spectra of IHSS fulvic acid exhibit about a two fold lower UV absorbance than that of Roth humic acid with a relative order of absorptivities at 254 nm as RHA (0.745) > IHSS HA (0.592) > NHA (0.565) > IHSS SHA (0.460) > AHA (0.432) > IHSS FA (0.364). This suggests that RHA has a relatively high amount of aromatic or polyphenolic organic compounds (Chen et al., 2002). In agreement with literature results, soil derived humic acids have higher absorbance values in the UV-vis region than the aquatic humic acids (Frimmel et al., 2002).

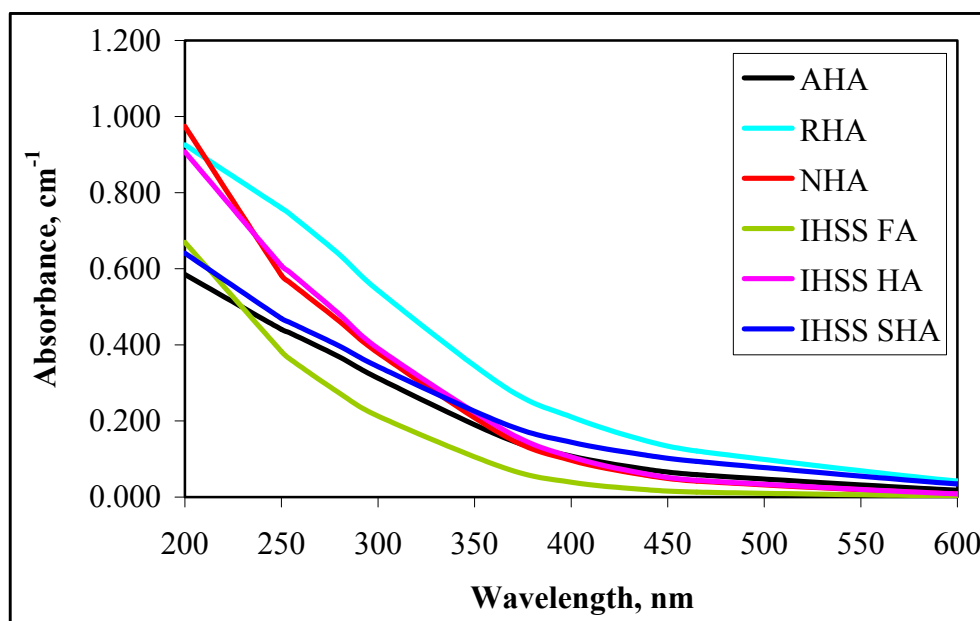


Figure 4.1. UV-vis spectra of humic substances (20 mg L⁻¹).

For the basic characterization and differentiation of humic substances, TOC values as well as the specified absorbance values were presented in Table 4.1.

Table 4.1. Basic spectroscopic characterization of humic substances (20 mg L⁻¹)

	Color₄₃₆ (m⁻¹)	Color₄₀₀ (m⁻¹)	UV₃₆₅ (m⁻¹)	UV₃₀₀ (m⁻¹)	UV₂₈₀ (m⁻¹)	UV₂₅₄ (m⁻¹)	TOC (mg L⁻¹)
AHA	7.5	10.8	15.7	31.2	36.8	43.2	6.21
RHA	15.2	21.1	29.2	54.3	63.8	74.5	7.81
NHA	5.9	9.7	16.2	37.9	46.2	56.5	9.23
IHSS FA	2.0	3.9	7.8	21.3	27.4	36.4	8.34
IHSS HA	6.4	10.6	17.7	39.1	48.1	59.2	7.56
IHSS SHA	11.2	14.4	19.4	34.3	39.7	46.0	6.46

Depending on the concentration of the working solutions, UV-vis and TOC parameters of the humic substances display certain values. Humic acids of terrestrial origin i.e. AHA, RHA and IHSS SHA exhibited Color₄₃₆ values in the range of 7.5–15.2, whereas humic acids of aquatic origin i.e. IHSS HA and NHA had a relatively lower content of color forming moieties. Obviously, IHSS FA displayed quite low color characteristics.

The UV_{365} parameter is found to be irrespective of the source of the samples as expressed in a decreasing order of $RHA > IHSS\ SHA > IHSS\ HA > NHA > AHA > IHSS\ FA$. Moreover, RHA has remarkably high UV_{280} and UV_{254} values with respect to the other samples emphasizing strong aromatic character. The order of UV_{300} , UV_{280} and UV_{254} absorbing moieties of the samples follows a decreasing trend as; $RHA > IHSS\ HA > NHA > IHSS\ SHA > AHA > IHSS\ FA$, reflecting non-source dependency. The TOC contents of the humic substances are ordered as follows; $NHA > IHSS\ FA > RHA > IHSS\ HA > IHSS\ SHA > AHA$. In order to compare the absorptivities of different humic substances, UV-vis spectra were normalized to the total organic carbon contents and presented as specific absorbance values (Table 4.2).

Table 4.2. Specific absorbance values of humic substances

	SUVA₂₅₄ m⁻¹ mg⁻¹ L	SUVA₃₆₅ m⁻¹ mg⁻¹ L	SCOA₄₃₆ m⁻¹ mg⁻¹ L
AHA	7.0	2.5	1.2
RHA	9.5	3.7	1.9
NHA	6.1	1.8	0.64
IHSS FA	4.4	0.94	0.24
IHSS HA	7.8	2.3	0.85
IHSS SHA	7.1	3.0	1.7

The values calculated at certain wavelengths point out the significant difference between humic substances (Table 4.2). The specific absorbance values at 254 nm, 365 nm and 436 nm are the highest for RHA sample whereas the lowest values were attained for the IHSS FA. $SUVA_{254}$ can be used to describe the composition of water in terms of hydrophobicity and hydrophilicity. Studied humic acids have higher $SUVA_{254}$ values than the fulvic acid indicating the presence of a higher amount of hydrophobic fraction (Edzwald et al., 1985).

In this study, the applicability of surrogate parameters such as E_{250}/E_{365} , E_{254}/E_{436} , E_{365}/E_{465} and E_{465}/E_{665} , which are widely used in the literature for NOM characterization (Buffle et al., 1982; De Haan et al., 1982; Traina et al., 1990; Chin et al., 1994; Peuravuori

and Pihlaja, 1997; Chen et al., 2002) were investigated in relation to aquatic and terrestrial model humic substances (Uyguner and Bekbölet, 2005c). The data of the absorbance ratios of the studied humic substances are presented in Table 4.3.

Table 4.3. Absorbance ratios of humic substances

	Absorbance ratios of humic substances						
	E_{250}/E_{365}	E_{254}/E_{365}	E_{280}/E_{365}	E_{254}/E_{436}	E_{300}/E_{400}	E_{300}/E_{436}	E_{465}/E_{665}
AHA	2.8	2.7	2.3	5.7	2.9	4.2	6.1
RHA	2.6	2.6	2.2	5.0	2.6	3.6	5.1
NHA	3.6	3.5	2.8	9.5	3.9	6.4	11.7
IHSS FA	5.0	4.8	3.6	17.6	5.2	10	12.7
IHSS HA	3.4	3.3	2.8	9.3	3.6	5.9	9.8
IHSS SHA	2.4	2.4	2.0	4.1	2.4	3.0	4.5

The spectroscopic ratios calculated for the different types of humic substances show differences depending on their source. As a general trend it could be observed from the table that terrestrial humic acids i.e., AHA, RHA and IHSS SHA exhibit the lowest absorbance ratios compared to those of aquatic humic substances i.e., IHSS HA, IHSS FA and NHA. Amongst the humic substances, IHSS SHA represents the lowest absorbance ratios for all of the studied parameters. In all cases, IHSS FA has the highest values. It was reported by several researchers that the quotient E_{250}/E_{365} , which is a property of aquatic humic substances, increases as the aromaticity and molecular size decreases (Peuravuori and Pihlaja, 1997). The higher E_{250}/E_{365} ratio of IHSS FA is in agreement with its lower aromatic content and lower molecular size as opposed to other humic substances studied.

The ratio of E_{254}/E_{436} gives a good impression about the intensity of the UV absorbing functional groups compared to the colored ones. The highest ratio was achieved for IHSS FA whereas the lowest was attained for IHSS SHA. E_{254}/E_{436} values in the range of 4-11 were reported for terrestrial dissolved organic matter which has a greater organic matter content associated with the presence of tannin like or humic like substances derived from plants and soil organic matter. Except for IHSS FA the results presented in Table 4.3 are in accordance with literature (Battin, 1998).

On the other hand, the slope of the absorption curves as measured by the absorbance ratio E_{465}/E_{665} is commonly used to indicate the degree of condensation of the aromatic carbon network and suggested to be inversely related to the degree of aromaticity, particle size and molecular weight (Chen et al., 1977). The ratio was reported to be dependent on the concentration of humic substances especially below a UV_{254} value of 0.4 and 5 $mg\ L^{-1}$ of TOC still being a characteristic of substances obtained from different sources (Summers et al., 1987). The absorbance ratios in Table 4.3 were calculated for humic substances with relative TOC values in the range of 15-21 $mg\ L^{-1}$. Hence, E_{465}/E_{665} concentration dependence was small enough that the rank should not change. For the samples examined, the highest E_{465}/E_{665} ratio was obtained for IHSS FA, while the lowest was measured for the IHSS SHA as 4.5. AHA has a ratio of 6.1. These results are similar to the findings of others where the ratios for IHSS SHA and AHA were reported as 4.3 and 6.3, respectively (Chen et al., 2002; Summers et al., 1987). In accordance with literature, E_4/E_6 ratios measured for aquatic humic substances were higher than those of their terrestrial counterparts. A low E_{465}/E_{665} ratio may be largely attributed to the absorption by aromatic C=C functional groups (Senesi et al., 1989). Moreover, the high degree of condensation as well as the large molecular weight of IHSS SHA is thought to contribute to its relatively high absorption in the visible range (Schnitzer and Khan, 1972).

4.1.2. UV-vis Spectroscopic Properties of the Molecular Size Fractions of Humic Substances

Aiming at focusing on a comparative basis, the molecular size, or parameters indirectly linked to molecular size, of five different types of (aquatic and terrestrial source) humic substances namely Aldrich humic acid, Roth humic acid, IHSS soil humic acid, IHSS humic acid and IHSS fulvic acid were measured by UV-vis spectroscopy after molecular size fractionation. The fast removal of both of the UV-absorbing moieties and color forming groups during the ultrafiltration process is noticeable. In order to utilize the spectroscopic properties of the lower size fractions, the concentration of humic substance was increased to 50 $mg\ L^{-1}$.

As previously reported, the UV-vis spectra of the molecular size fractions of humic substances exhibit a similar declining pattern, the absorbance values of which also

gradually decrease with the molecular size fractions (Uyguner and Bekbölet, 2005a). A representative illustration of the fractions of Aldrich humic acid (50 mg L^{-1}) is given in Figure 4.2.

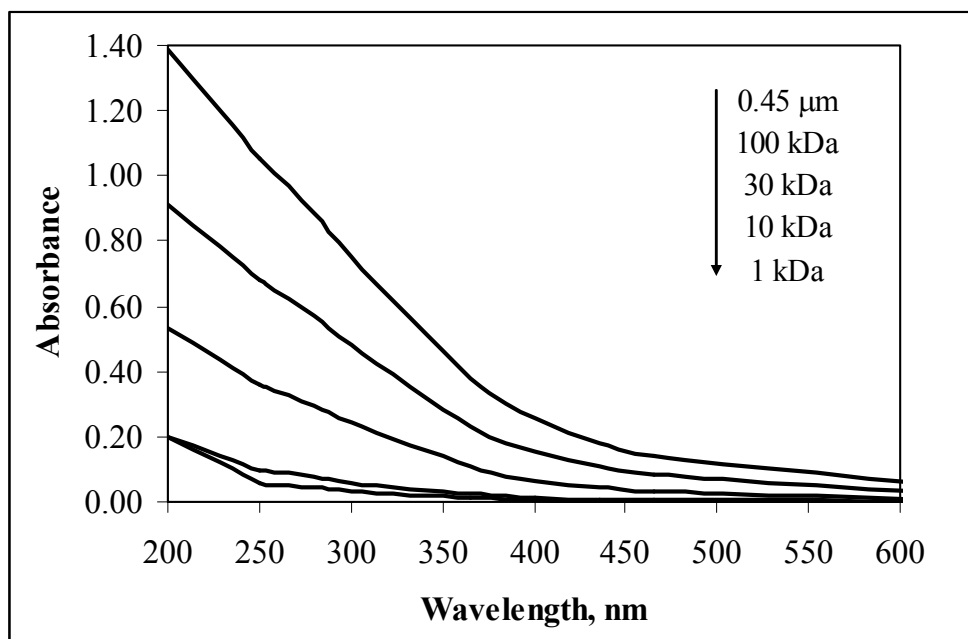


Figure 4.2. UV-vis spectra of the molecular size fractions of Aldrich humic acid.

The spectra of the higher molecular size fractions; $0.45 \mu\text{m}$, 100 kDa and 30 kDa exhibit Color_{436} values as 0.180, 0.107 and 0.044, respectively. The fractions $< 10 \text{ kDa}$ have relatively lower absorbance values that level off and approach zero for wavelengths $> 465 \text{ nm}$. On the other hand, UV_{254} absorbance values display relative order of absorptivities as 0.671, 0.355, 0.094, and 0.053 for the size fractions 100 kDa, 30 kDa, 10 kDa and 1 kDa fractions.

Since the ultrafiltration of humic substances for molecular size fractions $< 10 \text{ kDa}$, revealed very low Color_{436} values, the molecular size distribution data of humic substances were assessed only on the basis of SUVA_{254} and SUVA_{365} values.

The specific absorbance values of the molecular size fractions of Aldrich humic acid are presented in Figure 4.3.

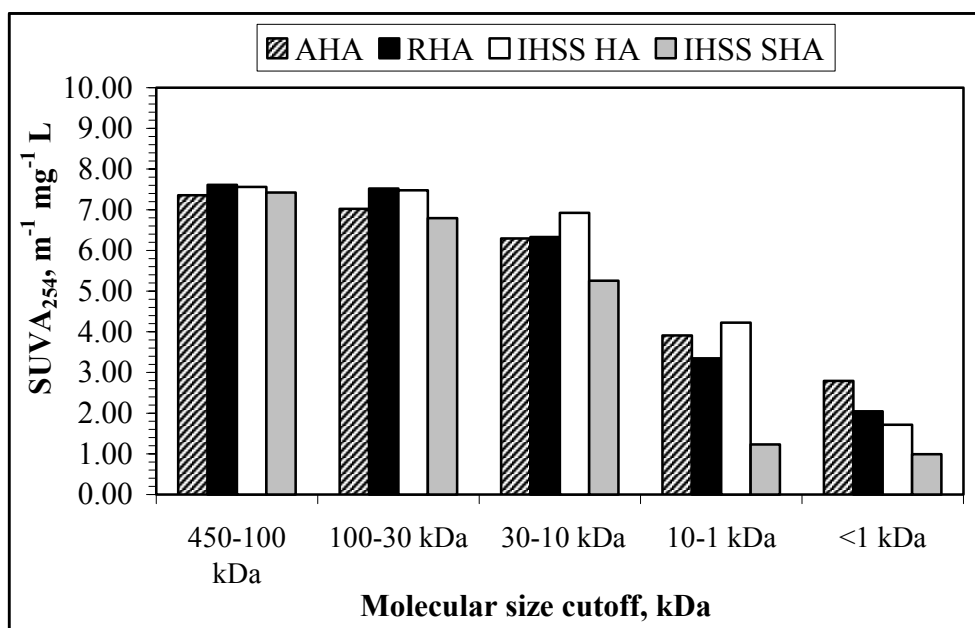


Figure 4.3. $SUVA_{254}$ values for raw humic substances with respect to molecular size distribution.

All of the studied humic acid samples exhibited close similarities in terms of $SUVA_{254}$ up to 30 kDa fraction (Figure 4.3). The medium size fractions of 30-10 kDa region of aquatic origin IHSS HA displayed comparatively higher $SUVA_{254}$ value than the others that are of terrestrial origin. IHSS SHA has quite low $SUVA_{254}$ value whereas IHSS HA still revealed higher $SUVA_{254}$ than the others for 10-1 kDa fraction. On the other hand, AHA sample acquired comparatively high $SUVA_{254}$ for fractions < 1 kDa.

It is known that the molecular size distribution profiles of the humic substances exhibit a general decreasing trend from high molecular size fractions to the lower molecular size fractions irrespective of the origin and source of the organic matter (Alberts et al., 2002).

Depending on the explanation that the specific UV absorbance ($SUVA_{254}$) parameter can be used to describe the composition of humic material in terms of hydrophobicity and hydrophilicity, the hydrophobic character predominates for humic samples up to 10 kDa size fraction whereas comparatively hydrophilic character could be

assessed for smaller molecular size fractions as 10-1 kDa and even smaller than 1 kDa fraction (Figure 4.3).

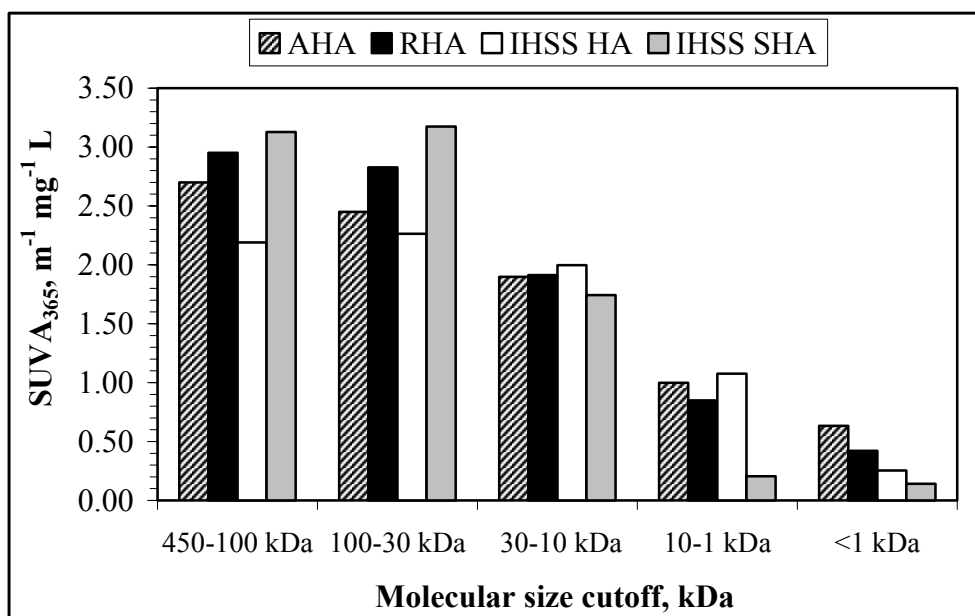


Figure 4.4. SUVA₃₆₅ values for raw humic substances with respect to molecular size distribution.

Although SUVA₂₅₄ values show similarities for the high molecular size fractions of humic acids, significant differences are observed between humic acids based on SUVA₃₆₅ data (Figure 4.4). It is noteworthy that the size fractions of the 450-30 kDa region of the aquatic origin IHSS HA displays comparatively lower SUVA₃₆₅ value than the others that are of terrestrial origin. However, a different trend prevails for the fractions <30 kDa and IHSS SHA reveals lower SUVA₃₆₅ values.

The specified absorbance ratios were also calculated for each size fraction and illustrated in the following tables (Table 4.4, Table 4.5, Table 4.6, Table 4.7 and Table 4.8). Due to the low absorbance capacities at 465 nm and 665 nm, E₄₆₅/E₆₆₅ ratios of smaller size fractions were lower than the limit of detection and has the lack sensitivity, and thus were not considered to be a suitable surrogate parameter. Therefore, humic substances differ from natural organic matter with respect to the use of E₄₆₅/E₆₆₅ parameter.

Table 4.4. Absorbance ratios of the molecular size fractions of AHA

	Absorbance ratios of AHA					
	E₂₅₀/E₃₆₅	E₂₅₄/E₃₆₅	E₂₈₀/E₃₆₅	E₂₅₄/E₄₃₆	E₃₀₀/E₄₀₀	E₃₀₀/E₄₃₆
0.45 μ m	2.78	2.73	2.34	5.74	2.92	4.18
100 kDa	2.91	2.87	2.45	6.27	3.07	4.50
30 kDa	3.36	3.27	2.78	7.95	3.61	5.50
10 kDa	3.96	3.92	3.17	10.44	4.29	6.67
1 kDa	4.75	4.42	3.50	26.50	6.20	15.50

Table 4.5. Absorbance ratios of the molecular size fractions of RHA

	Absorbance ratios of RHA					
	E₂₅₀/E₃₆₅	E₂₅₄/E₃₆₅	E₂₈₀/E₃₆₅	E₂₅₄/E₄₃₆	E₃₀₀/E₄₀₀	E₃₀₀/E₄₃₆
0.45 μ m	2.63	2.58	2.21	4.99	2.61	3.63
100 kDa	2.73	2.66	2.26	5.30	2.72	3.82
30 kDa	3.37	3.31	2.73	7.91	3.41	5.26
10 kDa	4.06	3.94	3.06	8.88	4.00	5.50
1 kDa	5.29	4.86	3.71	11.33	4.75	6.33

Table 4.6. Absorbance ratios of the molecular size fractions of IHSS FA

	Absorbance ratios of IHSS FA					
	E₂₅₀/E₃₆₅	E₂₅₄/E₃₆₅	E₂₈₀/E₃₆₅	E₂₅₄/E₄₃₆	E₃₀₀/E₄₀₀	E₃₀₀/E₄₃₆
0.45 μ m	4.96	4.77	3.58	17.56	5.24	10.03
100 kDa	5.03	4.85	3.63	18.45	5.42	10.55
30 kDa	5.01	4.82	3.62	18.57	5.47	10.62
10 kDa	5.90	5.65	4.13	25.60	6.53	13.93
1 kDa	6.40	6.00	4.40	30.0	6.20	15.50

Table 4.7. Absorbance ratios of the molecular size fractions of IHSS HA

	Absorbance ratios of IHSS HA					
	E₂₅₀/E₃₆₅	E₂₅₄/E₃₆₅	E₂₈₀/E₃₆₅	E₂₅₄/E₄₃₆	E₃₀₀/E₄₀₀	E₃₀₀/E₄₃₆
0.45 μ m	3.43	3.34	2.80	9.31	3.56	5.91
100 kDa	3.39	3.30	2.69	9.14	3.62	6.04
30 kDa	3.56	3.47	2.79	10.05	3.92	6.53
10 kDa	4.04	3.93	3.11	12.22	4.25	7.56
1 kDa	7.00	6.75	5.25	27.00	6.50	13.00

Table 4.8. Absorbance ratios of the molecular size fractions of IHSS SHA

	Absorbance ratios of IHSS SHA					
	E₂₅₀/E₃₆₅	E₂₅₄/E₃₆₅	E₂₈₀/E₃₆₅	E₂₅₄/E₄₃₆	E₃₀₀/E₄₀₀	E₃₀₀/E₄₃₆
0.45 μ m	2.40	2.37	2.05	4.09	2.37	3.05
100 kDa	2.63	2.14	1.84	4.76	3.23	4.21
30 kDa	3.08	3.02	2.58	6.03	3.17	4.29
10 kDa	6.33	6.00	5.00	18.00	10.00	10.0
1 kDa	7.50	7.00	5.50	14.00	6.00	6.00

As a general trend the absorbance ratios of the fractionated humic and fulvic acids increase with decreasing molecular size. Such a pattern suggests a shift in the UV-vis absorbance towards higher wavelengths with increasing molecular sizes as reported by Duarte et al., 2003. As compiled in Tables 4.4- 4.8, the molecular size fractions of the soil-derived RHA, AHA and IHSS SHA exhibit similar properties up to 30 kDa.

The absorbance of humic acid at 365 nm will more greatly increase with increasing molecular size than that at 250 nm, thus permitting an estimate for the relative degree of humification (Peuravuori and Pihlaja, 1997). The smallest size fraction (1 kDa) shows similar properties with that of IHSS SHA and IHSS HA. On the other hand, size fractions of Roth HA, AHA and IHSS SHA exhibit similar properties up to 30 kDa.

E_{254}/E_{436} ratio could be proposed to assess the composition of altered natural organic matter provided that the total removal of color-forming moieties is not required. Under conditions of total decolorization, the use of E_{250}/E_{365} is also recommended relevant to the spectroscopic changes acquired during treatment. Furthermore, E_{254}/E_{436} and E_{250}/E_{365} ratios exhibit molecular size-specific distribution with respect to the source and type of humic acid.

The polydisperse nature of humic acid unifying components of dissimilar molecular weights is clearly observed from the presented absorbance ratios of each size fraction. When ultrafiltration is applied, it does not involve explicit size or functional group separation however, it produces humic acid fractions of a certain size distribution that show spectroscopic evidence of distinct structural differences. The discrepancies between size fractions suggest that the molecular weight fractionation pattern for different humic substances may differ by source and type.

4.1.3. Fluorescence Spectroscopic Properties of Humic Substances

Preliminary studies were conducted on raw humic substances for the selection of the appropriate excitation wavelengths for the emission and the synchronous scan spectra and to observe the effect of concentration of humic substances on the fluorescence spectra.

Both aquatic and terrestrial humic substances have intrinsic fluorescence with the property of concomitant variation in the emission maximum when excited at different wavelengths. This per se indicates the presence of various fluorophores and illustrates the complexity of these materials.

A representative example for the emission scan spectra of Aldrich humic acid in the excitation wavelength range of 300 nm-370 nm was given in Figure 4.5. The effect of the excitation wavelength on the fluorescence intensity of Aldrich humic acid could be observed most significantly in the 400-500 nm region, whereas the effect could be considered insignificant in the longer wavelength region up to 600 nm. Similar results were obtained for the other humic substances (data not shown).

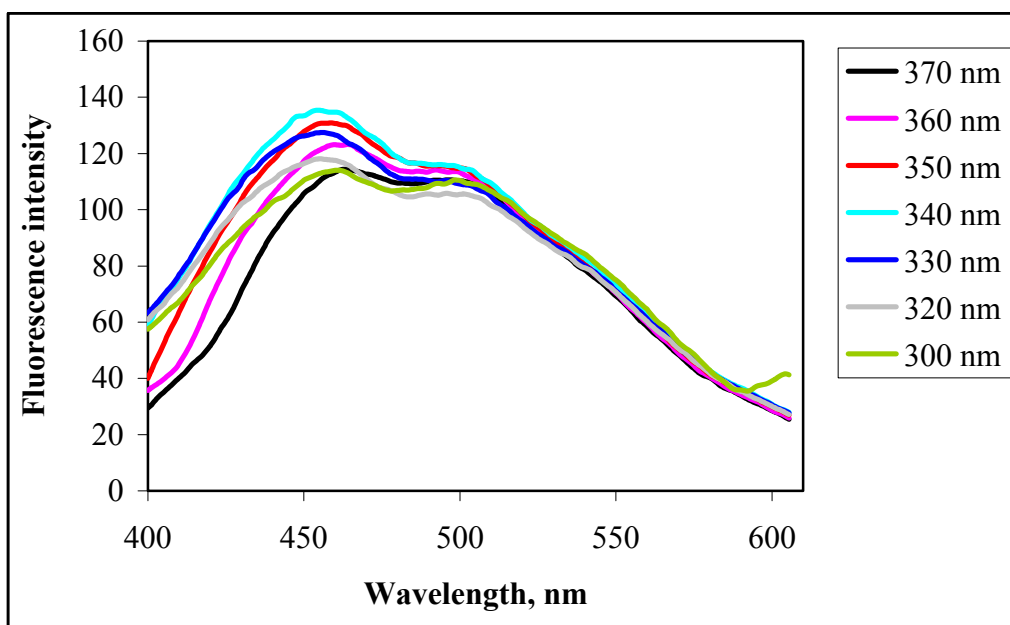


Figure 4.5. Effect of excitation wavelength on the emission spectra of AHA (50 mg L⁻¹).

Previous work, in the field of fluorescence spectroscopy for characterisation of humic substances has established that they typically fluoresce in the excitation range of 300–400 nm and the emission range of 400–500 nm (Goslan et al., 2004). The emission spectra of Aldrich humic acid exhibit different intensity values in the specified range (Figure 4.5). The fluorescence intensities at 450 nm are discriminative with 26 % difference between the lowest and the highest fluorescence intensity. On the other hand, at 500–550 nm wavelength range the intensities change with less 10 % difference.

Considering the complexity of the situation and to simplify the comparison of humic substances based on fluorescence properties, the use of a single emission wavelength at 350 nm was preferred throughout the study. This excitation wavelength has been widely used for similar studies in literature (Senesi, 1990; Hautala et al., 2000; Cho and Choi, 2002).

As a consequence humic substances exhibit featureless conventional emission spectra, but are well characterized by another type of fluorescence spectra-synchronous scan spectra (Senesi et al., 1989; Miano and Senesi, 1992). Similarly, the effect of varying $\Delta\lambda$ was investigated for Aldrich humic acid within a range of 5 nm to 60 nm (Figure 4.6).

In order to be comparable with literature results, $\Delta\lambda$ was chosen as 18 nm throughout this study (Senesi, 1990; Rivero et al., 1998; D'Orazio et al., 1999; Peuravuori et al., 2002).

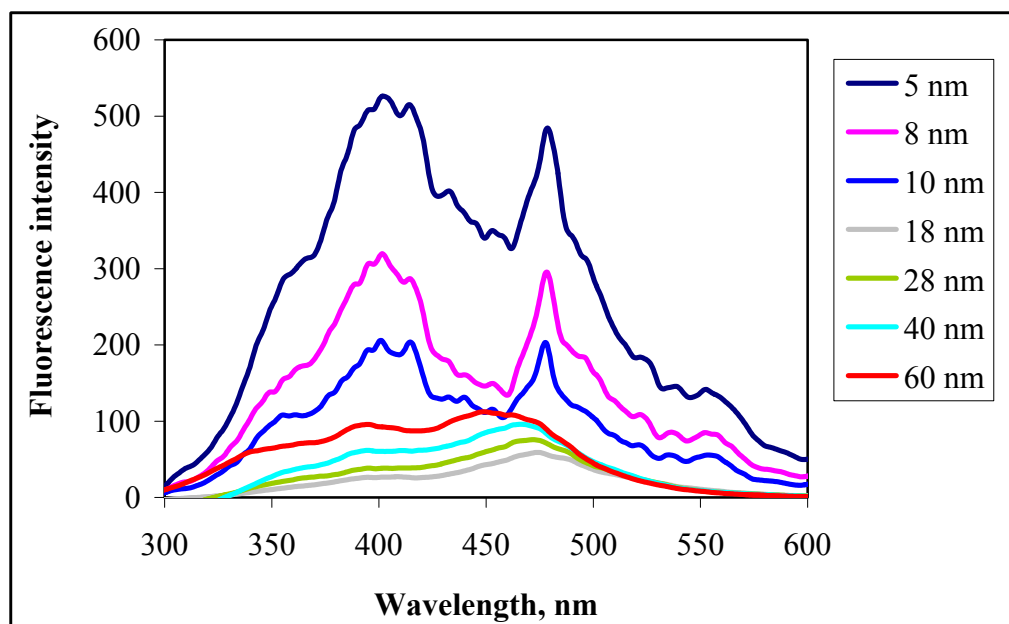


Figure 4.6. Synchronous scan fluorescence spectra of 50 mg L⁻¹ Aldrich humic acid for $\Delta\lambda=5$ nm, 8 nm, 10 nm, 18 nm, 28 nm, 40 nm and 60 nm.

The effect of concentration on the fluorescence spectra of humic substances has been studied in 10-100 mg L⁻¹ concentration range for Aldrich humic acid. Both the emission and synchronous scan fluorescence spectra of AHA were illustrated in Figure 4.7 and Figure 4.8 as representatives of the studied humic substances.

The figures illustrate the basic differences in the scan modes of the fluorescence spectra. On the other hand, emission scan spectra still reveals information that could be considered as minor with respect to the acquired spectra in the synchronous scan mode (Figure 4.8).

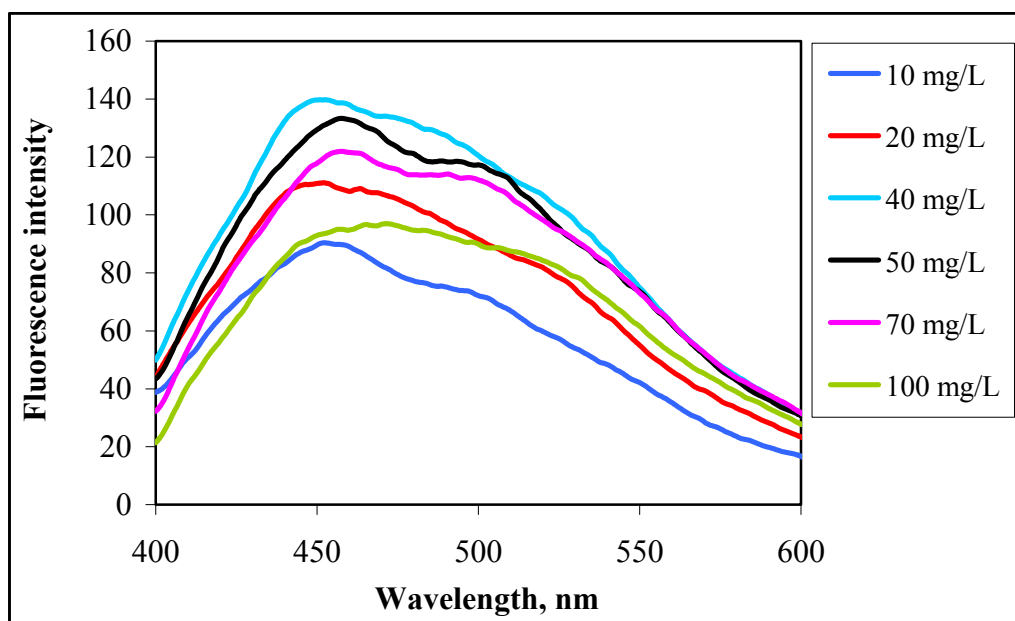


Figure 4.7. Effect of concentration on the emission scan spectra of Aldrich humic acid.

Up to humic acid concentration of 40 mg L^{-1} , the fluorescence maxima slightly shift to longer emission wavelengths. Further increase in concentration leads to a decrease of the fluorescence intensity.

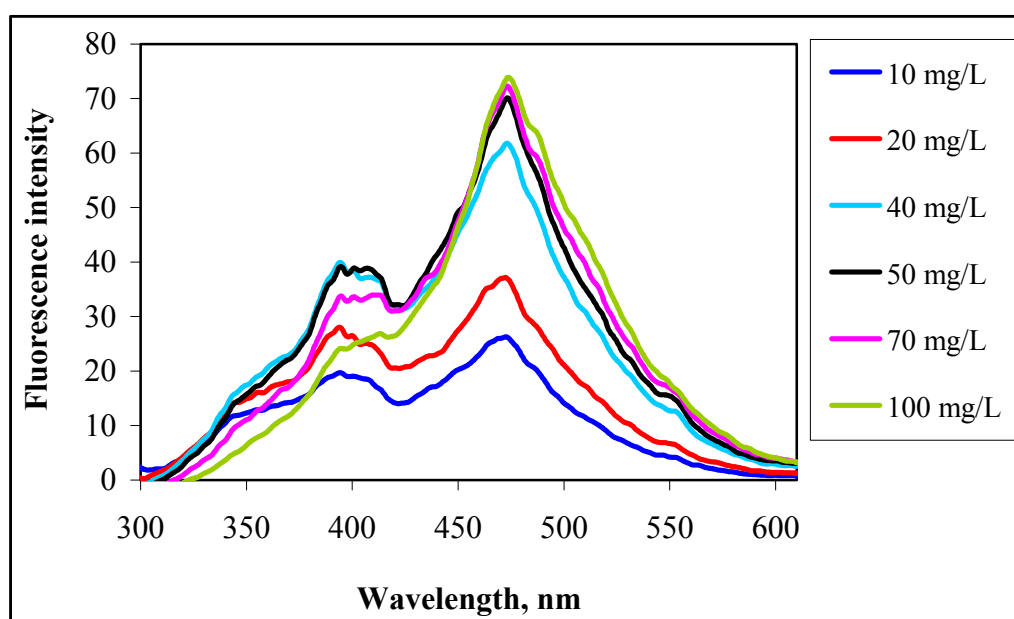


Figure 4.8. Synchronous scan spectra of Aldrich humic acid at different concentrations.

In the synchronous scan spectra of humic acid, varying the concentration had a minor effect on the position of bands and shoulders but altered their intensities. The overall fluorescence intensity of humic acid increases up to 50 mg L^{-1} and remains almost unchanged at higher concentration probably due to intermolecular aggregation (Figure 4.8). Similar results were obtained for the other humic substances in accordance with the literature (Senesi, 1990).

In order to avoid concentration effects and allow a more accurate comparison between the studied samples, both the emission and synchronous scan spectra were plotted as specific fluorescence (fluorescence intensity per unit total organic carbon) versus excitation wavelength. However, a plot of the fluorescence intensity per unit carbon of each of the humic substances does not significantly change the shape of the fluorescence spectra in our case, since the TOC values of humic substances (50 mg L^{-1} each) are close to each other ranging from 15.50 to 20.56 mg L^{-1} .

The TOC normalized fluorescence emission spectra of humic substances consist of a broad band of overall intensity and a maximum intensity wavelength varying according to the origin of the sample (Figure 4.9).

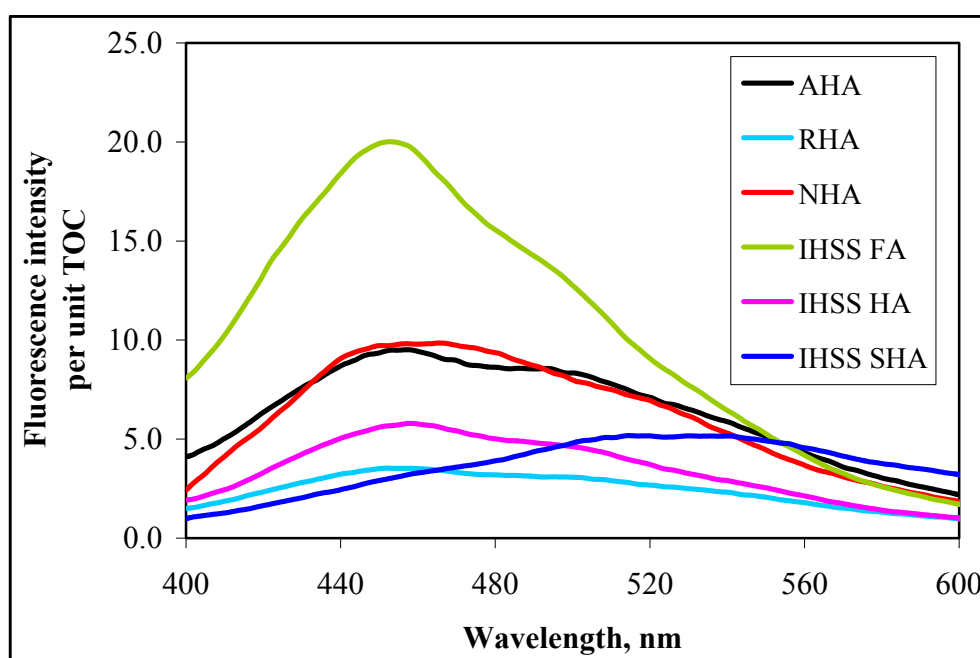


Figure 4.9. TOC normalized emission scan spectra of humic substances.

It is obvious from Figure 4.9 that IHSS FA presents a significantly different trend exhibiting a higher intensity peak than the other humic acids. On the other hand, the lowest emission intensity is observed for Roth humic acid.

Humic acid mixtures have on average larger molecular size than fulvic acids and it has been shown that mixtures of larger sized compounds, as defined by ultrafiltration, have lower fluorescence intensity per unit carbon than smaller materials (Miano and Alberts, 1999; Alberts et al., 2004). IHSS soil humic acid exhibits a broad emission band with a maximum around ~510 nm, this band is consistent with data published in literature (Senesi et al., 1989). The possible reason for the lower fluorescence intensity of Roth humic acid might be explained by its coal based origin and the possible presence of impurities. As a function of their structural differences, fluorescence spectra have different profiles.

Synchronous scan spectra not only offer a potential use to reduce overlapping interferences but also provide a possibility for each fluorescent component to be identified in a spectral range. The synchronous scan fluorescence spectra for HS are shown in Figure 4.10.

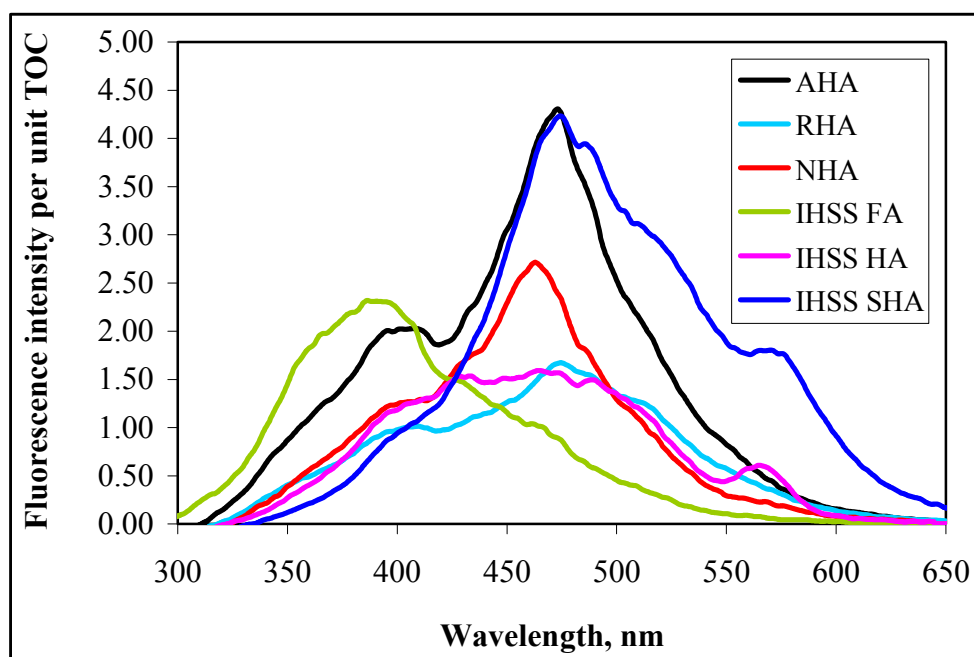


Figure 4.10. TOC normalized synchronous scan fluorescence spectra of humic substances.

The synchronous scan fluorescence spectra measured proved useful for highlighting similarities and differences in the composition of the studied HS. Although the UV-vis spectra of HS show similarities (Figure 4.10), significant differences were observed in the synchronous scan fluorescence spectra. In accordance with literature, the spectra of HAs are shifted to longer wavelengths compared to that of fulvic acid (Senesi, 1990). This is attributed to the presence of high molecular weight fractions, electron withdrawing substituents such as carbonyl and carboxyl groups and a higher degree of conjugation in humic acids. The synchronous scan spectrum of fulvic acid has a distinct profile in comparison to those of humic acids. It is broad and shifted to the blue region at shorter wavelengths with a maximum around 370 nm indicating simple structural features with fewer aromatic functional groups (Miano and Senesi, 1992). The terrestrial IHSS SHA and AHA have characteristic peaks around 470 nm with relatively higher intensities than those of RHA, IHSS HA and NHA. Additionally, a minor peak around 400 nm is observed for AHA. The fluorescence spectra of RHA and IHSS HA are very similar to each other and exhibit broad bands without any significant features although no such similarity was observed for the previously discussed UV-vis parameters.

4.1.4. Fluorescence Spectroscopic Properties of the Molecular Size Fractions of Humic Substances

The fluorescence properties of the fractionated humic substances, Aldrich humic acid, Roth humic acid, IHSS fulvic acid, IHSS humic acid and IHSS soil humic acid have been investigated. Both the emission and the synchronous scan spectra of each humic substance with respect to molecular size fractions were presented in a comparative approach.

4.1.4.1. Fluorescence Spectroscopic Properties of the Molecular Size Fractions of Aldrich Humic Acid. The emission scan spectra of Aldrich humic acid are broad and exhibit gradually declining fluorescence intensity pattern with decreasing size fraction (Figure 4.11). The maximum intensities range from as high as 140 for high molecular size fractions to lower values around 20 for 1 kDa fraction. The broad emission peaks of large fractions slightly shift to shorter wavelengths and the fluorescence intensity decreases with the decreasing molecular size.

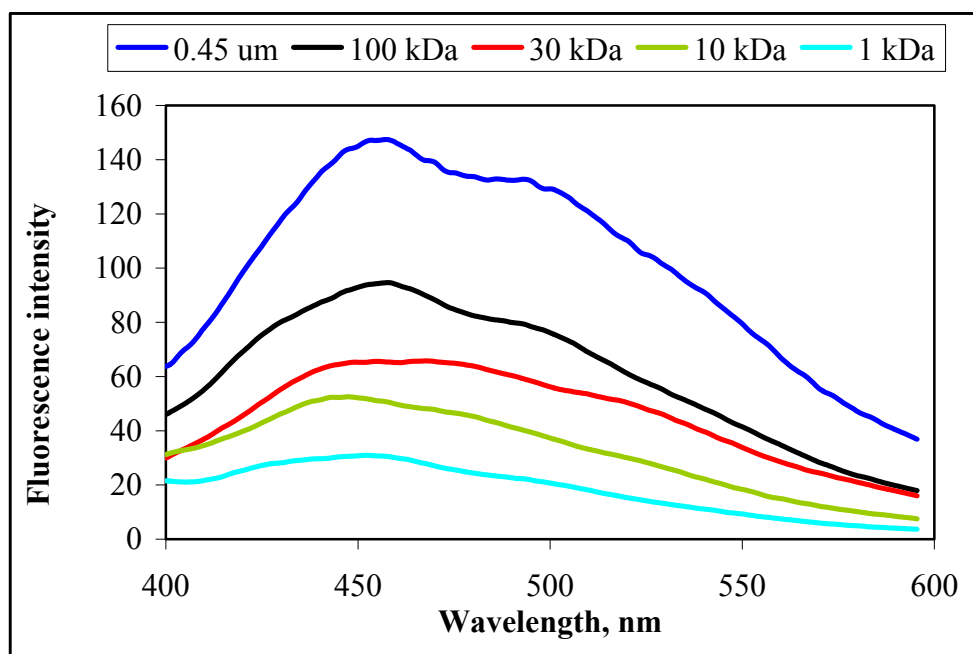


Figure 4.11. Emission scan spectra of the molecular size fractions of AHA.

According to McKnight et al. (2001), who proposed to use a fluorescence index as the ratio of the fluorescence emission intensity at 450 nm to that at 500 nm (with excitation at 370 nm), fluorescence index (F.I.) values of 1.9 are indicative of these autochthonous (microbial) sources, while values of 1.4-1.5 are indicative of allochthonous (terrestrial) sources. This ratio has been successfully used to differentiate dissolved organic matter from terrestrial and aquatic precursors in water samples.

Similarly, in this study a fluorescence index was calculated on a comparative basis as the quotient of fluorescence intensity at 450 nm and 500 nm as a relative measure of fluorescence changes between each fraction at an excitation wavelength of 350 nm. The respective values of the fluorescence index parameter of the molecular size fractions of AHA were presented in Table 4.9.

Although AHA was an example of a humic acid of terrestrial origin, molecular size fractions reflected the original significance in terms of fluorescence index parameters. The calculated F.I. values range from 1.12 to 1.48 and are in good agreement with the findings of others (Chen et al., 2003; McKnight et al., 2001). The values increase with decreasing molecular size fraction.

Table 4.9. Fluorescence index values of the molecular size fractions of AHA

Molecular size fraction	F.I. (450 nm/500 nm)
0.45 μm	1.12
100 kDa	1.16
30 kDa	1.22
10 kDa	1.42
1 kDa	1.48

In a comparative approach, the synchronous scan fluorescence spectra of molecular size fractions of raw humic acid were presented in Figure 4.12. The spectra of each size fraction exhibit a decreasing trend in terms of fluorescence intensity.

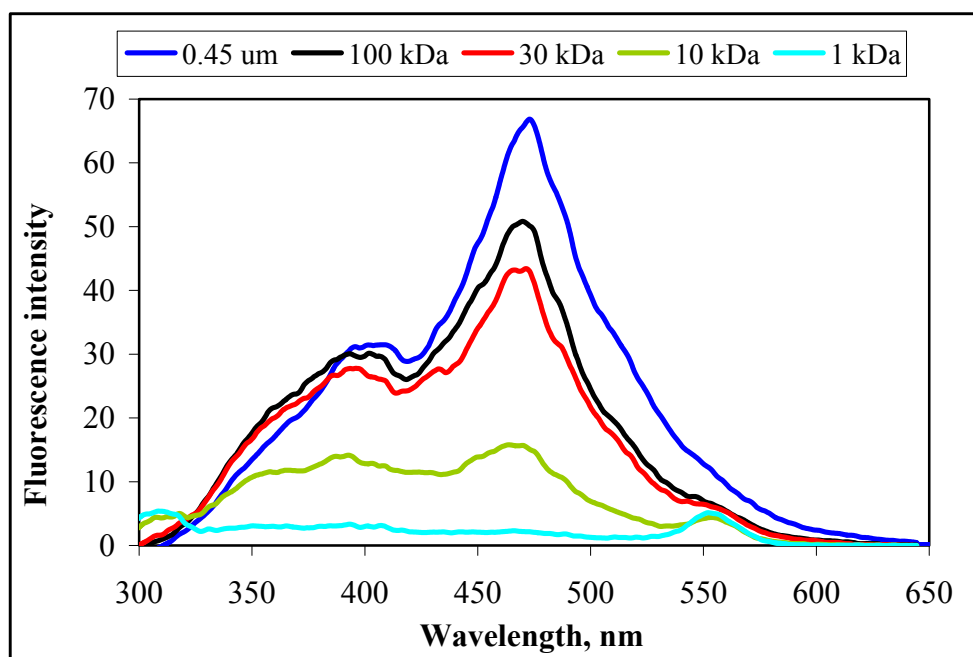


Figure 4.12. Synchronous scan spectra of the molecular size fractions of AHA

The decrease in the fluorescence intensity of lower molecular weight fractions of humic acid and the formation of a minor intensity peak around 550 nm could be due to the depletion of the aromatic and/or polyphenolic contents which are mostly of high molecular weight and also due to the enrichment of its carbohydrate materials which do not give fluorescence.

4.1.4.2. Fluorescence Spectroscopic Properties of the Molecular Size Fractions of Roth Humic Acid. The emission scan spectra of the molecular size fractions of Roth humic acid are presented in Figure 4.13.

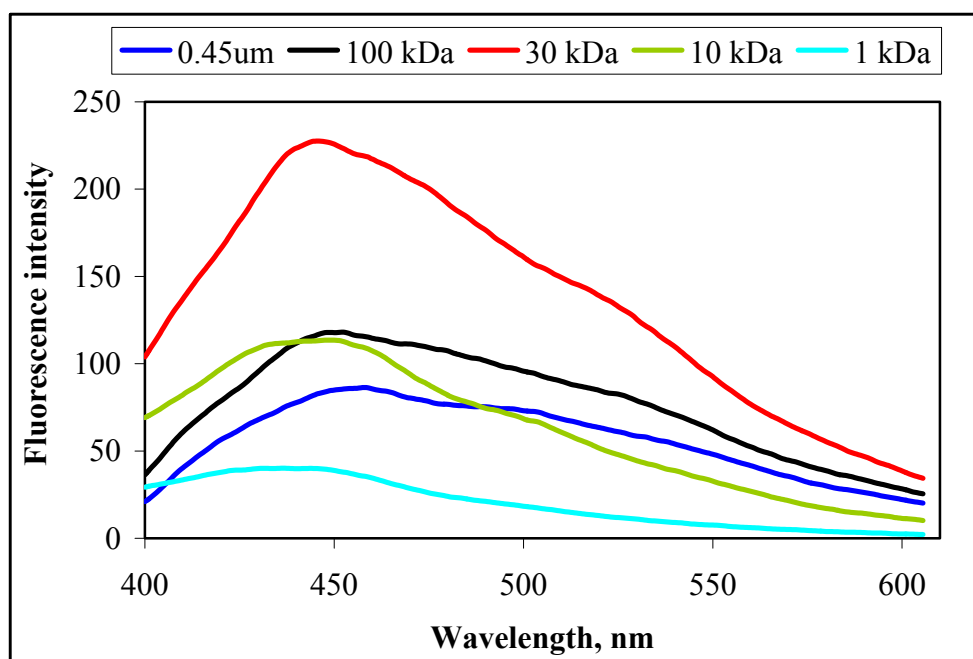


Figure 4.13. Emission scan spectra of the molecular size fractions of RHA.

The spectra of Roth humic acid are broad and exhibit a disordered pattern irrespective of the size fractions (Figure 4.13). The maximum intensities range from as high as 220 for 30 kDa to lower values around 20 for 1 kDa molecular size fraction. Initially, the increase of fluorescence intensity is observed up to 30 kDa fraction. However, the fluorescent moieties are being depleted through the ultrafiltration process leading to the formation of lower sized moieties with less fluorescent properties. The emission peaks of small size fractions that are less than 30 kDa slightly shift to shorter wavelengths and the fluorescence intensity decreases with the decreasing molecular size.

Based on the emission spectra presented in Figure 4.13, the fluorescence indexes were evaluated on a comparative basis for the molecular size fractions of Roth humic acid (Table 4.10). The calculated F.I. values range from 1.16 to 2.11.

Table 4.10. Fluorescence index values of the molecular size fractions of RHA

Molecular size fraction	F.I. (450 nm/500 nm)
0.45 μm	1.16
100 kDa	1.23
30 kDa	1.40
10 kDa	1.66
1 kDa	2.11

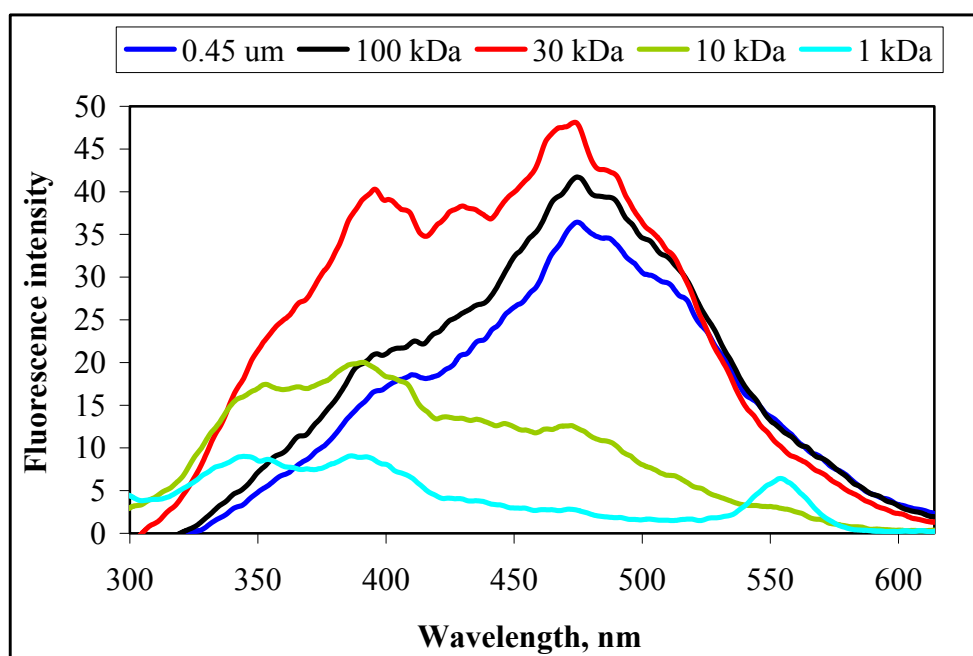


Figure 4.14. Synchronous scan spectra of the molecular size fractions of RHA.

The synchronous scan spectra of the molecular size fractions of RHA are presented in Figure 4.14. Up to 30 kDa fraction an initially increasing trend of the fluorescence intensity is observed. However, fractionation to lower molecular size fractions, leads to a decrease of the fluorescence intensity with a noticeable shift of the emission maxima to lower wavelength region.

4.1.4.3. Fluorescence Spectroscopic Properties of the Molecular Size Fractions of IHSS Fulvic Acid. The emission scan spectra of the molecular size fractions of IHSS FA is presented in Figure 4.15.

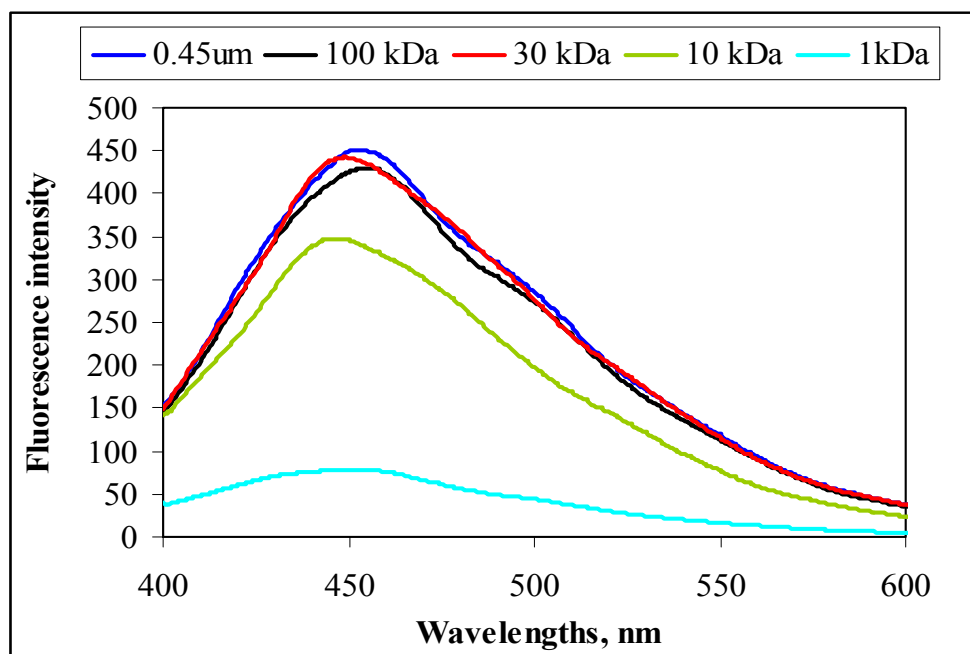


Figure 4.15. Emission scan spectra of the molecular size fractions of IHSS FA.

The emission scan spectra of IHSS FA display similar fluorescence properties up to 30 kDa size fraction. With decreasing molecular size, the fluorescence intensity gradually decreases, exhibiting an almost 95 % decrease in the emission maxima for 1 kDa molecular size fraction with respect to initial fluorescence intensity.

Compared to the other humic substances studied, it is notable that fulvic acids express substantially higher fluorescence intensities. However, the high fluorescence intensities are not reflected as high values to the calculated fluorescence index parameters that are presented in Table 4.11.

Table 4.11. Fluorescence index values of the molecular size fractions of IHSS FA

Molecular size fraction	F.I. (450 nm/500 nm)
0.45 μm	1.58
100 kDa	1.56
30 kDa	1.61
10 kDa	1.75
1 kDa	1.79

The results presented are comparable with the findings of McKnight et al., (2001) where the fluorescence indexes were reported to be in the range of 1.4 to 1.5 for IHSS FA at an excitation wavelength of 370 nm.

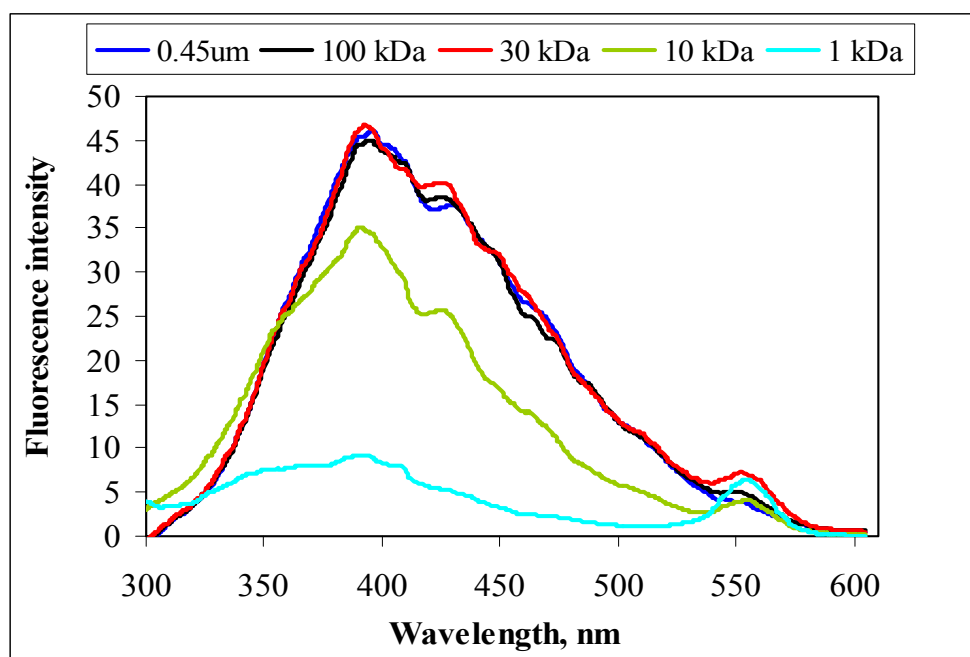


Figure 4.16. Synchronous scan spectra of the molecular size fractions of IHSS FA.

IHSS FA has a sharp peak around 395 nm. The fluorescence intensity exhibits the same emission maxima and the same fluorescence intensity up to 30 kDa fraction. For the lower size fractions of 10 kDa and 1 kDa, a rapid decline of the fluorescence intensity is observed.

4.1.4.4. Fluorescence Spectroscopic Properties of the Molecular Size Fractions of IHSS Humic Acid. The emission scan spectra of the molecular size fractions of IHSS humic acid are presented in Figure 4.17. A generally decreasing trend is observed with respect to the decreasing molecular size fractions. The emission maxima of the fractions are allocated around 450 nm.

The smaller molecular size fractions as 10 kDa and 1 kDa exhibited a smoother profile even 1 kDa size fraction could be considered as revealing nonsignificant fluorescence intensity changes with respect to wavelength.

The spectra are very similar in shape to those of IHSS FA except for the considerably high fluorescence intensities attained as shown in Figure 4.15. This is due to the fact both IHSS HA and IHSS FA are aquatic standards extracted from the same source.

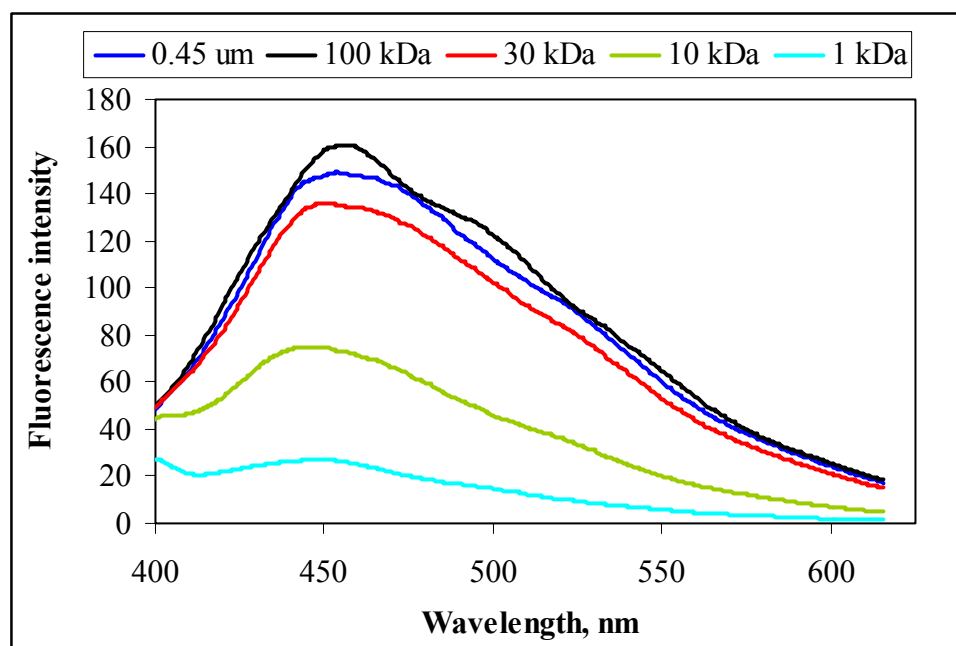


Figure 4.17. Emission scan spectra of the molecular size fractions of IHSS HA.

Based on the presented emission scan spectra, the respective fluorescence indexes for each size fraction are calculated and displayed in Table 4.12.

Table 4.12. Fluorescence index values of the molecular size fractions of IHSS HA

Molecular size fraction	F.I. (450 nm/500 nm)
0.45 μm	1.29
100 kDa	1.32
30 kDa	1.33
10 kDa	1.64
1 kDa	1.82

IHSS HA and its size fractions represent F.I. values typical to the values reported for aquatic humic substances (McKnight et al., 2001).

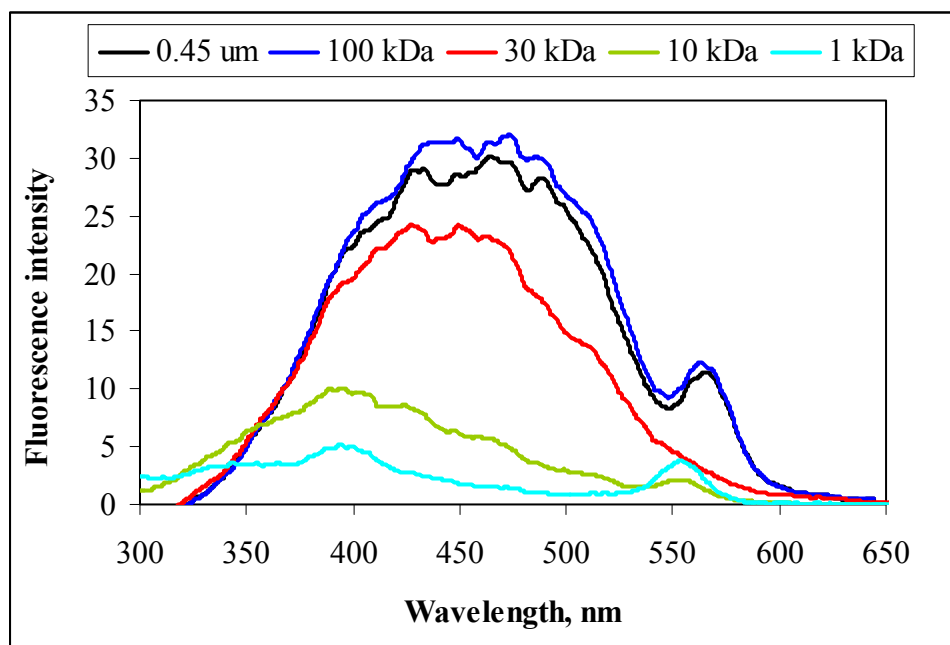


Figure 4.18. Synchronous scan spectra of the molecular size fractions of IHSS HA.

IHSS HA has one distinctly broad peak in the range of 400 to 500 nm and another minor shoulder around 560 nm that could be considered as a peak. The higher molecular size fractions (> 100 kDa) represent similar fluorescence properties. With decreasing molecular size, the broad peak of IHSS HA gradually decays in intensity and shifts to the blue region.

4.1.4.5. Fluorescence Spectroscopic Properties of the Molecular Size Fractions of IHSS Soil Humic Acid. The emission scan spectra of IHSS soil humic acid and its molecular size fractions are presented in Figure 4.19.

As a general trend the emission scan spectra of the molecular size fractions of IHSS soil humic acid display broader emission profiles with respect to the other humic substances studied. The emission maxima corresponding to the fluorophores of the high molecular size fractions of IHSS SHA are allocated at longer wavelengths around 520 nm. Up to 30 kDa, the emission intensities gradually increase with evident shift to lower wavelengths. For lower size fractions, the fluorescence intensity gradually decreases.

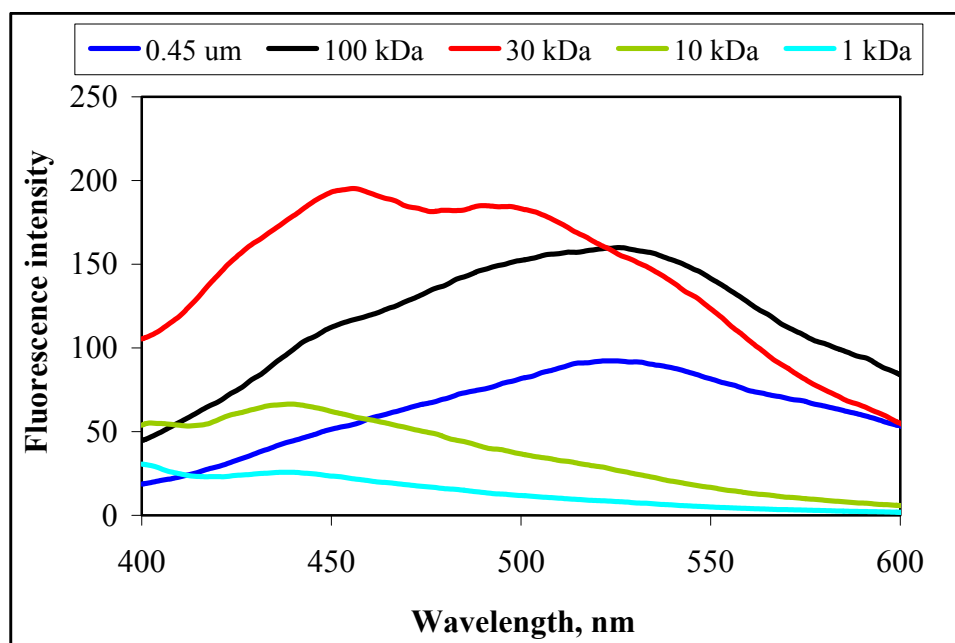


Figure 4.19. Emission scan spectra of the molecular size fractions of IHSS SHA.

The discrepancy of the emission scan spectra of the molecular size fractions of IHSS soil humic acid are reflected to the fluorescence index values as presented in Table 4.13.

The F.I. values of IHSS SHA are in the range of 0.63 to 1.98. Since the emission spectra of IHSS soil humic acid lies in a broad range with the peak position shifted to longer wavelength region (around 520 nm), the calculated indexes have values less than one in agreement with the results reported by Chen et al., (2003). Apparently, the fluorescence index may be best applied to aquatic humic and fulvic acids.

Table 4.13. Fluorescence index values of the molecular size fractions of IHSS SHA

Molecular size fraction	F.I. (450 nm/500 nm)
0.45 μm	0.63
100 kDa	0.74
30 kDa	1.05
10 kDa	1.69
1 kDa	1.98

As opposed to the broad and featureless emission scan spectra presented in Figure 4.19, the synchronous scan spectra of IHSS SHA provides a much better defined peak resolution in the synchronous scan spectra (Figure 4.20).

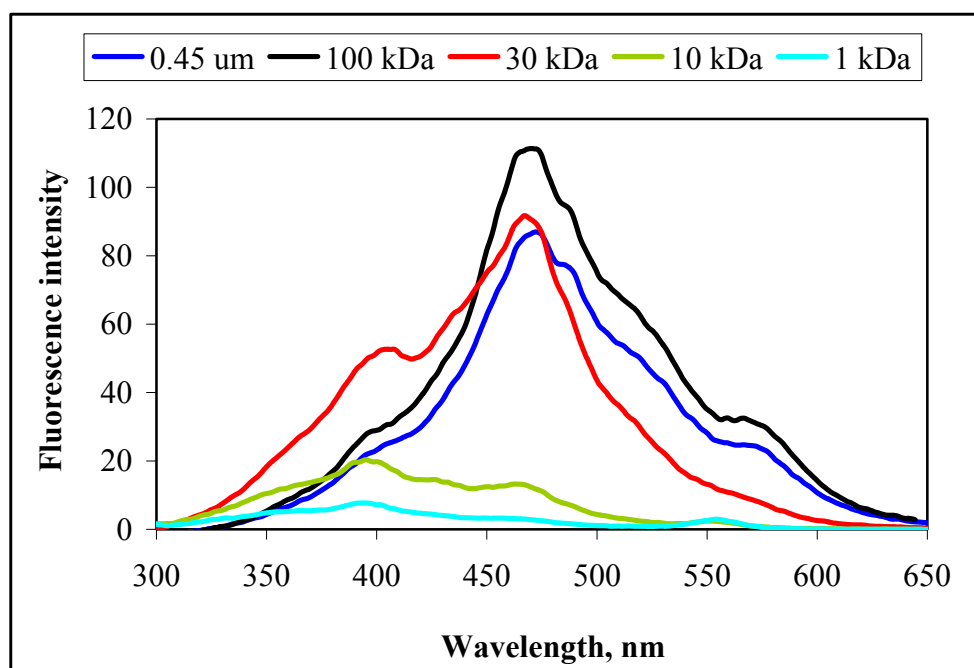


Figure 4.20. Synchronous scan spectra of the molecular size fractions of IHSS SHA.

The high molecular size fractions (0.45 μm and 100 kDa) of IHSS SHA exhibit similar patterns with slight variation in their fluorescence intensity. However, with the decreasing molecular size fraction, a slight shift to lower wavelength region is observed ($\lambda < 450$ nm). For molecular size fractions < 30 kDa, the initial increase of the fluorescence intensity is subsequently followed by the quenching of the intensity.

It is interesting to note that the synchronous scan spectra of all the humic substances show similarities for fractions < 10 kDa. The lowest size fractions have in common a broad peak around 400 nm and another peak formation around 550 nm. Although, humic size fractions are known to differ in their chemical composition (Christl et al., 2000), lower size fractions display similar fluorescence properties irrespective of their origin.

4.2. Oxidative Treatment of Humic Substances via Photocatalysis

In this section, different humic substances from aquatic and terrestrial origins namely, Aldrich humic acid, Roth humic acid, IHSS fulvic acid, IHSS humic acid and IHSS soil humic acid were treated by photocatalytic oxidation using TiO_2 Degussa P-25 as the photocatalyst. The reaction kinetics was followed in terms of the specified UV-vis parameters as Color_{436} , Color_{400} , UV_{365} , UV_{300} , UV_{280} and UV_{254} . Modeling of the photocatalytic removal of humic substances was based on pseudo first order and Langmuir Hinshelwood kinetic equations.

4.2.1. Pseudo First Order Kinetic Model

As already presented in Figure 4.1, the UV-vis spectra of humic substances are broad, featureless and monotonously decrease with increasing wavelength. A representative example of the time dependent UV-vis spectra is given for the photocatalytic oxidation of Aldrich humic acid (Figure 4.21). The oxidized humic acid also follow the same decreasing trend.

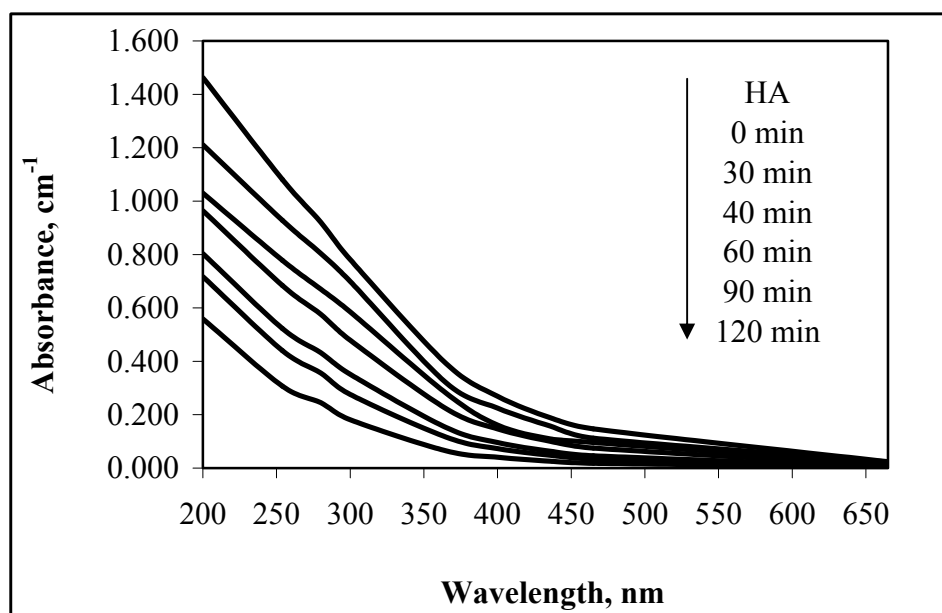
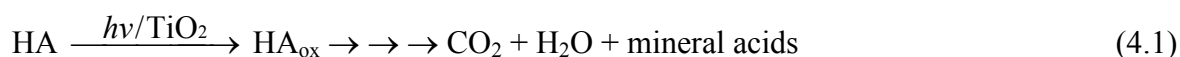


Figure 4.21. UV-vis spectra of the photocatalytic degradation of 50 mg L^{-1} Aldrich humic acid in the presence 0.25 mg mL^{-1} of TiO_2 .

Displaying the same basic pattern of declining trend, a decrease in the absorbance values was observed with the increasing irradiation time during photocatalytic oxidation.

The organic compounds (e.g. humic acid, HA) present in the illuminated titania slurry undergo many chain and consecutive reactions. In an ideal case, all intermediate compounds are fully mineralized to carbon dioxide and water.



The kinetics of the photocatalytic degradation of humic substances is known to obey pseudo first order as expressed by the equation (Bekbölet et al., 1998; Bekbölet et al., 2002);

$$R = -dA/dt = k A \quad (4.2)$$

where the terms represent the following meanings;

R: Pseudo first order rate in terms of the specified UV-vis parameters ($\text{m}^{-1} \text{min}^{-1}$) or in terms of TOC ($\text{mg L}^{-1} \text{min}^{-1}$)

A: Specified UV-vis parameters (m^{-1}) or TOC content of humic substances (mg L^{-1})

t: Irradiation time (min)

k: Pseudo first order reaction rate constant (min^{-1})

The evaluation of the kinetic data for the photocatalytic removal of different humic substances (50 mg L^{-1}) in the presence of 0.25 mg mL^{-1} Degussa P-25 TiO_2 revealed the following model parameters for Color_{436} , Color_{400} , UV_{365} , UV_{300} , UV_{280} and UV_{254} as presented in Table 4.14. The related half life values could also be assessed by the equation $t_{1/2} = 0.693/k$. But for comparison purposes, only rate and rate constants were calculated.

Table 4.14. Pseudo first order model parameters of different humic substances

Color₄₃₆	k, min⁻¹	Rate, m⁻¹ min⁻¹
AHA	1.60×10^{-2}	0.301
RHA	3.04×10^{-3}	0.115
IHSS FA	7.22×10^{-3}	0.0368
IHSS HA	8.30×10^{-3}	0.132
IHSS SHA	1.34×10^{-2}	0.376

Color₄₀₀	k, min⁻¹	Rate, m⁻¹ min⁻¹
AHA	1.52×10^{-2}	0.410
RHA	2.91×10^{-3}	0.153
IHSS FA	6.71×10^{-3}	0.0658
IHSS HA	7.93×10^{-3}	0.209
IHSS SHA	1.29×10^{-2}	0.466

UV₃₆₅	k, min⁻¹	Rate, m⁻¹ min⁻¹
AHA	1.49×10^{-2}	0.584
RHA	2.69×10^{-3}	0.196
IHSS FA	6.46×10^{-3}	0.125
IHSS HA	7.03×10^{-3}	0.311
IHSS SHA	1.24×10^{-2}	0.600

UV₃₀₀	k, min⁻¹	Rate, m⁻¹ min⁻¹
AHA	1.24×10^{-2}	0.968
RHA	2.24×10^{-3}	0.304
IHSS FA	4.87×10^{-3}	0.259
IHSS HA	5.79×10^{-3}	0.566
IHSS SHA	1.14×10^{-2}	0.977

UV₂₈₀	k, min⁻¹	Rate, m⁻¹ min⁻¹
AHA	1.09×10^{-2}	1.004
RHA	2.13×10^{-3}	0.339
IHSS FA	4.02×10^{-3}	0.275
IHSS HA	5.45×10^{-3}	0.655
IHSS SHA	1.10×10^{-2}	1.092

UV₂₅₄	k, min⁻¹	Rate, m⁻¹ min⁻¹
AHA	1.03×10^{-2}	1.113
RHA	2.01×10^{-3}	0.374
IHSS FA	3.76×10^{-3}	0.342
IHSS HA	4.90×10^{-3}	0.726
IHSS SHA	1.07×10^{-2}	1.229

The color forming chromophoric groups of humic substances are usually expressed with Color₄₃₆ and/or Color₄₀₀ values; hence, the removal of similar moieties might be considered. A comparison between the rate constants of Color₄₃₆ and Color₄₀₀ for the photocatalytic degradation of humic acid in diverse solution matrix indicate that the difference is < 10 % (Bekbölet et al., 1998). Accordingly in this study, the rate constants calculated for Color₄₃₆ and Color₄₀₀ differ from each other by 4 to 7 %. Recent results indicate the importance of absorbance ratios such as E₂₅₄/E₄₃₆, E₂₈₀/E₃₆₅ and their

respective changes during photocatalytic degradation of humic acid (Uyguner and Bekbölet, 2005b). In view of that, the removal of UV₃₆₅ forming moieties was also analyzed (Uyguner and Bekbölet, 2005c).

The degradation rates calculated for all the indicated parameters follow the general trend as IHSS SHA > AHA > IHSS HA > RHA > IHSS FA. However, the rate constants show differences depending on the UV-vis parameters used. The ordering is similar for the rate constants in terms of Color₄₃₆ and UV₃₆₅ and decreases in the following order as; AHA > IHSS SHA > IHSS HA > IHSS FA > RHA. The IHSS SHA and AHA exhibit quite similar UV₂₈₀ and UV₂₅₄ degradation rate constants that could be considered as differing insignificantly. The lowest degradation rate constant is obtained for RHA followed by IHSS FA and IHSS HA for both of the UV absorbing moieties which signify benzene like polycyclic aromatic hydrocarbons with two or more rings. On the other hand UV₃₀₀ removal rates are found to be relatively lower than UV₃₆₅ but exhibit higher removal rates than UV₂₈₀.

Although fulvic acids are generally characterized by their low molecular weight and small number of total and aromatic carbons than their humic acid counterparts, unexpectedly, their respective removal rates in terms of the specified UV-vis parameters are found to be relatively lower than the removal rates of humic acids which have longer chain fatty acid products and a higher hydrophobicity than fulvic acids. The structural and compositional characteristics of the samples could possibly affect the photocatalytic degradation pathway leading to various degradation products comprising composite UV-vis properties (Uyguner and Bekbölet, 2005c).

In view of the generally presented optimum loading of 1.0 mg mL⁻¹ of TiO₂, photocatalytic experiments were also carried out under higher photocatalyst conditions (Bekbölet, 1996). Color₄₃₆ degradation rate constants are found to be 4.30x10⁻², 4.19x10⁻², 2.58x10⁻², 1.52x10⁻², and 8.31x10⁻³ for IHSS SHA, AHA, IHSS FA, IHSS HA and RHA, respectively. Following the same decreasing trend, considerably lower UV₂₅₄ rate constants are calculated as 3.25x10⁻², 2.49x10⁻², 1.22x10⁻², 1.08x10⁻² and 6.22x10⁻³ for the photocatalytic degradation of the humic substances. The most prominent effect was observed for IHSS FA with a four fold increase of Color₄₃₆ removal rate. On the other

hand, the effect of increasing the photocatalyst loading by four fold affected the removal rate of IHSS HA by two fold increase which is comparatively less than the other samples investigated. A similar trend was observed for the UV_{254} removal rates. For IHSS FA and IHSS SHA, increasing TiO_2 loading has the effect of increasing UV_{254} removal rates by approximately three fold.

The standard protocol of Serpone (1997) suggests using phenol in photocatalyzed processes as a secondary standard actinometer to standardize process efficiencies of the degradation of various organic substrates for a given set of conditions.

$$\text{Relative photonic efficiency (RPE)} = \frac{\text{Initial rate of disappearance of substrate}}{\text{Initial rate of disappearance of phenol}} \quad (4.3)$$

where both initial rates are obtained under identical conditions.

The photocatalytic conversion of phenol was chosen as the standard process due to the fact that the molecular structure of phenol is present in many organic pollutants and is essentially by oxidation rather than reduction (Serpone, 1997).

Pseudo first order model parameters for the TOC removal of the photocatalytic oxidation of humic substances and their relative photonic efficiencies are presented in a comparative approach with respect to the photocatalytic oxidation of 1×10^{-3} M phenol using 0.25 mg mL^{-1} of Degussa P-25 TiO_2 under same experimental conditions (Table 4.15).

The relative photonic efficiencies calculated for the humic substances from different sources range from 0.257 to 1.738. Due to the lack of the available data relevant to the natural macromolecules, a substance exhibiting similar functional groups such as phenol was selected. Compared to the relative photonic efficiencies of the substituted phenols reported by Serpone (1997) which range from 1.0 ± 0.1 to 3.0 ± 0.2 , relatively lower values were obtained for AHA, RHA and IHSS HA. In accordance with its high TOC removal rate, IHSS SHA displays the highest relative photonic efficiency.

Table 4.15. Relative photonic efficiencies and pseudo first order model parameters for the TOC removal of humic substances

	TOC ₀ , mg L ⁻¹	k, min ⁻¹	t _{1/2} , min	Rate, mg L ⁻¹ min ⁻¹	RPE, ζ
AHA	15.50	9.75x10 ⁻³	71	0.151	0.774
RHA	19.53	3.02x10 ⁻³	230	0.0590	0.302
IHSS FA	20.59	1.07x10 ⁻²	65	0.220	1.128
IHSS HA	16.16	2.65x10 ⁻³	261	0.0501	0.257
IHSS SHA	15.48	2.10x10 ⁻²	33	0.339	1.738

Considering that UV₂₅₄ is generally used as a surrogate parameter for TOC (Najm et al., 1994), the rate constants calculated for TOC removal might be expected to follow the same trend observed for UV₂₅₄. However, UV₂₅₄ rate constants for IHSS FA, IHSS SHA, and RHA are relatively low compared to the TOC rate constants (Table 4.14). Contrary to that, lower values for the rate constants of TOC were obtained for IHSS HA and AHA with respect to the rate constants calculated for UV₂₅₄ removal. For IHSS FA, UV₂₅₄ rate constant is one third of the rate constant calculated for TOC. This indicates that low-molecular-weight and less UV-absorbing compounds are formed.

Due to the surface oriented nature of humic and fulvic acids, preferential adsorption of humic substances onto TiO₂ is a prerequisite to photocatalytic degradation. Upon irradiation through the introduction of light energy to the system, •OH radicals are generated that subsequently attack the surface adsorbed humic moieties initiating the degradation process. It is expected that during photocatalysis, humic and fulvic acids as well as the degradation products exhibit competitive adsorption and desorption phenomena on the surface.

The kinetic evaluation of the degradation profiles with respect to TOC removal (Table 4.15) reveal a rough correlation between the rate constants and the initial adsorption levels which were determined as 6, 7, 15, 30, 47 % for IHSS FA, IHSS HA, RHA, AHA and IHSS SHA, respectively. After 60 minutes of photocatalytic degradation 14.7 % TOC removal was achieved with IHSS HA. The highest TOC removal was achieved with IHSS SHA standard (70.85 %). As verified by the discrepancy between TOC values, the initial

adsorption of IHSS SHA onto TiO_2 is reflected as high removal rates in terms of the specified UV-vis parameters and TOC. However, for the other samples removal rates were not found to be in accordance with the initial adsorption trends. The reason might be explained by the conformational changes present in the adsorbed state of humic and fulvic samples on TiO_2 .

It is known that the degradation rate depends on the capacity of the molecules to adsorb at the catalyst surface. Since the TOC variation depends on the intermediate degradation rate and not on the target compound (humic substance), it seems normal to note a lower TOC removal with respect to irradiation time. Comparison of the results with rate constants calculated for UV_{254} showed that photocatalytic oxidation of IHSS HA and AHA led to a much larger decrease in UV absorbance with respect to the removal of TOC. The decrease of UV-vis absorbance at 254 nm and at 436 nm can be attributed to the degradation of the double bonds and oxidation of reactive chromophoric groups such as $\text{C}=\text{O}$ and $-\text{NO}_2$.

4.2.2. Langmuir-Hinshelwood Kinetic Model

Considering that photocatalysis occurs at the surface, the concentration of substrate adsorbed to the surface directly affects the overall rate of adsorption. Langmuir-Hinshelwood model is widely used to describe the kinetics of semiconductor photocatalysis (Matthews, 1988; Turchi and Ollis, 1989). The rate of degradation of humic substances in terms of Color_{436} , UV_{254} and other UV-vis parameters is expressed in the following empirical type relationship:

$$R_{\text{LH}} = k_{\text{LH}} K_{\text{LH}} A / (1 + K_{\text{LH}} A) \quad (4.4)$$

where the terms represent the following meanings;

R_{LH} : Rate of the reaction ($\text{m}^{-1} \text{min}^{-1}$),

k_{LH} : Reaction rate constant ($\text{m}^{-1} \text{min}^{-1}$)

K_{LH} : Adsorption coefficient of reactant onto TiO_2 under irradiation conditions (m)

A: Initial value of the specified UV-vis parameters of humic substances (m^{-1})

A new series of photocatalytic experiments were designed to assess the Langmuir-Hinshelwood kinetics utilizing 20-50 mg L⁻¹ humic substances in the presence of 0.25 mg mL⁻¹ TiO₂ Degussa P-25 as presented in Table 4.16.

Table 4.16. Langmuir Hinshelwood kinetic parameters of humic substances. Rate was calculated for 50 mg L⁻¹ of each humic substance (k_{LH} , m⁻¹ min⁻¹; K_{LH} , m; Rate, m⁻¹ min⁻¹)

AHA	Color₄₃₆	Color₄₀₀	UV₃₆₅	UV₃₀₀	UV₂₈₀	UV₂₅₄
k_{LH}	0.188	0.251	0.376	0.612	0.627	0.697
K_{LH}	0.584	0.364	0.360	0.157	0.122	0.109
Rate	0.172	0.228	0.351	0.566	0.576	0.642

RHA	Color₄₃₆	Color₄₀₀	UV₃₆₅	UV₃₀₀	UV₂₈₀	UV₂₅₄
k_{LH}	0.0698	0.0920	0.115	0.175	0.195	0.214
K_{LH}	0.217	0.148	0.0936	0.0452	0.0395	0.0332
Rate	0.0623	0.0815	0.100	0.150	0.168	0.184

IHSS FA	Color₄₃₆	Color₄₀₀	UV₃₆₅	UV₃₀₀	UV₂₈₀	UV₂₅₄
k_{LH}	0.0089	0.0209	0.0513	0.0991	0.131	0.189
K_{LH}	0.822	0.487	0.470	0.102	0.0701	0.0523
Rate	0.00742	0.0178	0.0469	0.0857	0.112	0.161

IHSS HA	Color₄₃₆	Color₄₀₀	UV₃₆₅	UV₃₀₀	UV₂₈₀	UV₂₅₄
k_{LH}	0.0903	0.141	0.206	0.366	0.435	0.469
K_{LH}	1.47	0.754	0.337	0.130	0.134	0.0838
Rate	0.0866	0.134	0.193	0.339	0.410	0.434

IHSS SHA	Color₄₃₆	Color₄₀₀	UV₃₆₅	UV₃₀₀	UV₂₈₀	UV₂₅₄
k_{LH}	0.244	0.313	0.399	0.656	0.714	0.805
K_{LH}	0.398	0.387	0.345	0.243	0.120	0.105
Rate	0.224	0.292	0.376	0.626	0.659	0.743

For all of the humic acids, the L-H rate constants increase in the order of Color₄₃₆, Color₄₀₀, UV₃₆₅, UV₃₀₀, UV₂₈₀ and UV₂₅₄ revealing that UV absorbing centers may be removed more selectively than the color forming moieties. However, within the studied sample pool, the ordering of the rate constants follows the general trend IHSS SHA > AHA > IHSS HA > RHA > IHSS FA for all of the parameters. Contrary to the rate constants, the adsorption coefficients display a decreasing trend of Color₄₃₆, Color₄₀₀, UV₃₆₅, UV₃₀₀, UV₂₈₀ and UV₂₅₄ for all of the samples (Uyguner and Bekbölet, 2005c).

A comparison within the samples results in different orders such that; Color₄₃₆ values exhibit a trend as, IHSS HA > IHSS FA > AHA > IHSS SHA > RHA and UV₂₅₄ are ordered as AHA > IHSS SHA > IHSS HA > IHSS FA > RHA. The effect of the model parameters on the overall rate equation is expressed by the L-H rates following the same trend of the L-H rate constants. Considerably higher L-H degradation rates are achieved for UV₂₅₄ than Color₄₃₆ for all of the samples.

The ordering of the L-H rates are similar to that of first order rates however, the calculated L-H rates for the humic acids are approximately half the value of the pseudo first order rates for all of the studied parameters.

4.3. Characterization of Oxidized Humic Substances

4.3.1. UV-vis Spectroscopic Changes of Humic Substances during Photocatalytic Oxidation

In this section, the changes in the UV-vis properties of humic substances during photocatalytic oxidation were analyzed. As a representative to the terrestrial humic substances, time dependent UV-vis spectra of the photocatalytic oxidation of AHA were illustrated in Figure 4.21 in Section 4.2. As an example for the aquatic humic substances, UV-vis spectroscopic changes of IHSS HA are presented with respect to photocatalytic irradiation time (Figure 4.22).

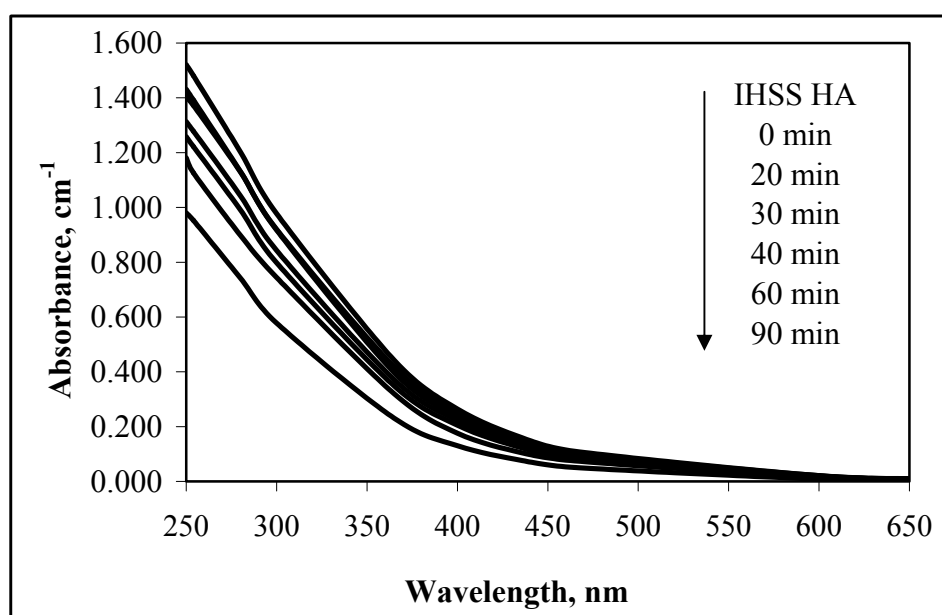


Figure 4.22. UV-vis spectra of the photocatalytic degradation of 50 mg L⁻¹ IHSS HA in the presence of 0.25 mg mL⁻¹

As expected, the UV-vis spectra of humic substances monotonously decrease with increasing wavelength. Displaying the same basic pattern of declining trend, a decrease in the absorbance values was observed with the increasing irradiation time during photocatalytic oxidation. Depending on the source of humic substances, differences are

observed in the UV-vis properties of the oxidized samples as denoted in Table 4.17 in terms of selective parameters as Color_{436} , UV_{365} and UV_{254} .

Table 4.17. Basic spectroscopic characterization of humic substances (50 mg L^{-1}) during photocatalytic oxidation

	$\text{Color}_{436} (\text{m}^{-1})$			$\text{UV}_{365} (\text{m}^{-1})$			$\text{UV}_{254} (\text{m}^{-1})$		
	initial	30 min	60 min	initial	30 min	60 min	initial	30 min	60 min
AHA	18.8	10.9	6.3	39.2	28.1	15.2	108.1	77.8	52.3
RHA	38	33.2	31.6	72.9	65.2	62.1	186.3	173.1	166.2
IHSS FA	5.1	4.0	3.0	19.4	14.9	11.6	91.1	75.5	64.9
IHSS HA	15.9	13.3	10.4	44.3	37.2	32.0	148.1	127.8	112.6
IHSS SHA	28.1	14.7	12.0	48.4	26.2	22.1	114.9	67.4	58.3

Since the removal of organic carbon content was apparently accomplished by the diminishing of UV-vis absorption intensities during the course of the photocatalysis, the interpretation of the specific absorbance parameters such as SUVA_{254} , SUVA_{365} and SCOA_{436} was presented in Table 4.18.

Table 4.18. Specific absorbance ratios of humic substances during photocatalytic oxidation

	SUVA_{254}			SUVA_{365}			SCOA_{436}		
	initial	30 min	60 min	initial	30 min	60 min	initial	30 min	60 min
AHA	7.5	5.9	4.7	2.7	1.8	1.1	1.3	0.7	0.5
RHA	9.5	10.8	10.4	3.7	4.0	3.9	1.9	2.1	2.0
IHSS FA	4.4	6.0	5.9	0.94	1.2	1.1	0.2	0.3	0.3
IHSS HA	7.8	7.3	7.0	2.3	2.1	2.0	0.8	0.8	0.6
IHSS SHA	7.1	10.1	12.4	3.0	3.9	4.7	1.7	2.2	2.5

The initial SUVA_{254} , SUVA_{365} and SCOA_{436} values for Aldrich humic acid were found to be 7.5, 2.7 and 1.3 respectively. Considerably insignificant decrease ($\leq 10\%$) was

observed for all of the specific absorbance parameters after the introduction of TiO_2 . On the other hand, after 30 minutes of irradiation and in the presence of abundant source of organic carbon in the reaction medium, substantial decreases as 21 % SUVA_{254} , 33 % SUVA_{365} and 46 % SCOA_{436} were attained.

SUVA_{254} has been reported to be proportional to both the apparent molecular weight and the aromaticity of humic substances (Edzwald et al., 1985). Compared to that of raw humic acid approximately 37 % decrease of SUVA_{254} and 59 % decrease of SUVA_{365} were achieved for AHA after 60 minutes of photocatalytic degradation revealing lessened degree of aromaticity in relation to the removal of organic carbon. At this stage the reaction medium was composed of unreacted humic acids and the degradation products expressing almost 55 % removal of TOC. Considering relatively higher removal rate of Color_{436} with respect to the removal rates of UV_{254} and UV_{365} the importance of the changes observed in SCOA_{436} would be limited by the irradiation time. Therefore, SCOA_{436} parameter probably would not display a discriminative significance due to the lower Color_{436} attained during extended periods of photodegradation. As verified by the decrease in the specific absorbance values, humic substances are degraded to less UV-vis absorbing compounds through the formation of a series of intermediates and their consequent reactions. The decreasing trend of TOC and the relevant parameters also indicate the mineralization of humic acid to carbon dioxide and water.

Bearing in mind the differences in the chemical and physical properties of humic substances from various origins, each humic substance is expected to follow different photocatalytic degradation mechanisms based on spectroscopic evidence presented in Table 4.14. Hence, it is plausible to observe dissimilar trends for the SUVA/SCOA values calculated for the different photocatalytic irradiation times.

As a representative to the studied humic substances and due to the fact that Aldrich humic acid is widely used in literature, it was selected as the model humic acid and investigated in detail. The selected absorbance ratios of oxidized humic substances as a function of irradiation time were evaluated for Aldrich humic acid and illustrated in Figure 4.23.

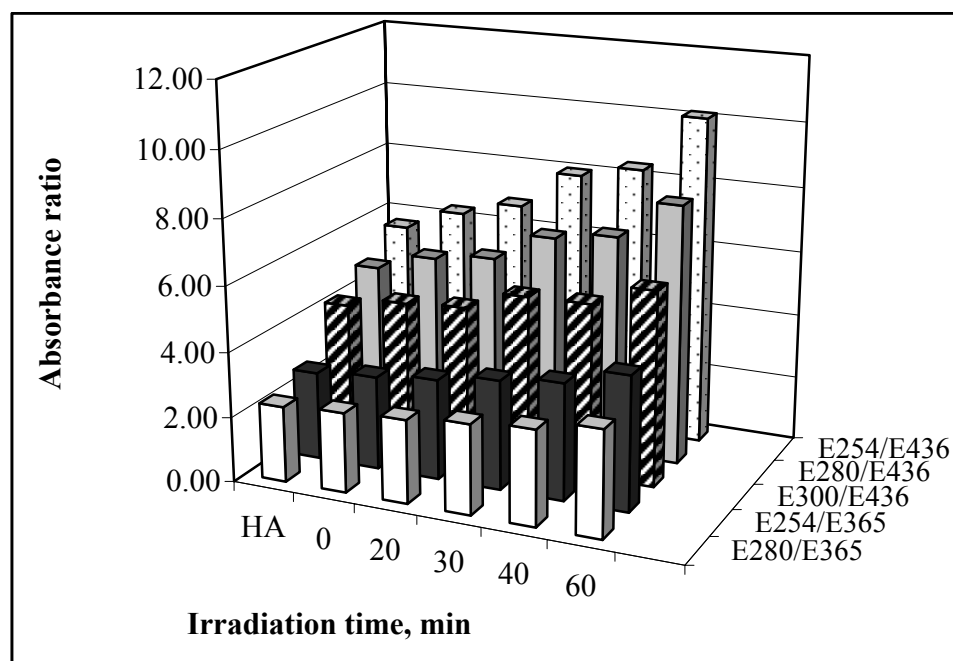


Figure 4.23. UV-vis parameters of Aldrich humic acid as a function of the irradiation time

Irradiation time dependent changes are observed in spectroscopic properties during each stage of humic acid photocatalytic degradation starting with $t=0$ min which is the initial titanium dioxide introduction to humic acid solution in the absence of irradiation. No preadsorption equilibrium was attained prior to photocatalysis and the absorbance ratios were instantaneously measured after TiO_2 addition. This point represents the initial adsorption effect prior to photocatalysis. The values of the absorbance ratios during photocatalytic oxidation are higher than that of raw humic acid gradually increasing as irradiation time increases.

Although the absorbance measurements at wavelengths 254 nm and 280 nm were interchangeably used in literature to represent aromatic content of humic acids, the values for E_{254}/E_{365} deviate from E_{280}/E_{365} ratios with increasing irradiation time by 14 % to 20 % difference denoting the significance of 280 nm as a discrete wavelength.

Dahlen et al., (1996) reported that E_{254}/E_{365} ratio increased from 4.5 to 4.9 and TOC of fulvic acid decreased by 20 % after UV-vis irradiation. Accordingly, in this study E_{254}/E_{365} value for humic acid increased from 2.71 to 3.37 after 30 minutes of photocatalysis with a subsequent 48 % reduction of TOC. The spectroscopic changes

during photocatalysis could be better visualized in terms of quotients calculated with respect to Color_{436} rather than with UV_{365} . The reason may be attributed to the higher removal rate of color forming moieties revealing the quantitative assessment more reliable. Consequently, the ratio of UV_{254} to Color_{436} may represent an alternative indicative parameter for the elucidation of the photocatalytic degradation efficiency.

Concomitantly, the use of similar absorbance ratios was also introduced by several researchers. Eggins et al. (1997) reported that during photocatalytic degradation of humic acid the absorbance ratio 254 nm/400 nm and colour in Hazen units was reduced by 50 % after irradiation for 12 min. However, in their study the possible adsorption of humic acid onto TiO_2 was increased with the equilibration period (30 minutes) that was applied before irradiation. Additionally, instead of 0.45 μm Millipore membrane filters, humic acid was filtered through 0.22 μm filters that would possibly lead to the alteration of the preadsorption and molecular size distribution of the humic acid sample.

Lipski et al. (1999) attributed the increase of absorbance quotient (Q) as $Q_{270/400}$ ratio of humic acids during UV irradiation to the degradation of long-wavelength absorbing chromophores such as quinoid and semiquinoid arrangements, and aromatic and/or heterocyclic zwitteranions i.e. aminochromes. Similarly the observed increase in the ratios of E_{280}/E_{436} or E_{254}/E_{436} might be ascribed to the same reason.

4.3.2. UV-vis Spectroscopic Properties of the Molecular Size Fractions of Partially Oxidized Humic Substances

Humic substances were partially oxidized with respect to their 50 % photocatalytic degradation in terms of Color_{436} so that a significant and meaningful evaluation of the studied parameters after molecular size distribution will still be possible. The related rate constants of photocatalytic oxidation have already been presented in Section 4.2.1. The half life values for Aldrich humic acid, Roth humic acid, IHSS fulvic acid, IHSS humic acid and IHSS soil humic acid correspond to 43 min, 228 min, 96 min, 83 min and 52 min, respectively. The molecular size fractionation of the partially oxidized humic substances were performed using ultrafiltration stirred cell by filtering through membranes with pore sizes in the range of 100 to 1 kDa. The UV-vis spectroscopic properties of each size

fraction were presented in Table 4.19, Table 4.20, Table 4.21, Table 4.22 in comparison to the data presented for raw humic substances in Section 4.1.2.

Table 4.19. Absorbance ratios for the molecular size fractions of partially oxidized Aldrich humic acid

	Absorbance ratios of AHA					
	E₂₅₀/E₃₆₅	E₂₅₄/E₃₆₅	E₂₈₀/E₃₆₅	E₂₅₄/E₄₃₆	E₃₀₀/E₄₀₀	E₃₀₀/E₄₃₆
0.45 μ m	3.41	3.34	2.77	8.02	3.55	5.41
100 kDa	3.76	3.70	3.03	9.47	3.78	5.98
30 kDa	4.13	4.03	3.23	11.2	4.17	7.00
10 kDa	4.83	4.75	3.75	19.0	4.86	11.33
1 kDa	5.57	5.43	4.00	19.0	5.00	10.0
AHA	2.78	2.73	2.34	5.74	2.92	4.18

Ratios for the molecular size fractions of Aldrich humic acid follow a generally increasing trend with decreasing molecular size. It can be seen from the table that, the lowest values were attained for the absorbance ratios of E₂₈₀/E₃₆₅ for all of the calculated molecular size fractions. On the other hand, E₂₅₄/E₄₃₆ exhibited comparatively higher values.

Table 4.20. Absorbance ratios for the molecular size fractions of partially oxidized Roth humic acid

	Absorbance ratios of RHA					
	E₂₅₀/E₃₆₅	E₂₅₄/E₃₆₅	E₂₈₀/E₃₆₅	E₂₅₄/E₄₃₆	E₃₀₀/E₄₀₀	E₃₀₀/E₄₃₆
0.45 μ m	3.08	3.02	2.53	6.18	3.01	4.29
100 kDa	3.43	3.35	2.74	7.33	3.26	4.79
30 kDa	3.99	3.88	3.13	9.70	3.88	6.09
10 kDa	4.85	4.74	3.85	12.80	4.75	7.60
1 kDa	5.31	5.06	4.00	16.20	5.22	9.40
RHA	2.63	2.58	2.21	4.99	2.61	3.63

Table 4.21. Absorbance ratios for the molecular size fractions of partially oxidized IHSS fulvic acid

	Absorbance ratios of IHSS FA					
	E₂₅₀/E₃₆₅	E₂₅₄/E₃₆₅	E₂₈₀/E₃₆₅	E₂₅₄/E₄₃₆	E₃₀₀/E₄₀₀	E₃₀₀/E₄₃₆
0.45 μ m	5.48	5.30	4.01	19.27	5.61	10.55
100 kDa	5.90	5.60	4.13	20.57	5.83	11.58
30 kDa	6.19	5.99	4.40	23.96	6.33	12.67
10 kDa	6.57	6.39	4.74	29.40	6.91	15.20
1 kDa	7.50	7.00	5.25	28.00	7.00	14.00
IHSS FA	4.96	4.77	3.58	17.56	5.24	10.03

Depending on the structural properties of IHSS FA, the absorbance ratios of the molecular size fractions are considerably higher than the ratios of other humic substances. The effect of oxidation on the molecular size fractions of aquatic fulvic acid is reflected as the increase of the E₂₅₄/E₄₃₆ and E₃₀₀/E₄₃₆ absorbance ratios for the 10 kDa fraction.

Table 4.22. Absorbance ratios for the molecular size fractions of partially oxidized IHSS humic acid

	Absorbance ratios of IHSS HA					
	E₂₅₀/E₃₆₅	E₂₅₄/E₃₆₅	E₂₈₀/E₃₆₅	E₂₅₄/E₄₃₆	E₃₀₀/E₄₀₀	E₃₀₀/E₄₃₆
0.45 μ m	4.24	4.13	3.21	12.54	4.45	7.61
100 kDa	4.20	4.11	3.19	12.54	4.48	7.61
30 kDa	4.17	4.04	3.18	12.71	4.39	7.77
10 kDa	4.85	4.69	3.62	15.25	5.05	8.83
1 kDa	6.14	6.00	4.43	14.00	5.50	7.33
IHSS HA	5.10	3.45	2.80	9.31	3.56	5.91

For the aquatic origin IHSS HA the effect of photocatalysis on the 10 kDa fraction is observed as an increase of the absorbance ratio for E₂₅₄/E₄₃₆ and E₃₀₀/E₄₃₆. The similar result has been observed for the IHSS FA. Hence, molecular size specific distribution of the absorbance ratios could be assessed for the oxidized fractions of aquatic humic substances.

Table 4.23. Absorbance ratios for the molecular size fractions of partially oxidized IHSS soil humic acid

	Absorbance ratios of IHSS SHA					
	E₂₅₀/E₃₆₅	E₂₅₄/E₃₆₅	E₂₈₀/E₃₆₅	E₂₅₄/E₄₃₆	E₃₀₀/E₄₀₀	E₃₀₀/E₄₃₆
0.45 μ m	2.98	2.92	2.50	5.66	3.01	4.08
100 kDa	3.65	3.56	2.91	7.63	3.50	4.95
30 kDa	4.34	4.21	3.32	11.0	4.21	6.78
10 kDa	5.53	5.33	4.13	16.00	5.88	9.40
1 kDa	8.67	8.00	5.67	24.0	12.0	12.0
IHSS SHA	2.40	2.37	2.05	4.09	2.37	3.05

It is known that the molecular size distribution profiles of the humic substances exhibit a general decreasing trend from high molecular size fractions to the lower molecular size fractions irrespective of the origin and source of the organic matter (Table 4.19-Table 4.23). As a general trend, the absorbance ratios of the partially oxidized humic substances also follow the same pattern of increasing absorbance ratios with respect to decreasing molecular size fractions. Similar results were also reported in literature for humic substances (Peuravuori and Pihlaja, 1997). Substantial differences among the fractions of different molecular sizes point out the importance of characterizing the size fractions properly to better understand the spectroscopic properties of oxidized humic substances. The structural as well as the conformational changes occurring in humic substances would be better assessed in view of the absorbance ratios.

As a representative to the other absorbance ratios already presented, a comparative elucidation of the E₂₅₄/E₄₃₆ ratio of raw and oxidized humic substances with respect to molecular size fractions are given in Figure 4.24.

The molecular size fractions of partially oxidized AHA exhibit higher E₂₅₄/E₄₃₆ values than raw humic acid in the range of 450-1 kDa. Only for the small size fractions that are < 1 kDa, raw IHSS HA and raw AHA display higher E₂₅₄/E₄₃₆ ratios compared to the corresponding oxidized fraction.

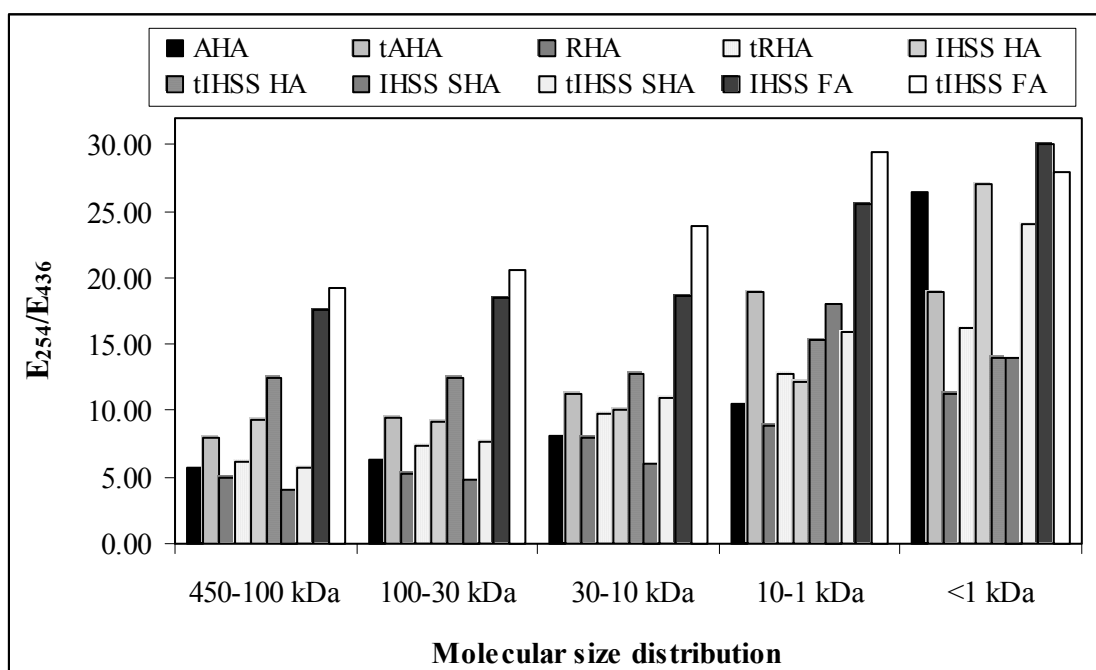


Figure 4.24. E_{254}/E_{436} absorbance ratios of the molecular size fractions of raw and treated humic substances. (i.e. AHA represents raw Aldrich humic acid, tAHA represents oxidized humic acid)

As confirmed with the photocatalytic degradation rate constants presented in Table 4.14, the removal efficiencies of UV absorbing centers could be ascribed as relatively slower with respect to the color forming moieties. Hence, the decrease in Color_{436} and the increase in UV_{254} values during the photocatalytic degradation time course can not compensate for the generally increasing trend of E_{254}/E_{436} ratios. Such a pattern suggests a higher degree of aromaticity of the highest size fractions of humic substances.

In general, noteworthy changes in molecular size distribution are recorded during water treatment by physicochemical methods, namely coagulation–flocculation, and by oxidation methods either for disinfection purposes or for the removal of natural organic matter by advanced oxidation processes. The formation of lower molecular size fractions is followed by UV_{254} removal efficiency. Hence, it seems probable to monitor the removal of natural organic matter during oxidation processes by the changes in the E_{254}/E_{436} quotient considering that the preferential removal of color-forming moieties leads to residual color above detection limits. Consequently, the use of E_{254}/E_{436} is recommended as a tool for the assessment of structural changes of NOM during treatment relevant to the removal

efficiency under conditions of incomplete destruction of color. On the other hand, for the size fractions lower than 10 kDa, differences could still be observed in terms of E_{250}/E_{365} , E_{254}/E_{365} and E_{280}/E_{365} ratios with respect to each molecular size fraction. Under conditions of total decolorization, E_{250}/E_{365} , E_{254}/E_{365} and E_{280}/E_{365} ratios could also be used relevant to the degradation of humic substances.

The molecular size distribution data of raw humic substances in terms of $SUVA_{254}$ and $SUVA_{365}$ values were presented in Section 4.1.2, Figure 4.3 and Figure 4.4, respectively. Consequently, the molecular size distribution data of oxidized humic acids with respect to the specific absorbance parameter as $SUVA_{254}$ and $SUVA_{365}$ values are illustrated in Figure 4.25 and Figure 4.26, respectively.

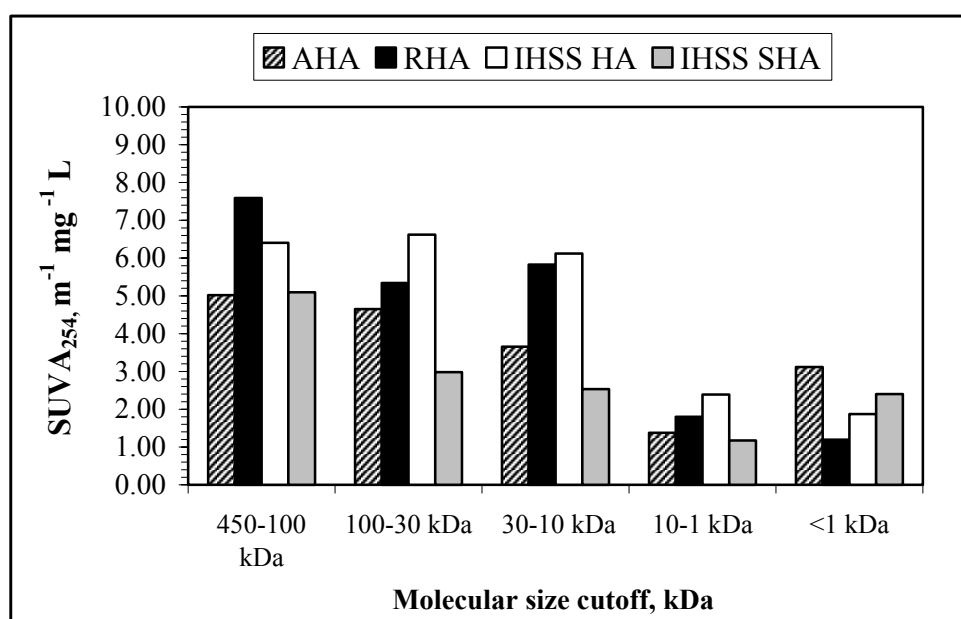


Figure 4.25. $SUVA_{254}$ values for partially oxidized humic substances with respect to molecular size distribution.

As a general trend for all of the humic acids investigated, $SUVA_{254}$ and $SUVA_{365}$ values decrease after photocatalytic treatment indicating that the degradation efficiency is irrespective of the molecular size fractions (Figure 4.25, Figure 4.26). For AHA, an overall increase is observed for the size fractions < 1 kDa.

With the effect of oxidation, shift of $SUVA_{254}$ to lower values is observed. In accordance with the presented results of photocatalytic degradation kinetics, the most pronounced effect is observed on IHSS SHA and AHA.

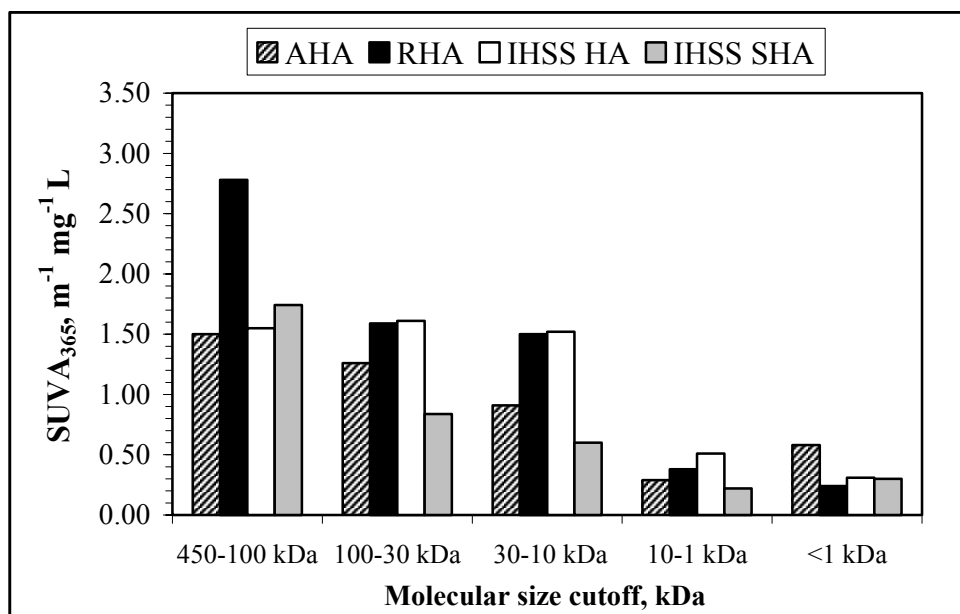


Figure 4.26. $SUVA_{365}$ values for partially oxidized humic substances with respect to molecular size distribution

In terms of the specified $SUVA$ parameters, the primary effect of photocatalytic oxidation was observed in the 450-100 kDa fraction for all of the humic acids except for RHA. The probable resistance displayed by the 450-100 kDa fraction of RHA to photocatalytic degradation could also be explained by the formation of high molecular weight fractions (450-100 kDa) via the combination of the photoproducts originating from lower molecular size fractions (Uyguner and Bekbölet, 2005c). The removal of $SUVA_{365}$ for AHA and IHSS SHA are more likely in accordance with their high photocatalytic removal rates.

For comparative purposes, $SUVA_{254}$ and $SUVA_{365}$ values of raw and oxidized Aldrich humic acid as a representative to terrestrial humic substances and IHSS humic acid as a representative to aquatic humic substances compiled from Figure 4.3 and Figure 4.4 were presented in Figure 4.27 and Figure 4.28.

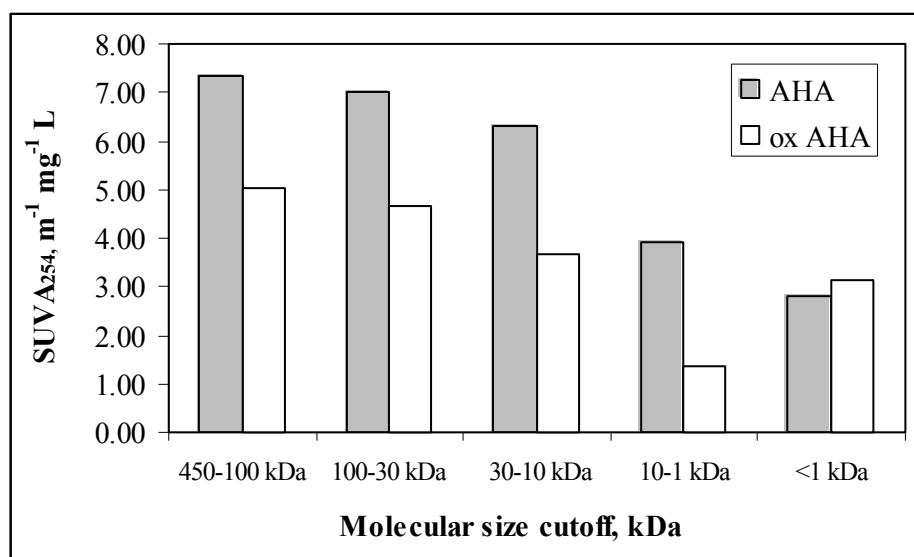


Figure 4.27. SUVA₂₅₄ values for raw and oxidized AHA with respect to molecular size distribution.

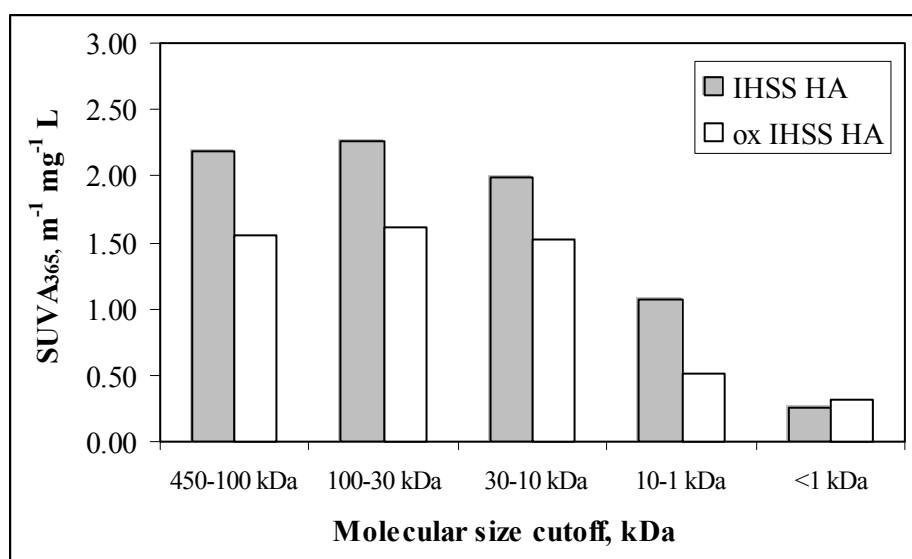


Figure 4.28. SUVA₃₆₅ values for raw and oxidized IHSS HA with respect to molecular size distribution.

A slight change is observed for SUVA₂₅₄ of IHSS HA irrespective of the size fraction up to 10 kDa. IHSS HA and RHA retained a hydrophobic character up to the 10 kDa size fraction whereas AHA displayed more hydrophilic character for size fractions smaller than 30 kDa. IHSS SHA expressed quite different photocatalytic reactivity towards

the formation of lower molecular size fractions that could be considered as possessing more hydrophilic properties since $SUVA_{254}$ values are found to be between 2 to 3. For AHA and IHSS SHA samples, increased $SUVA_{254}$ values are noticed for smallest size fractions. The size fractions < 1 kDa for IHSS SHA exhibits a two fold increase in $SUVA_{254}$ relative to that of the 10 kDa fraction. Oxidation reactions taking place during photocatalysis may lead to a shift as an increase in lower molecular size fractions that is apparently observed in the case of IHSS SHA and AHA. This could be explained by relatively high pseudo-first-order and L-H UV_{254} , UV_{365} degradation rates as well as the TOC removal rates of the IHSS SHA and AHA (Table 4.14 and Table 4.15). Similarly, an increase in the percentage of smallest molecules (500-300 Da) of natural organic matter fractions after ozonation and oxidation with chlorine dioxide was reported (Swietlik et al., 2004).

4.3.3. Fluorescence Spectroscopic Changes of Humic Substances during Photocatalytic Oxidation

In this section fluorescence changes of humic substances during photocatalytic oxidation were analyzed. As a representative to the studied humic substances, both the emission and synchronous scan spectra of Aldrich humic acid were investigated in detail. Aldrich humic acid was selected as the model humic acid due its availability and wide use in literature and in order to be comparable with recent studies (Eggins et al., 1997; Cho and Choi, 2002; Wiszniowski et al., 2004; Bekbölet et al., 2002; Uyguner and Bekbölet, 2004a). The related UV-vis spectroscopic properties and the photocatalytic degradation kinetics of Aldrich humic acid have been explained in Section 4.2.1.1 and 4.2.3. Due to the fact that the emission scan spectra do not reveal significant differences, only the synchronous scan spectra were presented for the other humic substances.

4.3.3.1. Fluorescence Spectra of Aldrich Humic Acid. Fluorescence is a typical property of humic and fulvic acids (Senesi, 1990). The emission scan spectra of Aldrich humic acid were presented with respect to photocatalytic irradiation time (Figure 4.29).

The emission scan spectra of oxidized humic acids are commonly broad and featureless. A gradual decrease in fluorescence intensity is observed with increasing

photocatalytic irradiation time (Figure 4.29). The respective broad emission maximum for raw humic acid is found to be approximately around 450 nm. After 60 minutes of irradiation the characteristic peak of the spectrum completely disappears.

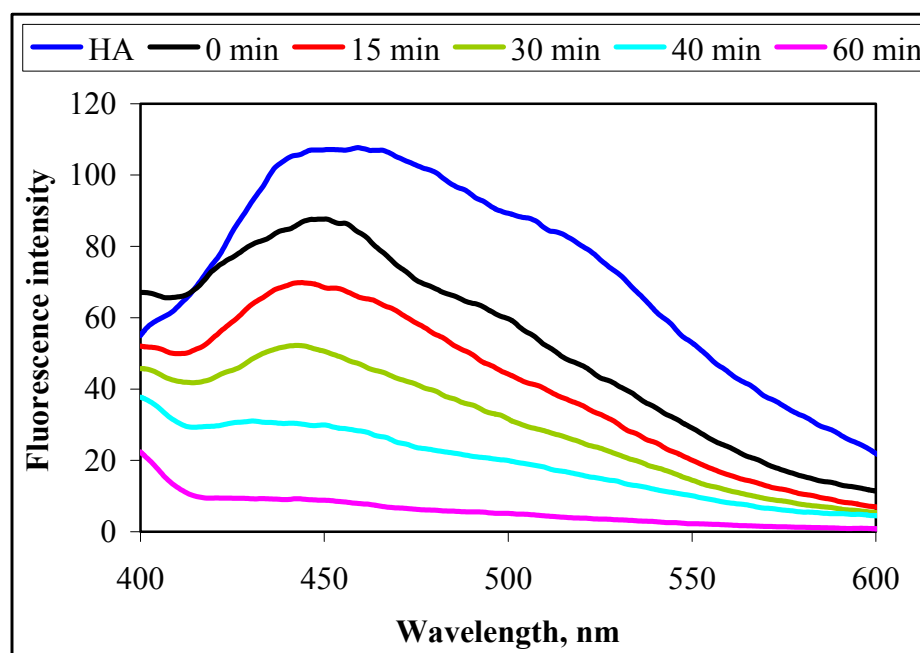


Figure 4.29. Emission scan spectra of Aldrich humic acid (50 mg L^{-1}) with respect to irradiation time. HA represents raw humic acid.

In a recent study by Cho and Choi, (2002) the emission spectra of the photocatalytic degradation of a humic acid solution with a lower concentration (10 mg L^{-1}) and higher TiO_2 loading (0.5 mg mL^{-1}) was presented. The similar declining trend of the fluorescence intensities was observed. In particular, the fluorescence intensity was drastically quenched within 1 h irradiation. Since the fluorescence is largely due to $\pi \rightarrow \pi^*$ transitions in humic molecules, its rapid extinction under UV irradiation was ascribed to the destruction of aromatic structures of humic acid by the nonselective action of hydroxyl radicals generated on UV-illuminated TiO_2 . The loss of fluorescence intensity could also be related to the loss of organic carbon thereby to the degree of mineralization.

However, due to overlapping and peak broadening linked to the multicomponent nature of humic acids, straightforward identification and interpretation of fluorescence signatures in emission spectroscopy was found difficult (Chen et al., 2002).

The synchronous scan fluorescence spectroscopy is more structured and has been extensively used for the characterization of humic substances (Senesi, 1990; Pullin and Cabaniss, 1995; Santos et al., 2001; Peuravuori et al., 2002). Synchronous scan excitation spectra not only offer a potential use to reduce overlapping interferences but also provide a possibility for each fluorescent component to be identified in a spectral range.

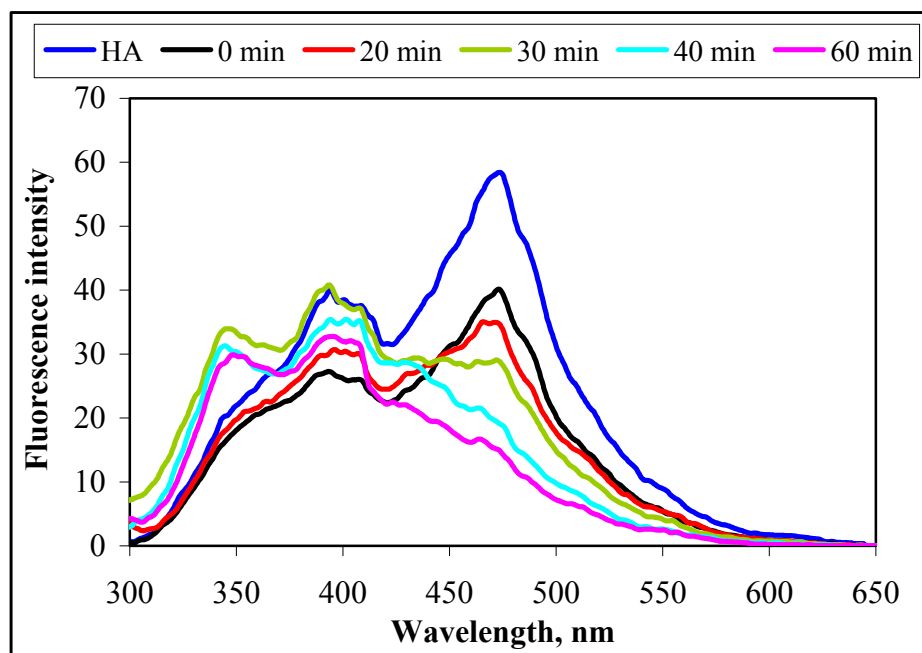


Figure 4.30. Synchronous scan excitation spectra of photocatalytically oxidized Aldrich humic acid in relation to the irradiation time. HA represents raw humic acid.

The spectra of all oxidatively treated humic substances are characterized by broad peaks the intensities of which relatively decrease with increasing photocatalytic irradiation time (Figure 4.30). Raw humic acid has a sharp peak around 473 nm, a moderate peak around 400 nm and a minor peak around 340 nm. Peuravuori et al., (2002) ascribe the maximum around 460 nm to more complex systems such as polycyclic aromatics consisting about seven fused benzene rings.

It is noteworthy that the characteristic peak of humic acid is quenched even with the sole action of TiO_2 without the effect of light ($t=0$ min). The significant decrease of the sharp peak of humic acid at $t=0$ min is accompanied by approximately 30 % TOC removal. This indicates the importance of adsorption of humic acid onto TiO_2 taking into

consideration that the carboxylic functional groups of humic acids attach to the TiO_2 surface (Tunési and Anderson, 1991).

After 60 minutes of irradiation, the characteristic sharp peak of raw humic acid completely disappears and the moderate peak around 400 nm decays in intensity. Since humic acid contains a variety of aromatic moieties and carboxylic groups, the molecular and structural characters within the molecule may be substantially changed as a result of $\text{HO}\cdot$ attack via photocatalytic oxidation. The train-loops attachment and folding of humic acid on TiO_2 might also lead to the masking of the fluorescent moieties in humic acid structure (Uyguner and Bekbölet, 2004b). Similar results were presented in literature and the rapid extinction of the fluorescence intensity of humic acid during photocatalysis was ascribed to the destruction of aromatic structures of humic acid (Cho and Choi, 2002). The findings of Carvalho et al. (2004) and Korshin et al. (1999) also indicate a reduction of fluorescence intensity upon chlorination. It was reported that in large humic molecules, intrafluorophore energy transfer shortens the apparent decay lifetimes of the fluorophores whereas in smaller molecules, lifetimes of the fluorophores increase due to weakening of excitation energy dissipation. Since the photocatalytic oxidation decrease the size of humic acid molecule and destroys some of the aromatic chromophores involved in the energy transfer, the decrease of fluorescence intensity in oxidized humic acid samples correlates well with this hypothesis.

Conversely, increasing relative fluorescence intensity after oxidative treatment via chlorination, ozonation and UV photolysis of humic substances has also been reported in literature (Korshin et al., 1999; Win et al., 2000). Eggins et al. (1997) stated that humic acids are photocatalytically degraded to lower molecular weight compounds and the intermediates of the reaction can be highly fluorescent. However, considering the diverse reaction mechanisms and different experimental conditions, contrary results would also be plausible. Another major point would be the source of humic substance reflecting the structural diversity.

The UV-vis spectra of light absorption of humic acid assumed the same basic pattern of monotonous decline with the increase of wavelength regardless of the oxidative treatment methods (Kerç et al., 2004; Uyguner and Bekbölet, 2004a). Supported by the

decrease in UV-vis parameters and the corresponding TOC values, the decrease in fluorescence intensities also point out the degradation of the humic acid and the formation of simpler degradation by-products.

In order to present the fluorescence spectra on a comparative basis, the fluorescence intensities were normalized with respect to the corresponding TOC values.

TOC normalized synchronous scan spectra of 50 mg L⁻¹ Aldrich HA that has been photocatalytically treated using 0.25 mg mL⁻¹ Degussa P-25 as function of irradiation time are given in Figure 4.31.

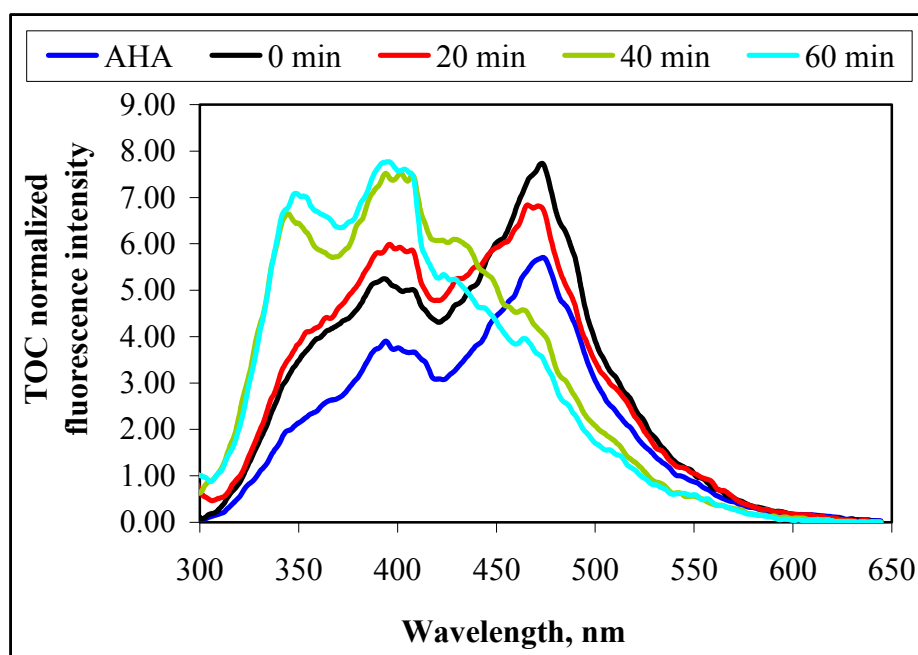


Figure 4.31. TOC normalized synchronous scan fluorescence spectra of AHA during photocatalytic oxidation.

The related rate constants of the reaction ($1.03 \times 10^{-2} \text{ min}^{-1}$ and $9.75 \times 10^{-3} \text{ min}^{-1}$ in terms of UV_{254} and TOC removal) have already been discussed in Section 2.2.1. Considering the degradation of the humic acid molecule and the subsequent decline of the TOC values, the specific fluorescence intensities during photocatalysis exhibit different trends. During the course of photocatalysis, the blue shift of the spectra is observed with a gradual increase in fluorescence intensity per unit carbon. After 60 minutes the

fluorescence maxima shift to lower wavelength region. Two moderate peaks around 400 nm and 340 nm are observed. Patel-Sorrentino et al. (2003) reported the initial increase of fluorescence intensity after solar UV irradiation of natural organic matter. The subsequent enhancement was attributed to the change of the macromolecule structure from a spherocollidal to a linear conformation. In relation to that explanation, the photocatalytic degradation of the humic acid molecule with subsequent structural change can be considered. The oxidation of the macromolecule probably leads to the formation of intermediate degradation products that have higher relative fluorescence properties.

4.3.3.2. Fluorescence Spectra of Roth Humic Acid. The TOC normalized synchronous scan spectra of the photocatalytic oxidation of RHA is presented in Figure 4.32.

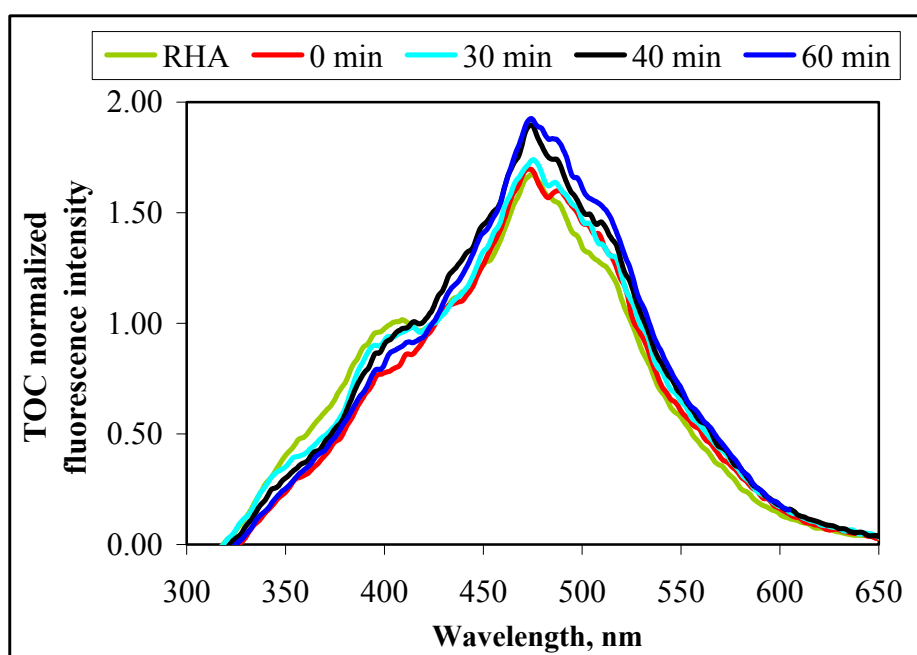


Figure 4.32. TOC normalized synchronous scan fluorescence spectra of RHA during photocatalytic oxidation

As confirmed by the slow initial degradation rate of Roth humic acid in terms of both pseudo first order and Langmuir-Hinshelwood kinetic models, no significant change in the fluorescence spectra of RHA was observed. With increased photocatalytic irradiation time, the fluorescence intensities slightly increase. However, the spectra keep the same

characteristic peak around 460 nm. This might be due to the slow oxidative removal of humic acid and related structural changes that occurs during photocatalytic degradation.

4.3.3.3. Fluorescence Spectra of IHSS Fulvic Acid. The TOC normalized synchronous scan spectra of 50 mg L⁻¹ IHSS FA that has been photocatalytically oxidized using 0.25 mg mL⁻¹ TiO₂ Degussa P-25 as a function of irradiation time is presented in Figure 4.33.

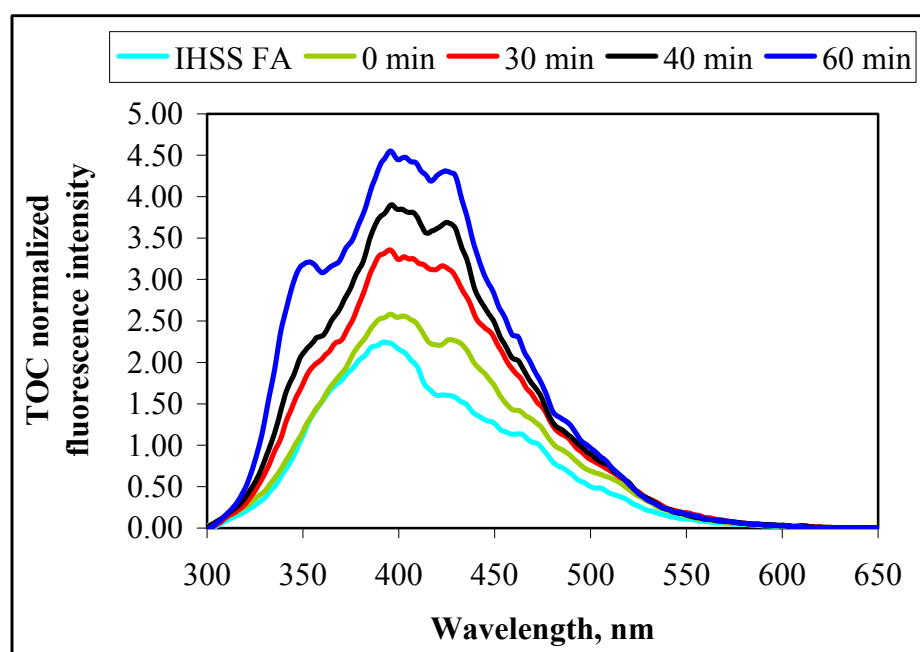


Figure 4.33. TOC normalized synchronous scan fluorescence spectra of IHSS FA during photocatalytic oxidation.

TOC normalized fluorescence intensity of IHSS fulvic acid increases with the increasing irradiation time. After 60 minutes of photocatalytic oxidation a minor peak formation around 350 nm is observed. Similar results were presented in literature for fulvic acid oxidation (Gül, 2002). The fluorescence intensities of an ozonated (18.94 % and 51.52 % for 5 min and 20 min ozonation times) aquatic fulvic acid sample were reported to exhibit an increase of the fluorescence intensities for both ozonation times (Gül, 2002). The increase of fluorescence of ozonated samples was attributed to the association of the fluorescent structures, which may be produced by the partial oxidation of nonfluorescent chromophores with ozone.

4.3.3.4. Fluorescence Spectra of IHSS Humic Acid. The TOC normalized synchronous scan spectra of 50 mg L⁻¹ IHSS humic acid that has been photocatalytically oxidized using 0.25 mg mL⁻¹ of TiO₂ Degussa P-25 as a function of irradiation time is presented in Figure 4.34.

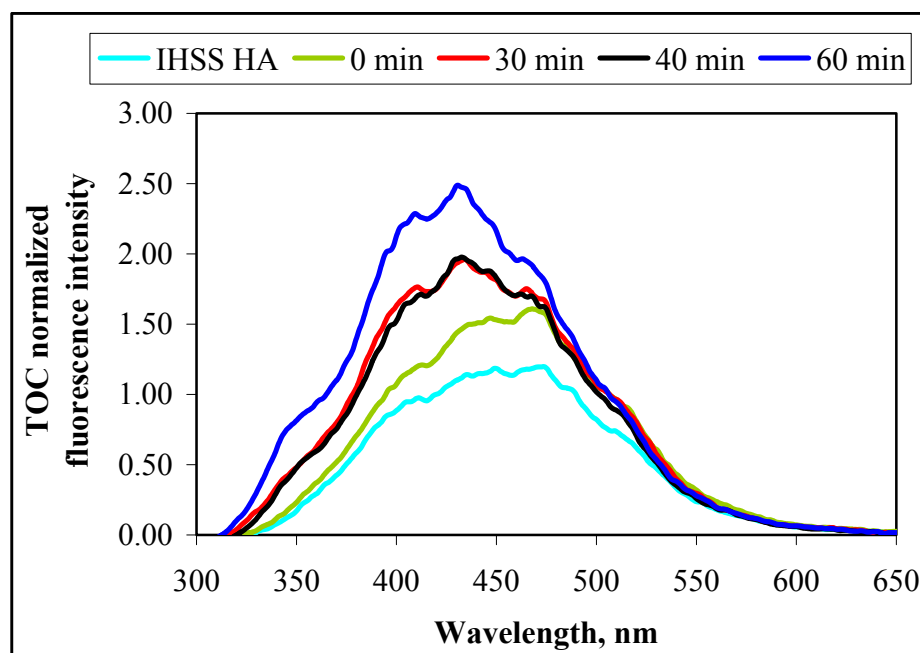


Figure 4.34. TOC normalized synchronous scan fluorescence spectra of IHSS HA during photocatalytic oxidation.

TOC normalized fluorescence intensity of IHSS humic acid increases with the increasing photocatalytic irradiation time. The broad peak of unoxidized IHSS humic acid resides in the range of 400-500 nm. After photocatalytic irradiation for 60 minutes, the blue shift of the fluorescence spectra to lower wavelength region is observed with the formation of a narrower peak that has an emission maxima at 430 nm.

4.3.3.5. Fluorescence Spectra of IHSS Soil Humic Acid. The TOC normalized synchronous scan spectra of 50 mg L⁻¹ IHSS soil humic acid that has been photocatalytically oxidized using 0.25 mg mL⁻¹ TiO₂ Degussa P-25 as a function of the irradiation time is illustrated in Figure 4.35.

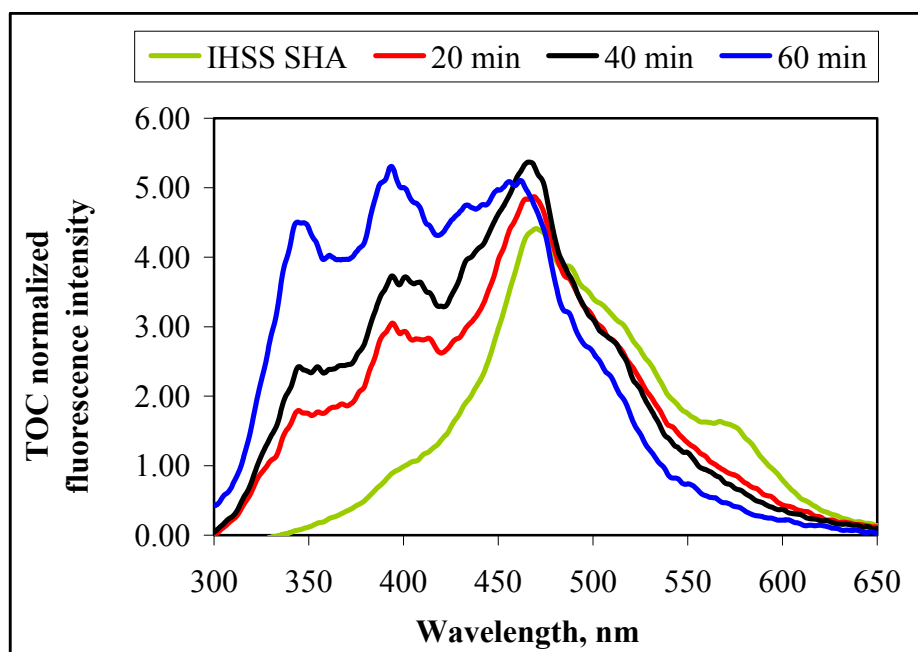


Figure 4.35. TOC normalized synchronous scan fluorescence spectra of IHSS SHA during photocatalytic oxidation.

Patel-Sorrentino et al. (2003) reported an initial increase of fluorescence intensity after solar UV irradiation of natural organic matter for a short time. The subsequent enhancement was attributed to the change of the macromolecule structure from a spherocollodial to a linear conformation exhibiting different fluorophores. After longer irradiation times, a decrease in fluorescence intensity was also observed. Photodegradation of humic substances causes drastic changes in the UV-vis absorption and fluorescence properties of humic acids. In order to fulfill the lack of knowledge about the spectral changes of humic acids during photocatalytic oxidation process and elucidate the effects observed on the molecular size distribution of humic acid, each molecular size fraction of humic substances was analyzed by UV-vis and fluorescence spectroscopy.

4.3.4. Fluorescence Spectroscopic Properties of the Molecular Size Fractions of Partially Oxidized Humic Substances

Aqueous solutions of humic substances are generally polydisperse, with size ranges differing according to their origins, thereby strongly affecting treatment efficiencies. The

oxidative behavior of humic substances towards photocatalytic degradation could also be evaluated by the use of molecular size distribution profiles.

Molecular size fractionation of the photocatalytically treated humic substances (50 % degradation with respect to Color_{436} according to rate constants given in Table 4.14) was performed by ultrafiltration through a sequence of membranes of decreasing pore sizes in the range of 100 to 1 kDa. After partial oxidation significant changes in molecular size distribution in all size fractions were noted in the synchronous scan fluorescence spectra of humic substances.

4.3.4.1. Fluorescence Spectra of the Molecular Size Fractions of Partially Oxidized Aldrich Humic Acid. The fluorescence spectra of the molecular size fractions of oxidized Aldrich humic acid are presented in Figure 4.36.

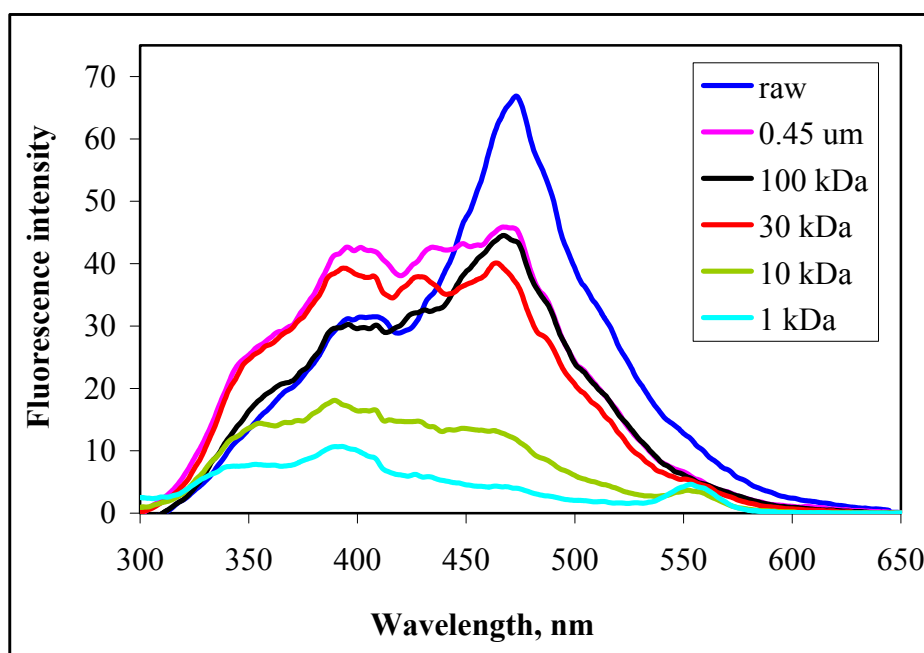


Figure 4.36. Synchronous scan spectra of the molecular size fractions of photocatalytically oxidized AHA

Compared to raw humic acid fractions shown in Figure 4.12, oxidation leads to changes both in terms of structural properties as well as the fluorescence properties in the molecular size fractions of AHA. The most significant difference is the shift of the

fluorescence peaks of oxidized size fractions to lower wavelength region. Furthermore, for the oxidized fractions, the decay of the characteristic peak of humic acid (at 470 nm) is more apparent than the decrease observed for the fractions of raw samples. The diminishing of the fluorescence intensity is more specific for small molecular size fractions.

Another observable difference is the peak broadening in the 350-470 nm region for the oxidized size fractions in the range of 0.45 μm to 30 kDa. This is indicative to the oxidation of fractions that are of higher molecular size. The size fraction < 1 kDa is higher in intensity than the corresponding size fraction of raw humic acid fraction.

4.3.4.2. Fluorescence Spectra of the Molecular Size Fractions of Partially Oxidized Roth Humic Acid. The molecular size fractions and their fluorescence properties of oxidized Roth humic acid are illustrated in Figure 4.37.

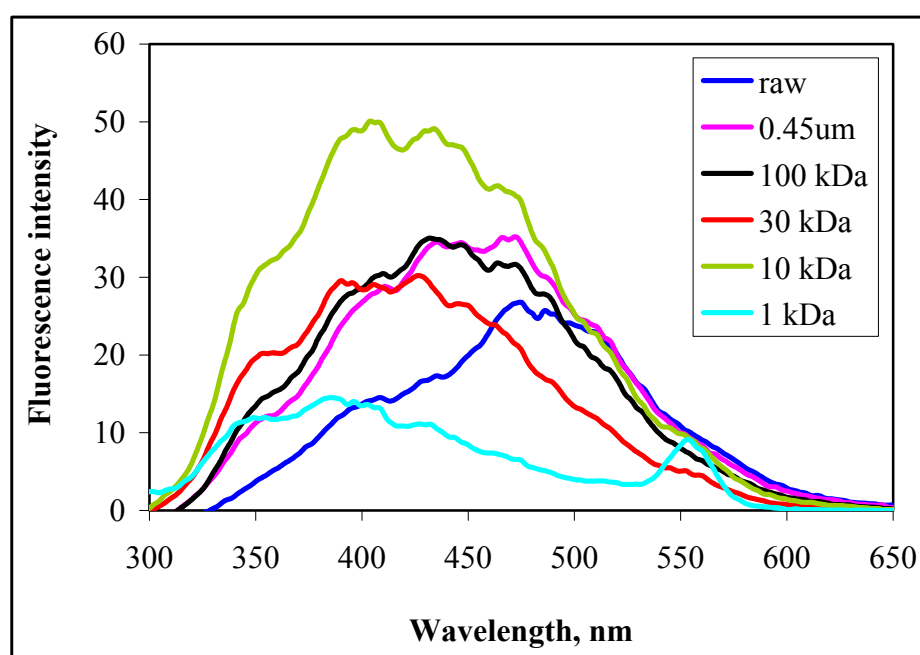


Figure 4.37. Synchronous scan spectra of the molecular size fractions of photocatalytically oxidized RHA.

Roth humic acid exhibits structurally diverse features in both untreated and oxidized samples. The shape of the spectra reveals a continuum of fluorophores with the

exception of separate peaks. Compared to raw molecular size fractions of RHA presented in Figure 4.14, slight shift of the emission maxima to blue region is observed for the oxidized fractions of RHA. The size fractions > 100 kDa exhibit similar fluorescence properties. This indicates that the structure has not been degraded to different structural components, the UV-vis spectroscopic properties and the slow degradation kinetics of Roth HA also confirms with this result.

4.3.4.3. Fluorescence Spectra of the Molecular Size Fractions of Partially Oxidized IHSS Fulvic Acid. The fluorescence spectra of the molecular size fractions of oxidized IHSS fulvic acid is presented in Figure 4.38.

The effect of oxidation on the molecular size fractions of fulvic acid could easily be observed by comparing the fractions of raw fulvic acid (Figure 4.16) with the spectra presented in Figure 4.38.

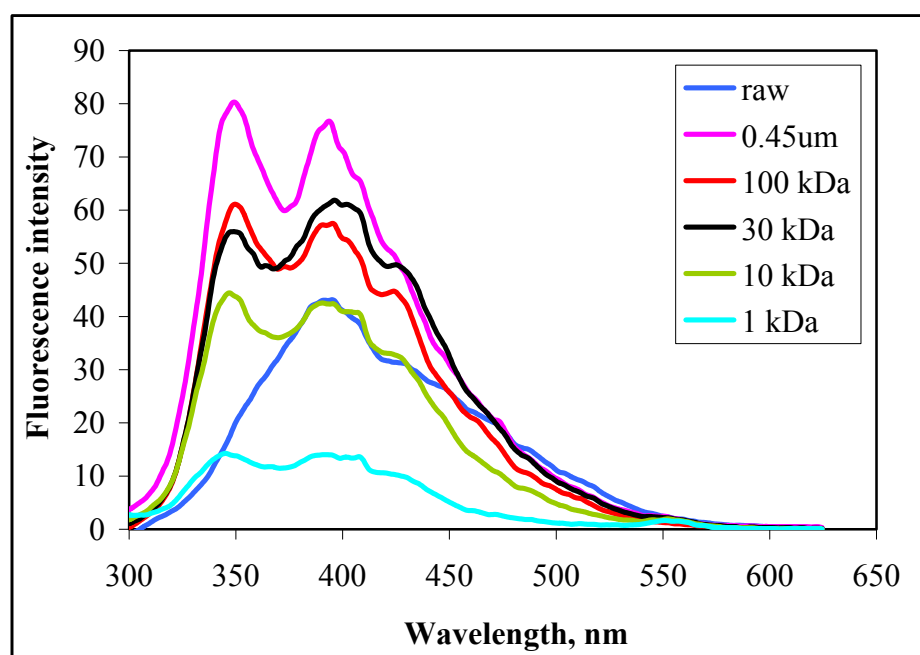


Figure 4.38. Synchronous scan spectra of the molecular size fractions of photocatalytically oxidized IHSS FA.

As a general trend, oxidation leads to a shift of the fluorescence intensity to lower wavelengths. An initial increase with two peak formation is evident with the effect of

partial oxidation. After partial oxidation, in addition to the presence of peak maxima at 400 nm, the formation of another peak around 350 nm is observed. The fluorescence intensity decreases up to 10 kDa keeping the twin peak shape of the spectra.

4.3.4.4. Fluorescence Spectra of the Molecular Size Fractions of Partially Oxidized IHSS Humic Acid. The synchronous scan fluorescence spectra of the molecular size fractions of oxidized IHSS HA is presented in Figure 4.39.

With the effect of oxidation, the broad peak of IHSS HA gets narrower, increases in intensity and slightly shifts to shorter wavelengths around 440 nm. The fractions of 0.45 μm , 100 kDa and 30 kDa have similar fluorescence properties. Further decrease of molecular size leads to subsequent quenching of fluorescence intensity.

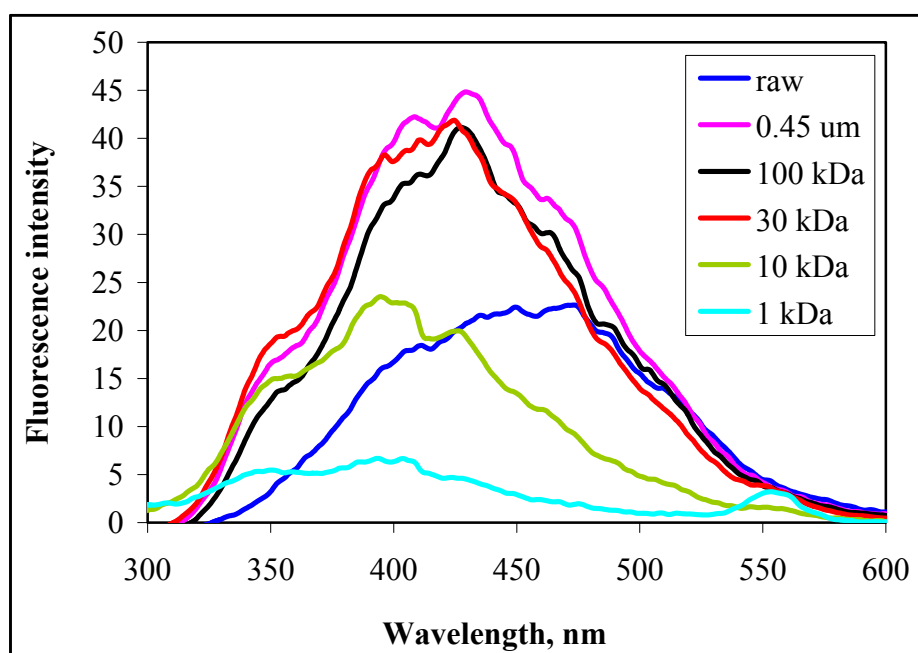


Figure 4.39. Synchronous scan spectra of the molecular size fractions of photocatalytically oxidized IHSS HA.

With the effect of oxidation, the broad peak of IHSS HA gets narrower, increases in intensity and slightly shifts to shorter wavelengths around 440 nm. The fractions of 0.45 μm , 100 kDa and 30 kDa have similar fluorescence properties. Further decrease of

molecular size leads to subsequent quenching of fluorescence intensity in relation to the different oxidative degradation mechanism with respect to the molecular size fractions.

Compared to the 10 kDa size fraction of raw humic acid, the corresponding oxidized humic acid fraction has higher fluorescence intensity (Figure 4.18). The effect of oxidation on the different molecular sizes of humic acid is the blue shift of the spectra and increase in the fluorophoric groups. The most significant effect is observed on the 10 kDa size fraction of humic acid.

4.3.4.5. Fluorescence Spectra of the Molecular Size Fractions of Partially Oxidized IHSS Soil Humic Acid. The synchronous scan properties of the molecular size fractions of IHSS SHA were presented in Figure 4.20. In a comparative approach the fluorescence properties of partially oxidized IHSS SHA is illustrated in Figure 4.40.

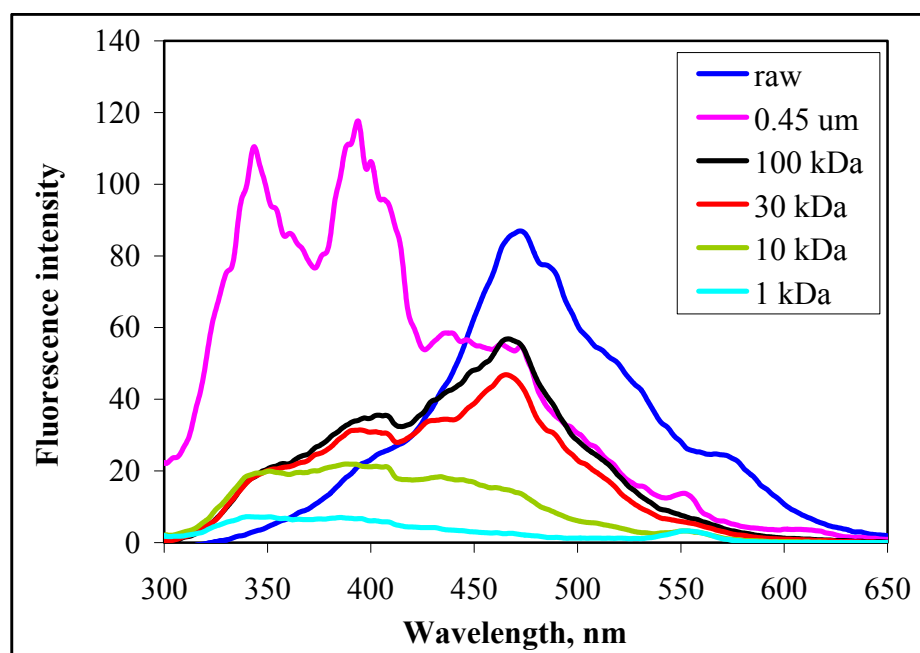


Figure 4.40. Synchronous scan spectra of the molecular size fractions of photocatalytically oxidized IHSS SHA

As a general trend, oxidation leads to a shift of the fluorescence intensity to lower wavelengths. An initial increase with two peak formation is evident with the effect of partial oxidation (Figure 4.40). For the size fractions of 100 kDa and 30 kDa similar

fluorescence properties are observed. Compared to the corresponding fractions of raw humic acid, decrease in fluorescence intensity is observed although the characteristic peak of IHSS SHA around 470 nm still prevails.

4.3.5. Fourier Transform Infrared (FTIR) Spectroscopy of Humic Substances

The FTIR analysis of raw and oxidized humic acid samples were performed after lyophilization of the samples. The results of Aldrich humic acid (50 mg L^{-1}) that has been photocatalytically oxidized using 1.0 mg mL^{-1} for certain reaction periods were presented in Figure 4.41.

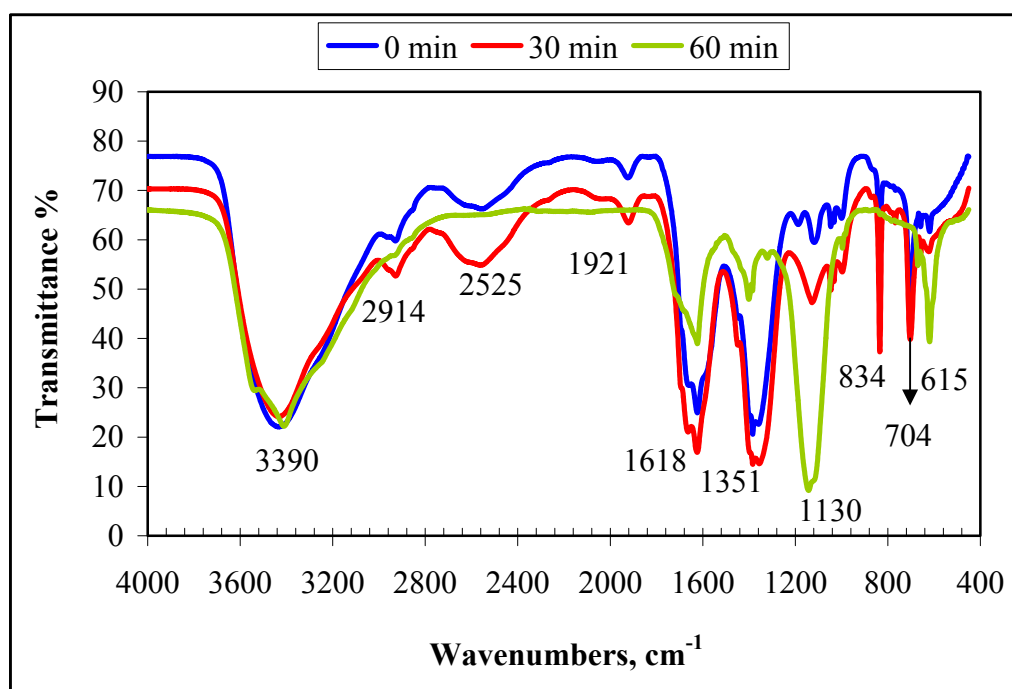


Figure 4.41. FTIR spectra of 50 mg L^{-1} of AHA during photocatalytic oxidation.

Aldrich humic acid, the FTIR spectra of which are shown above has a photocatalytic degradation rate constant of $2.49 \times 10^{-2} \text{ min}^{-1}$ in terms of UV_{254} . The broad band around $3400\text{--}3200 \text{ cm}^{-1}$ corresponds to OH stretching. No particular difference is observed for the OH band between the specified photocatalytic irradiation times. The minor peak around $2850\text{--}2950 \text{ cm}^{-1}$ is related to the valence vibrations of the CH_2 and CH_3 groups hence, designate that humic acid contain only a little amount of aliphatic

hydrocarbons. The absorption between 1620 to 1600 cm^{-1} region is attributable to aromatic C=C vibrations, symmetric stretching of COO^- groups, and H-bonded C=O of conjugated ketones. Therefore, the absorption bands 1618 cm^{-1} and 765 cm^{-1} confirm the aromatic character of the humic acid samples. However, a decrease in intensity is observed with the increasing photocatalytic irradiation time indicating the oxidation of humic acid. This finding is in accordance with the declining trend of the corresponding UV-vis parameters, fluorescence spectra and TOC values. Moreover, the formation of a sharp peak around 1130 cm^{-1} is significant after oxidation which might be related to the C-O vibrations. Considering the oxidation of humic macromolecule, this peak could be attributed to the formation of C-O containing organic compounds such as aldehydes, ketones, ethers or alcohols.

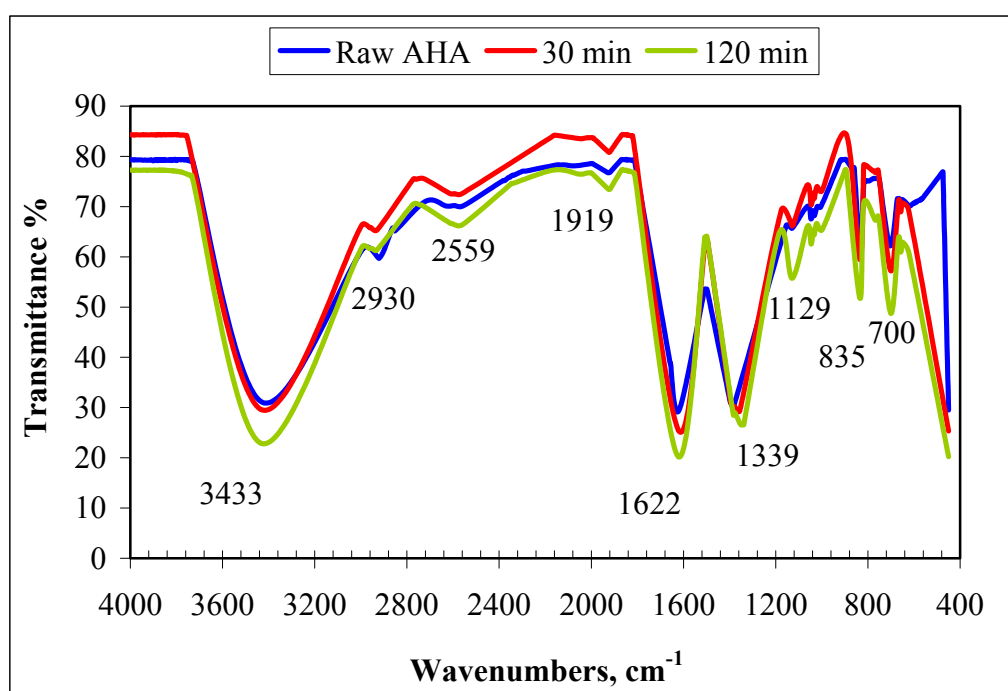


Figure 4.42. FTIR spectra of 100 mg L^{-1} AHA during photocatalytic oxidation.

The FTIR spectra were also analyzed for a higher humic acid concentration of 100 mg L^{-1} and presented in Figure 4.42. The related photocatalytic degradation rate constants correspond to $1.34 \times 10^{-2} \text{ min}^{-1}$ and $6.98 \times 10^{-3} \text{ min}^{-1}$ in terms of Color_{436} and UV_{254} absorption parameters. Compared to that of Figure 4.41, with a two fold increase of the humic acid concentration, no significant differences are observed in the FTIR spectra of

humic acid although degradation of the molecule is indicated with respect to the removal kinetics specified in terms of UV-vis parameters. A peak around 1130 cm^{-1} was well pronounced for humic acid treated for 120 min but was generally very weak or absent in untreated humic acid. It may be attributed to C–O of alcohol and ether groups indicating the degradation of the target compound. After 120 minutes of photocatalytic irradiation, approximately 65 % of UV_{254} removal has been attained. Since the humic acid molecule contains a large number of functional groups, the effect of photocatalysis can not be differentiated. Slight changes observed might be due to the formation of intermediate compounds.

Additionally, the concentration of the ions formed during photocatalysis were measured by ion chromatography coupled with a conductivity meter. The results are presented in Table 4.24.

Table 4.24. Concentration of common anions obtained from the photocatalytic degradation of 100 mg L^{-1} AHA using 1.0 mg mL^{-1} of TiO_2

	Chloride (mg L^{-1})	Nitrate (mg L^{-1})	Nitrite (mg L^{-1})	Sulfate (mg L^{-1})
AHA raw	0.277	0.091	-	0.760
60 min	1.464	0.460	0.133	1.676
120 min	1.086	0.228	0.148	1.795

The increase of nitrate, nitrite and sulfate ions are indicative to the degradation of humic acid. Bearing in mind that the humic acid contains N and S groups, the conversion of organically bond atoms to inorganic species as a result of mineralization is very likely. The oxidized forms of nitrogen in terms of nitrite and nitrate were detected after 60 min of irradiation. Degree of formation of SO_4^{2-} ions exceeded the background concentration after 60 min and increased up to 1.795 mg L^{-1} after prolonged irradiation for 120 min.

Various researchers monitored the conductivity changes during photocatalytic degradation of humic substances (Eggins et al., 1997; Palmer et al., 2002). The drop in conductivity was accepted as an indicator for mineralization. In our study, for raw humic acid the conductivity was measured as $7.82\text{ }\mu\text{S cm}^{-1}$. However, after photocatalytic

irradiation for 60 and 120 minutes, 8 % and 19 % increase of conductivity were measured respectively.

The conductivity measurements of raw and oxidized humic acid also confirm the degradation of the bulk molecule and formation of inorganic species during photocatalysis.

Similarly, the FTIR spectra of IHSS FA (100 mg L^{-1}) that has been photocatalytically oxidized for certain reaction periods using 1.0 mg mL^{-1} of TiO_2 were presented in Figure 4.43. The removal rate constant of the reaction was found to be $k=1.09 \times 10^{-2}$ for Color_{436} , and $k=5.50 \times 10^{-3}$ for UV_{254} .

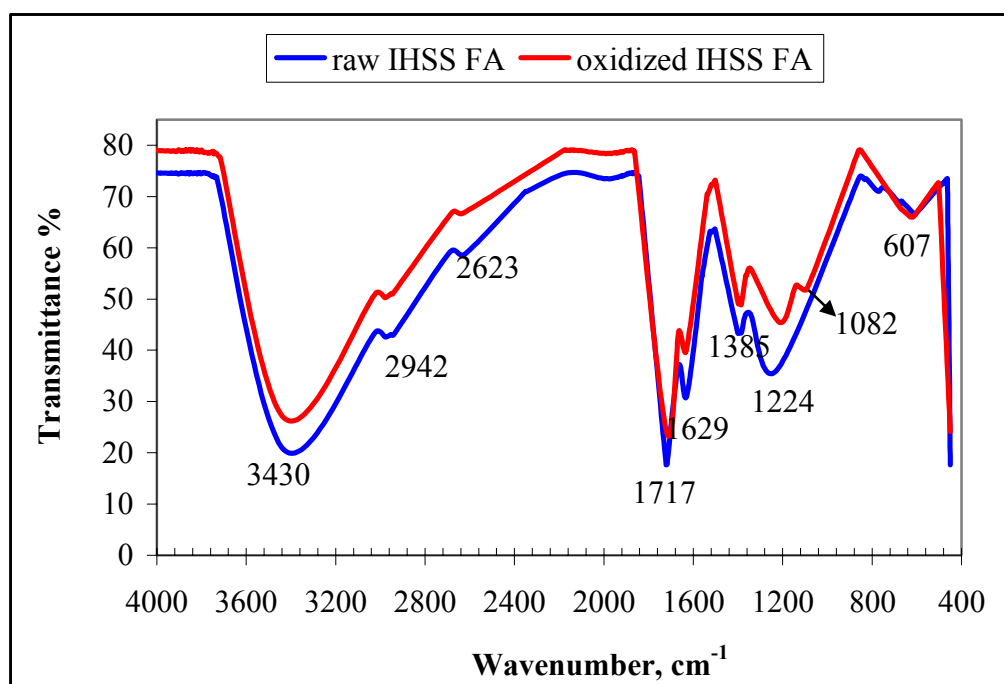


Figure 4.43. FTIR spectra of 100 mg L^{-1} IHSS FA during photocatalytic oxidation.

It is known that the features of the band for the spectra of humic substances are at $3700\text{-}3200 \text{ cm}^{-1}$ for phenolic O-H stretching and at $1720\text{-}1700 \text{ cm}^{-1}$ for carboxylic C=O stretching (aldehyde and ketonic carbonyl as well). The intensities decreased after photocatalytic oxidation with 1 mg mL^{-1} of TiO_2 for 120 min. At this stage of oxidation approximately 50 % UV_{254} removal has been achieved. The reason for the phenolic O-H stretching can be attributed to the formation of reaction products via phenoxy radicals.

Moreover, the small decrease in peak intensities corresponding to carboxylic C=O stretching can be attributed to carboxylic sites which are converted to CO₂ as recently stated by Fukushima et al., (2001) who have studied the degradation characteristics of humic acid during photo-fenton processes.

The absorption bands in the 2830 to 2965 cm⁻¹ region are assigned to the stretching vibrations of aliphatic -CH, -CH₂ and -CH₃ side chain groups of the aromatic nuclei. The peaks at 1381-1387 cm⁻¹ for (C-H deformation of -CH₂ and -CH₃ salts of carboxylic acid and/or aliphatic -CH) and 1222-1288 cm⁻¹ for C-O stretching vibrations of esters, ethers and phenols. For the photocatalytically oxidized fulvic acid a minor peak formation is observed around 1080 cm⁻¹ which is attributable to the formation of alcohols and carbohydrates (Chien et al., 2003). This is a result of the oxidation of fulvic acid.

4.3.6. Nuclear Magnetic Resonance Spectroscopy of Humic Substances

NMR spectroscopy is a non destructive technique that can be used to provide structural information for humic samples in both the solution and solid state. To prepare samples for NMR analysis, 100 mg L⁻¹ of Aldrich humic acid was photocatalytically oxidized for 120 min using 1 mg mL⁻¹ of TiO₂. The oxidation of humic acid corresponded to approximately 85 % of Color₄₃₆ and 65 % of UV₂₅₄ removal. The related FTIR spectra of raw and oxidized humic acid has been presented in Figure 4.42. Prior to NMR analysis, the collected samples were first lyophilized and then dissolved in D₂O.

Traditionally the presence of water in the sample to be analyzed causes a major problem due to the dynamic range limitations of the electronics needed for the collection of water's large NMR signal. Thus the signal of water must be attenuated to allow for the detection of the signal of interest. This attenuation was solved by the use of a deuterated solvent such as D₂O (Cook, 2004). Even in this case there is still the problem of a substantially large water signal due to the exchangeable protons of natural organic matter. In the spectra illustrated in Figure 4.44 the sharp peak around 4.8 ppm corresponds to D₂O signal.

^1H NMR spectrum consists of broad lines and peaks which arise from macromolecules or macromolecular assemblies of molecules that are overlaid by a series of sharp lines that may arise either from small molecules or highly mobile segments of large molecules or macromolecular assemblies of molecules. ^1H NMR is used for estimating the relative abundance of various humic substance functional groups. However, the obtained spectra are also extremely complex because of the chemical complexity of humics and due to the narrow spectral window brought about by the small chemical shift dispersion of protons. This indicates that the chemical group assignments of humic acid samples can only be made at the macroscopic level when proton spectrum is considered (Cook, 2004).

As illustrated in Figure 4.44, the interpretation of the ^1H NMR of the photocatalytically oxidized humic acids reveals more definite peaks when compared to the raw ^1H NMR spectra of raw humic acid. This could be explained by the formation of highly oxidized low molecular weight segments from the large macromolecular assemblies of humic substances.

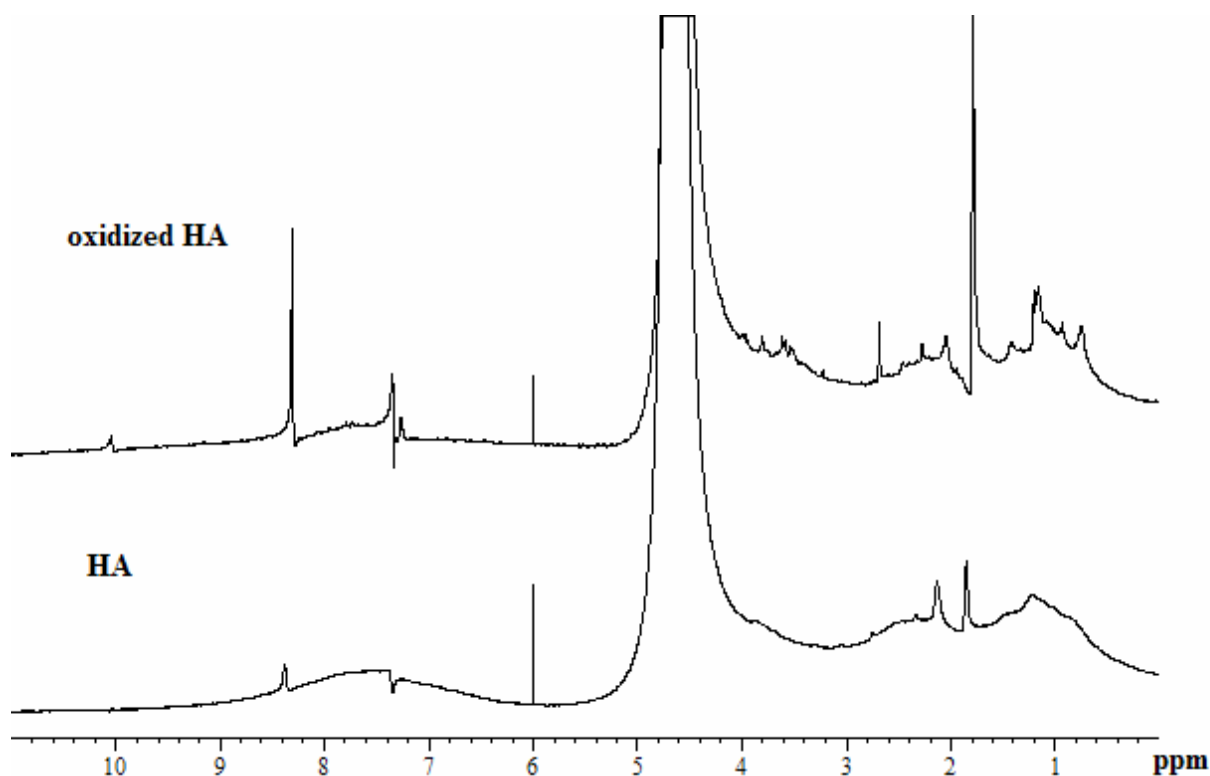


Figure 4.44. ^1H NMR (400 MHz) spectrum of raw and oxidized humic acid in D_2O .

In general, for humic acids the chemical shift at 0.8 ppm is assigned to the methyl protons in aliphatic structure. The other chemical shift assignments for ^1H NMR spectrum of an extracted fulvic acid fraction were reported as 1.2 ppm for methylene protons, 1.4 ppm for methine protons, 2.4 ppm for $\text{CH}_3\text{-CO-OAr}$ or $\text{R-CH}_2\text{-CO-R}$ (R: representing aliphatics, Ar: representing aromatics) and 2.6-2.7 ppm for $\text{CH}_3\text{-CO-Ar}$ or $\text{R-CH}_2\text{-Ar}$ (Davies and Ghabbour, 1998). Very few papers exist in literature related to the NMR characterization of oxidized humic acids. Schmitt-Kopplin et al., (1998) have investigated the photochemical degradation of soil extracted humic acids focusing on the structural changes. The main difference in ^1H NMR spectra of raw and irradiated samples was reported as a relative decrease in aliphatic and an increase in carbohydrate constituents. As can be observed from Figure 4.44, the peak around 1.8 ppm is intense for the oxidized humic acid sample. The other peaks with chemical shifts in the range of 0.8 ppm to 2.8 ppm also indicate the presence of higher content of aliphatic moieties in oxidized humic acid. Considering that photocatalytic oxidation leads to the removal of color forming moieties (85 % Color_{436} removal) mainly comprising of the conjugated double bonds systems that are susceptible to $\bullet\text{OH}$ radical attack, the formation of new aliphatic compounds are expected in the oxidized humic acid.

The region of 3-5.5 ppm represents a wide range of protons associated with the oxygen containing functional groups such as ethers, alcohols etc. which are easily exchangeable with the solvent D_2O . Hence, broadening of the water signal and loss of information due to the exchangeable protons of natural organic matter is unavoidable in this region.

The resonances in 6-7 ppm region are generally attributed to polymeric associations. Considering the degradation of the molecule to smaller size fractions, the presence of a lower intensity peak is likely for the oxidized humic acid sample. Moreover, in literature, the aromatic protons of humic substances were assigned chemical shifts in the range of 6-8.5 ppm (Cardoza et al., 2004; Schmitt-Kopplin et al., 1998; Dignac et al., 2000). Accordingly, the broadening of this region for raw humic acid indicates the high aromatic content due to the presence of benzenoid protons. On the other hand, the high intensity peak at 8.5 ppm for oxidized humic acid could be related with the presence of nonbenzenoid protons. However, the aromatic region is difficult to interpret because of the

diversity of compounds present. The peak at 10 ppm which is not observed in raw humic acid but apparent in oxidized humic acid might be associated with the aldehyde groups (α , β unsaturated RCHO) that might have formed as intermediate products of oxidation.

The ^{13}C NMR spectra has been useful when sufficient amount of humic substance is available hence spectral overlap is reduced and minor structural changes can be detected more readily. ^{13}C has a larger chemical shift dispersion than ^1H . However, in the presented ^{13}C spectra of raw and oxidized humic acids no clear peak resolution could be attained (Figure 4.45). The differences may be most easily observed by overlaying the spectra of raw and oxidized humic acid. This might be related with the presence of insufficient amount of humic acids and low signal strength of ^{13}C .

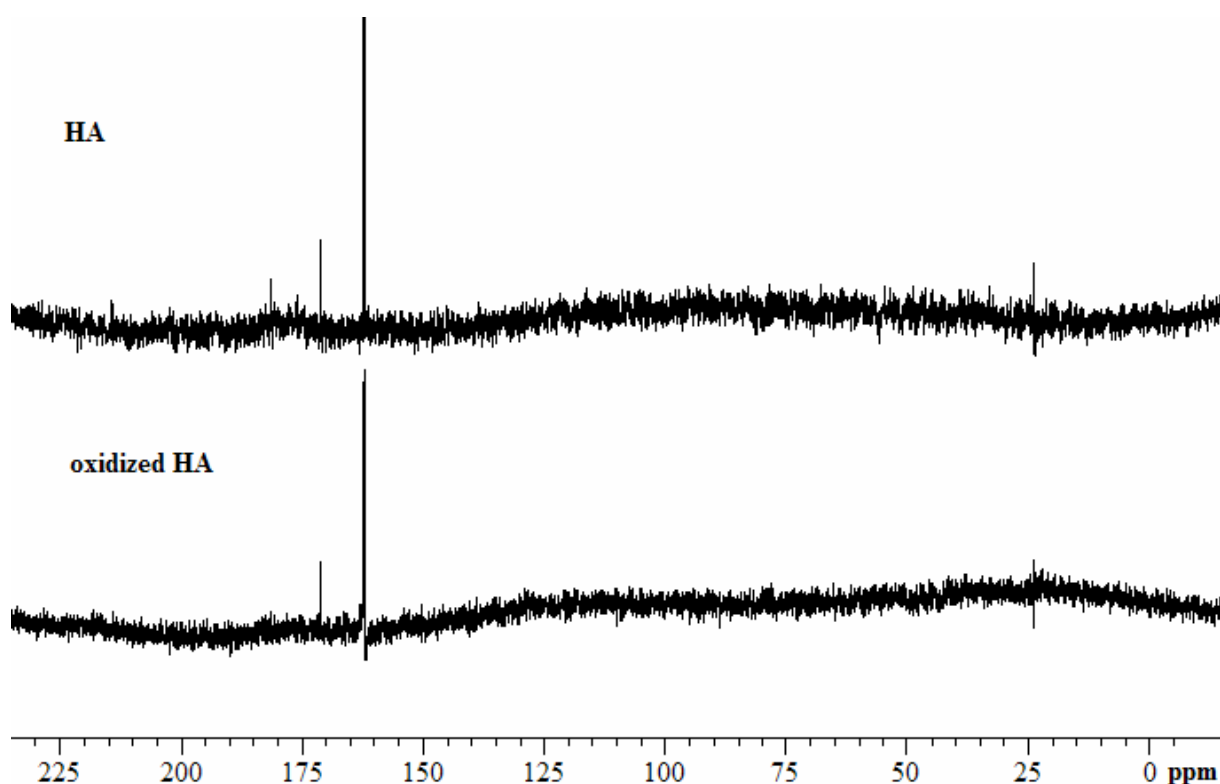


Figure 4.45. ^{13}C NMR spectra of raw and oxidized humic acids.

The spectra of raw and oxidized humic acids do not show significant differences except for the 165-185 ppm region where more peaks are observed in raw humic acid (Figure 4.45). The peak around 165 ppm could be related with the higher aromatic content

of raw humics whereas the resonances around 165-185 ppm can be due to the presence of carboxylate carbons, esters and amides (Schmitt-Kopplin, 1998). It can be assumed that the photocatalytic degradation of humic acid leads to the oxidation of carboxylate carbons to CO₂. This is also in accordance with the decrease of TOC values during photocatalytic oxidation.

There is a lack in the literature on the characterization of oxidized humic acids via various oxidation methods. Although the data acquired from the NMR spectra of humic substances can not directly be related to information as to determine the structure of the macromolecule, still reveal hints about the structural changes after oxidation. Therefore, even though limited information has been set forward through liquid state ¹H NMR and ¹³C NMR spectra of raw and oxidized humic acid systems, the findings presented here still serve as an important contribution to the area.

Furthermore, the recent advances in the application of NMR and recent studies relevant to NOM as well as humic acids reveal that the sensitivity of the method should be improved along with analytical innovations such as the direct coupling of NMR to separation methods such as high performance liquid chromatography (HPLC) or mass spectroscopy (MS) (Cardoza et al., 2004).

5. CONCLUSIONS

In this study, model humic and fulvic acids of aquatic and terrestrial origins were investigated with respect to their UV-vis and fluorescence properties as representatives to natural organic matter in drinking water supplies. Considering the complexity of the humic macromolecules, they were fractionated into well defined subcomponents of defined molecular sizes using ultrafiltration through membranes in the range of 1-100 kDa. Moreover, the spectroscopic properties of each size fraction were characterized and compared by UV-vis and fluorescence spectroscopy. Furthermore, the structural characteristics of the humic substances relative to changes during photocatalytic oxidation were monitored by fluorescence spectroscopy in emission and synchronous scan excitation modes, Fourier transform infrared (FTIR) and liquid state nuclear magnetic resonance spectroscopy (NMR) utilizing ^1H NMR and ^{13}C NMR.

The summary of the results can be listed in terms of the specified headings as follows:

Spectroscopic characterization of humic substances and the molecular size fractions

Specific absorbance values calculated at certain wavelengths as well as the spectroscopic ratios calculated for the studied humic substances show differences depending on the source. As a function of their structural differences, synchronous scan fluorescence spectra also exhibit different profiles.

According to the molecular size distribution results, the samples exhibited similar patterns irrespective of the source between size fractions 0.45 μm to 30 kDa for the ratio of E_{254}/E_{436} . As a general trend the absorbance ratios of the fractionated humic substances increase with decreasing molecular size. Such a pattern suggests a shift in the UV-vis absorbance towards high wavelength with increasing molecular sizes. On the other hand, for the size fractions lower than 10 kDa, differences were observed for E_{250}/E_{365} ratios with respect to each molecular size fraction. In general, the size fractions < 30 kDa express different fluorescence and UV-vis characteristics.

The E_{254}/E_{436} ratio can be proposed to assess the composition of altered humic matter provided that the total removal of color-forming moieties is not required. Under conditions of total decolorization, the other parameters such as E_{250}/E_{365} , E_{254}/E_{365} and E_{280}/E_{365} could also be used relevant to the spectroscopic changes acquired during treatment.

Photocatalytic Oxidation of Humic Substances

On the basis of their diverse chemical and physical properties such as molecular weight, molecular size, elemental composition and source of origin, substantial differences are observed in photocatalytic removal efficiencies of humic and fulvic acids. The pseudo first order rate constants calculated for Color_{436} of each of the humic substances show a decreasing trend as; AHA > IHSS SHA > IHSS FA > IHSS HA > RHA. On the other hand, the order of the rate constants of aromaticity removal (UV_{254}) is as follows; IHSS SHA > AHA > IHSS HA > IHSS FA > RHA. The rate constants calculated for TOC removal exhibit a different trend as IHSS SHA > IHSS FA > AHA > RHA > IHSS HA. Higher removal rates have been achieved with UV_{254} values compared to that of Color_{436} values. All of the studied humic acids exhibit higher pseudo first order removal rates compared to that for fulvic acid.

The ordering of the L-H rates is similar to that of first order rates. However, the calculated L-H rates for the humic acids are approximately half the value of the pseudo first order rates for all of the studied UV-vis parameters. Hence, it seems to be probable that the removal of humic substances during photocatalytic oxidation show differences depending on the source.

Considering that the humic acid comprises a variety of subcomponents with different chemical physical properties, molecular sizes and reactivities, oxidative degradation of the macromolecule is assumed to occur through an unselective pathway, by the reaction of $\bullet\text{OH}$ radicals with the various moieties within each fraction. Hence, the photocatalytic degradation of humic acid is achieved through the summation of a series of reactions.

During the course of photocatalysis, the blue shift of the spectra is observed with a gradual increase in fluorescence intensity per unit carbon. The initial increase of fluorescence intensity can be related to formation of intermediate degradation products that have a higher content of fluorophores. The blue shift of the spectra after long photocatalytic irradiation times and decrease of the fluorescence maxima is indicative to the destruction of the high degree of conjugation and aromatic structures of humic acids.

Specific absorbance values during photocatalytic oxidation exhibit a decreasing trend in relation to irradiation time. Hence it is assumed that degradation products have less UV-vis absorbing moieties with higher fluorescence properties.

The declining trend of the corresponding UV-vis parameters, the related changes in the fluorescence spectra (initial increase of the fluorescence intensities, formation of new fluorophores, blue shift of the spectra and decline of intensity after long photocatalytic irradiation times, decrease of TOC content during degradation, the spectral changes in FTIR and NMR indicate the oxidative degradation of humic acid.

Effect of photocatalytic oxidation on molecular size distribution profiles of humic substances

Comparison of the molecular size distribution profiles of the photocatalytically treated humic acids with those of raw untreated samples reveals the confirmation of the formation of lower molecular size fractions exhibiting low $SUVA_{254}$ values.

Based on the comparison between the synchronous scan fluorescence spectra of the molecular size fractions of raw and oxidized humic substances, it could be concluded that oxidative cleavage of the molecule leads to a blue shift of the spectra which is indicative to the formation of new fluorophores.

For the aquatic origin IHSS HA the effect of photocatalysis on the 10 kDa fraction is observed as an increase of the absorbance ratio for E_{254}/E_{436} and E_{300}/E_{436} ratio. The similar result has been observed for the IHSS FA. Hence, molecular size specific distribution of the absorbance ratios could be assessed for the oxidized fractions of aquatic humic substances.

With the effect of oxidation, the broad peak of IHSS HA gets narrower, increases in intensity and slightly shifts to shorter wavelengths around 440 nm. The molecular size fractions < 30 kDa have lower fluorescence intensity. However, 10 kDa fraction of oxidized humic acid has higher fluorescence intensity than the corresponding size fractions of raw humic acid. The effect of oxidation on the different molecular sizes of humic acid is the blue shift of the spectra and increase in the fluorophoric groups. The most significant effect is observed on the 10 kDa size fraction of humic acid.

The following points could be specified as a general approach;

The application of UV-vis spectra to NOM is a well known, widely used technique for the characterization of organic structure in natural waters. This study combines this information to the elucidation of structural and compositional alterations in degradation products of photocatalytically oxidized humic substances.

Considering the polydisperse nature and source dependent properties of humic substances, molecular size fractionation is proved to be a versatile tool to evaluate the photocatalytically oxidized degradation products and to assess the oxidation pathway.

UV-vis absorbance ratios reveal information regarding the molecular size fractions of the photocatalytically oxidized humic moieties as well as unoxidized humic and fulvic acids. Moreover, $SUVA_{254}$ parameter that is widely used for NOM characterization in drinking water treatment and the other specific UV-vis absorbance parameters presented as $SUVA_{254}$, $SUVA_{280}$, $SUVA_{365}$ and $SCOA_{436}$ are found to be informative for the characterization of photocatalytically oxidized humic substances.

Fluorescence spectroscopy in synchronous scan excitation mode gives further insight to the understanding of the molecular size dependent properties of the photocatalytically oxidized humic substances.

Although the presented data covers limited application for the photocatalytically oxidized humic substances, other advanced spectroscopic techniques like FTIR and NMR reveal vital information with regards to the induced structural changes during oxidation.

In conclusion, it is widely known that properties of NOM are site specific and the model compounds of different origins obviously express different properties. Spectroscopic techniques such as UV-vis and fluorescence spectroscopy give hints about the differences between humic substances and their size fractions; however, more clues can be obtained with the use of more powerful instrumental techniques such as NMR, FTIR etc. The continued development and evaluation of new analytical methods are required to achieve a fundamental understanding of humic substance structure and chemistry.

6. RECOMMENDATIONS

The understanding of the UV-vis properties of humic substances and their size fractions might improve the general knowledge about the spectroscopic properties of natural water samples. Studying humic substances as composite heterogeneous materials and evaluating their UV-vis properties might be helpful for further studies that cover treatment of natural water samples by any oxidative or conventional treatment methods.

Humic substances have been widely studied in the literature as a representative to NOM in drinking water supplies due to their composite heterogeneous nature comprising a variety of different molecular weight compounds. The most important point is the understanding of the use of model compounds such as humic substances of different origins that allows a comparative basis between different studies conducted in literature and to assess the simple spectroscopic properties of NOM. Thereby a conjunction between the studies using model compounds and the studies depending on the extraction of the individual NOM could be recognized. Another consideration is the extraction of humic substances i.e., as algal NOM, aquatic NOM and terrestrial NOM from the drinking water supplies and characterization of NOM in relation to advanced treatment technologies. However, taking into account the diversity of the components of the matrix of the aquatic systems, spectroscopic characterization and investigation of structural changes during oxidation processes still remain elusive. Instrumental methods such as UV-vis, fluorescence and FTIR can only provide qualitative information for the structural changes of humic substances. Further qualitative research can be conducted by the evaluation of fluorescence spectroscopy in quenching studies of humic substances with metal ions or organic compounds. The use three dimensional emission excitation matrix (3D EEM) might be useful to differentiate fluorophores of oxidized humic substances. For quantitative analysis other sophisticated instruments such as GC-MS, HPLC can be coupled with NMR to extend the research to the monitoring and analysis of intermediate products.

Considering the polydispersity of humic substances and based on data obtained from the spectroscopic properties of each size fraction, further research can be conducted on the oxidative degradation of each molecular size fraction of humic substances and the relative trihalomethane formation potentials (THMFPs) at each stage of oxidation can be investigated. The evaluation of the molecular size dependent photocatalytic oxidation pathway is recommended. However, the drawback is the sample preparation. Hence, the use of an ultrafiltration stirred cell system with a higher capacity is suggested.

The application of simple UV-vis spectroscopic parameters for estimating the characteristics of the treated water sample may provide a practical method especially after verification with much larger data sets.

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