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Titelbild: DOC chromatograms of sludge acquired with size exclusion chromatography

Sludge disintegration techniques - assessment of their impacts on solubilization of organic carbon and methane production

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Abstract

Anaerobic stabilization of excess sludge is the favored biological process in wastewater treatment with a capacity of more than 50000 people. The main benefits of this process are reduction of sludge solids and production of biogas as a renewable energy. However, this biological process is very slow due to the hydrolysis of bacteria and large molecular size polymers embedded in a matrix formed by extracellular polymer substances (EPS).

To accelerate the rate of hydrolysis, the first step in anaerobic digestion is destruction of particulate organic matter (bacteria and EPS matrix) and release of the organic compounds into the aqueous phase. For this reason, sludge pretreatment before anaerobic digestion would be an appropriate option to solubilize particulate organic compounds and make them more accessible to anaerobic bacteria. Several chemical, mechanical and biological sludge pretreatment methods have been proposed with the aim of increasing biodegradability of organic substances, thus enhancing methane production. The other beneficial aspect of sludge disintegration is utilization of the released organic carbon as an internal carbon source in denitrification process.

In the present thesis, ozone, sodium hydroxide and ultrasound were conducted to disintegrate the excess sludge prior to anaerobic digestion. Excess sludge was ozonated for short, moderate and long time intervals and the consumptions of ozone per gram total solids in the sludge were obtained. Solubilization and mineralization of dissolved organic carbon (DOC) during sludge ozonation were studied to determine the optimum ozone consumption. Degree of solubilization depended greatly on ozone dosage. However, mineralization of the released organic carbon during ozonation of excess sludge, was a limitation in ozone optimization process. The effects of different sodium hydroxide dosages and treatment durations on the solubilization of DOC were examined. Sludge treatment with low dosage of sodium hydroxide for short duration was the most efficient treatment condition. A low ultrasound frequency was conducted to disintegrate the excess sludge and to release approximately the same amount of DOC solubilized with optimum ozone consumption and sodium hydroxide dosage.

The impacts of different sludge disintegration methods on the molecular size distribution of DOC solubilized after disintegration were investigated using size exclusion chromatography with online organic carbon detection (SEC-OCD). Based on the treatment method, significant differences on the relative distribution of chromatographable DOC (cDOC) were obtained. Short sludge ozonation increased the percentage of large molecular size compounds in the supernatant, while longer sludge ozonation did not significantly change the relative cDOC distribution compared to untreated sludge. Small molecular size compounds were the dominant constituents of the DOC solubilized by sodium hydroxide treatment. Ultrasound disintegrated the sludge flocs and dissolved large molecules into the sludge liquid phase.

To assess the anaerobic biodegradability of DOC after different sludge pretreatments, batch anaerobic experiments were performed for five days. Methane production and DOC degradation during anaerobic digestion of the supernatant of untreated and pretreated sludge were analyzed at the beginning, after one day and five days. Sludge pretreatment with sodium hydroxide and ultrasound enhanced methane production twice higher than ozonation. To comprehensively assess the performance of three different pretreatment methods in enhancing methane production, changes in the relative cDOC distribution during anaerobic digestion of the supernatant of the untreated and pretreated sludge were studied in detail. Differences in the methane production due to the different disintegration mechanisms were explained by the transformation of DOC fractions during anaerobic digestion.

The potential of the disintegrated sludge to be used as an internal carbon source for the biological removal of nitrate was investigated in batch denitrification experiments. The nitrate utilization rate was improved after addition of the disintegrated sludge. The type of soluble carbon present in the supernatant of sludge treated with sodium hydroxide appeared to be more suitable for denitrification applications when compared with sodium acetate commonly used as an external carbon source.

SEC-OCD in combination with an online UV at λ= 254 nm detection (SEC-UV_{254}) and an online fluorescence detection at an excitation wavelength of $\lambda_{EX=}$ 275 nm and an emission wavelength of λ_{EM}= 335 nm (SEC-$Fl_{EX:275/EM:335}$) coupled with three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy were conducted to provide more detailed information on the characteristics of organic carbon released after sludge disintegration. The results of SEC-UV_{254} analysis showed a substantial decrease in the aromaticity of the large-intermediate compounds which were solubilized after sludge ozonation. SEC-$Fl_{EX:275/EM:335}$ in combination with EEM proved that large molecular size fraction of DOC was composed of proteins-like substances which was raised significantly after sludge disintegration with sodium hydroxide and ultrasound.

Zusammenfassung

Die anaerobe Schlammstabilisierung ist das bevorzugte Verfahren für Abwasseranlagen mit einer Ausbaugröße von mehr als 50000 EW. Die wichtigsten Vorteile dieses Verfahrens sind die Verminderung des Schlammvolumens und die Erzeugung von Biogas als erneuerbare Energie. Aufgrund der Hydrolyse von Bakterie und großen Polymermolekülen, die in einer Matrix aus extrazellulären polymeren Substanzen (EPS) eingebettet sind, läuft dieser biologische Prozess jedoch sehr langsam ab.

Um die Geschwindigkeit der Hydrolyse zu beschleunigen, werden im ersten Schritt der anaeroben Schlammfaulung aus partikulärem organischen Material (Bakterien und EPS) organische Verbindungen in die wässrige Phase freigesetzt. Aus diesem Grund ist eine Behandlung des Schlammes vor der anaeroben Schlammfaulung eine geeignete Methode, um organische Feststoffe zu lösen und für anaerobe Bakterien besser verfügbar zu machen. Es wurden verschiedene chemische, mechanische und biologische Verfahren zur Vorbehandlung von Schlamm vorgeschlagen, um die biologische Abbaubarkeit organischer Substanzen und dadurch die Methanproduktion zu erhöhen. Ein weiterer Aspekt der Schlammdesintegration ist die Nutzung der freigesetzten organischen Verbindungen als interne Kohlenstoffquelle in der Denitrifikationsstufe.

In der vorliegenden Studie wurden Ozon, Natriumhydroxid und Ultraschall für die Desintegration von Überschussschlamm vor der anaeroben Schlammfaulung verwendet. Überschussschlamm wurde für kurze, mittlere und lange Zeitintervalle mit Ozon behandelt, wobei der Verbrauch von Ozon pro Gramm Trockensubstanz des Schlammes bestimmt wurde. Während der Ozonierung des Schlammes wurden die Solubilisierung und Mineralisierung des gelösten organischen Kohlenstoffs (DOC) untersucht, um den optimalen Ozonverbrauch zu bestimmen. Der Grad der Solubilisierung war stark von der Ozonzugabe abhängig. Allerdings wurde die Optimierung des Ozonverbrauchs durch die Mineralisierung des freigesetzten organischen Kohlenstoffs während der Ozonierung des Überschussschlammes limitiert. Die Wirkung von Natriumhydroxid auf die Solubilisierung von DOC wurde anhand verschiedener Dosierungen und Behandlungsdauern untersucht. Eine Behandlung des Schlammes mit einer niedrigen Natriumhydroxid-Dosierung von kurzer Dauer war am effizientesten. Die Behandlung mit einer niedrigen Ultraschallfrequenz wurde durchgeführt, um bei der Desintegration des Überschussschlammes etwa die gleiche Menge an DOC freizusetzen, wie mit einer optimalen Ozon- bzw. Natriumhydroxid-Dosierung.

Die Auswirkungen der verschiedenen Desintegrationsverfahren auf die molekulare Größenverteilung des freigesetzten DOC wurden unter Verwendung der Größenausschluss-Chromatographie mit Online-Detektion des organischen Kohlenstoffs (SEC-OCD) untersucht. Für die verschiedenen Behandlungsverfahren wurden erhebliche Unterschiede der relativen Verteilung des chromatographierbaren DOCs (cDOC) festgestellt. Die kurzzeitige Ozonierung des Schlamms erhöht

den Anteil großer Moleküle im Überstand, während die länger andauernde Ozonierung des Schlammes die relative cDOC Verteilung im Vergleich zum unbehandelten Schlamm nicht wesentlich verändert hat. Hingegen waren kleine Molekülgrößen die dominierenden Bestandteile des DOC, der durch die Behandlung mit Natriumhydroxid freigesetzt wurde. Durch die Behandlung mit Ultraschall werden die Schlammflocken desintegriert und große Moleküle in die flüssige Schlammphase freigesetzt.

Um die anaerobe Bioabbaubarkeit des DOC, der durch die verschiedenen Vorbehandlungsverfahren freigesetzt wurde, zu bewerten, wurden anaerobe Batchexperimente mit einer Dauer von fünf Tagen durchgeführt. Die Methanproduktion und der Abbau des DOC während der anaeroben Faulung des Überstandes von unbehandeltem und vorbehandeltem Schlamm wurde zu Beginn des Experiments, nach einem Tag und nach fünf Tagen analysiert. Die Vorbehandlung des Schlammes mit Natriumhydroxid und Ultraschall führte zu einer zweifach höheren Methanproduktion im Vergleich zur Ozonierung. Um die Leistung der drei verschiedenen Vorbehandlungsverfahren bei der Verbesserung der Methanproduktion besser beurteilen zu können, wurden Veränderungen in der relativen cDOC-Verteilung während der anaeroben Faulung des Überstandes der unbehandelten und vorbehandelten Schlämme im Detail untersucht. Die Unterschiede in der Methanproduktion aufgrund der verschiedenen Verfahren der Vorbehandlung konnten durch die Transformation von DOC-Fraktionen während der anaeroben Schlammfaulung erklärt werden.

Das Potential des desintegrierten Schlammes als interne Kohlenstoffquelle für die biologische Entfernung von Nitrat wurde in Denitrifikations-Batchexperimenten untersucht. Die Denitrifikationsrate wurde nach Zugabe des desintegrierten Schlammes verbessert. Die Art des löslichen Kohlenstoffs im Überstand von Schlämmen, die mit Natriumhydroxid behandelt wurden, war offenbar besser für die Denitrifikation geeignet als die Zugabe von Natriumacetat, das typischerweise als künstliche Kohlenstoffquelle verwendet wird.

SEC-OCD in Kombination mit einer Online-UV-Detektion bei λ = 254 nm (SEC-UV_{254}), einer Online-Fluoreszenz-Detektion bei einer Anregungswellenlänge von λ_{EX} = 275 nm und einer Emissionswellenlänge von λ_{EM}= 335 nm (SEC-$Fl_{EX:275/EM:335}$), gekoppelt mit einer dreidimensionalen Anregungs-Emissionsmatrix (EEM) Fluoreszenzspektroskopie wurde durchgeführt, um detailliertere Informationen über die Eigenschaften des organischen Kohlenstoffs, der nach der Schlammdesintegration freigesetzt wurde, zu erhalten. Die Ergebnisse der SEC-UV_{254}-Analyse zeigte eine erhebliche Verringerung der Aromatizität der großen Zwischenprodukte, die nach der Ozonisierung des Schlammes freigesetzt wurden. Die Kombination von SEC-$Fl_{EX:275/EM:335}$ mit EEM bewies, dass große molekulare Fraktionen des DOC aus proteinartigen Substanzen zusammengesetzt sind und dass diese nach der Schlammdesintegration mit Natriumhydroxid und Ultraschall signifikant erhöht waren.

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Publication

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Abbreviations

AMPTS	automatic methane potential test system	
AOB	ammonium oxidizing bacteria	
BMP	biochemical methane potential test	
cDOC	chromatographable dissolved organic carbon	(mg/L)
$cDOC_{t0}$	chromatographable dissolved organic carbon at the beginning of anaerobic digestion	(mg/L)
$cDOC_{t1}$	chromatographable dissolved organic carbon after one day of anaerobic digestion	(mg/L)
$cDOC_{t5}$	chromatographable dissolved organic carbon after five days of anaerobic digestion	(mg/L)
COD	chemical oxygen demand	(mg/L, g/L)
DD	degree of disintegration	(%)
DGGE	denaturing gradient gel electrophoresis	
DNA	deoxyribonucleic acid	
DOC	dissolved organic carbon	(mg/L)
DOC_0	dissolved organic carbon of untreated sludge	(mg/L)
DOC_{O3}	dissolved organic carbon of ozonated sludge	(mg/L)
$DOC_{proteins}$	equivalent DOC concentration of proteins	(mg/L)
$DOC_{carbohydrates}$	equivalent DOC concentration of carbohydrates	(mg/L)
DOM	dissolved organic matter	
E_S	specific energy	(kJ/g TS)
EEM	excitation-emission matrix	
EPS	extracellular polymer substances	
FCM	flow cytometry	
FT-IR	Fourier transform infrared spectroscopy	
IHSS	International Humic Substance Society	
IHSS-HA	Humic acid standard substances derived from the International Humic Substances Society	
IHSS-FA	Fulvic acid standard substances derived from the International Humic Substances Society	
IFE	inner-filter effect	
NDIR	non-dispersive infrared	

NO_3-N_{t0}	initial nitrate nitrogen	(mg/L)
NOB	nitrite oxidizing bacteria	
NUR	nitrate utilization rate	(g NO_3-$N_{removed}$/g TS.d)
OC	organic carbon	
OCD	organic carbon detection	
PCR	polymerase chain reaction	
PUF	polyurethane foam	
SCOD	soluble chemical oxygen demand	(mg/L)
$SCOD_{t0}$	initial soluble chemical oxygen demand	(mg/L)
$SCOD_{t5}$	soluble chemical oxygen demand after five days of anaerobic digestion	(mg/L)
SEC	size exclusion chromatography	
SEC-$Fl_{EX:275/EM:335}$	size exclusion chromatography with online fluorescence detection at excitation wavelength of λ_{EX}= 275 nm and emission wavelength of λ_{EM}= 335 nm	
SEC-$Fl_{EX:254/EM:450}$	size exclusion chromatography with online fluorescence detection at excitation wavelength of λ_{EX}= 254 nm and emission wavelength of λ_{EM}= 450 nm	
SEC-OCD	size exclusion chromatography with online organic carbon detection	
SEC-UV_{254}	size exclusion chromatography with ultraviolet absorbance detection at wavelength of λ= 254 nm	
SRT	solid retention time	
SUVA	specific ultraviolet absorbance at wavelength of λ= 254 nm	(L/(mg.m))
TC	total carbon	
TCOD	total chemical oxygen demand	(g/L)
$TCOD_0$	total chemical oxygen demand of untreated sludge	(g/L)
$TCOD_{O3}$	total chemical oxygen demand of ozonated sludge	(g/L)
TOC	total organic carbon	
TS	total solids	(g/L)
UV_{254}	ultraviolet absorbance at wavelength of λ= 254 nm	
VFA	volatile fatty acid	
V_o	exclusion volume	(mL)
V_p	permeation volume	(mL)
VS	volatile solid	(g/L)

Mathematical symbols

A	ultraviolet absorbance at wavelength of λ= 254 nm	
α	specific ozone molar absorption coefficient	(m^2/mol)

c	concentration	(mg/L)
I	light intensity after absorption by ozone	
I_0	light intensity before absorption by ozone	
L	path length	(cm)
$CH_{4_{blank}}$	accumulated methane produced from the blank reactor	(mL)
$CH_{4_{sample}}$	accumulated methane produced from the samplereactor	(mL)
O_3	amount of ozone consumed per liter of sludge	(g/L)
O_{3in}	inlet ozone concentration	(g/L)
O_{3out}	outlet ozone concentration	(g/L)
P	power	(kW)
Q	flow rate	(L/h)
t	time	(s, hr, day)
V	volume	(L)
$V_{In_{sample}}$	volume of the inoculum in the sample reactor	(L)
$V_{In_{blank}}$	volume of the inoculum in the blank reactor	(L)

1 Introduction

1.1 Treatment of excess sludge: a bottleneck in wastewater treatment plants

Sludge is produced as a result of the biological wastewater treatment process. The continuous increase in the number of wastewater treatment plants all over the world creates a new challenge: sludge management (Braguglia et al., 2012). In recent years, the need to achieve a sustainable sludge management strategy has become of great concern, due to the new regulation and restriction, human health and direct sludge utilization in agriculture (Smith, 2009b).

The treatment of sludge is especially important because of possible existence of undesirable toxic contaminants which are discharged in urban wastewater from industrial sources (Clarke and Smith, 2011, Smith, 2009a). These toxic substances tend to concentrate in the sludge during the treatment process (Mathney, 2011). In wastewater treatment the pathogenic organisms mainly attached to the solid particles, thus might be accumulated in sludge and can cause serious diseases in human being (Arthurson, 2008). Therefore, sludge has to be stabilized before an environmentally safe utilization and/or disposal can occur.

Considering the sludge as a resource, it contains valuable matters such as water, organic matter, energy, and nutrients. Sustainable sludge management emphasis on developing innovative systems with the aim of maximizing the recovery and reuse of valuable products from the sludge (Spinosa et al., 2011). Anaerobic digestion is a sustainable biological process which stabilize the sludge components and reduce the organic content of the solid waste. Stabilized sludge contains organic carbon and nutrients such as nitrogen and phosphorus which can be used as fertilizer or suitable soil conditioner (Senthilkumar et al., 2014). Thus, sludge treatment aims to close the nutrient loops to ensure the retention of nutrients to agricultural lands. Nevertheless, in many industrialized countries agricultural application of sludge poses a great concern, regarding the potential content of persistent pollutants such as heavy metals as well as pathogens contamination (Khalid et al., 2012). With respect to the possible hazardous impacts of the contaminations present in the sludge, in industrialized countries energy recovery from sludge, mainly through biodigestion, is gaining considerable attentions (Lorenz et al., 2013).

Energy production and efficiency are the important issues regarding the sludge stabilization. Anaerobic sludge stabilization provides the benefit of energy recovery by converting waste into valuable products such as biogas (Batstone and Jensen, 2011). Biogas produced from the anaerobic sludge digestion could save the electricity and the heat consumption in the wastewater treatment process. About half of the organic matter in sludge is susceptible to anaerobic biodegradation to form biogas (Elliot and Mahmood, 2007). Thus applying innovative treatment processes to gain more biodegradable organic substances out of sludge, certainly develops more efficient and sustainable sludge management systems.

1.2 Principals in anaerobic digestion of excess sludge

Anaerobic digestion is a natural process during which organic matter, in the absence of oxygen, is degraded to methane (60 – 70 %), carbon dioxide (30 – 40 %), water vapor and traces of nitrogen, hydrogen sulfide and ammonia (Borjesson and Berglund, 2007). Anaerobic digestion of excess sludge is considered to be a sequential processes; hydrolysis, acidogenesis, acetogenesis and methanogenesis (Fig. 1.1).

Hydrolysis: In this step, particulate organic compounds are hydrolyzed into soluble constituent parts such as sugars, long-chain fatty acids and amino acids. This is carried out by different groups of obligate or facultative fermentative bacteria through excreting extracellular enzymes (Kaseng et al., 1992).

The hydrolysis of carbohydrates to monomeric saccharides is done by the cellolytic and xylanolytic bacteria. The proteolytic bacteria produce proteases that catalyze the hydrolysis of proteins into amino acids. These can then be absorbed through the cytoplasmic bacterial membranes. Lipids are hydrolyzed to glycerin and long chain fatty acids through lipases and phospholipases. Hydrolysis is a relatively slow process and generally it limits the rate of the overall anaerobic digestion process. Conversion of non-structural carbohydrates to smaller molecules occurs within few hours, however the hydrolysis of proteins and lipids is more time-consuming and may take a few days (Chandra et al., 2012). Structural carbohydrates like cellulose or lignocellulose are the most difficult to transformation and therefore hydrolysis of lignocellulosic material represents a significant rate-limiting step in anaerobic digestion.

Acidogenesis: The dissolved sugars, long-chain fatty acids and amino acids produced by hydrolysis are used in this step either by fermentative bacteria or by anaerobic oxidizers (Siegrist et al., 2002), forming volatile fatty acids (VFAs) and alcohols, along with hydrogen and carbon dioxide. The concentration of hydrogen formed as an intermediate product in this stage influences the type of final product produced during the fermentation process. A full acidification of amino acids is dependent on low partial pressure of hydrogen, which can be provided by a coupling with hydrogen consuming methanogenic bacteria.

Acetogenesis: In the third step, acetogenesis, the products of acidogenic phase such as VFAs and alcohols are further degraded to acetate, hydrogen and carbon dioxide. Homoacetogenic bacteria produce acetate from a mixture hydrogen-carbon dioxide while obligate hydrogen producing acetogenic bacteria oxidize VFA in acetate, hydrogen and carbon dioxide.

Anaerobic oxidation of mixture of hydrogen-carbon (eq. 1.1), propionate and butyrate (eq. 1.2, eq. 1.3) in acetate are as follows:

$$4H_2 + 2\,HCO_3^- + H^+ \rightarrow\ CH_3COO^- + 4\,H_2O \qquad (\Delta G^{\circ\prime} = -98.7\ kJ\ per\ mole) \qquad eq.1.1$$

$$CH_3CH_2COO^- + 3\,H_2O\ \rightarrow 2\,CH_3COO^- + 3\,H_2 + HCO_3^- + H^+$$

$$(\Delta G^{\circ\prime} = +71.8\ kJ\ per\ mole) \qquad eq.1.2$$

$$CH_3CH_2CH_2COO^- + 2\,H_2O \rightarrow 2\,CH_3COO^- + 2\,H_2 + H^+$$

$$(\Delta G^{\circ\prime} = +44.80\ kJ\ per\ mole) \qquad eq.\,1.3$$

The hydrogen produced by these organism plays an important role in this process, as the reaction will only occur if the hydrogen partial pressure is low enough to thermodynamically allow the conversion of all the acids. In order to remove the resulting hydrogen, acetogenesis (hydrogen producing bacteria) needs to live in a syntrophic relationship with hydrogen consuming methanogens. Such a symbiotic cooperation provides favorable conditions for the degradation of fatty acids to acetate and methane.

Methanogenesis: The final step of anaerobic digestion is methanogenesis carried out by three groups of methanogens which belong to Archae. Aceticlastic methanogens degrade acetate, hydrogentrophic methanogens (hydrogen utilizing methanogens) synthesize methane from hydrogen and carbon dioxide and methylotrophic methanogens which degrade methyl group of acetate. Only a few species of methanogens are capable of utilizing acetate. It is estimated that about 70 % of methane produced in anaerobic digesters is formed from the methyl group of acetate, and about 30 % is produced by hydrogen utilizing methanogens. The symbiotic relationship between acetogenic bacteria and hydrogen utilizing methanogens provides favorable conditions for both groups of microorganism. Methanogens consume hydrogen produced by acetogenic bacteria thereby keep the hydrogen partial pressure low. Under this condition VFA can be degraded to acetate. If the partial pressure of hydrogen was high acetogenic bacteria cannot degrade VFA. Build-up of VFAs tends to reduce the pH resulting in significant inhibition of the methanogens activity.

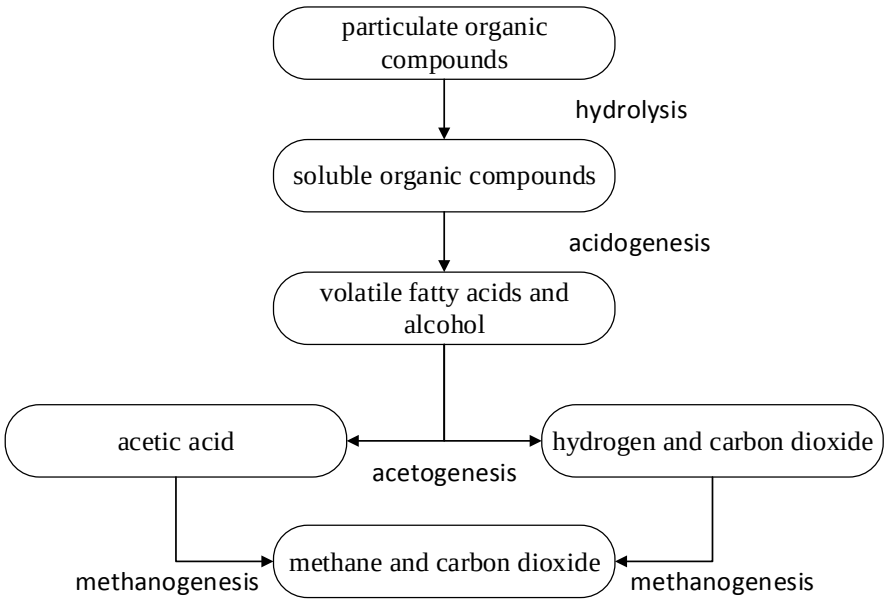


Fig. 1.1 Sequential anaerobic digestion processes; hydrolysis, acidogenesis, acetogenesis and methanogenesis, taken from Appels et al. (2008).

1.3 Sludge pretreatment methods

The anaerobic digestion of excess sludge has been shown to be a valuable treatment due to the reduction of sludge volume, destruction of pathogenic organisms and stabilization of the sludge. Attractive benefits of anaerobic digestion are not only sludge stabilization, but involved potential biogas production. However, this process is very slow (Kim et al., 2010). Anaerobic digesters have retention times in the range of 20 – 30 days and approximately only half of the organic material fed to anaerobic digestion could be degraded and subsequently transformed to methane (Rani et al., 2014). These limitations are associated to the disintegration and hydrolysis of sludge flocs containing microbial cells embedded in extracellular polymer substances (EPS) (Quan et al., 2013). EPS consist of large organic polymers such as carbohydrates, proteins and lipids and is a semi-rigid structure causing resistance to the subsequent microbial biodegradation (Vu et al., 2009).

To enhance the degradation of organic matter, hence production of higher biogas various pretreatment methods have recently been investigated in order to accelerate the rate of hydrolysis in the anaerobic digestion process (Carrère et al., 2010). These methods include mechanical, thermal, chemical and biological interventions. The aim of all pretreatments is to disintegrate the sludge flocs, disrupting the cell wall, thus releasing and solubilizing intracellular material into the liquid phase. Sludge disintegration enhance transformation of particulate organic compounds into more readily biodegradable substances and subsequently accelerate the process of anaerobic methane production (Wang et al., 2016). Among different disintegration methods ozone, ultrasound and alkaline treatment with sodium hydroxide have been reported to be the very promising techniques to increase the solubilzation degree and enhance anaerobic digestion performance (Neyens et al., 2003, Zhang et al., 2009, Zhang et al., 2007).

This section provides an introduction to the three methods of sludge disintegration; ozonation, alkaline and ultrasound pretreatment and the review of previous works done to investigate the effect of each disintegration method on sludge properties and performance of anaerobic digestion.

1.3.1 Ozone pretreatment

1.3.1.1 Mechanism of ozone reaction

Ozone is a molecule comprising of three oxygen atoms. It is a relatively unstable and reactive gas. Therefore, ozone is always generated on-site by an ozone generator. Ozone is generated by applying energy to break the bonds between the oxygen atoms in an oxygen molecule. This allows the oxygen atoms to dissociate and then reform as ozone (von Sonntag and von Gunten, 2012). The reaction of formation of ozone is as follow:

$$3O_2 \longrightarrow 2O_3 \qquad \Delta H = +\ 286\ \text{kJ/mol} \qquad \text{eq. 1.4}$$

The two main principles of ozone generation are UV-light and corona-discharge (Qasim, 1999). Ozone generation by corona-discharge is most common nowadays. Based on the corona discharge principle, ozone produced when high voltage passes through an air gap to break the oxygen molecule. This allows the formation of a three-atom oxygen molecule ozone.

Ozone reacts with organic matter based on the effect of direct oxidation and indirect free hydroxyl radical oxidation, both proceeding simultaneously. It is stated that direct oxidation of organic matter by ozone is a quite selective reaction mechanism, during which ozone reacts quickly with organic matter that contains double bonds, activated aromatic groups or amines (von Sonntag and von Gunten, 2012). Criegee mechanism and electrophilic reactions are the main mechanisms of direct ozonation (Atkinson and Arey, 2003, Miao et al., 2015).

Based on the Criegee mechanism, ozone reacts with unsaturated compounds such as double bonds leads to the formation of aldehydes, ketones and carboxyl compounds (Waring and Wells, 2015). Electrophilic reactions occur mainly in solutions that contain a high level of aromatic compounds. Aromatic compounds react relatively quickly with ozone (Naumov and von Sonntag, 2010).

1.3.1.2 Changes in sludge properties after ozonation

Ozone as a strong oxidant can oxidize a wide range of organic and inorganic compounds. Ozone is very effective in cell lysis. The most important mechanism of ozonation is proposed to be damages of microorganism cells. Ozone penetrates the cell wall and damages the cell membrane of microorganisms. As a result, the solid organic components of sludge are transformed to soluble substances.

The effects of various ozone doses on the solubilization of organic matter in terms of an increase in soluble chemical oxygen demand (SCOD) and a decrease in the total solids (TS), were investigated by several studies (Chu et al., 2008, Dogruel et al., 2007). Organic matter solubilization is linearly correlated to ozone consumption, until mineralization occurs. At this stage higher sludge ozonation is not successful in further release of organic matter. Ozonation lower than 0.02 g O_3/g TS was shown to be insufficient to penetrate the cell wall of the microorganisms and resulted in low degree of solubilization. It was reported that during sludge ozonation in the range of 0.02 to 0.09 g O_3/g TS, abundant of organic matter was solubilized mainly due to bacteria cell lysis. Some researchers showed that 0.05 g O_3/g TS was the optimum ozonation for cell lysis. Further increase in ozonation slightly improved the sludge lysis which might be attributed to the oxidation of the released organic compounds by ozone (Deleris et al., 2000, Park et al., 2004, Zhang et al., 2009).

The application of sludge ozonation prior to anaerobic digestion was investigated by many researchers (Cesaro and Belgiorno, 2013, Silvestre et al., 2015). The effect of ozonation on the biodegradability of waste activated sludge depends largely on the ozone dose. Sludge treatment with ozone has two counteracting effects: Enhancing the hydrolysis of macromolecules which are not easily degradable by

methanogenic bacteria, oxidation of the soluble organic molecules and generation of byproducts during ozonation.

During sludge ozonation, no correlation was reported between sludge solubilization and biogas production. Effect of various ozone doses, 0.04, 0.06, 0.08 and 0.1 g O_3/g TS on anaerobic degradability of excess sludge was studied by Silvestre et al. (2015). The results revealed that lower ozonation increased biogas production, while the sludge digestibility was not improved with higher ozonation. The increase in biogas production obtained from pretreated sludge at low ozonation, might be due to the changes in structure of proteins during ozonation. On the other hand, generation of aldehydes during anaerobic digestion of excess sludge treated with high ozonation was observed. It was proposed that reaction of ozone with the long chains of fatty acids possibly generated aldehydes which might cause an inhibition for improving methane production. Cesaro and Belgiorno (2013) studied the effect of high ozonation on the anaerobic biodegradability of the organic fraction of municipal solid waste. They found that ozonation above 0.16 g O_3/g TS led to the production of biogas lower than untreated sludge. It could be concluded that sludge pretreatment with ozone has some limitations in improving anaerobic digestibility. Ozonation of excess sludge may convert hardly degradable compounds to easily degradable ones which can consequently impact the anaerobic digestibility of the sludge. Kianmehr et al. (2010) found that ozone appeared to preferentially solubilize nitrogenous compounds and increase the formation of NH_4^+. They showed that ozone improved hydrolysis of nitrogenous organics such as proteins. In addition to ozone dose, solid retention time (SRT) of the sludge was shown to have high influence in sludge digestibility. The results of the biochemical methane potential tests (BMP) demonstrated that when ozonation of sludge with short SRTs did not improve methane production, applying high dose of ozone to the sludge with long SRTs, substantially increased anaerobic digestibility of the sludge.

1.3.2 Alkaline pretreatment

1.3.2.1 Changes in sludge properties after alkaline pretreatment

Alkalization is a commonly examined method owing to its easy operation, simplicity and high efficiency. At high pH values, hydroxyl anions result in the saponification of lipids, loose of the natural shapes of proteins, and the transformation of extracellular and intracellular polymeric substances into the aqueous phase (Neyens et al., 2003). However, pH values higher than 10 in anaerobic digestion could inhibit anaerobic microorganisms. Therefore it is necessary to neutralize the alkaline pretreated sludge prior to anaerobic digestion. It was reported that 0.2 g NaOH/g TS with the pH of 13 was the optimum concentration for sludge solubilization and methane production (Li et al., 2012). Although solubilization of organic matter increased as a result of alkali addition, methane yield is not consistent with the sludge solubilization. Accumulation of cations present in the alkali-solution increases the solubilization but does not always enhance anaerobic digestion performance. The inhibitory byproducts

of the pretreatment conditions result in the low biodegradability of the solubilized substances in anaerobic digestion (Kim et al., 2013).

Li et al. (2016) showed that organic matter solubilized after alkaline treatment contained readily degradable compounds which could be directly converted to methane. The result of Fourier transform infrared spectroscopy (FT-IR) analysis proved that the released low molecular weight proteins after alkaline treatment were highly degradable during the anaerobic process. The removal efficiency of organic matter during anaerobic digestion of the alkaline pretreated sludge was significantly higher than that of the untreated sludge.

Alkaline pretreatment has been widely combined with other pretreatment methods to overcome their limitations. When 0.3 g NaOH/g TS was combined by thermal treatment (130 °C) sludge solubilization was 50 % higher than the sludge solubilization obtained after thermal treatment at 180 °C. Moreover, the thermochemical pretreated sludge improved methane production by 150 % compared to the thermal treatment alone (Tanaka et al., 1997). Significant solubilization of protein and high acidification efficiency of waste activated sludge in a fermentation was observed due to the combination effect of ultrasound and sodium hydroxide (Liu et al., 2008a).

1.3.3 Ultrasound pretreatment

1.3.3.1 Brief background of sonication

Ultrasound is a sound wave at a frequency above the normal hearing range of humans (> 20 kHz). When the ultrasound is applied to a medium, it generates repeating high and low pressure waves creating the cavitation bubbles in the medium. The cavitation bubbles expand to the unstable size and then suddenly collapse. The violent collapse produces very powerful mechanical shear forces (Tiehm et al., 2001, Wang et al., 2005).

The temperature and pressure inside the collapsing cavitation bubbles rise up to about 5000 K and 180 MPa. Under such extreme conditions hydroxyl radicals are generated which can degrade pollutants through sonochemical reactions (Riesz et al., 1985). In the case of excess sludge pretreatment, both the hydromechanical shear forces and the sonochemical reactions can contribute to the sludge disintegration. Wang et al. (2005) found that mechanical shear forces are predominantly responsible for sludge disintegration and the effects of hydroxyl radicals on oxidizing sludge particulate matter are more pronounced at high ultrasound intensity.

1.3.3.2 Main parameters affecting ultrasound sludge disintegration

Regarding excess sludge pretreatment with ultrasound Tiehm et al. (2001) and Zhang et al. (2008) found that higher sludge disintegration degree can be achieved at the lower ultrasound frequency that range of 20 - 40 kHz. In theory, the cavitation bubble radius is inversely proportional to the ultrasound frequency. Therefore, low ultrasound frequencies create larger cavitation bubbles (Leighton, 2007). Mechanical

shear forces produced as a result of bubble implosion are responsible for sludge disintegration instead of free radicals. On the other hand, at high frequency more radicals are produced which subsequently promote chemical reactions (Crum, 1995). Sludge disintegration is highly effective at low frequencies which have been restricted to around 20 - 25 kHz (Carrère et al., 2010, Pilli et al., 2011, Wang et al., 2006).

Ultrasound intensity is defined by dividing the ultrasound power to the surface area of the probe (W/cm^2). It is proved that increasing the power or intensity can improve sludge disintegration degree. For instance, degree of disintegration was double by increasing the intensity from 8 to 16 W/cm^2 (Neis et al., 2000). On the other hand, Contamine et al. (1994) explained that very high power results in the formation of dense bubbles that significantly restricts the energy transmission to the medium.

Ultrasound sludge disintegration depends on several factors such as solid content of the sludge, sludge volume, ultrasound power, frequency and duration of sonication. Ultrasound specific energy represents the amount of energy consumed by the solid content of the sludge. The specific energy is a function of ultrasonic power, duration, volume of sonicated sludge and TS concentration, and can be calculated using the following equation (Bougrier et al., 2005):

$$E_S = \frac{P \times t}{V \times TS} \qquad eq.1.5$$

where E_S is the specific energy in kJ/g TS, P the ultrasonic power in kW, t the ultrasonic duration in seconds, V the volume of sonicated sludge in liters, and TS the total solids concentration in g/L.

Generally higher sludge solubilization, in terms of reduction in total solid of the sludge or an increase in SCOD, can be achieved by applying higher energy. In other words higher sludge solubilization positively correlated to the ultrasound specific energy. Nevertheless, release of SCOD slows down at a certain specific energy. This is probably due to the depletion of easily disintegrable particles in the sludge (Salsabil et al., 2009).

Interestingly, Kidak et al. (2009) demonstrated that at a given ultrasound specific energy, a higher ultrasound power for a shorter duration results in the higher sludge disintegration degree than a lower power for a longer duration. This explained that the particles in the excess sludge are resistant to ultrasound disintegration. Therefore higher ultrasound power should be applied to break these particles.

Sludge characteristics such as sludge total solid and pH have significant impacts on ultrasound disintegration. Khanal et al. (2006) reported that the SCOD increased linearly by increasing TS from 1.5 % to 3 % and by increasing E_S to 35 kJ/g TS. However, even for a thickened sludge higher E_S did not always solubilize higher SCOD in the sludge liquid phase. Wang et al. (2005) studied the effect of different parameters that have significant impacts on sludge disintegration. He established a kinetic model which was defined based on the sludge concentration, pH, ultrasound density and intensity. According to the model pH has the highest influence on sludge disintegration.

Many studies have shown that combination of alkaline with ultrasound improve solubilization of microbial cells (Chu et al., 2001, Kim et al., 2010, Liu et al., 2008b). Alkaline treatment of sludge increases the pH, thereby modifying the structure of proteins and sonification of lipids (Cassini et al., 2006). Once the substances structures are modified by alkaline treatment, ultrasound mechanical shear forces can easier disrupt the cell walls and solubilize intra-cellular components into the liquid phase of the sludge.

1.3.3.3 Changes in sludge properties after sonication

Excess sludge contains EPS, bacteria and a large amount of water. Sonication disintegrates the EPS and loses the floc structure. This is confirmed by the release of cations such as Ca^{2+} and Mg^{2+} which play a key role in binding the EPS (Keiding and Nielsen, 1997). Wang et al. (2006) examined the release of main components of EPS such as protein, polysaccharide, and deoxyribonucleic acid (DNA) in the aqueous phase, during ultrasonic disintegration of excess sludge at different specific energy. Protein was predominant in the aqueous phase of sonicated sludge. The released protein after sludge sonication was 5.4 times higher than the released polysaccharide (Feng et al., 2009). Mechanical shear forces produced by ultrasound leads to sludge floc disintegration and microbial cell lyses, according to the specific energy applied. Foladori et al. (2010) showed that specific energy about 20 kJ/g TS extensively disaggregates the sludge flocs.

Ultrasound has widely been applied as pretreatment prior to anaerobic digestion. Ultrasound increases the specific surface area by reducing the particle size of solids in the sludge (Chu et al., 2002). Therefore, ultrasound increases the substrate utilization rate of anaerobic bacteria, thus enhances anaerobic degradability process. Grönroos et al. (2005) studied the effect of 2.5 min and 10 min ultrasound (27 kHz, 200 W/L) treatment of excess sludge on methane production. The results demonstrated that 2.5 min treatment improved methane production 2.5 times higher compared to untreated sludge. While for long sonication of 10 min methane production was not further improved. It was demonstrated by Tian et al. (2015a) that methane production per gram COD added increased 15 % after 11.5 kJ/g TS ultrasound specific energy. However, higher energy of about 21 kJ/g TS did not improved methane production. The results suggested that there is a certain point at which increase in specific energy is not efficient for sludge anaerobic biodegradability. It is often reported that specific energy range of about 1 - 16 kJ/g TS, is optimal for sludge solubilization by ultrasound (Carrère et al., 2010).

1.3.3.4 Comparison of sludge disintegration methods in terms of energy demand

A very good overview on various disintegration methods is provided by DWA-M 302 (DWA, 2015). In Table 1.1 various disintegration methods, treatment conditions and the corresponding degrees of disintegration are shown. It can be seen that the knowledge about the effectiveness of the sludge disintegration techniques is still very incomplete. Degree of sludge disintegration is conventionally evaluated based on the changes in SCOD after disintegration (increase of SCOD concentration after

disintegration divided by maximum SCOD obtained by alkaline hydrolysis) or degradation of total solids (TS) after sludge pretreatment. It seems that degree of disintegration depends heavily on the method of treatment, sludge composition, operating and ambient conditions.

Table 1.1 Sludge disintegration methods with energy demand, degree of disintegration (DD) in terms of the increase in COD and degradation of total solids (TS). The table is taken and translated from DWA-M 302 (DWA, 2015).

	Treatment condition	Degree of disintegration	comments
Mechanical	0.5 – 20 kWh/m^3	DD_{COD}: 20 % DD_{TS}: 50 %	• Only electrical energy demand • Chemical properties of sludge hardly changed • Ultrasound treatment has been used in some plants
Thermal	130 – 180 °C 130 – 190 kWh/m^3	DD_{COD}: 50 % DD_{TS}: 70 %	• Thermal energy is cheaper than electrical • Heat recovery up to 95 % • Increase in the formation of persistent compounds • Europe has a large number of implemented thermal treatment
Chemical	Hydrolysis	After addition of chemicals DD_{COD}: 100 %	• Neutralization of sludge after treatment with acid and base is necessary • Inhibition effect on the subsequent process is possible • The application is especially in industrial wastewater treatment
	Oxidation (ozone) 0.05 – 0.2 g O_3/g TS 0.19 – 2.5 kWh/kg TS	After ozonation DD_{COD}: 80 % DD_{TS}: 20 %	• Positive effects especially at very low doses of ozone, no recent studies on ozone and anaerobic degradation
Biological	Hydrolysis	DD_{TS}: 20 %	• Low investment costs, easy handling • In some operated systems a positive impact on floating covers and ragging • Enzyme activity can be limited by sludge components

Table 1.2 illustrates the classification of the disintegration methods based on the degree of disintegration and energy requirement. It is likely that oxidation with ozone, thermal and thermochemical sludge pretreatments provide substantial increase in COD degree of disintegration (30 % - 50 %), with a high consumption of energy (> 50 kWh/m^3). On the other hand, low impact pretreatments such as biological treatment with enzyme, hydrocavitation and electrokinetic disintegration required low energy input.

Table 1.2 Estimations of the required energy for various disintegration degree. The table is taken and translated from DWA-M 302 (DWA, 2015).

Degree of disintegration DD_{COD}	Typical energy input for sludge disintegration	Disintegration method
< 10 %	Low about 0 – 10 kWh/m³	• Electrokinetic disintegration • Hydrocavitation • Enzyme • Ultrasound disintegration
10 – 30 %	Middle about > 10 – 50 kWh/m³	• Ultrasound disintegration • High pressure homogenization • High performance pulse technology • Electroporation
30 – 50 %	High about > 50 kWh/m³	• Thermal process • Oxidation (Ozone, H_2O_2) • Thermochemical process

Degree of disintegration and increase in methane production after sludge disintegration with ultrasound and ozone with respect to the energy demand are summarized in Table 1.3. It can be seen that 8 kWh/m^3 energy is consumed by ultrasound to increase DD_{COD} to 20 % and methane production to 250 %, while applying higher energy (33 kWh/m^3) did not further enhanced methane production (Grönroos et al., 2005). With regard to the cost of energy, the optimum energy demand to increase degree of disintegration and subsequently methane production was reported in the range of 1.4 – 22 kWh/m^3 (Carrère et al., 2010), which is in agreement with the values provided by DWA-M 302 (DWA, 2015).

Ozone consumption between 0.05 to 0.1 g O_3/g TS is more effective in improving sludge disintegration and methane production, compared to ozone consumption above 0.1 g O_3/g TS (Weemaes et al., 2000, Yan et al., 2009). According to DWA-M 302 (DWA, 2015) sludge disintegration with ozone is a high energy consumption method (energy requirement > 50 kWh/m^3). Since the energy input to the pretreatment systems appears obviously too high compared to the recoverable biogas yield, it is strongly recommended to apply low ozone consumption in case of sludge disintegration.

Table 1.3 Energy requirement to increase the COD degree of disintegration (DD_{COD}) and methane production, for sludge disintegration with ultrasound and ozone.

	Energy requirement	Degree of disintegration DD_{COD}	Increase in methane production	Reference
Ultrasound	0.5 – 20 kWh/m^3	20 %		DWA (2015)
	16 kWh/m^3 32 kWh/m^3	23 % 33 %		Tian et al. (2015a)
	8 kWh/m^3 33 kWh/m^3	20 % 47 %	250 % 250 %	Grönroos et al. (2005)
	1.4 kWh/m^3 22 kWh/m^3	2 % 40 %	 140 %	Carrère et al. (2010)
Ozone	0.05 – 0.2 g O_3/g TS > 50 kWh/m^3	80 %		DWA (2015)
	0.05 – 0.1 g O_3/g TS 0.1 – 0.2 g O_3/g TS	20 % – 35 % 35 % – 40 %		Yan et al. (2009)
	0.05 g O_3/g TS 0.1 g O_3/g TS	25 % 34 %	150 % 180 %	Weemaes et al. (2000)

1.3.4 Biological denitrification process

Wastewater is rich in nitrogen in the form of ammonia or organic nitrogen. Discharge of these compounds into the environment promotes eutrophication which results in intensive growth of algae and depletion of oxygen in the aquatic environments (Guerrero et al., 2016). In the wastewater treatment nitrogen is commonly removed through biological nitrification and denitrification process. During nitrification, ammonium is converted to nitrite by ammonium oxidizing bacteria (AOB), in the next step nitrite is oxidized to nitrate which is commonly done by nitrite oxidizing bacteria (NOB). Nitrification is an aerobic process and both AOB and NOB are considered to be autotrophs since they drive energy from inorganic compounds such as CO_2 (Sedlak, 1991).

Reaction for oxidation of ammonium to nitrite by AOB:

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2H^+ + \ H_2O \qquad eq.1.6$$

Reaction for oxidation of nitrite to nitrate by NOB:

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \qquad eq.1.7$$

Denitrification is a biological reduction of nitrate to nitrite and nitrogen gas, primarily performed by heterotrophic bacteria. Denitrification proceeds in the absence of oxygen. In general to have a successful denitrification process, dissolved oxygen concentration should not exceed 0.2 – 0.5 mg/L. In this case, heterotrophic bacteria respires nitrate as an electron acceptor. A complete biological denitrification process requires a suitable electron donor, which is usually an organic compound (Payne, 1981).

Reaction for denitrification when organic compounds in wastewater are used as an electron donor:

$$C_{10}H_{19}O_5N + 10\ NO_3^- \rightarrow 5N_2 + 10CO_2 + \ 3H_2O + \ NH_3 + 10\ OH^- \qquad eq.1.8$$

The concentration of organic carbon relative to the concentration of nitrogen in the wastewater influent highly affects the biological nitrogen removal. A wide range of chemical oxygen demand to nitrogen (COD/N) ratio from 3 to 7 has been reported by several studies (Park et al., 2004). However, to achieve a complete denitrification, in practice this ratio varied in the range from 4 to 15 (Kujawa and Klapwijk, 1999). This is because COD/N ratio largely depends on the biodegradability of organic carbon and nitrogen compounds in wastewater influent. Since most of the organic carbon in the influent are oxidized in nitrification process, there is a lack of electron donor in the following denitrification process. In wastewater treatment plants external carbon sources such acetic acid, sodium acetate, and methanol are commonly added to keep the COD/N ratio in an acceptable range. Disintegrated sludge can be used as an internal carbon source to improve denitrification, since it is assumed to have a high content of available organic carbon.

Suitability of organic carbon released after mechanical disintegration in biological removal of nitrate was studied by Kampas et al. (2009). It was found that organic carbon in form of VFA released after

sludge disintegration, is a suitable type of carbon to improve denitrification. The disintegrated sludge and sodium acetate exhibited nearly the same rate of nitrate consumption. It was shown that ozonated sludge can enhance nitrogen removal, when an optimum COD/N ratio of 5.13 was chosen for denitrification process (Park et al., 2004). In this condition, denitrification rate of 3.66 mg NO_3-N/ (g TS. h) was achieved. The denitrification rate was reported to be in the range of 0.6 – 1 mg NO_3-N/ (g TS. h) when wastewater was used as a carbon source, while it varied between 2 – 7 mg NO_3-N/ (g TS. h) for sodium acetate (Henze, 1991, Kujawa and Klapwijk, 1999, Park et al., 2004).

1.4 Methods of dissolved organic carbon characterization

Dissolved organic carbon (DOC) refers to the fraction of organic compounds that passed through 0.45 µm filter. Organic substances in surface water and groundwater are produced from physical, chemical, biochemical processes taking place in water or originated from the wastewater effluent discharged into the aquatic systems (Awad et al., 2016, Zheng et al., 2016). Organic matter present in the wastewater effluent or sludge are the products of biological degradation in the wastewater treatment process (Navalon et al., 2011). Depending on the source of origination DOC comprised of several organic carbon with various structures. Knowing DOC concentrations of organic matter do not provide any indications of their degradability. Hence, compositions of DOC need to be taken into account, since its characteristics can highly influence water, wastewater and sludge treatment process (Chen et al., 2013, Chow et al., 2004, Rosenberger et al., 2006). Thus advanced methods are required in order to characterize the DOC in aquatic environments.

1.4.1 Ultraviolet absorption

Ultraviolet (UV) absorption is one of the favorable methods to characterize DOC. Organic compounds that contain aromatic structures and unsaturated carbon bonds have a strong UV absorbance at λ= 254 nm (UV_{254}) (Frimmel and Abbt-Braun, 2011, Guo et al., 2016). Specific UV absorbance (SUVA) is defined as UV_{254} absorbance divided by DOC concentration:

$$\mathrm{SUVA} = \frac{\mathrm{A}}{\mathrm{L} \times \mathrm{c}} \times 100 \qquad eq.\,1.9$$

where A is the UV_{254} absorbance, L is the path length of the quartz cuvette (cm), and c is the DOC concentration (mg/L).

SUVA is a useful parameter for estimating the dissolved aromatic carbon content in aquatic systems (Weishaar et al., 2003). For instance, to control the aromaticity of organic compounds during tertiary treatment of wastewater effluent, SUVA is one of the parameters which has been commonly investigated (Gonzalez et al., 2013). UV_{254} is only absorbed by the chromophore part of a molecule. Therefore, to characterize all components of DOC, UV_{254} detector has been coupled with chromatographic techniques such as size exclusion chromatography (Her et al., 2002, Kawasaki et al., 2011).

1.4.2 Size exclusion chromatography

An advanced technique to characterize DOC is size exclusion chromatography with online organic carbon detection (SEC-OCD), where molecules are separated by their physical and chemical differences. In this analytical system determination of the molecular size of DOC occurred based on the retention of the organic molecules eluting from the column. Larger molecules elute more quickly than smaller ones, while small molecules are penetrated into the pores of the resin in the column. In the SEC method exclusion limit refers to the molecular size of compounds which are too large to penetrate the pores of the resin. Conversely, permeation limit is defined by the small molecules which completely penetrate in the pores of the resin. SEC system is calibrated to get the exclusion and the permeation values (Huber and Frimmel, 1994). Ideally the resin material in a SEC system should be hydrophilic. In practice, most resins exhibit hydrophobicity and ionic charge (Trathnigg, 2004). Because of that as the organic compounds pass the column, interactions such as adsorption or ion exchange between the organic substances and the resin lead to the longer retention time. Thus it is appeared that organic compounds have smaller molecular size (Lankes et al., 2009). Selection of an appropriate eluent is important to minimize such interactions. For instance for ionic samples it is recommended to use salt/buffer solution as an eluent. To suppress the hydrophobic interactions, depending on the resin material, addition of organic modifiers to an eluent is suggested (Trathnigg, 2004). Using unappropriated eluent could cause physical deterioration of the resin (Ngoc Han et al., 2015).

In SEC-OCD oxidation of organic matters relies on the photochemical oxidation by UV and the resulting CO_2 is detected by infrared spectrometry. The oxidation efficiency of the online OCD has been discussed by several researchers and has been compared with the oxidation efficiency provided by thermal catalytic oxidation method which is able to oxidize all the DOC components (Aiken et al., 2002, Ogawa and Ogura, 1992, Specht et al., 2000). It was shown that depending on the structure of organic compounds up to 70 % of the DOC could not be detected with the online OCD system. It was concluded that oxidation efficiency of organic carbon measured by the online OCD was lower than that of measured by the thermal catalytic oxidation method (Lankes et al., 2009). Despite the restrictions of SEC-OCD system, this technique has been widely used to characterize DOC, based on the molecular weight of the organic components.

In addition to the online OCD, SEC can be coupled with online UV at λ= 254 nm detection (SEC-UV_{254}) and with online fluorescence detection at excitation wavelengths of λ_{EX}= 278 nm, 337 nm and emission wavelengths of λ_{EM}= 353 nm, 423 nm (SEC-$Fl_{EX:278/EM:353}$ and SEC-$Fl_{EX:337/EM:423}$). For instance, SEC-UV_{254} provides information about the aromaticity of DOC fractions, SEC-$Fl_{EX:278/EM:353}$ detects protein-like substances and SEC-$Fl_{EX:337/EM:423}$ identified fulvic acid-like substances e.g. in the groundwater samples (Her et al., 2003). The SEC system with multiple online detectors has been previously used to characterize organic substances in water and wastewater samples. As expected, waters from different

origin such as groundwater, surface water and wastewater showed substantial differences (Frimmel and Abbt-Braun, 2011, Her et al., 2003, Kumke et al., 2001).

Fig. 1.2 shows the organic carbon (OC) and UV_{254} chromatograms of a surface water (river Rhine). This figure is taken from Figure 6 of a work done by Frimmel and Abbt-Braun (2011), in which OC and UV_{254} chromatograms of different river waters, wastewater and wastewater effluent are presented.

The water samples were filtered through 0.45 µm filter, thus the OC chromatogram showed in Fig. 1.2 is the same as DOC chromatogram. Elution volume is the volume of the eluent required to elute the organic compounds from the column. As can be seen in Fig. 1.2 a relatively low OC and no UV_{254} responses were detected around the exclusion volume (V_o). These substances could be attributed to hydrophilic compounds with high molecular weight such as polysaccharides (Huber et al., 2011). The dominant OC and UV_{254} responses were obtained for the organic compounds eluted approximately between 40 and 60 mL of elution volume (Fig. 1.2). Significant UV_{254} absorbance reflects the presence of aromatic and unsaturated structures (Guo et al., 2016).

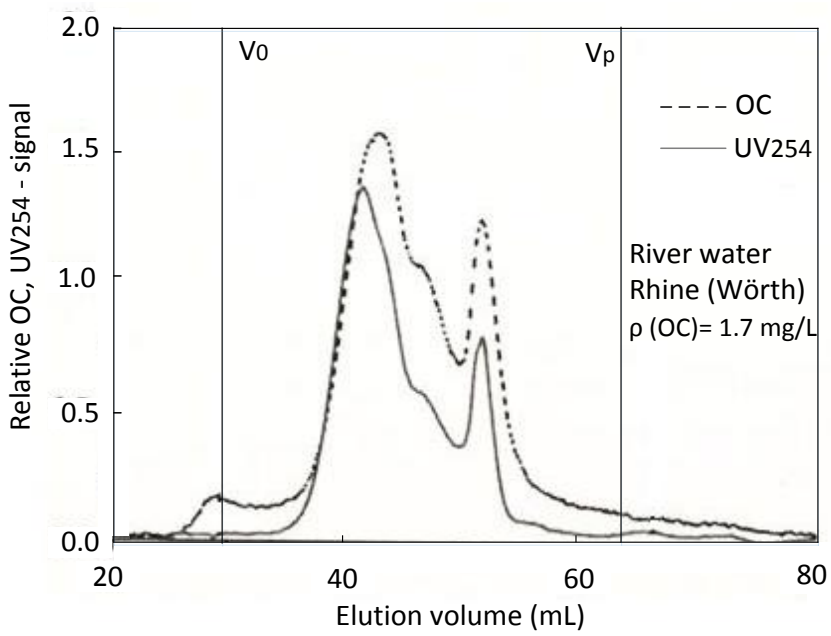


Fig. 1.2 OC and UV_{254} chromatograms of river Rhine, taken form Frimmel and Abbt-Braun (2011). In the obtained chromatograms the elution volume is equal to the retention time of organic compounds in the column because the eluent velocity was 1 mL/min.

Fig. 1.3 represents the OC and the UV_{254} chromatograms of wastewater and wastewater treatment plant effluent which is taken from Figure 6 of the work done by Frimmel and Abbt-Braun (2011). Comparison of the OC chromatograms obtained from the wastewater and wastewater treatment plant effluent (Fig. 1.3) revealed that the OC response for the small molecular weight compounds around the permeation volume (V_p) was very low in the wastewater effluent compared to the wastewater (Frimmel and Abbt-Braun, 2011). This illustrates the degradation of small molecular weight substances during biological wastewater treatment. On the other hand, large molecular weight substances around the exclusion

volume (V_o) with low UV_{254} response were dominant in the wastewater effluent, compared to the wastewater. This points to the generation of large molecules after biological treatment. Based on the classification of DOC explained by (Huber et al., 2011), the large molecular compounds with low UV_{254} can be attributed to polysaccharides. However, for detail interpretation of the components, combination of SEC-OCD with spectrometry methods such as Fourier transform ion cyclotron resonance mass spectrometry, has to be conducted (Kunenkov et al., 2009, Reemtsma et al., 2008).

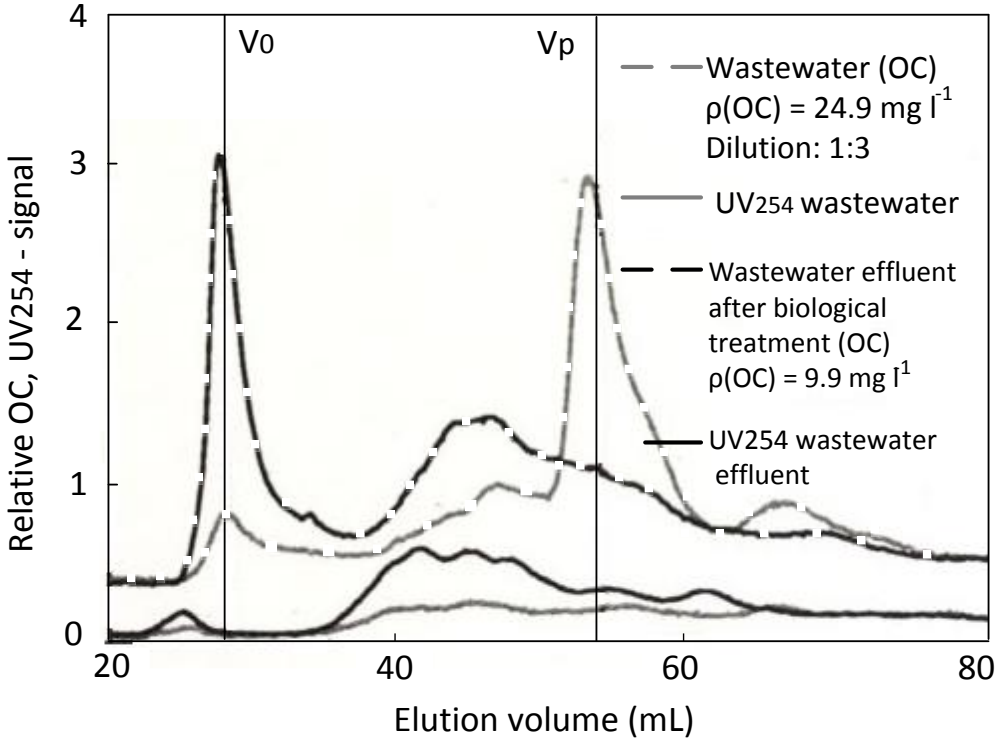


Fig. 1.3 OC and UV_{254} chromatograms of wastewater and wastewater effluent after biological treatment, taken from Frimmel and Abbt-Braun (2011).

Fig. 1.4 shows the DOC, UV_{254}, $Fl_{EX:337/EM:423}$ and $Fl_{EX:278/EM:353}$ chromatograms of a groundwater (Irvine Ranch groundwater). This figure is taken from Figure 5 of the work done by Her et al. (2003), in which DOC, UV_{254} and fluorescence chromatograms of waters with different origin such as groundwater, surface water and the secondary effluent of wastewater are shown.

As can be seen in Fig. 1.4a for the groundwater sample (Irvine Ranch groundwater) no $Fl_{EX:337/EM:423}$ intensity related to fulvic acid-like substances was obtained in large molecular weight fraction (F.1). The $Fl_{EX:278/EM:353}$ intensity related to the protein-like substances was also not detected in F.1 (Fig. 1.4a). Furthermore, relatively low UV_{254} response was observed in F.1, see Fig. 1.4b (Her et al., 2003). It seems that large molecular weight substances in Irvine Ranch groundwater were mostly composed of polysaccharides. Due to the high UV_{254} and high $Fl_{EX:337/EM:423}$ intensities in F.2, this fraction might composed of aromatic and double bound structures (Fig. 1.4a-b).

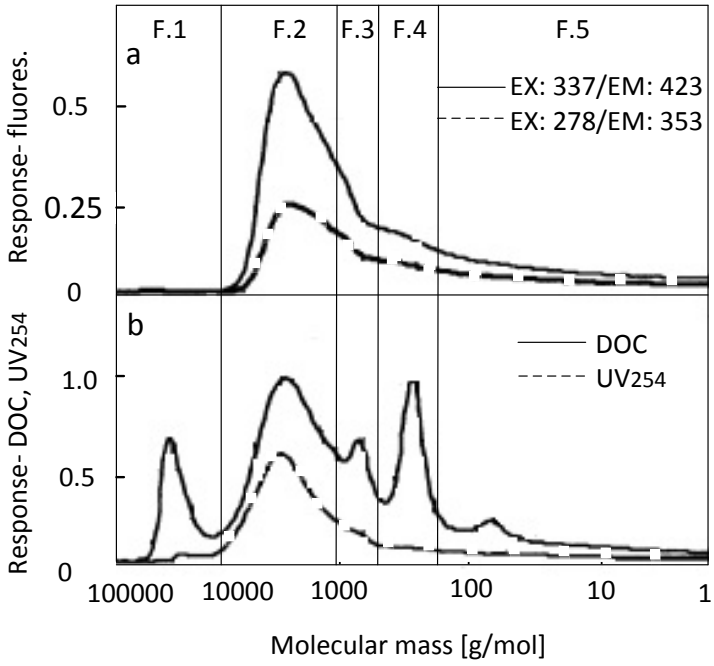


Fig. 1.4 Characterization of Irvine Ranch groundwater a) $Fl_{EX:337/EM:423}$ chromatogram related to fulvic acid-like substances and $Fl_{EX:278/EM:353}$ chromatogram related to protein-like substances, b) DOC and UV_{254} chromatograms, taken from Her et al. (2003).

Dissolved organic matter (DOM) from various origin contains substances with unknown structures and unknown molecular weights. Standard substances with the known molecular weights are used to find the relation between the molecular weight and the retention time of the standards. This relation is named as calibration standard. The apparent molecular weight of DOM is determined by comparing the retention time of the DOM with the retention time of the standards. Moreover, the separation in SEC is not only based on the size of the molecules and is influenced by the non-ideal interactions between DOM and the resin in the column. Therefore, the accurate molecular weights and molecular weight distributions of DOM is not possible.

SEC-OCD has been recently conducted to illustrate the difference in the molecular weight of substances solubilized after sludge disintegration methods (Li et al., 2016, Tian et al., 2015b). Pretreatment of excess sludge with ultrasound contributed in the release of high molecular weight components in the sludge liquid phase. However, combination of ozone with ultrasound utilizing in sludge disintegration showed that low molecular weight components were the main products of the combination treatment. This implies that high molecular weight products generated by ultrasound were effectively degraded in the subsequent oxidation process by ozone (Tian et al., 2015b).

Molecular weights of the solubilized substances can be considered as an important factor to evaluate the performance of sludge pretreatment method. Due to the changes in the molecular weight of the organic compounds after sludge disintegration, possible promotion on the solid reduction and methane

production in the anaerobic digestion of the disintegrated sludge can be assumed. SEC-OCD was conducted to illustrate the improvement in anaerobic digestion performance when solubilization products by alkaline sludge pretreatment was fed as a substrate in to the anaerobic reactor. The chromatogram of the alkali-solubilized DOC primarily composed of low molecular weight substances. These components were assigned to low molecular weight polysaccharides and amino acids which are easily degradable carbon sources for the anaerobic bacteria (Li et al., 2016).

1.4.3 Excitation-emission matrix fluorescence spectroscopy

Although characterization of DOC by SEC in combination with multiple online detectors has been widely used and accepted, packing material of the column and the eluent could highly influence the results. Therefore, development of alternative techniques to characterize DOC in aqueous samples have been taking attentions (Baghoth et al., 2011, Goldman et al., 2012).

Fluorescence spectroscopy is a reliable and highly sensitive technique to characterize DOC in aqueous samples. Simple sample analysis coupled with rapid data acquisition, have made fluorescence spectroscopy an attractive tool to analyze the fluorescence components of organic matter. The sensitivity of this method is typically 1000-fold more than UV absorption spectroscopy (Henderson et al., 2009). Furthermore, accurate sample analysis for low concentrations less than 1 mg/L DOC is applicable by fluorescence spectroscopy (Tran et al., 2015).

DOC can be classified into chromophoric and nonchromophoric fractions, based on the optical properties. Chromophoric fractions of DOC absorb light in the ultraviolet and visible range. This fraction of DOC is commonly attributed to fluorophores which have fluorescent signals (Coble, 1996). Thus only chromophoric fractions of DOC are detected by fluorescence spectroscopy. Therefore, similar to UV absorbance not necessarily all the DOC fractions can be characterized by this method.

To measure the fluorescence intensity of the soluble fluorescent products, continues light from an excitation source such as a xenon arc lamp, passes through a monochromator and strikes the sample. The monochromator separates the incident light and the fluorescent light. The excitation light is absorbed by the molecules in the sample which emits fluorescence. The fluorescent light reaches an emission detector which reports the fluorescence spectrum (Chen et al., 2003, Coble, 1996).

The most common fluorescent organic components of DOC in natural aqueous samples are related to fulvic acid-like and humic acid-like substances derived from the decomposition of plant material by biological and chemical processes. Amino acids in protein-like substances such as tryptophan and tyrosine are the other common fluorescence compounds. Protein-like, fulvic acid-like, humic acid-like substances represent fluorescence signals with distinct locations in an excitation-emission matrix (EEM) (Henderson et al., 2009, Patel-Sorrentino et al., 2002, Stedmon et al., 2003), see Fig. 1.5.

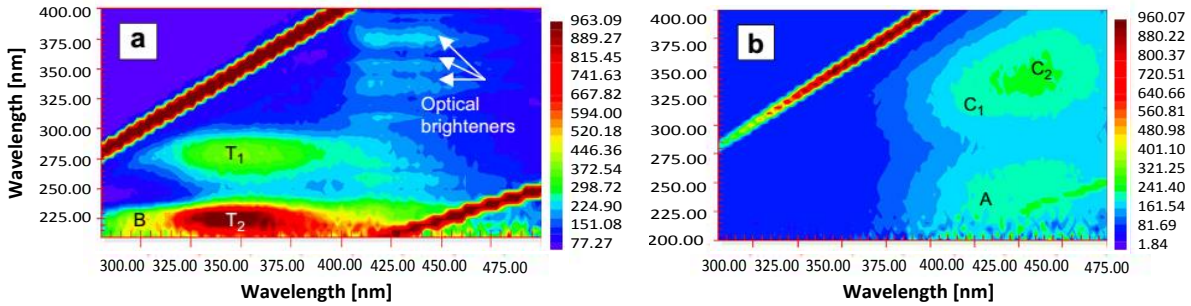


Fig. 1.5 Fluorescence excitation–emission matrices for a) raw sewage and b) river water, where B: tyrosine-like, T_1 and T_2: tryptophan-like, A: fulvic acid-like, $C_1$and C_2: humic acid-like substances, taken from Henderson et al. (2009).

Chen et al. (2003) isolated DOM fractions including hydrophobic and hydrophilic acids, bases and neutrals from a broad range of water sources such as surface water, groundwater, drinking water, wastewater effluent and standard substances. Suwanne River was used as fulvic acid and humic acid standard substances. Tryptophan, a standard for aromatic amino acid, was used for EEM confirmation of samples containing aromatic amino acids. While, tyrosine is used as a standard for aromatic proteins. The dominant EEM peak locations were obtained from the analysis of DOM fractions. Based on that regional excitation-emission wavelength boundaries were determined, which has been widely used for interpretation of the data obtained from fluorescence spectroscopy analysis. The regional excitation-emission wavelength boundaries were defined as follows (Fig. 1.6):

- Region I and II: peaks at shorter excitation wavelengths (< 250 nm) and shorter emission wavelengths (< 380 nm) are related to simple aromatic proteins such as tyrosine.

- Region III: peaks at shorter excitation wavelengths (< 250 nm) and longer emission wavelengths (> 380 nm) are related to fulvic acid-like substances.

- Region IV: peaks at intermediate excitation wavelengths (250 nm - 280 nm) and shorter emission wavelength (< 380 nm) are related to soluble microbial byproduct-like material and protein-like substances such as tryptophan.

- Region V: peaks at longer excitation wavelengths (> 250 nm) and longer emission wavelengths (> 380 nm) are related to humic acid-like substances.

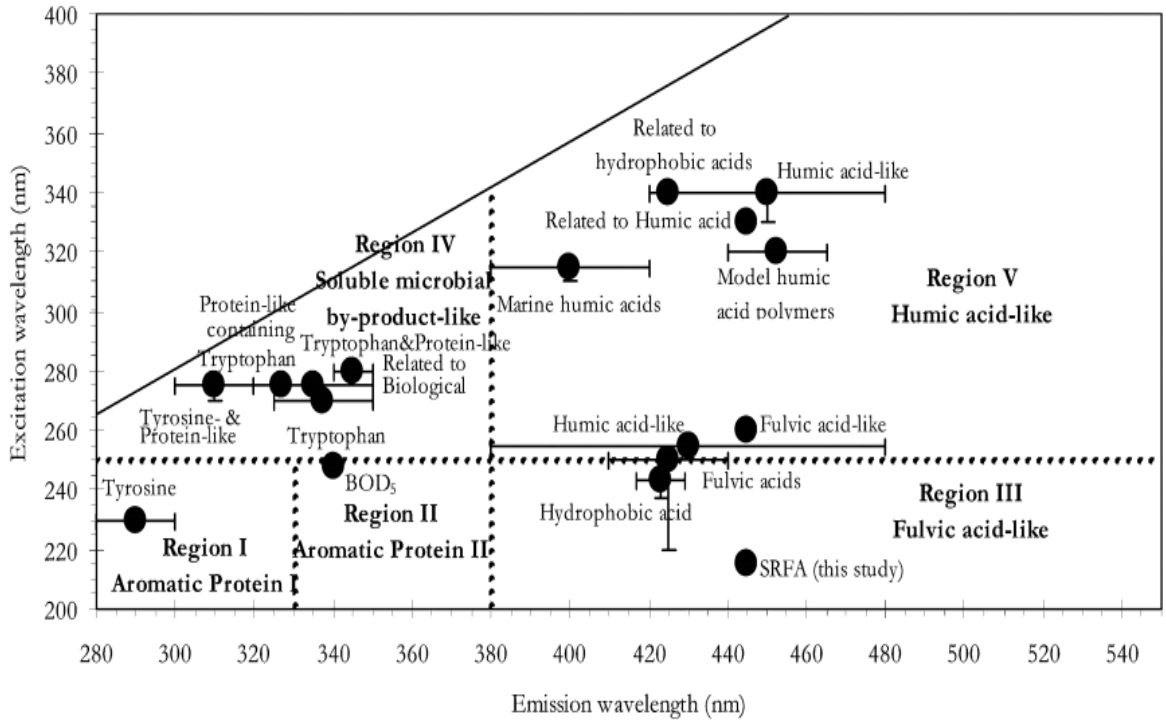


Fig. 1.6 Excitation and emission (EEM) wavelength boundaries and the location of EEM peaks, based on the dissolved organic matter fractions derived from rivers, drinking water, and wastewater effluent, along with model organic substances, taken from Chen et al. (2003).

Fluorescence spectroscopy has received extensive attention to identify and characterize DOM for water quality application. During drinking water treatment process organic matter can react and form disinfection byproducts which are classified as highly hazardous compounds. This indicates the importance of water quality monitoring by online, rapid and sensitive methods such as fluorescence spectroscopy (Hudson et al., 2007, Kuzniz et al., 2007). The quality of wastewater has been usually evaluated by off-line methods which are not always reliable. Fluorescence spectroscopy has been recently used for assessing the quality of wastewater for discharge in surface water and for process control in wastewater treatment plants (Carstea et al., 2016). DOC in wastewater influent comprises abundance of tryptophan-like substances. After biological treatment high fluorescence intensities related to soluble microbial products, aromatic amino acids, humic-like and fulvic-like substances were observed in the wastewater effluent (Henderson et al., 2009, Hudson et al., 2007).

Composition of DOC in the wastewater influent highly affects excess sludge properties. Fluorescence spectroscopy can be a useful tool to study the sludge components characteristics which are an important factors in the subsequent anaerobic digestion process. Soluble microbial byproduct such as tyrosine-like and humic acid-like substances were found to be the major fluorescence components of DOC in excess sludge. On the other hand, tyrosine-like and humic acid-like substances have been known to be hardly degradable (Zhang et al., 2013). Thus performance of anaerobic digestion process and methane production are highly influenced by presence and accumulation of these compounds during anaerobic

degradation (Wan et al., 2012, Zhen et al., 2012). Application of fluorescence spectroscopy in characterization of DOC may facilitate the detection of hardly biodegradable compounds in the digesters which further helps to find practical solutions to intensify their degradation. For instance, thermophilic hydrolysis of excess sludge successfully degraded tryptophan, tyrosine and soluble microbial by-product substances (Luo et al., 2013). However, fluorescence analysis demonstrated that thermophilic bacteria are not capable of removing humic acid-like substances.

1.5 General objectives and scopes of this work

As described so far, sludge disintegration is a favorable technique to increase the bioavailability of organic carbon in biological treatment processes such as anaerobic digestion and denitrification. In the present work, disintegration of excess sludge by means of ozone, sodium hydroxide and ultrasound is discussed and different aspects of disintegration methods are comprehensively investigated. The context and objectives of this work are explained in the following stages:

- Impacts of sludge pretreatment with ozone, sodium hydroxide and ultrasound on the solubilization and molecular size distribution of DOC were studied in section 3.1 which is comprised on three subsections:
  - First, the effect of ozone on the disintegration of sludge particles was studied during long time of ozone reaction with sludge varied from 2 min to 120 min. The DOC solubilization and mineralization of sludge were considered as the main processes to achieve the optimum DOC released into the sludge supernatant. SEC-OCD was conducted to separate the DOC components into different fractions based on their molecular size. The aim was to illustrate the impacts of ozone consumptions on the relative cDOC distribution (section 3.1.1).

  - Disintegration of excess sludge with various sodium hydroxide dosages and different duration treatments were examined to find out the optimum treatment condition. Effect of sodium hydroxide dosages and treatment time on the relative cDOC distribution was discussed (section 3.1.2).

  - Low ultrasound frequency was performed to disintegrate the sludge to the same degree of DOC solubiliaztion obtained by optimum ozone consumption and sodium hydroxide dosage. Furthermore, changes in the relative cDOC distribution after sludge sonication was studied (section 3.1.3).

- Comparison of sludge disintegration methods was done in section 3.2 with respect to the following aspects:
  - Molecular size distribution of DOC after sludge pretreatments (section 3.2.1).

- Batch anaerobic digestion experiments were conducted to evaluate the biodegradability of the released DOC after sludge pretreatments and subsequently the effect of disintegration on the methane production (section 3.2.2).
- To study the impacts of the molecular size of DOC released after sludge pretreatments on methane production during anaerobic digestion, samples were analyzed with SEC-OCD. Changes in the relative cDOC distribution and transformation of DOC fractions during anaerobic digestion experiments were comprehensively investigated. Furthermore, characteristics of the hardly biodegradable DOC were analyzed using SEC-OCD, SEC-UV_{254}, SEC-$Fl_{EX:254/EM:450}$ (section 3.2.3).
- The potential of the disintegrated sludge to be used as an internal carbon source for biological removal of nitrate was investigated in batch denitrification experiments. The denitrification test aimed to establish the nitrate utilization rates using different carbon sources (section 3.2.4).

❖ EEM fluorescence spectroscopy was coupled with SEC-OCD, SEC-UV_{254}, SEC-$Fl_{EX:275/EM:335}$ to gain more detailed information on the changes occurred in the aromaticity and fluorescence characteristics of DOC after sludge disintegration (section 3.3).

2 Material and methods

2.1 Sludge sources and collection

Samples of excess sludge, anaerobic sludge and denitrification sludge used in this study were obtained from a municipal wastewater treatment plant in Heidelsheim, Germany. The plant works on the principle of conventional activated sludge. The sludge samples were collected weekly and stored in refrigerator (4 °C) until use within 24 hours. The anaerobic digested sludge was collected from anaerobic digestion plant working under mesophilic conditions (37 °C) and the denitrification sludge from the anoxic denitrification basin. General characteristics of sludge samples are presented in Table 2.1.

Table 2.1 General characteristics of excess sludge, anaerobic sludge and denitrification sludge used in this study.

		Excess sludge	Anaerobic digested sludge	Denitrification sludge
TCOD	[g/L]	6 ± 1	23 ± 6	4 ± 0.3
SCOD	[mg/L]	37 ± 7	410 ± 50	27 ± 2
TS	[g/L]	6 ± 1	21 ± 1	4 ± 0.3
VS	[g/L]	4 ± 1	11 ± 1	3 ± 0.2
pH	-	7 – 7.1	7 – 7.2	7 – 7.1

2.2 Experimental setup and procedure

2.2.1 Ozonation setup

Ozone was generated from pure oxygen (99.5 % purity) with an Ozomat COM ozone generator (ANSEROS Company, Germany). A schematic of the ozonation set up is shown in Fig. 2.1. Excess sludge sample with the volume of 0.5 L (TS: 5 - 6.8 g/L) was placed in a reactor (reactor volume = 1.5 L) and ozonated for different time intervals from 0 to 120 min. To provide an effective contact between ozone and substrate to favor the oxidation of organic matter a diffuser was installed at the bottom of the reactor. Ozone gas with a concentration of 40 mg/L and a flow rate of 45 L/h was fed to the reactor. Ozone concentration in the gas phase after a certain time of reaction with sludge was measured with UV analyzers. The analytical principle of the UV analyzers is based on the absorption of UV light by the ozone molecule. The light is largely absorbed by ozone at λ= 254 nm. UV light at λ= 254 nm is passed through the sample cell. UV absorption is proportional to the amount of ozone present in the sample (Gottschalk et al., 2009). Ozone concentration is determined by means of Beer-Lambert law:

$$I = I_0 \exp(-\alpha L c) \qquad \text{eq. 2.1}$$

where:

I: light intensity after absorption by ozone

I_0: light intensity at zero ozone concentration

α: specific ozone molar absorption coefficient (m^2/mol)

L: path length (cm)

c: ozone concentration (mg/L)

First, the air sample passed through a scrubber. Here ozone is removed with manganese dioxide. Then air sample which is now free of ozone enters the sample absorption cell to establish a light intensity at zero ozone concentration (I_0). In the next step, the sample air does not pass the scrubber, thus directly enters the sample absorption cell. Here the light intensity after absorption by ozone (I) is measured. The difference between I and I_0 is correlated to the ozone concentration according to the Beer-Lambert law (eq. 2.1).

Ozone consumption was determined from the difference in the amount of ozone concentration at the inlet and outlet of the reactor per dry weight of initial total solid in the sludge and was calculated according to the following equation:

$$\text{Ozone consumption} = \frac{(O_{3_{in}} - O_{3_{out}}).Q.t}{TS\,.\,V} \qquad \text{eq. 2.2}$$

where O_{3in} is the inlet ozone concentration (g/L), O_{3out} the outlet ozone concentration (g/L), Q the ozone flow rate (L/h), t the ozonation time (h), TS the total solids concentration (g/L), V the volume of the sludge (L). The residual of ozone was destructed in a potassium iodide solution (KI, Merck, Germany).

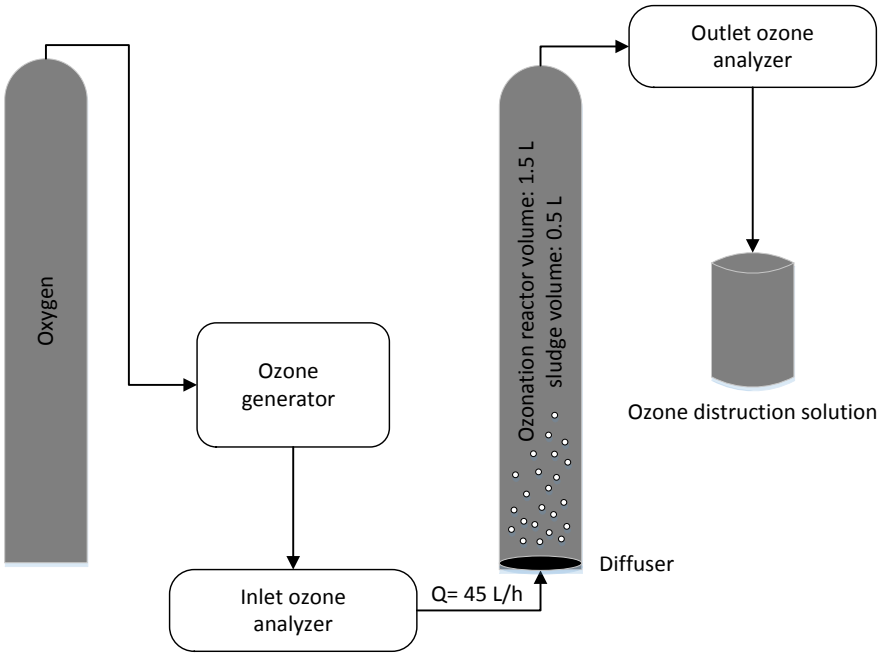


Fig. 2.1 Schematic diagram of ozonation setup in which ozone gas produced by ozone generator, enters the reactor of 1.5 L volume, with the flow rate (Q) of 45 L/h.

Excess sludge was disintegrated at different ozone consumptions varied from 0.005 to 0.16 g O_3/g TS depending on the time of ozonation varied from 0 to 120 min (Table. 2.2).

Table. 2.2 Ozonation time and corresponding ozone consumption

Ozonation time [min]	Ozone consumption [g O_3/g TS]
0	0
2	0.005
5	0.02
15	0.06
30	0.08
60	0.10
90	0.14
120	0.16

2.2.2 Alkaline treatment setup

In this study sodium hydroxide was used as an alkaline source. Sodium hydroxide (NaOH, VWR, Germany) was dissolved in deionized water to make 0.1 and 1 mol/L sodium hydroxide solutions. 200 mL of each solution was introduced to 0.5 L of excess sludge, resulted in 0.2 and 2 g NaOH/g TS dosage or 0.03 and 0.3 mol/L concentration, pH of 12.5 and 13.5, respectively. The sludge was mixed at around 200 rpm for the treatment periods of 2 and 24 hours. Prior to sample analyses, pH of the alkalized sludge was adjusted to about 7, using hydrochloric acid (HCL, 30 %, Brend Kraft, Germany).

2.2.3 Ultrasound treatment setup

Sonication was performed with ultrasound of type SONOPULS ultrasonic homogenizer HD 3200 (Bandelin electronic GmbH & Co.KG, Germany). The SONOPULS ultrasonic homogenizer is essentially made up of four components: generator, ultrasonic transducer, booster horn and the probe (Fig. 2.2). The generator transforms energy (with frequency of 50 or 60 Hz) into high-frequency energy with a frequency of 20 kHz. The ultrasonic transducer converts the high-frequency energy into ultrasound and thus into mechanical energy. The booster horn amplifies the ultrasound vibrations and then transmits them to the probe. The probe transfers the ultrasound amplitudes into the liquid media. A Rosett cell, RZ5, was used for an intensive and uniform sonication of the liquid media (Bandelin electronic GmbH & Co.KG, Germany). The sludge (volume = 0.5 L) was filled in the Rosett cell, ultrasound probe was placed in the center and immersed 1to 2 cm into the sludge. The sludge samples were pretreated for 7 min at 20 kHz frequency with the maximum input power of 120 W. This resulted in the specific energy input of 16.8 kJ/g TS.

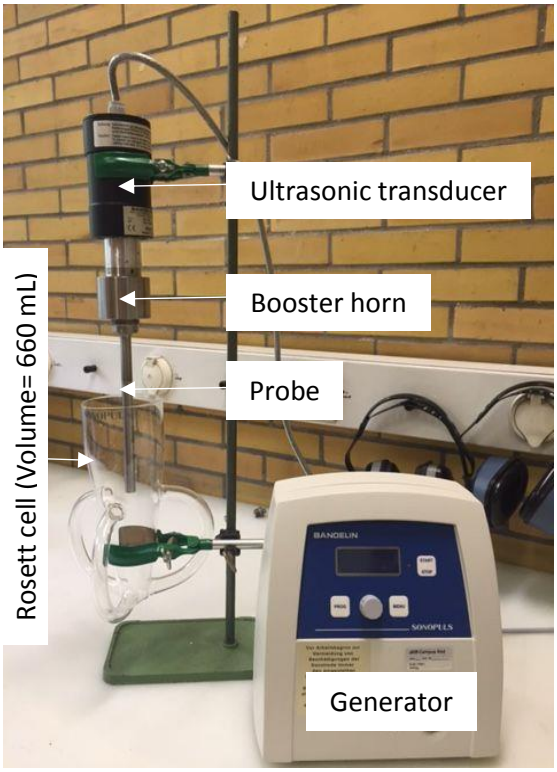


Fig. 2.2 Ultrasound setup which is made up of a generator, an ultrasonic transducer, a booster horn, a probe and a Rosett cell.

2.2.4 Methane measurement system

An automatic methane potential test system (AMPTSII, Bioprocess Control AB, Sweden) was conducted for anaerobic digestion experiment. A detailed description of the system can be found in (Badshah et al., 2012).

The anaerobic digested sludge collected from anaerobic digestion plant was used as an inoculum. Inoculum was pre-incubated for five days at test temperature (37 °C) to deplete residual biodegradable organic matter present in it. The activity of inoculum was checked by performing activity tests on standard substances such as gelatin, glucose and cellulose (Angelidaki et al., 2009). These assays give an idea of the inoculum response toward the standards substances. Methane yield is given in mL CH_4/g VS, where VS is the volatile solid of the sludge and represents the estimation of organic substances in the substrate. Methane yields by gelatin, glucose and cellulose as substrates were comparable with the theoretical values (Table 2.3). This demonstrates that the inoculum was active and qualified to treat the standard substrates. As can be seen in Table 2.3, the experimental methane yields obtained for standard substances are lower than the theoretical values. This explains that a fraction of substrate is used for cellular growth, maintenance and biomass formation.

Table 2.3 Experimental and theoretical methane yields of gelatin, glucose and cellulose as standard substances to check the activity of the inoculum in anaerobic digestion assay.

Substrate	Experimental yield [mL CH_4/g VS]	Theoretical yield [mL CH_4/g VS]	Reference
Gelatin	368 ± 20	433	Raposo et al. (2011)
Glucose	338 ± 2	380	Badshah et al. (2012)
Cellulose	347 ± 6	414	Raposo et al. (2011)

After the quality of the inoculum was proved, batch anaerobic digestion procedure was conducted for excess sludge and disintegrated sludge as the substrates. Excess sludge and disintegrated sludge (substrate) were centrifuged at 8000 rpm for 10 min, 300 mL of the supernatant from each substrate was mixed with 100 mL anaerobic digested sludge (inoculum) in a reactor. In order to prevent the inhibition effect of high pH on anaerobic bacteria, pH of disintegrated sludge with sodium hydroxide was adjusted to about 7 by adding hydrochloric acid before feeding to the digestion.

The blank reactors were filled with 400 mL of inoculum and without added substrate were assayed to subtract inoculum's methane production from the sample reactors (contain substrate and inoculum). The content of the reactors were flushed with nitrogen to remove oxygen and make the condition anaerobic. The experiments were carried out in triplicates for each substrate under mesophilic conditions (37 °C) and daily methane production was monitored for 5 days. To study the effect of each disintegration method on the biodegradability of the released organic carbon and methane production, samples were taken from each reactor at the beginning, after one day and after five days of anaerobic digestion and were analyzed by SEC-OCD. The amount of methane which was produced from the substrate after one day or five days was normalized to the amount of chromatographable DOC $(cDOC)_{t0}$ of the sample in the reactor (substrate and inoculum) at the beginning of the anaerobic digestion experiment and is calculated as follow:

$$\frac{mL\ CH_4}{g\ cDOC_{t0}} = \frac{(CH_4)_{sample} - [(CH_4)_{\ blank} \times \frac{V_{In_{sample}}}{V_{In_{blank}}}]}{g\ cDOC_{t0}} \qquad eq.\ 2.3$$

CH_4: volume of methane produced from the substrate (mL).

$cDOC_{t0}$: amount of the cDOC of the sample in the reactor (sample is a mixture of substrate and inoculum) at the beginning of the anaerobic digestion experiment (g).

$CH_{4_{sample}}$: accumulated methane produced from the sample reactor (contains substrate and inoculum) (mL).

$CH_{4_{blank}}$: accumulated methane produced from the blank reactor (contains inoculum) (mL).

$V_{In_{sample}}$: volume of the inoculum in the sample reactor (contains substrate and inoculum) (L).

$V_{In_{blank}}$: volume of the inoculum in the blank reactor (contains inoculum) (L).

2.2.5 Denitrification test

To assess the impact of disintegrated sludge as the internal carbon source, a series of experiments were designed to investigate the nitrate removal rate and compare it with external carbon source such as sodium acetate. Sludge collected from denitrification tank in Heidelsheim wastewater treatment plant, was used as a medium which contains denitrifier bacteria. Untreated and pretreated sludge were centrifuged at 8000 rpm for 10 min and the supernatant of the samples were used as a carbon source in the denitrification process.

Vessels of 650 mL volume containing 200 mL of denitrification sludge (contain denitrifiers), were filled with 200 mL supernatant of untreated sludge, sludge pretreated with ozone, sodium hydroxide and ultrasound. 200 mL solution of sodium acetate ($C_2H_3NaO_2$, Merck, Germany) was mixed with 200 mL denitrification sludge in a vessel. Nitrogen gas was continuously supplied to the headspace of the vessels to ensure anaerobic conditions. The vessels were mixed with magnetic stirrers at 200 rpm for 6 hours of the denitrification process. To compare the effect of different carbon source on the nitrate removal rate during denitrification experiments, approximately the same amount of SCOD released after ozonation, sonication and sodium hydroxide pretreatment and SCOD provided by sodium acetate solution was added to each vessel. The initial nitrate nitrogen concentration (NO_3-N) was fixed to 80 ± 6 mg/L in the form of potassium nitrate (KNO_3, Merck, Germany). During 6 hours of denitrification experiments, samples were taken every one hour and analyzed for NO_3-N.

2.3 Analytical methods

2.3.1 Physical and chemical analyses

TS and VS of the sludge were carried out according to the DIN 38 414 (DIN, 1985). TCOD, SCOD was measured according to the German DIN ISO 15705 (DIN, 2002) and NO_3-N was analyzed based on (DIN, 2011). SCOD and NO_3-N were determined after centrifugation of the sample at 8000 rpm for 10 min and filtration of the supernatant through 0.45 µm polyether sulfone (PES) membrane filter.

The carbohydrates concentration in the supernatant of the sludge samples were measured using the anthrone method (DuBois et al., 1956), which is a simple colorimetric method. The carbohydrate concentration in the sample is estimated via reading the absorbance of the resulting solution against a glucose standard curve.

Lowry protein assay was used to determine the protein concentration in the liquid phase of the sludge (Lowry et al., 1951). The protein concentration is exhibited by a color change of the sample solution in proportion to proteins concentration, which can then be measured using colorimetric techniques.

The soluble protein and carbohydrate concentrations were converted to equivalent DOC concentration by assuming stoichiometric conversion factors of 0.53 for protein and 0.36 for carbohydrate, which were derived from the protein formula $C_{16}H_{24}O_5N_4$ and carbohydrate formula $C_5H_{10}O_6$, represented by Rittmann and McCarty (2001)

2.3.2 Ultraviolet absorbance

UV absorbance at λ= 254 nm (UV_{254}) was measured using the Carry 50 Spectrophotometer (Varian, United States of America). The samples were filtered through 0.45 μm PES membrane filter and were diluted with deionized water to reach the UV_{254} absorbance lower than 1. The measurement was done through a quartz cuvette of 1 cm path length. This measurement is representative of the average UV_{254} absorbance of all organic molecules. SUVA value for each sample is determined by dividing the UV_{254} absorbance value to the concentration of DOC based on the eq. 1.9 explained in section 1.4.1.

2.3.3 Particle size analysis

Changes in the particle size of the sludge before and after ozonation were measured with dynamic image analysis (Camsizer XT, Retsch). Principally the particles are dispersed by means of the compressed air when passing the measurement area. Moreover, due to the effects of Van-der-Waals forces or electrostatic charges, small particles tend to make aggregates. To avoid such impacts, shear forces are applied to break up particles agglomerations. Dispersed particles passed through the light sources and two cameras. The basic camera analyzes the large particles, whereas the zoom camera detects the smaller particles. With Camsizer XT, it is possible to measure a wide range of particles from 1 μm to 3 mm and to determine the size distribution based on the width, length and average diameter of the particles. The advantage of this mode is that the measurement is simple and fast. Relatively small volume of sample, about 5 mL, is required for analysis and each measurement takes approximately 2.5 min.

2.3.4 Thermal catalytic oxidation

Total components of DOC were oxidized through thermal catalytic oxidation and the concentration of DOC was analyzed using a Shimadzu TOC-V CSN analyzer (Shimadzu, Japan). First, samples were filtered through 0.45 μm PES membrane filter. Total carbon (TC) in the filtered sample is composed of inorganic and organic carbons. In this technique, the inorganic carbon is volatized as CO_2 by acidifying the sample to a pH of 2 - 3. The remaining carbon (organic carbon) is introduced into the combustion tube, which is filled with an oxidation catalyst and heated to 680 °C. As a result, the organic carbon is converted to carbon dioxide. Carrier gas, which flows at a rate of 150 mL/min to the combustion tube, carries the sample combustion products from the combustion tube to an electronic dehumidifier, where the gas is cooled and dehydrated. The gas then carries the sample combustion products through a halogen

scrubber to remove chlorine and other halogens. Finally, the carrier gas delivers the sample combustion products to the cell of a non-dispersive infrared (NDIR) gas analyzer, where the carbon dioxide is detected. The NDIR outputs form a peak area is proportional to the DOC concentration of the sample. The detection limit of the method is 0.1 mg/L, the standard variation is ± 0.1 mg/L and potassium hydrogen phthalate (KHP, VWR, Germany) is used for the DOC calibration.

2.3.5 Size exclusion chromatography coupled with online organic carbon, UV and fluorescence detections

In this study SEC was carried out using three different online detectors, OC, UV and fluorescence detectors (Frimmel and Abbt-Braun, 2011). SEC was combined with online UV at λ= 254 nm detection (SEC-UV_{254}) and with online fluorescence detection at excitation wavelengths of λ_{EX}= 275 nm, 245 nm and emission wavelengths of λ_{EM}= 335 nm, 450 nm (SEC-$Fl_{EX:275/EM:335}$ and SEC-$Fl_{EX:254/EM:450}$).

The chromatographic column was packed with Toyopearl HW 50S resin (Tosoh Corp., Japan). The exclusion volume (at the retention time = 28 min) of the SEC column was determined using a solution of dextran blue. For the detection of the permeation volume (at the retention time = 71 min) methanol was used. The calibration was done with polyethylene glycols (molecular weights from 2 × 100000 to 2 × 100 g/mol), diethylene glycol (106 g/mol) and ethylene glycol (62 g/mol), which results in several nominal size fractions ranging from 2 × 100000 g/mol (at the retention time = 30.5 min) to 62 g/mol (at the retention time = 65.5 min), (nominal molecular weight calibration), see Fig. 2.3. A phosphate eluent (1.5 g L^{-1} $Na_2HPO_4 \cdot 2H_2O$ + 2.5 g L^{-1} KH_2PO_4; flow rate of 1 mL min^{-1}) was used as mobile phase with a flowrate of 1.0 mL/min. The dimensions of the column were: length = 250 mm, inner diameter= 20 mm (Novogrom columns, Alltech Grom, Germany).

Prior to SEC analysis sludge samples were centrifuged at 8000 rpm for 10 min and the supernatant was filtered through 0.45 µm PES membrane filter. In order to obtain optimal results, samples were diluted within a range of 6 - 15 mg/L. To separate the components of DOC by their molecular size, a sample of 500 µL was injected to the SEC column. The main components of OCD are the Graentzel Thin-Film Reactor and non-dispersive IR-Detector (Siemens, Ultramat 6, Germany). The organic carbon which passes the chromatographic column enters the reactor and exposed to UV radiation, thereby oxidized to carbon dioxide. The carbon dioxide is then detected by IR-detector. Considering the interactions between the organic compounds and the resin, some compounds are retained by the resin in the column. To determine the oxidation of organic carbon by the online OCD, a bypass line is designed in which a sample directly enters the Thin-Film Reactor without passing the column. A sample of 50 µL was injected to the bypass.

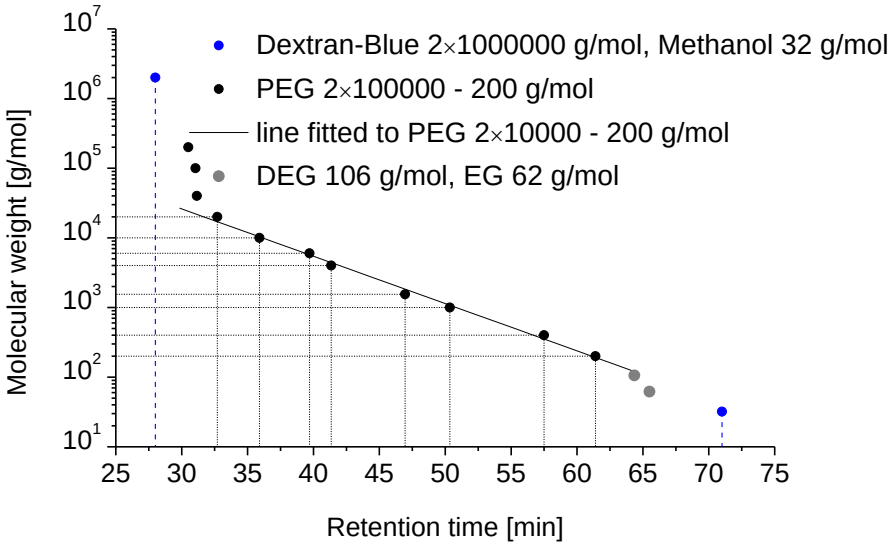


Fig. 2.3 SEC calibration with polyethylene glycols (PEG), diethylene glycol (DEG) and ethylene glycol (EG). Dextran blue and methanol were used to detect the exclusion volume (28 min) and the permeation volume (71 min).

In this study the chromatograms were divided into four fractions (Huber et al., 2011), see Fig. 2.4:

- Fraction 1 (F.1): this fraction refers to the large molecules with retention time of 26 - 36 min. Molecular weight of the organic carbon in F.1 can be possibly varied in the range of 2 × 100000 to 1 × 10000 g/mol compared to the calibration standard obtained for PEG, DEG and EG (Fig. 2.3).

- Fraction 2 (F.2): organic carbon in this fraction contains intermediate molecular size compounds elute between 36 - 52 min retention time. The approximate molecular weight of these compounds are in the range of 1 × 10000 to 1 × 1000 g/mol compared to the calibration standard obtained for PEG, DEG and EG (Fig. 2.3).

- Fraction 3 (F.3): intermediate molecular size compounds in this fraction pass the column between 52 - 60 min retention time. They are composed of molecules with the approximate molecular weight in the range from 1 × 1000 to 200 g/mol compared to the calibration standard obtained for PEG, DEG and EG (Fig. 2.3).

- Fraction 4 (F.4): molecules in F.4 are the smallest molecules and elute later from the column between 60 - 100 min retention time. Organic carbon in this fraction has the molecular weight of approximately lower than 200 g/mol compared to the calibration standard obtained for PEG, DEG and EG (Fig. 2.3).

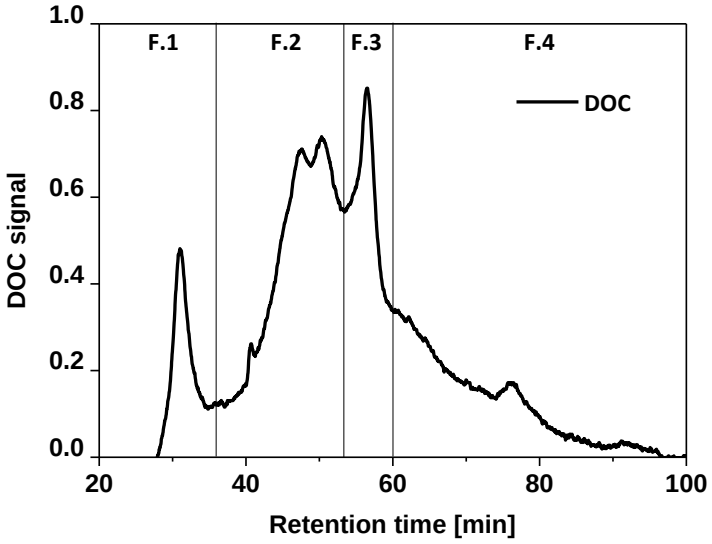


Fig. 2.4 DOC chromatogram divided into the four fractions based on the retention time of organic compounds in the SEC column.

SEC-OCD provides information about the concentration of the chromatographable DOC (cDOC) and its molecular size distribution. The concentrations of cDOC and bypass DOC were determined by converting the area of the cDOC chromatogram (retention time 26 -100 min) and bypass DOC signal to the DOC concentrations based on the calibration equation. Potassium hydrogen phthalate was used as a standard substance to determine the calibration equation.

The relative cDOC distribution in each fraction is proportional to the ratio between the area of the specific fraction to the area of the chromatogram (26 – 100 min).

2.3.6 Three-dimensional excitation-emission matrix fluorescence spectroscopy

Three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy was conducted to characterize DOC before and after sludge disintegration using fluorescence spectrophotometer (Aqualog, Horiba scientific, Germany). The spectrometer used an ozone-free xenon arc-lamp excitation source. Supernatant of untreated and pretreated sludge were filtered through 0.45 µm PES membrane filter. The filtered samples were analyzed by the thermal catalytic oxidation and DOC concentrations varied from 15 to 360 mg/L. In order to avoid the measured fluorescence intensity exceeding the maximum level, filtered samples were diluted with deionized water to reach DOC concentrations lower than 10 mg/L.

To obtain an acceptable EEM and fluorescence intensity, spectral and instrumental corrections were considered. To achieve the spectral responsivity, the reference detector and the emission detector signals were corrected by the correction-factor files which were applied to the data through Aqualog software. Considering the Raman scattering, the fluorescence spectra of the blank was subtracted from the

fluorescence spectra recorded for samples containing DOC. The blank solution was prepared from deionized water. Applying a Rayleigh-masking algorithm implemented in the Aqualog software, nullifies the signal intensities for the Rayleigh line. To account for the absorbance of light from the lamp by DOC molecules, an inner-filter effect (IFE) correction was applied by IFE algorithms provided by the Aqualog software.

To obtain fluorescence EEMs, the emission spectra in the range from 250 nm to 500 nm were detected at 4.5 nm steps for each excitation wavelength, which was incrementally increased from 250 nm to 450 nm at 3 nm intervals. The resultant EEMs of the samples were plotted as contour maps using Origin Pro9.3 program. In this study fluorescence components of DOC were identified based on the regional excitation-emission wavelength boundaries defined by Chen et al. (2003), see Fig. 1.6, section 1.4.3.

3 Results and discussion

3.1 Sludge pretreatment with ozone, sodium hydroxide and ultrasound

In section 3.1 changes in the solubilization and molecular size distribution of DOC were investigated under different disintegration conditions:

- Long time reaction of ozone with excess sludge (section 3.1.1)
- Various sodium hydroxide dosages and durations of treatment (section 3.1.2)
- Low ultrasound frequency (section 3.1.3)

In this study DOC of the samples was analyzed by thermal catalytic oxidation and SEC-OCD. The correlation between the DOC concentrations measured by different methods are represented in Fig. 3.1. It can be seen that for the samples analyzed by SEC-OCD, bypass DOC is 1.10 ± 0.15 times higher than cDOC (Fig. 3.1a). It means that up to 25 % of the DOC was retained by the resin. DOC analyzed by thermal catalytic oxidation is 1.32 ± 0.37 times higher than bypass DOC (Fig. 3.1b). This difference is due to the different oxidation methods. It seems that oxidation efficiency of the online OCD is lower than the oxidation efficiency of the thermal catalytic oxidation. Moreover, the DOC values determined by thermal catalytic oxidation are higher compared to the cDOC values obtained by SEC-OCD (Fig. 3.1c). This can be explained first by the interaction effects of organic compounds and the resin and second by the lower oxidation efficiency of the online OCD compared to the thermal catalytic oxidation. Despite this limitation, SEC-OCD is still a valuable method which was used in this study to investigate the changes in the molecular size distribution of DOC after sludge disintegration.

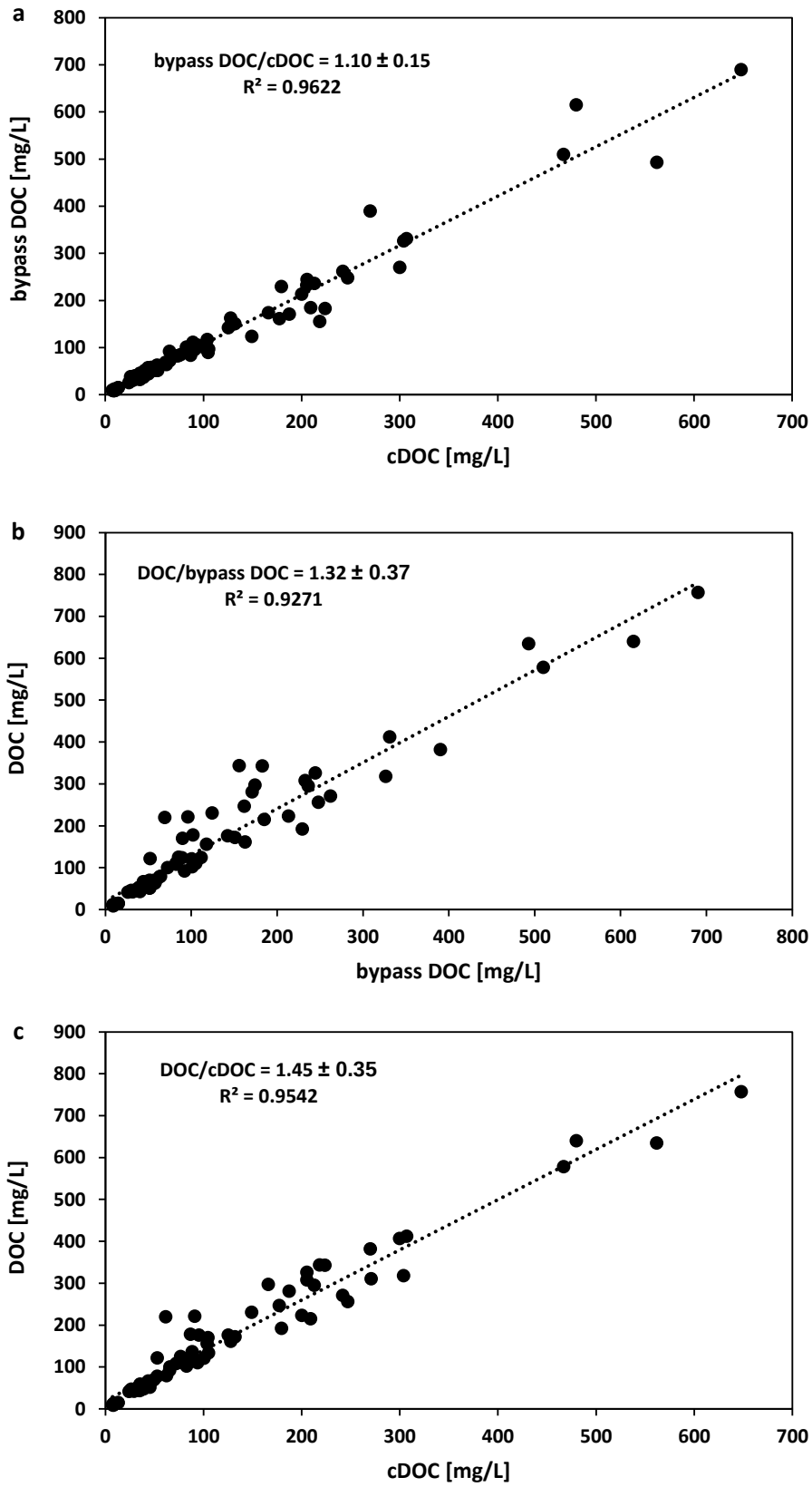


Fig. 3.1 Correlation between the concentrations of a) bypass DOC and the cDOC, b) DOC analyzed by thermal catalytic oxidation and bypass DOC and c) DOC analyzed by thermal catalytic oxidation and cDOC.

3.1.1 Sludge pretreatment with ozone

3.1.1.1 Effect of ozone consumption on DOC solubilization

To assess the solubilization of organic carbon as a result of sludge ozonation, excess sludge was disintegrated with different ozone consumptions varied from 0.005 to 0.16 g O_3/g TS depending on the time of ozonation 0 - 120 min. Table 3.1 shows the variation of DOC and TCOD concentrations corresponding to the ozone consumptions. To measure the concentration of DOC released before and after sludge ozonation, samples were filtered through 0.45 μm PES membrane filter and were analyzed by thermal catalytic oxidation.

Table 3.1 Effect of sludge ozonation on the concentration of DOC released in the supernatant of sludge and changes in the TCOD concentration in the whole sludge.

Ozone consumption [g O_3/g TS]	Ozonation time [min]	DOC [mg/L]	SD	TCOD [g/L]	SD
0	0	14	± 4	7.5	± 0.14
0.005	2	48	± 2	7.4	± 0.10
0.02	5	116	± 8	7.4	± 0.13
0.06	15	360	± 42	7.0	± 0.20
0.08	30	520	± 44	6.8	± 0.27
0.10	60	640	± 40	6.6	± 0.22
0.14	90	710	± 40	6.4	± 0.25
0.16	120	750	± 30	6.2	± 0.23

SD: standard deviation

Reaction of ozone with the particles in the sludge, solubilized high amount of organic carbon in the sludge liquid phase, reflected in a significant increase in DOC concentration (Table 3.1). It is stated in literatures that mechanism of sludge disintegration is mainly via the damages of cells. Ozone effectively penetrates into the microorganisms and damages the cell walls. This process contributes to the increase of total nitrogen, and protein. Thus the increase in DOC concentration is mainly due to the solubilization of intra-cellular organic substances (Cesbron et al., 2003, Paul and Debellefontaine, 2007, Zhang et al., 2009).

To study the effect of different ozone consumptions on the disintegration of sludge flocs and disaggregation of the solid particles in the sludge, particle size analysis was conducted. Changes in the size of the particles during sludge ozonation can represent the changes accrued in the sludge flocs and can help to better understand the contribution of sludge floc disintegration on the solubilzation of DOC.

The result of the particle size distribution before and after sludge ozonation is shown in Fig. 3.2. It is represented in Fig. 3.2 that the size distribution of the particles in the sludge was relatively stable when ozone consumptions varied from 0.02 to 0.1 g O_3/g TS. The behavior of ozone in solubilization of high amount of DOC and its effect on the size distribution of the particles, demonstrated that ozone preferentially attacked the bacteria cells, while it was less effective in sludge floc disintegration. Similar observation has been reported before (Bougrier et al., 2006, Zhang et al., 2007).

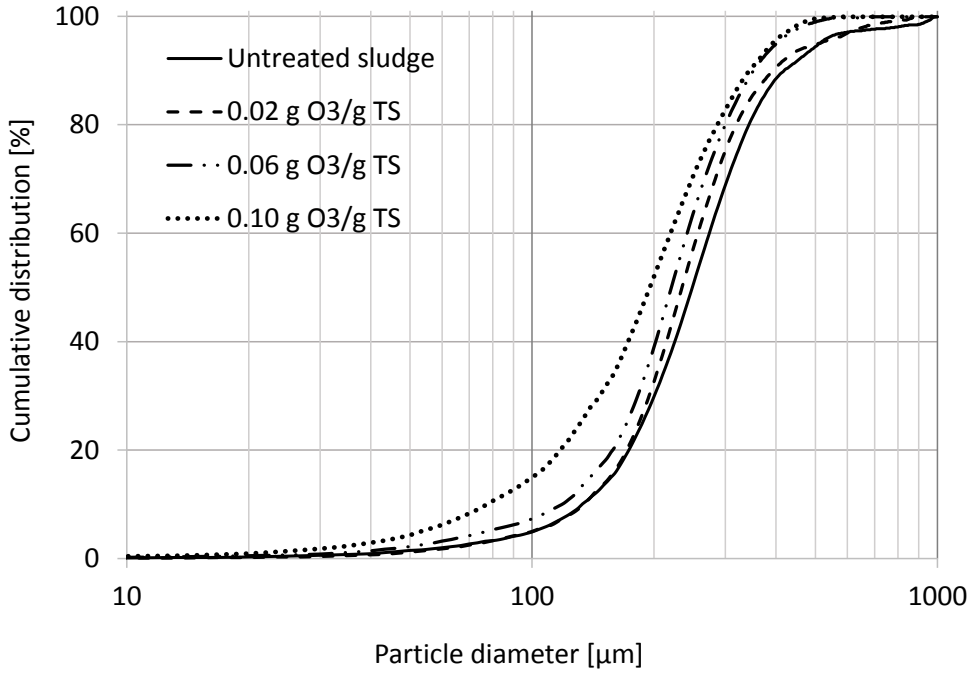


Fig. 3.2 Particles size distribution before and after sludge ozonation with 0.02, 0.06 and 0.1 g O_3/g TS.

Optimization of ozone process is necessary due to the high cost of energy consumption for generating ozone. Sludge solubilization and mineralization are the two important processes that should be considered to achieve optimum release of organic carbon.

Sludge solubilization was represented by the amount DOC released after disintegration, per amount of ozone consumed by total solids in the sludge and calculated as follows:

$$\text{Sludge solubilization: mg DOC}_{\text{released}}\text{/g O}_3\text{.g TS} = \frac{\text{DOC}_{O_3} - \text{DOC}_0}{O_3 \,.\, \text{TS} \,.\, \text{V}} \qquad eq.\,3.1$$

Where DOC_{O_3} and DOC_0 are the DOC concentration of the filtered supernatant of ozonated sludge and untreated sludge (mg/L), measured by thermal catalytic oxidation. O_3 is the ozone consumed by sludge during the reaction process (g/L), TS is the total solid of sludge before ozonation (g/L) and V is the sludge volume (L).

Due to the oxidation effect of ozone there is a reduction in TCOD which is represented as sludge mineralization and calculated as follows:

$$\text{Sludge mineralization: } \% = \frac{\text{TCOD}_0 - \text{TCOD}_{O_3}}{\text{TCOD}_0} \times 100 \qquad eq.3.2$$

Where TCOD_0 and TCOD_{O_3} are total chemical oxygen demand before and after sludge ozonation.

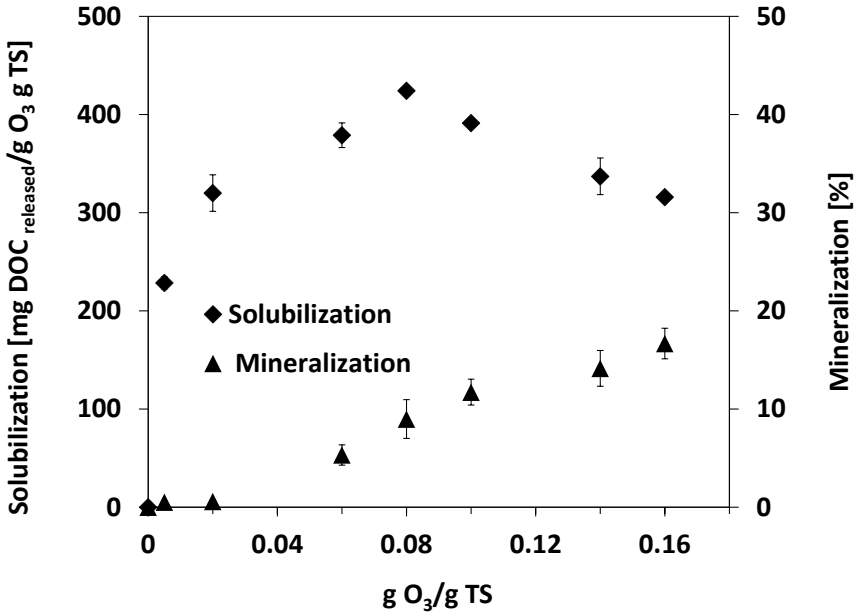


Fig. 3.3 The effect of ozonation on the DOC solubilization ($\frac{\text{DOC}_{O_3} - \text{DOC}_0}{\text{g O}_3 \text{ . g TS. V}}$) and mineralization ($\frac{\text{TCOD}_0 - \text{TCOD}_{O_3}}{\text{TCOD}_0} \times 100$) of sludge.

As can be seen in Fig. 3.3 DOC solubilization increased linearly by increasing ozone consumption from 0.005 to 0.08 g O_3/g TS. However, further ozone consumption from 0.08 to 0.16 g O_3/g TS oxidized the released organic matter which is represented by decrease in solubilization and increase in mineralization. The results showed that degree of solubilization depends greatly on ozone consumption, higher ozonation oxidized the organic substances released from the cell (Cui and Jahng, 2004, Saktaywin et al., 2005). When ozone consumption changed from 0.06 to 0.08 g O_3/g TS, sludge solubilization slightly increased, on the other hand increase in sludge mineralization was observed. This indicates that part of the released organic carbon could be oxidized by ozone. In order to reduce the loss of organic carbon and energy consumption 0.06 g O_3/g TS was determined as an optimum ozone consumption in this study. The recommended ozone dose to achieve a balance between sludge reduction efficiency and cost of energy ranges from 0.03 to 0.05 g O_3/g total suspended solid (Chu et al., 2009). Low ozonation was recommended in order to remain in an economical accepted range of about 0.375 Euro total costs per kg total solid (Weemaes et al., 2000). Ozonation at 0.05 g O_3/g TS was found to be the optimum dose for cell lysis while 0.08 g O_3/g TS did not significantly improved cell decomposition (Zhang et al., 2009). Results obtained in this study and in previous works indicate that mineralization of the released

organic carbon during ozonation of excess sludge is a limitation in ozone optimization process (Lee et al., 2005, Yan et al., 2009).

3.1.1.2 Molecular size distribution of DOC after ozonation

In the previous section effect of ozonation on the solubilization of organic carbon was demonstrated by changes in the concentration of DOC. Disintegration of excess sludge with ozone led to the release of organic substances resulting in an increase in DOC concentration. The organic carbon consists of molecules with various sizes. In this section, SEC-OCD was conducted to study the changes in the relative cDOC distribution after sludge ozonation to have a better understanding of the sludge disintegration mechanism with ozone.

Excess sludge was pretreated with ozone for different durations which resulted in different ozonation modes; low ozonation 0.005 - 0.02 g O_3/g TS, moderate ozonation 0.06 - 0.08 g O_3/g TS and high ozonation 0.10 - 0.16 g O_3/g TS. The supernatants of the untreated and ozonated sludge were analyzed by SEC-OCD. The DOC chromatograms for the supernatant of the sludge pretreated with various ozone consumptions are shown in Fig. 3.4. The DOC intensities raised by increasing ozone consumptions indicating the increase in the solubilization of organic carbon in the sludge liquid phase. Moreover, the DOC intensities in the fractions were changed, as the ozone consumptions increased. For instance, as demonstrated in Fig. 3.4a, the peak in the large molecular size DOC fraction (F.1) increased significantly from about 0.5 for untreated sludge to about 5, 10 after 0.005, 0.02 g O_3/g TS, respectively and to approximately 20 after 0.06 g O_3/g TS (Fig. 3.4b). While at ozone consumptions higher than 0.06 g O_3/g TS, the DOC intensity in F.1 was not significantly changed (Fig. 3.4b). It can be observed in Fig.3.4 that the peaks obtained in the intermediate molecular size DOC fractions (F.2 and F.3) raised by increasing the ozone consumptions from 0.005 to 0.16 g O_3/g TS.

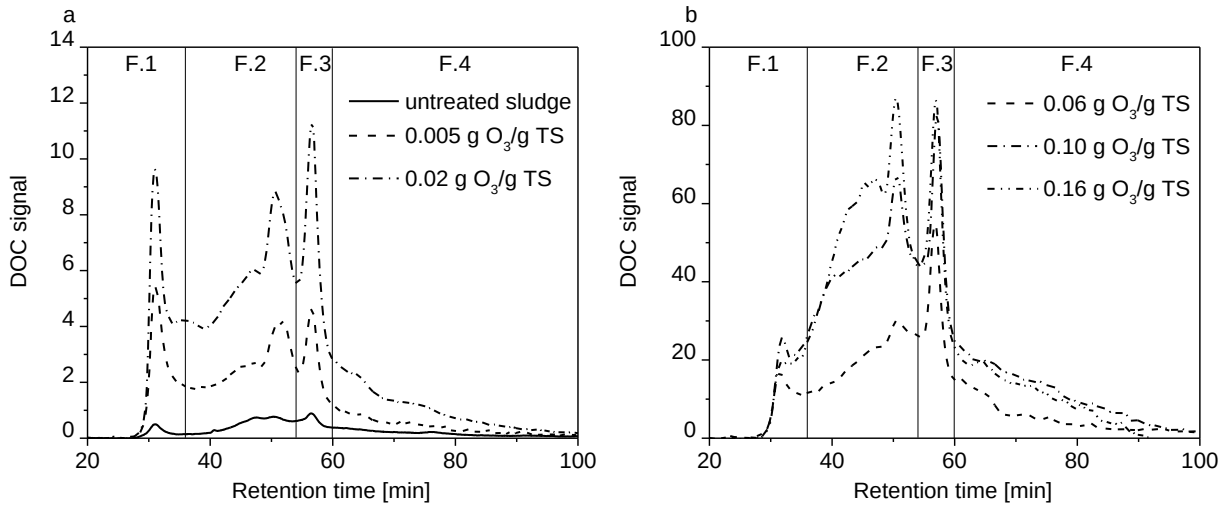


Fig. 3.4 DOC chromatograms a) for the supernatants of untreated sludge (cDOC= 9 mg/L) and sludge pretreated with low ozonation 0.005 - 0.02 g O_3/g TS (cDOC= 39 – 87 mg/L) and b) for the supernatants of sludge pretreated with moderate ozonation 0.06 g O_3/g TS (cDOC= 345 mg/L) and high ozonation 0.10 - 0.16 g O_3/g TS (cDOC= 693 – 720 mg/L).

Table 3.2 shows the cDOC concentrations and their relative distributions in four fractions for the supernatant of sludge ozonated at various ozone consumptions. The relative cDOC distribution in each fraction is correlated to the ratio between the area of the specific fraction to the area of the chromatogram (26 – 100 min).

Table 3.2 Relative cDOC distribution in the four fractions for the supernatant of sludge ozonated at various ozone consumptions.

Ozonation mode	Ozonation time	Ozone consumption	cDOC	F.1: 26-36 min	F.2: 36-55 min	F.3: 55-60 min	F.4: 60-100 min
	[min]	[g O_3/g TS]	[mg/L]	[%]	[%]	[%]	[%]
No ozonation	0	0	9	12	45	17	25
Low ozonation	2	0.005	39	23	48	13	16
	5	0.02	87	17	47	19	18
Moderate ozonation	15	0.06	345	10	46	21	24
	30	0.08	506	10	50	18	23
High ozonation	60	0.10	693	8	50	17	25
	90	0.14	688	9	56	16	20
	120	0.16	720	7	58	17	19

It is exhibited in Table 3.2 that the relative cDOC distribution in F.1 increased from 12 % before treatment to 23 % and 17 % after 0.005 g O_3/g TS and 0.02 g O_3/g TS, respectively. At moderate sludge ozonation, 0.06 - 0.08 g O_3/g TS, cDOC concentrations highly increased in the sludge liquid phase. At this stage a change in the relative cDOC distribution can be observed compared to the low sludge ozonation. At moderate ozonation the relative cDOC distribution in F.1 decreased compared to low ozonation, while it raised slightly in F.2, F.3 and F.4. It seems that high ozonation was not very effective in further solubilization of organic matter in the sludge matrix, since the cDOC concentration was not considerably increased at above 0.10 g O_3/g TS. In addition, transformation of cDOC from F.1 to F.2 occurred during high sludge ozonation.

It is stated in the literatures that sludge floc disintegration is the main process when ozonation is less than 0.02 g O_3/g TS (Meng et al., 2015, Yan et al., 2009, Zhang et al., 2009). It can be concluded that in the first stage low sludge ozonation primarily disintegrated the sludge flocs and enhanced the hydrolysis of EPS that protects the microbial cell against ozone. Reaction of ozone with EPS resulted in the release of large molecular compounds (F.1) into the liquid phase.

Further reaction of ozone with the sludge matrix at 0.06 - 0.08 g O_3/g TS solubilized a high amount of organic carbon which is reflected in a remarkable increase in cDOC concentration. It is supposed that once the sludge floc is disintegrated, ozone attacks quickly the bacteria cells, disrupts the cell wall and releases intra-cellular substances. Therefore, the increase of cDOC concentration at moderate ozonation could be associated with the solubilization of large molecules released from the sludge floc and the bacteria cells (Table 3.2). On the other hand, a decrease in the relative cDOC distribution in F.1 and an increase in F.2, F.3 and F.4 during moderate ozonation compared to low ozonation, indicate a slight transformation of macromolecules into the intermediate and smaller molecules. It is expected that ozone at moderate range, effectively solubilized high amount of organic matter by disintegrating the sludge floc and disrupting the bacteria cells, while simultaneously reacts with the released large molecular compounds and slightly transformed them into the smaller molecules. Numeration of the bacterial cell number analysis by Yan et al. (2009) demonstrated that rapid destruction of the bacteria cells occurred when ozonation was greater than 0.02 g O_3/g TS. They showed that sludge ozonation in the range of 0.02 to 0.10 g O_3/g TS oxidized macromolecules such as proteins and DNA released from the sludge matrix.

The effect of ozone on the bacteria cells in the excess sludge was shown by Meng et al. (2015). They revealed that the number of the live cells decreased and the permeabilized cells increased at ozonation in the range of 0.01 - 0.09 g O_3/g TS. Meanwhile, a rapid increase in the concentration of SCOD, carbohydrates and proteins was observed. The results of both chemical and microbial analysis indicated that during ozonation, solubilization of macromolecules was mainly due to the cell lysis.

Yan et al. (2009) stated that reaction of ozone with the sludge matrix and the bacteria cells became slow at levels higher than 0.1 g O_3/g TS. At this stage ozone preferentially reacted with the released macromolecules and transformed them into the smaller molecules.

These findings are in consistence with the results of this study, obtained from SEC-OCD analysis. At above 0.1 g O_3/g TS ozone consumption, the relative cDOC distribution exhibits a transformation of large molecular fraction of cDOC (F.1) into the intermediate molecules (F.2). Furthermore, it was presented in Fig. 3.3 (section 3.1.1) that the effect of mineralization was remarkable at high ozonation 0.1 - 0.16 g O_3/g TS. Therefore, the decrease of the relative cDOC distribution in F.1 could be also attributed to the mineralization of the solubilized organic compounds which may have a screening effect on further cell lysis. Yan et al. (2009) showed that ozonation higher than 0.14 g O_3/g TS failed to enhance sludge disintegration. The explanation was that after the disintegration of the sludge matrix and leakage of macromolecules from the disrupted cells, ozone began to oxidize the macromolecules into small organic substances such as lactic acid and inorganic compounds like SO_4^{2-}. These compounds might have acted as radical scavengers, hence inhibited the further oxidation process by ozone.

Fig. 3.5 presents the relative cDOC distribution in four fractions, before and after sludge ozonation. It is depicted that low sludge ozonation increased the release of large molecular fraction of cDOC (F.1). Whereas, moderate and high sludge ozonation did not change the relative cDOC distribution significantly, compared to untreated sludge.

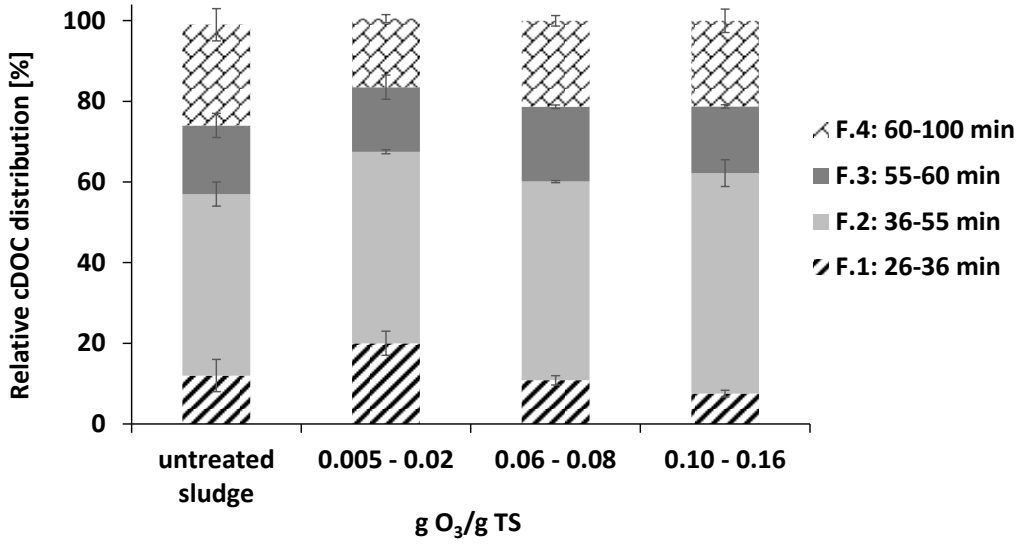


Fig. 3.5 Relative cDOC distribution in four fractions for the supernatant of untreated sludge, and sludge pretreated with different ozone consumption ranges: 0.005 – 0.2 g O_3/g TS, 0.06 – 0.08 g O_3/g TS and 0.10 – 0.16 g O_3/g TS.

An increase in the large molecular fraction of cDOC (F.1) occurred at short sludge ozonation was followed by a slight transformation of large molecular compounds to smaller molecules at moderate

ozonation. It can be related to the reaction of ozone with the sludge floc and microbial cells. Functional groups such as double bounds and aromatic compounds present in excess sludge have a high affinity for direct reaction with ozone. Aromaticity of organic carbon can be assessed through the SUVA parameter.

Changes in DOC concentration measured by thermal catalytic oxidation as well as changes in SUVA values at different ozone consumptions are demonstrated in Fig. 3.6. An increase in DOC concentration by ozonation occurred concomitantly with a decline in SUVA values, implying a change in the character of organic carbon.

A significant decline in the SUVA values at low ozone consumptions of 0.005 – 0.02 g O_3/g TS indicates a decrease in aromaticity of the organic carbon. This suggests that ozone quickly reacts with the released organic compounds, modifies the structure to form compounds with lower aromaticity and more aliphatic structures. The SUVA values slightly decreased as excess sludge was ozonated with ozone consumption above 0.02 g O_3/g TS.

Based on the relative cDOC distribution obtained from SEC-OCD analysis of ozonated sludge (Fig 3.5), low sludge ozonation released large molecules (F.1) into the sludge supernatant. While, moderate ozonation slightly transformed the released large molecules to the intermediate and small molecules (F.2, F.3, F.4). However high sludge ozonation did not further produce smaller molecules. These results in combination with the slight decrease in SUVA at ozone consumption above 0.02 g O_3/g TS indicate that ozone preferentially reacts with the released organic compounds and transformed them into smaller molecules or oxidized them to CO_2, rather than reacting with the sludge flocs and aromatic compounds in the sludge.

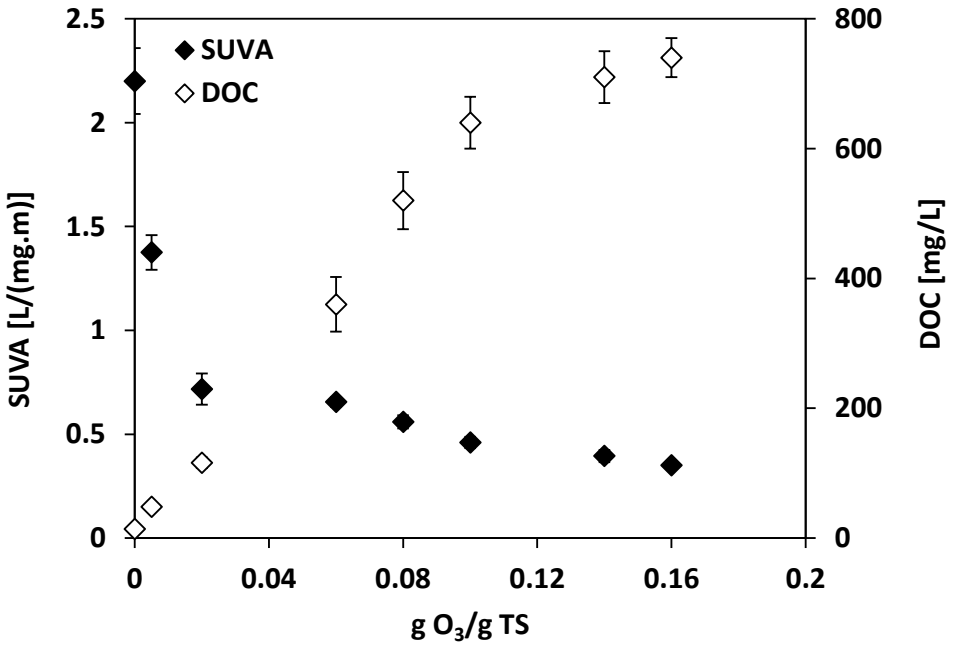


Fig. 3.6 An increase in the DOC concentration in the supernatant of the sludge followed by a decrease in SUVA values during sludge ozonation, ozone consumption varied from 0 to 0.16 g O_3/g TS.

3.1.2 Sludge pretreatment with sodium hydroxide

3.1.2.1 Effect of sodium hydroxide dosage on DOC solubilization

This section comprises the effect of sodium hydroxide dosage and treatment duration on the sludge disintegration. Excess sludge was treated with 0.2 and 2 g NaOH/g TS for 2 and 24 hours and the supernatants of the samples were analyzed by SEC-OCD.

The effects of sodium hydroxide dosage and treatment duration on the concentration of the cDOC is represents in Table 3.3. In general, the cDOC concentrations in the supernatants of the sludge increased by applying higher sodium hydroxide dosage and longer duration of treatment. Sludge treatment with 0.2 g NaOH/g TS for 2 hours, released 215 ± 2 mg/L cDOC which is about 70 % of the cDOC (310 ± 14 mg/L) solubilized when sludge was treated with 2 g NaOH/g TS for 2 hours. Moreover, independent of the applied sodium hydroxide dosage, increase of cDOC concentration in the first two hours of treatment was about 60 % of the cDOC concentration obtained after 24 hours of treatment. The results imply that sludge treatment with low dosage of 0.2 g NaOH/g TS for short treatment duration of 2 hours was the most efficient treatment condition. Li et al. (2008) studied sludge treatment with different sodium hydroxide dosages varied from 0.16 to 3.2 g NaOH/g TS. The results showed that 0.16 g NaOH/g TS was the most efficient dose and 60 – 70 % solubilization of the organic matter was achieved in the first 30 min of pretreatment.

Table 3.3 Effects of various sodium hydroxide dosages and treatment durations on the concentration of the cDOC in the supernatant of untreated and pretreated sludge.

Sodium hydroxide dosage	Treatment duration [hour]	cDOC [mg/L]
Untreated sludge	0	9 ± 1
0.2 g NaOH/g TS	2	215 ± 2
	24	350 ± 90
2 g NaOH/g TS	2	310 ± 14
	24	560 ± 70

3.1.2.2 Molecular size distribution of DOC after alkaline pretreatment

DOC chromatograms for the supernatants of untreated sludge and sludge pretreated with 0.2 g NaOH/g TS during 2 and 24 hours of treatment is shown in Fig. 3.7a. Whereas Fig. 3.7b depicts DOC chromatograms for the supernatants of sludge treated with 2 g NaOH/g TS for 2 and 24 hours. Comparison of the chromatograms shows that for the sludge treated with 0.2 g NaOH/g TS (Fig. 3.7a) the DOC signals obtained in the fraction of large and intermediate molecular size compounds

(F.1 and F.2) are significantly lower than the corresponding signals for the supernatant of the treated sludge with 2 g NaOH/g TS (Fig. 3.7b). On the other hand, the chromatogram obtained for the supernatant of the sludge treated with the low sodium hydroxide dosage (Fig. 3.7a) demonstrates a higher DOC signal in the fraction of small molecular size substances (F.4) compared to the signal obtained in F.4 for the supernatant of the treated sludge with high dosage of sodium hydroxide (Fig. 3.7b). It seems that low sodium hydroxide dosage first solubilized large compounds and then hydrolyzed them into the small molecules. While high dosage only solubilized large molecules into the sludge liquid phase. Two additional peaks are observed in F.4, when sludge was treated with 0.2 g NaOH/g TS for 24 hours compared to 2 hours treatment (Fig. 3.7a). This suggests that longer time of treatment led to the formation of more small molecular size substances.

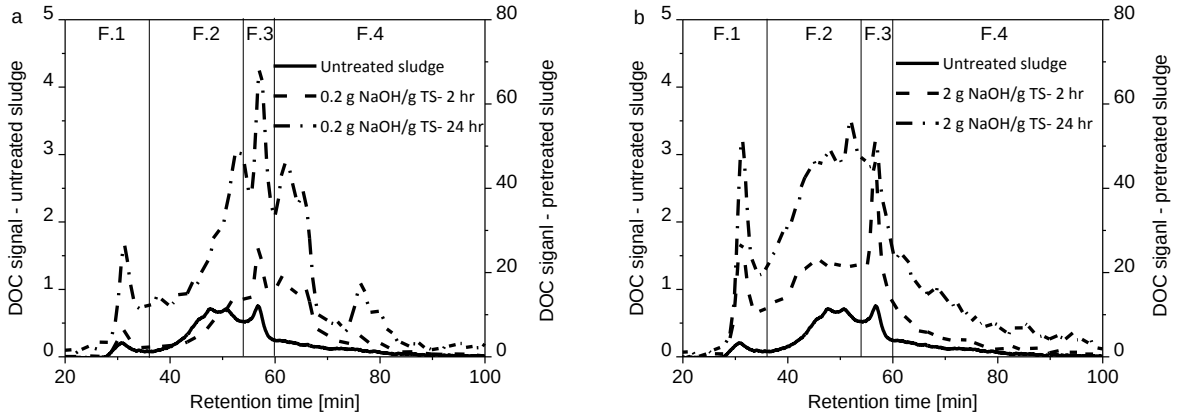


Fig. 3.7 DOC chromatograms a) for the supernatants of untreated sludge (cDOC= 9 ± 1 mg/L) and sludge pretreated with 0.2 g NaOH/g TS for 2 and 24 hours (cDOC= 215 ± 2 and 350 ± 90 mg/L) and b) for the supernatants of sludge pretreated with 2 g NaOH/g TS for treatment duration of 2 and 24 hours (cDOC= 310 ± 15 and 560 ± 70 mg/L).

It can be seen in Table 3.4 that the relative cDOC distribution in F.1 decreased from 12 % up to 9 % and it reduced from 48 % up to 36 % after sludge was pretreated with 0.2 g NaOH/ g TS. Whereas, the relative cDOC distribution in F.4 increased from 25 % up to 33 % in the supernatant of pretreated sludge with 0.2 g NaOH/g TS. Applying high dosage of sodium hydroxide to the excess sludge increased the relative cDOC distribution in F.1 to nearly 15 %. While it was about 9 % in the supernatant of pretreated sludge with the low sodium hydroxide dosage. Furthermore, the relative cDOC distribution in F.4 decreased from about 33 % in the supernatant of the sludge pretreated with low sodium hydroxide dosage to about 23 % for pretreatment with high dosage of sodium hydroxide.

Table 3.4 Relative cDOC distribution in four fractions for the supernatants of untreated sludge and sludge pretreated with different sodium hydroxide dosages and different treatment durations.

Sodium hydroxide dosage	Treatment duration [hour]	cDOC [mg/L]	F.1: 26-36 min [%]	F.2: 36-54 min [%]	F.3: 54-60 min [%]	F.4: 60-100 min [%]
Untreated sludge	0	9 ± 1	12	48	15	25
0.2 g NaOH/g TS	2	215 ± 2	9	36	23	33
0.2 g NaOH/g TS	24	350 ± 90	10	38	19	30
2 g NaOH/g TS	2	310 ± 15	15	44	17	24
2 g NaOH/g TS	24	560 ± 70	14	48	15	23

It seems that 2 g NaOH/g TS did not significantly change the relative cDOC distribution, compared to untreated sludge. The high sodium hydroxide dosage was not effective in hydrolyzing large molecules into smaller fractions compared to the low dosage. Besides, sludge treatment with low and high dosages of sodium hydroxide for 24 hours solubilized more organic carbon in the supernatant, compared to 2 hours of treatment. However, it did not increase the relative cDOC distribution in the fraction of small molecules (F.4), compared to the short sludge treatment duration.

The effect of sodium hydroxide dosages on the relative cDOC distribution after 2 hours of treatment is depicted in Fig. 3.8. It can be concluded that treating the sludge with 0.2 g NaOH/g TS for 2 hours hydrolyzed the large molecular fractions (F.1 and F.2) into small molecular compounds (F.4) and was more effective than high dosage. It was reported that lower sodium hydroxide dosage was more preferred for pretreating the excess sludge prior to anaerobic digestion, due to the inhabitation effect of high cation concentration on the methanogens and the performance of anaerobic treatment process (Kim et al., 2013, Li et al., 2012). Knowing the changes in the molecular size distribution of organic carbon released after sludge treatment with various sodium hydroxide dosages and durations of treatment, can accelerate the optimization of sludge treatment process. The knowledge gained by SEC-OCD analysis not only help to reach the optimum sludge solubilization degree, but it can also be very helpful in selecting the optimum dosage and the treatment time to enhance the performance of anaerobic digestion in terms of methane production and solid reduction.

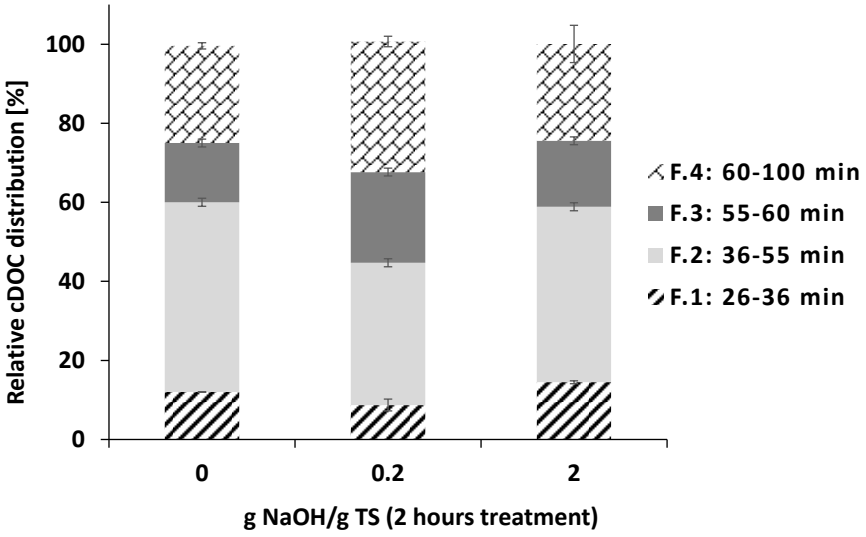


Fig. 3.8 Relative cDOC distribution in the four fractions for the supernatant of untreated sludge and sludge pretreated with low and high sodium hydroxide dosages for 2 hours.

3.1.3 Sludge pretreatment with ultrasound

3.1.3.1 Molecular size distribution of DOC after sonication

To compare ultrasound disintegration method with ozone and sodium hydroxide, ultrasound conditions were adjusted to achieve approximately the same concentration of cDOC obtained at optimum ozone consumption of 0.06 g O_3/g TS and sodium hydroxide dosage of 0.2 g NaOH/g TS for 2 hours treatment. Excess sludge was sonicated at low frequency of 20 kHz for 7 min resulted in specific energy of 16.8 kJ/g TS. DOC chromatograms for the supernatants of untreated and sonicated sludge are shown in Fig. 3.9. The peak observed in F.1 has the most obvious increase after sludge sonication. Organic compounds in this fraction contain large molecules.

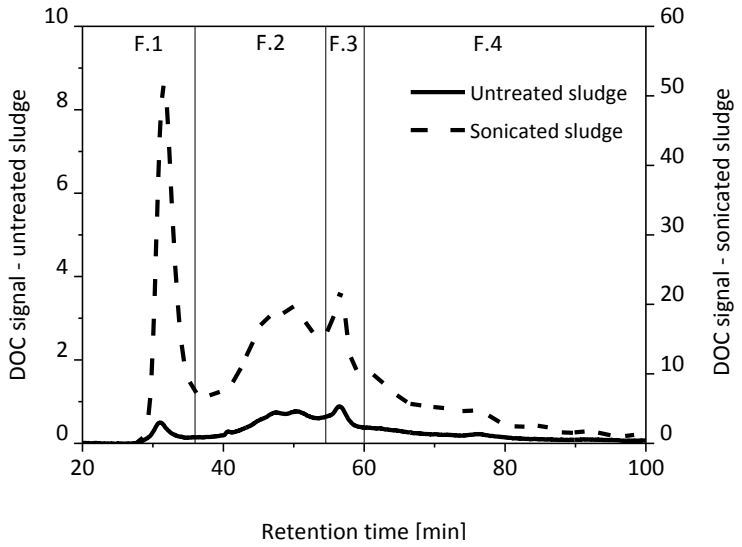


Fig. 3.9 DOC chromatogram for the supernatants of untreated sludge and sludge sonicated at 16.8 kJ/g TS specific energy.

The relative cDOC distribution in the four fractions for the supernatants of untreated and sonicated sludge is shown in Table 3.5. The relative cDOC distribution in F.1 increased significantly from 12 % before treatment to 36 % after sludge sonication. Moreover, 32 % of cDOC released after sonication, is in F.2. It means 68 % of cDOC which is distributed in F.1 and F.2 contains large-intermediate molecular size compounds. The rest of cDOC in F.3 and F.4 (32 %) belongs to the smaller molecules. The results obtained from SEC-OCD analysis revealed that ultrasound pretreatment solubilized large-intermediate organic compounds into the sludge liquid phase. This finding is in consistent with the results of previous work which used polyether sulfone UF membranes with molecular weight cut-offs of 300 kilo Dalton (kDa), 30 kDa and 5 kDa, to fractionate the total organic carbon (TOC) released after sludge pre-treatment at 21 kJ/g TS ultrasound (Tian et al., 2015a). The TOC fractions with molecular weight larger than 300 kDa was about 8 % before treatment which raised to 60 % after sludge sonication, indicating the solubilization of large molecular compounds after sludge treatment with ultrasound.

Table 3.5 Relative cDOC distribution in the four fractions for the supernatants of untreated sludge and sonicated sludge, E_s refers to the ultrasound specific energy.

	Treatment time [min]	E_S [kJ/g TS]	cDOC [mg/L]	F.1: 26-36 min [%]	F.2: 36-54 min [%]	F.3: 54-60 min [%]	F.4: 60-100 min [%]
Untreated sludge	0	0	9 ± 1	12	45	17	25
Sonicated sludge	7	16.8	270 ± 0	36	32	15	17

Based on the literatures, ultrasound energy level of 16.8 kJ/g TS produced mechanical shear forces which are mainly responsible for sludge floc disintegration rather than cell lysis (Tiehm et al., 2001, Zhang et al., 2008). Therefore, the large molecules released after sludge sonication are supposed to be extra-cellular substances solubilized from the EPS of the sludge floc. EPS contains variety of large organic matter such as proteins, carbohydrates, humic acids and nucleic acids. Proteins and carbohydrates have been identified as the predominant constitutes of EPS (Wei et al., 2012). Once the sludge floc was disintegrated, such substances in EPS would be released into the aqueous phase. The release of proteins and carbohydrates after sludge sonication led to an increase in the concentration of DOC. The equivalent DOC concentrations of proteins ($DOC_{proteins}$) and carbohydrates ($DOC_{carbohydrates}$) in the soluble phase before and after sonication were calculated and shown in Table 3.6. It can be seen that the concentrations of $DOC_{proteins}$ and $DOC_{carbohydrates}$ represented 33 % and 8 % of the DOC concentration in the soluble phase of untreated sludge. The DOC concentration of untreated sludge might be attributed to the soluble organic matter which was not degraded during the biological activated sludge process and remained in the sludge.

Table 3.6 The increase in the DOC concentration after sludge sonication, as well as the increase in the concentrations of $DOC_{proteins}$ and $DOC_{carbohydrates}$ in the soluble phase of sonicated sludge, compared to untreated sludge.

	DOC	$DOC_{Proteins}$	$DOC_{carbohydrates}$	$\frac{\text{mg } DOC_{proteins}}{\text{mg DOC}}$	$\frac{\text{mg } DOC_{carbohydrates}}{\text{mg DOC}}$
	[mg/L]	[mg/L]	[mg/L]	[%]	[%]
Untreated sludge	12 ± 2	4 ± 1	1 ± 0.2	33	8
Sonicated sludge	320 ± 9	152 ± 4	38 ± 2	50	12

After sludge floc disintegration induced by ultrasound, sludge floc components were hydrolyzed, which resulted in an increase in DOC of about 320 mg/L (Table 3.6). As indicated in Table 3.6, the released organic carbon after sludge sonication was mainly comprised of proteins. The concentrations of $DOC_{proteins}$ and $DOC_{carbohydrates}$ were about 152 and 38 mg/L, respectively, which were responsible for 50 % and 12 % of the DOC concentration. These results are consistent with the results of other research studies which demonstrated the high solubilization of proteins after sludge pretreatment with ultrasound (Feng et al., 2009, Wang et al., 2006, Zhang et al., 2007).

The chemical analysis of protein and carbohydrate concentrations in the sludge shows that 62 % of the DOC concentration (analyzed by thermal catalytic oxidation) after sludge sonication were composed of proteins and carbohydrates which have been defined as macromolecules (Table 3.6). The results obtained from the chemical analyses are in agreement with the results of SEC-OCD, representing that large to intermediate molecular size compounds (F.1 and F.2) contained 68 % of the cDOC released after sludge sonication (Table 3.5).

3.2 Comparison of sludge disintegration methods

*Part of the results presented in sections 3.2.1, 3.2.2 and 3.2.3 have been submitted on 6th June 2016 in Water Research, in Revision Process.

Based on the optimum conditions of sludge disintegration with ozone, sodium hydroxide and ultrasound (section 3.1), in this section performances of the sludge disintegration techniques are assessed in different aspects; changes in the molecular size distribution of DOC, suitability of the released DOC for methane production during anaerobic digestion and for nitrate removal in the denitrification processes.

To accomplish this goals, based on the limitation of sludge oxidation by ozone (section 3.1.1), two ozone consumptions of 0.02 and 0.06 g O_3/g TS were selected for comparison. Optimum sodium hydroxide dosage of 0.2 g NaOH/g TS for 2 hours of treatment and ultrasound specific energy of 16.8 kJ/g TS were proposed to disintegrate the sludge and solubilize approximately the same amount of organic carbon released after sludge ozonation with 0.06 g O_3/g TS. Impacts of each disintegration technique on the molecular size distribution of DOC (section 3.2.1), methane production in anaerobic digestion (section 3.2.2 and 3.2.3) and denitrification process (section 3.2.4) are discussed in detail.

3.2.1 Comparing disintegration techniques based on molecular size distribution of DOC

Concentrations of cDOC in the supernatant increased from 9 ± 1 mg/L for untreated sludge to 86 ± 11 mg/L after 0.02 g O_3/g TS and to 234 ± 34 mg/L after 0.06 g O_3/g TS. Sludge disintegration with ultrasound released 270 ± 0 mg/L cDOC and pretreatment with sodium hydroxide solubilized 215 ± 26 mg/L cDOC into the liquid phase of the sludge. The DOC chromatograms for the supernatant of untreated sludge, sludge disintegrated with ozone and sodium hydroxide are illustrated in Fig. 3.10a. Whereas Fig. 3.10b represents the DOC chromatogram for the supernatant of sonicated sludge compared to untreated sludge. It can be observed that the shape and the peak locations of the DOC chromatogram was changed after disintegration compared to the DOC chromatogram of the untreated sludge (Fig. 3.10a-b). By ozonating the sludge all DOC fractions increased approximately linear, compared to untreated sludge (Fig. 3.10a). Although cDOC concentrations obtained after 0.06 g O_3/g TS, 0.2 g NaOH/g TS and 16.8 kJ/g TS ultrasound were nearly the same, there are distinct differences in their DOC chromatograms.

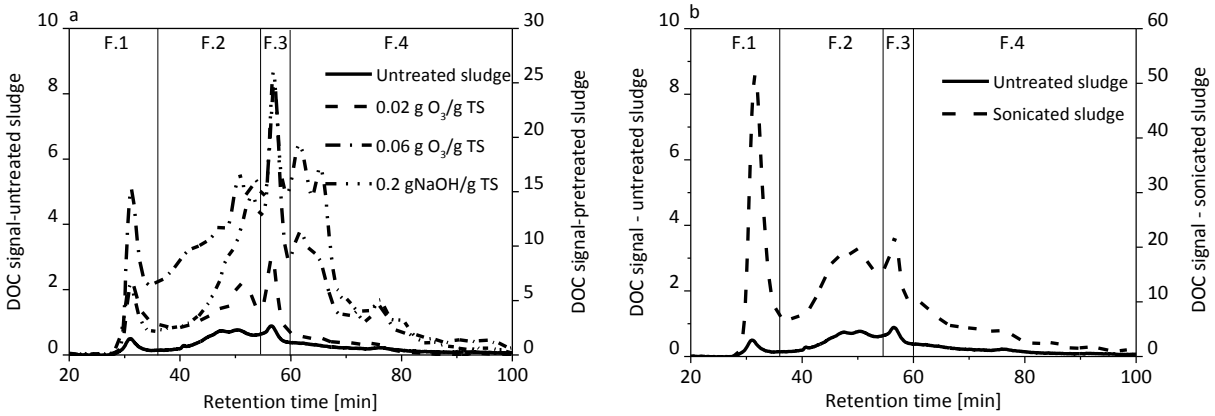


Fig. 3.10 DOC chromatograms a) for the supernatants of untreated sludge (cDOC= 9 ± 1 mg/L) and sludge treated with 0.02, 0.6 g O_3/g TS (cDOC= 86 ± 11, 234 ± 34 mg/L) and 0.2 g NaOH/g TS (cDOC= 215 ± 26 mg/L) and b) for the supernatant of sonicated sludge (cDOC= 270 ± 0 mg/L), compared to untreated sludge.

The main fractions of the DOC chromatogram related to sludge treated with sodium hydroxide were shifted to the higher retention time, compared to the DOC chromatograms of the ozonated sludge (Fig. 3.10a). However, ultrasound disintegration mainly dissolved large to intermediate molecules (F.1 and F.2) into the supernatant (Fig. 3.10b).

The relative cDOC distribution for the supernatant of untreated and pretreated sludge is given in Fig. 3.11. Intermediate and large compounds were found to be the main solubilized substances after ozone and ultrasound sludge pretreatment. However, the relative cDOC distribution in F.1 and F.2 were different for the supernatant of ozonated and sonicated sludge. By ozonation 12 % and 49 % of cDOC were distributed in F.1 and F.2. While by ultrasound 36 % of cDOC was in F.1 and 32 % in F.2 (Fig. 3.11). The differences in the relative cDOC distribution after ozonation, ultrasound and sodium hydroxide can be explained by the differences in the mechanism of disintegration.

Ozone is very effective in cell lysis. It reacts with sludge flocs, attacks the cell walls and released large molecular compounds such as proteins and polysaccharides (the main components of EPS and cell membranes) into the supernatant (Meng et al., 2016). Chemical reaction of ozone with high molecular weight compounds containing double bonds and aromatic groups, effectively changes their structure to smaller molecules (Khan and Jung, 2016). Therefore, ozone partially oxidized large molecules (F.1) and transformed them into intermediate molecules (F.2). However, smaller molecules (F.3 and F.4) were not dominant in the cDOC in the supernatant after sludge ozonation. It could be proposed that during sludge ozonation part of the released large molecules were transformed to smaller molecules which were then mineralized to CO_2.

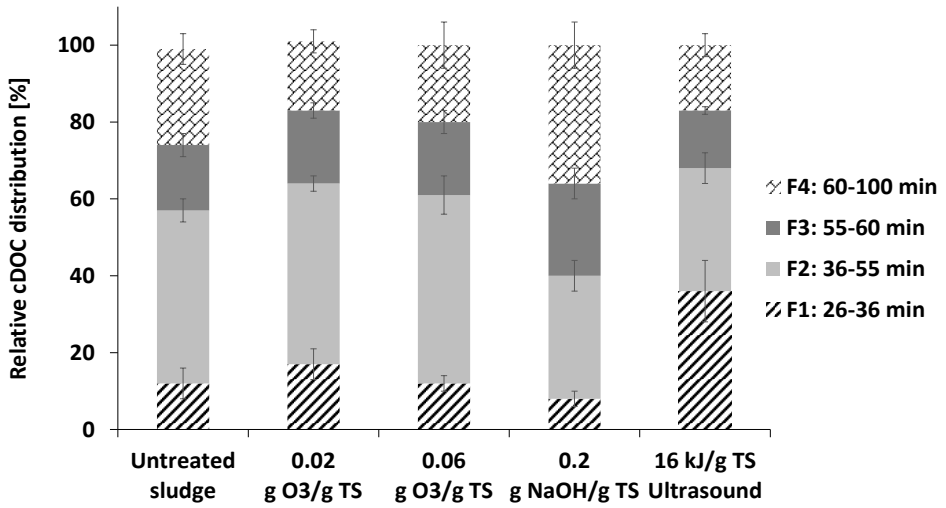


Fig. 3.11 Relative cDOC distribution in the four fractions for the supernatants of untreated sludge and sludge pretreated with ozone, sodium hydroxide and ultrasound.

Mechanical shear force produced by low ultrasound frequency of 20 kHz, effectively damaged the sludge flocs and solubilized large-intermediate molecular compounds in F.1 and F.2. The relative cDOC distribution in F.1 and F.2 are much higher than the amount in F.3 and F.4. Tiehm et al. (2001) stated that at high ultrasound frequencies between 200 to 1000 kHz sonochemical process leads to the production of hydroxyl radicals which are very strong oxidizers. Since ultrasound frequency of 20 kHz was applied to disintegrate the sludge in this study, oxidation of large molecules to the smaller molecules through chemical reaction of hydroxyl radicals was not predominant.

0.2 g NaOH/g TS increased the pH of the sludge to 12.5. At high pH, cell membrane was disrupted and led to the release of large molecular size compounds such as proteins and carbohydrates (Ruiz-Hernando et al., 2013). These compounds were chemically degraded by hydroxyl ions during hydrolysis which results in the formation of smaller substances. He et al. (2008) stated that sodium hydroxide changes the structure of high molecular weight compounds such as lignin to ones with smaller molecular weight. He showed that during pretreatment with sodium hydroxide, functional groups of the lignin such as carbonyls were obviously changed. Sodium hydroxide breaks some intermolecular hydrogen bonds through hydrolysis reaction.

3.2.2 Impacts of sludge disintegration techniques on methane production

This section evaluates sludge disintegration techniques by analyzing the anaerobic biodegradability of the released organic carbon and methane production. Moreover, contribution of cDOC fractions, based on their molecular size, in methane production is discussed in detail.

Effects of three sludge disintegration methods, ozone, alkaline treatment and ultrasound on biodegradability of organic carbon and methane production were investigated in anaerobic batch reactors for five days. To study the influence of the soluble organic carbon released after sludge

disintegration on methane production, the supernatant of the sludge before and after disintegration was used as a substrate in each anaerobic reactor. The supernatant was separated from the particulate part of the sludge by centrifugation of the sludge samples and was mixed with the anaerobic digested sludge. The samples were taken from each reactor at the beginning, after one day and five days and were analyzed with SEC-OCD. Anaerobic digestibility experiments for the supernatants of untreated sludge and ozonated sludge were repeated four times and for sludge pretreated with sodium hydroxide and ultrasound were repeated two times. The accumulated methane production was monitored during five days (Fig. 3.12).

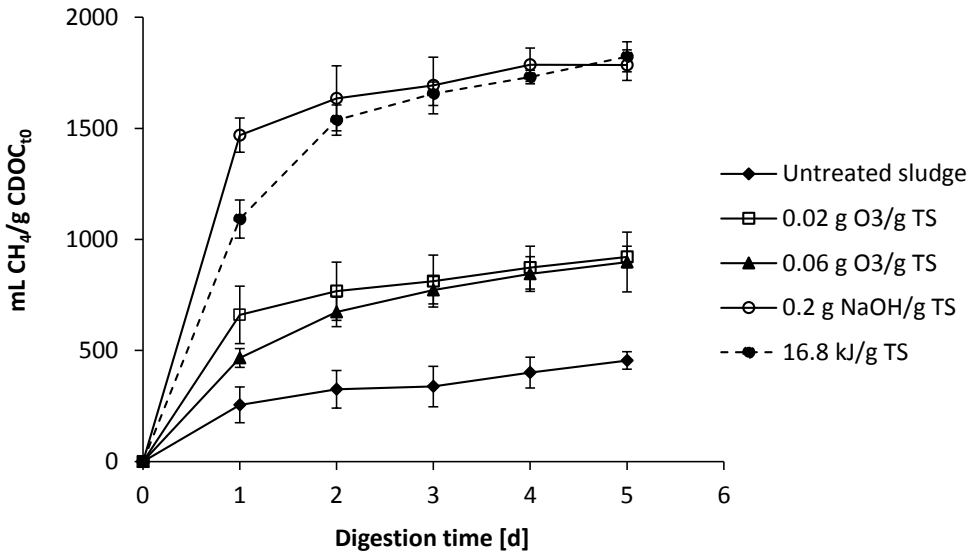


Fig. 3.12 Methane production obtained from the anaerobic digesters fed with the supernatant of untreated sludge, sludge pretreated with 0.02 g O_3/g TS and 0.06 g O_3/g TS, 0.2 g NaOH/g TS and 16.8 kJ/g TS ultrasound specific energy.

It can be seen in Fig. 3.12 that pretreating the sludge had a positive effect on the performance of the anaerobic digester. In all cases, methane production was improved when the supernatant of the pretreated sludge was fed into the anaerobic reactor, compared to untreated sludge. Methane production was highly influenced by the biodegradability of the organic matters present in the sludge. The anaerobic biodegradation was limited due to the aggregation of the organic matters in the sludge flocs. Disintegrating the sludge was able to provide more soluble carbon source, enhance hydrolysis, and thus improve methane production.

Anaerobic biodegradability and methane production during five days of anaerobic digestion are presented in Table 3.7. After one day of anaerobic digestion, cDOC removal increased from 11 % when the supernatant of untreated sludge was fed to the reactor, to 35 % when supernatant of ozonated sludge with 0.02 g O_3/g TS was used as a substrate. Consequently, methane production improved 2.5 times

higher, compared to untreated sludge. On the other hand, methane production in the reactor fed with the supernatant of disintegrated sludge with 0.06 g O_3/g TS was lower, compared to lower ozonation and only 12 % of the cDOC was removed from the reactor.

Table. 3.7 cDOC concentrations at the beginning (t_0), after one day (t_1) and after five days (t_5), cDOC removal and methane production after one day and five days of anaerobic digestion fed with the supernatant of untreated and pretreated sludge.

	$cDOC_{t0}$ [mg/L]	$cDOC_{t1}$ [mg/L]	$cDOC_{t5}$ [mg/L]	$cDOC_{removal\ t0\text{-}t1}$ [%]	$cDOC_{removal\ t0\text{-}t5}$ [%]	$(\frac{mL\ CH_4}{g\ cDOC_{t0}})_{t1}$	$(\frac{mL\ CH_4}{g\ cDOC_{t0}})_{t5}$
Untreated sludge	35±7	32±6	30±6	11	14	256±80	454±40
0.02 g O_3/g TS	86±9	57±12	45±10	35	48	660±43	920±135
0.06 g O_3/g TS	190±28	166±29	101±25	12	47	466±130	900±50
0.2 g NaOH/g TS	116±15	48±1	36±2	59	69	1470±77	1790±70
16.8 kJ/g TS	157±52	88±17	34±9	44	78	1090±86	1820±70

After five days of anaerobic digestion, sludge ozonation at 0.02 g O_3/g TS enhanced cDOC removal from 14 % to 48 % and increased methane production twice higher, compared to untreated sludge. In the reactor fed with the supernatant of 0.06 g O_3/g TS ozonated sludge, cDOC removal increased from 12 % in the first day to 47 % after five days. The substantial increase in cDOC removal, improved methane production in this reactor. Despite, abundance of dissolved organic carbon released after 0.06 g O_3/g TS, methane production was not improved further, compared to low sludge ozonation. The results demonstrated an obvious difference in the anaerobic degradation of organic compounds released at low and high ozonation. It suggested that depending on the range of ozonation, new compounds with different characteristics could be generated, which highly influenced the biodegradability and the methane production. The same result was found by Cesaro and Belgiorno (2013) who studied the effect of ozone pretreatment on the anaerobic biodegradability of organic fraction of municipal solid waste. They explained that low biodegradability of organic compounds might be attributed to the generation of byproducts during high ozonation, which were hardly degradable in anaerobic digestion process. Weemaes et al. (2000) pointed out that sludge ozonation at 0.05 and 0.1 g O_3/g COD increased methane production by the factor of 1.5 and 1.8, while formation of carboxylic group at higher ozonation hampered methane production. In the study done by Weemaes et al. (2000) the whole sludge (soluble and particulate fraction of sludge) after ozonation was used as a substrate in an anaerobic digestion test. While in our study after ozonating the whole sludge, the particulate part was separated by centrifuge, thus only supernatant mainly contains of soluble organic carbon was fed into the anaerobic digester.

The results imply that high sludge ozonation is ineffective in further improving methane production, either the whole or the supernatant of the ozonated sludge was fed as a substrate into the anaerobic digester.

In general, sludge pretreatment with 0.02 and 0.06 g O_3/g TS, enhanced methane production relative to untreated sludge. This implies that ozone can oxidize the organic compounds into smaller ones, thereby improving their biodegradability and methane production. Proteins are the main organic compounds in the excess sludge. Silvestre et al. (2015) showed that amino acids are potential targets for oxidation by ozone. Ozone reacts with the amino acids components of the proteins and changes them into the secondary structure proteins. The higher methane production obtained from the pretreated sludge with ozone, could be explained by the changes occurred in the structure of proteins during ozonation.

The initial cDOC concentration ($cDOC_{t0}$) in the reactors fed with 0.06 g O_3/g TS, 0.2 g NaOH/g TS and 16.8 kJ/g TS were approximately the same. However, in the first day of anaerobic digestion the cDOC removal from the reactor fed with the supernatant of pretreated sludge with ozone, sodium hydroxide and ultrasound was 12 %, 59 % and 44 %, respectively. Consequently, sludge treatment with sodium hydroxide and ultrasound significantly improved methane production compared to ozonated sludge (Table 3.7). Nevertheless, in the first day of anaerobic digestion, methane production of the sodium hydroxide treated sludge reactor was higher, compared to ultrasound. It could be proposed that sludge disintegration with sodium hydroxide enhanced hydrolysis rate in the anaerobic digester, by breaking the chains of large organic polymers and dissolving the smaller molecules into the solution. This process made organic compounds readily bioavailable to anaerobic bacteria. On the other hand, large molecular compounds released after ultrasound treatment seems to be slowly degraded compared to the compounds solubilized by sodium hydroxide.

After five days of anaerobic digestion, sludge pretreatment with sodium hydroxide and ultrasound, enhanced cDOC removal to 69 % and 78 %, which was 47 % for the ozonated sludge. Moreover, methane production from sonicated sludge and sludge treated with sodium hydroxide were doubled compared to the value obtained from sludge disintegration with ozone. It is clearly shown in Fig. 3.13 that after the first day of anaerobic digestion, daily methane production in the reactor fed with sonicated sludge exceeded the amount obtained from sludge treated with sodium hydroxide. It suggests that hydrolysis of large molecular compounds released as a result of ultrasound disintegration, was a limiting step for the production of methane in the first day. Once the large molecules were hydrolyzed, methane production rate increased.

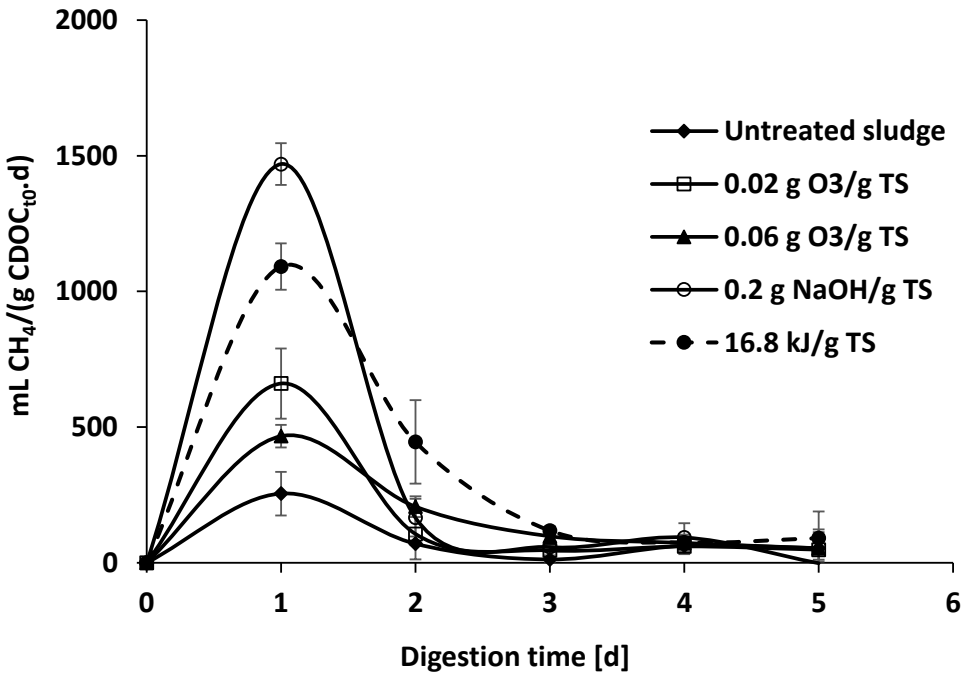


Fig. 3.13 Daily methane production during anaerobic digestion of the supernatant of untreated sludge and pretreated sludge with ozone, sodium hydroxide and ultrasound.

It has to be stressed that the values of methane productions shown in Table 3.7 were normalized to the cDOC concentrations and thereby should not be understood as absolute numbers. The purpose of this work was to study the changes in the cDOC fractions during anaerobic digestion and their impacts on methane production. Therefore, methane production is presented in terms of mL CH_4/g $cDOC_{t0}$ (Table 3.7). To have an estimation of the amount of methane produced per gram DOC provided in the reactor, one should normalize the methane production to the DOC concentration measured by thermal catalytic oxidation. Because it is assumed that this method is able to oxidize all of the organic compounds. It is also common to show the methane production per gram SCOD of the organic matter. SCOD for quantitative determination of oxidizable organic carbon does not necessarily lead to the equivalent result as DOC measurement, even though in both cases the end product of the reactions is CO_2 (Frimmel and Abbt-Braun, 2011). The average ration of SCOD/(DOC analyzed by thermal catalytic oxidation) for the samples examined in this study was around 3 (ranging from 2 – 4). The methane production was calculated based on the DOC analyzed by thermal catalytic oxidation and SCOD and are available in the appendix (Table A.1 and Table A.2).

3.2.3 Changes in molecular size distribution of DOC during anaerobic digestion and the effect on methane production

It was shown in the previous section that methane production is highly influenced by the mechanism of sludge disintegration. This can be related to the changes in the characteristics of organic matter released after each pretreatment method and during anaerobic digestion. To illustrate the influence of pretreatment methods on the digestibility of organic carbon, changes in the relative cDOC distribution during anaerobic digestion of the sludge were studied.

The relative cDOC distribution during anaerobic digestion of the supernatant of untreated sludge, sludge treated with ozone, sodium hydroxide and ultrasound at the beginning of anaerobic digestion is shown in Fig. 3.14a. Changes in the relative cDOC distribution during sludge anaerobic digestion and their impacts on methane production after one day (1d) and five days (5d) are presented in Fig. 3.14b-c and Fig. 3.14d-e, respectively. Total cDOC concentrations are written above the columns of the figures. The relative cDOC distribution in the four fractions was calculated according to the concentration of cDOC at the beginning of the digestion process ($cDOC_{t0}$). For instance, digestion of untreated sludge after one day (Fig. 3.14b) showed that cDOC in F.1 was 20 % of $cDOC_{t0}$.

For untreated sludge cDOC degradability was very low (cDOC decreased from 35 to 30 mg/L) even after five days of digestion. This referred to the limiting stage of sludge floc disintegration (Fig. 3.14a-b-d). Fig. 3.14a shows that the amount of cDOC removed during anaerobic digestion of the supernatants of low and high ozonated sludge were approximately the same after one day. 29 mg/L cDOC was removed during anaerobic digestion of the supernatant of the low ozonated sludge (cDOC decreased from 86 to 57 mg/L) and 24 mg/L was removed during anaerobic digestion of the supernatant of high ozonated sludge (cDOC decreased from 190 to 166 mg/L). However, the relative cDOC distributions during anaerobic digestion of the supernatant of low and high ozonated sludge were different (Fig. 3.14b). After one day in the reactor fed with the supernatant of sludge ozonated at 0.02 g O_3/g TS, most of the cDOC removal was in F.2, while in the reactor fed with the supernatant of 0.06 g O_3/g TS ozonated sludge, after one day cDOC decreased in F.2 and it increased in F.3. Smaller compounds in F.3 could be generated from the hydrolysis of polymers in F.2 during anaerobic digestion. The difference in the relative cDOC distribution during anaerobic digestion of the supernatant of low and high ozonated sludge, resulted in different methane productions after one day. It can be seen in Fig. 3.14c that anaerobic digestion of the supernatant of the low ozonated sludge resulted in the higher methane production compared to the anaerobic digestion of the supernatant of high ozonated sludge. It seems that ozonation at 0.02 g O_3/g TS enhanced the hydrolysis of organic compounds in F.2, made them easier degradable and convertible to methane by anaerobic bacteria. On the other hand, organic compounds produced as a result of oxidation at high ozonation were not easily degradable and were transformed to smaller molecules in the first day. Therefore 0.06 g O_3/g TS ozonation did not improve methane production compared to lower ozonation.

After five days of anaerobic digestion approximately 50 % of $cDOC_{t0}$ was degraded in both reactors fed with the supernatants of the ozonated sludge (Fig. 3.14d). Between day one and day five cDOC degradation was low in the reactor fed with the supernatant of 0.02 g O_3/g TS ozonated sludge and no considerable changes occurred in the relative cDOC distribution. On the other hand, a significant amount of cDOC was degraded during anaerobic digestion of the supernatant of sludge ozonated at 0.06 g O_3/g TS. The cDOC degradation was improved from 12 % in the first day to about 50 % after five days (Fig. 3.14b-d). An increase in the cDOC removal was related to the considerable degradation of organic compounds in F.3. These compounds are the transformation products of the large molecular compounds (F.2) in the first day of anaerobic digestion. After five days the methane production during anaerobic digestion of the supernatant of 0.06 g O_3/g TS ozonated sludge increased two times compared to the first day (Fig. 3.14e). This might be due to the degradation of smaller molecules (F.3).

Low degradation of cDOC during anaerobic digestion of the supernatant of ozonated sludge (except high cDOC degradation in F.3 during anaerobic digestion of the supernatant of sludge ozonated with 0.06 g O_3/g TS) between day one and day five might be attributed to the chemical effect of ozone. Oxidation of excess sludge by ozone resulted in the formation of substances which could be possibly converted to hardly biodegradable products during anaerobic digestion. Tian et al. (2015a) stated that ozonation of sludge could provide different degradation pathways by chemically breaking down the high molecular weight biopolymers into smaller fragments.

The distinct differences in the relative cDOC distribution during anaerobic digestion of the supernatant of low and high ozonated sludge illustrate the oxidation effect of ozone in the biodegradability of organic carbon. Although anaerobic biodegradability was improved by oxidizing the hardly degradable components of the sludge during ozonation, some biodegradable substances were also oxidized during ozonation. Short sludge ozonation would not only save some energy but also reduce the oxidation of biodegradable organic matter. Since higher ozonation did not further improve cDOC degradation or methane production, lower ozonation would be more applicable when it is chosen as a sludge disintegration method in a real wastewater treatment plant.

At the beginning of the anaerobic digestion of the supernatant of the sludge pretreated with sodium hydroxide 42 % of the $cDOC_{t0}$ was distributed in F.4 (Fig. 3.14a). These substances were small molecules produced as a result of the hydrolysis of the large molecules which were solubilized by sodium hydroxide. During the anaerobic digestion process the removal efficiency of the small molecules was generally higher than the removal of large molecules. High methane production achieved in the first day (Fig. 3.14c) suggests that the compounds in F.4 were easily biodegradable. The relative cDOC distribution in this fraction decreased from 42 % to 14 % after one day of digestion (Fig. 3.14a-b). After five days cDOC degradation increased to 70 % which was related to the further degradation of smaller molecules in F.4 (Fig. 3.14d).

It was explained in section 3.2.1 that ultrasound disintegration released large compounds into the liquid phase. At the beginning of the digestion process the supernatant of sonicated sludge was mixed with inoculum in an anaerobic reactor. 32 % of the $cDOC_{t0}$ was distributed in F.1 and as well in F.2 (Fig. 3.14a). After one day of digestion the relative cDOC distribution in F.1 and F.2 reduced to 20 % and 17 %, respectively (Fig. 3.4b). Consequently methane production was enhanced compared to untreated sludge (Fig. 3.14c). It could be postulated that ultrasound effectively disintegrated the sludge flocs and dissolved large compounds into the solution. These compounds were easily hydrolyzed to smaller molecules by anaerobic bacteria and were converted to methane. A decrease of the relative cDOC distribution in all fractions and an increase in methane production after five days of digestion might be attributed to the enhancement in the hydrolysis of the large molecules due to the effect of sonication (Fig. 3.14d-e).

Three sludge disintegration methods, ozone, sodium hydroxide and ultrasound had different impacts on the relative cDOC distribution in the fractions and the biodegradation of the cDOC fractions during anaerobic digestion process. Mechanism of disintegration with ozone based on the oxidation of sludge particles leads to the formation of by-products at high ozonation which could inhabit methanogens (Weemaes et al., 2000). While sodium hydroxide solubilizes organic matter which then are hydrolyzed to smaller molecules. These compounds could be easily converted to methane. Large molecules released as a result of mechanical disintegration by ultrasound were easier hydrolyzed by anaerobic bacteria than large molecules produced during sludge ozonation. The results explain that the production of methane is highly influenced by the characteristic of organic matter provided to anaerobic digestion.

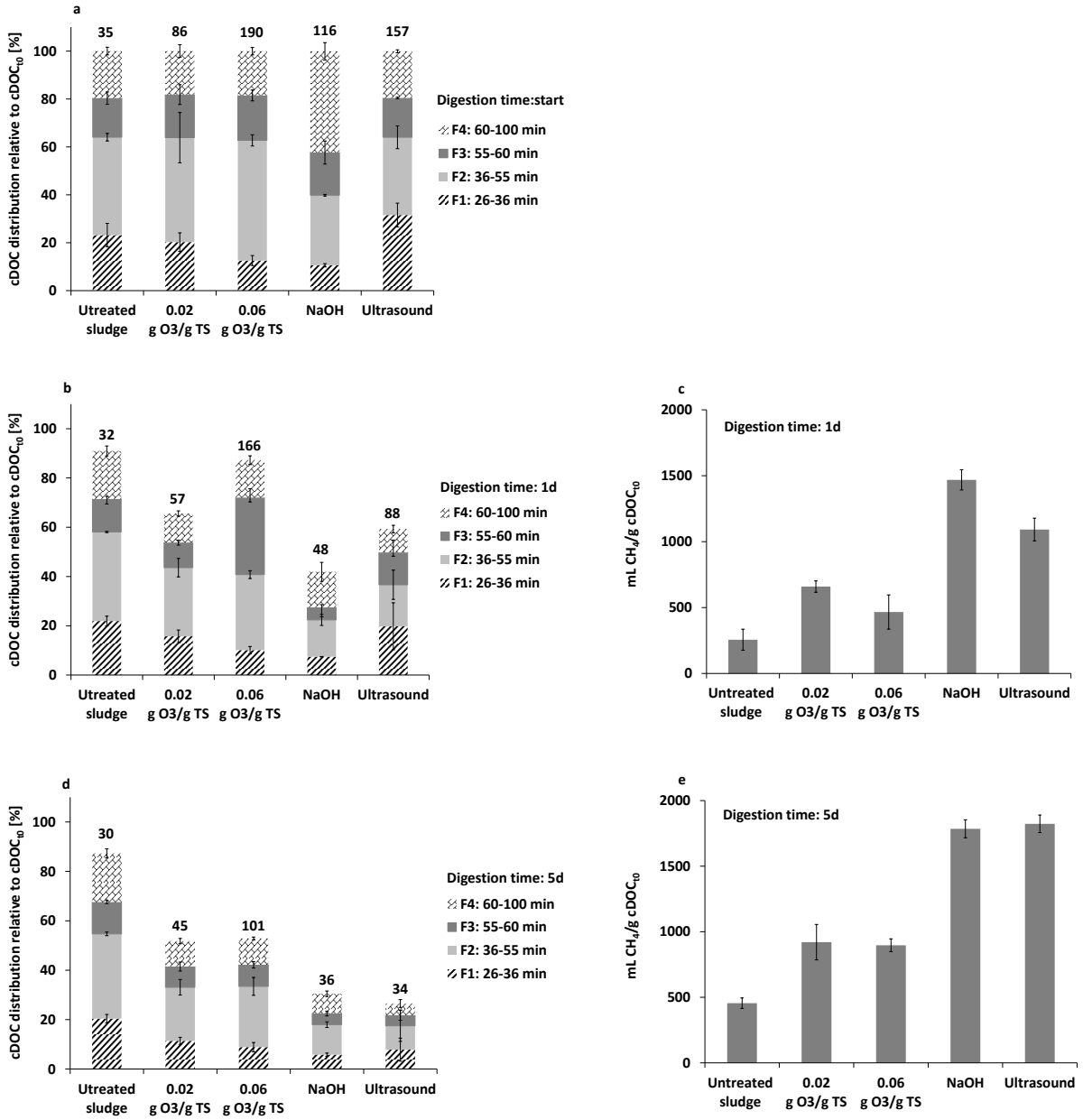


Fig. 3.14 cDOC distribution in the four fractions relative to the $cDOC_{t0}$ during the anaerobic digestion of the supernatants of untreated sludge and pretreated sludge a) at the beginning b) after one day and d) after five days of the anaerobic digestion experiments. The cDOC distribution in each fraction was calculated relative to the concentration of cDOC at the beginning of the digestion process ($cDOC_{t0}$). Total cDOC concentrations in mg/L are written above the columns. Methane production during anaerobic digestion of the supernatants of untreated and pretreated sludge c) after one day and e) after five days.

Although sludge pretreatments enhanced degradation of organic matter during the anaerobic digestion process, considerable amount of organic carbon was not degraded after five days. More than 50 % of the cDOC which was not degraded during five days contained organic compounds of intermediate molecular size (F.2 and F.3). Theses fractions were hardly biodegradable and could be the degradation products of the sludge pretreatment or the anaerobic digestion process. About 50 % of the $cDOC_{t0}$ which was distributed in F.2 and F.3 $(cDOC_{F.2\text{-}3})_{t0}$, was not degraded after five days of the anaerobic digestion of the supernatant of the ozonated sludge (Fig. 3.14a and Fig. 3.14d). Furthermore, approximately 30 % of $(cDOC_{F.2\text{-}3})_{t0}$ was not degraded after five days of the anaerobic digestion of the supernatant of the sludge pretreated with sodium hydroxide and ultrasound. To better understand the characteristics of the organic carbon which was not degraded during the anaerobic digester process, SEC in combination with an online UV at λ= 254 nm detection (SEC-UV_{254}) and with an online fluorescence detection at excitation wavelength of λ_{EX}= 245 nm and emission wavelength of λ_{EM}= 450 nm (SEC-$Fl_{EX:254/EM:450}$) was performed.

The DOC, UV_{254} and $Fl_{EX:254/EM:450}$ chromatograms during anaerobic digestion of the supernatants of the untreated and pretreated sludge are given in Fig. 3.15. At the beginning of the anaerobic digestion experiments (t_0), intermediate molecular size substances (F.2-3) show a relatively high UV_{254} and $Fl_{EX:254/EM:450}$ intensities (Fig. 3.15a-d). After five days the intensities of the DOC chromatograms were decreased due to the degradation of organic carbon during anaerobic digestion process (Fig. 3.15e-h). Moreover, the comparison of the shapes of the DOC chromatograms at t_0 and t_5 demonstrates the changes in the quality of the DOC. These changes are significant during anaerobic digestion of the supernatant of the pretreated sludge. This indicates that sludge pretreatment has changed the composition of the DOC compared to the DOC of the untreated sludge. However, the intensities of the UV_{254} and $Fl_{EX:254/EM:450}$ chromatograms were not changed significantly after five days of anaerobic digestion (Fig. 3.15e-h). The DOC in F.2-3 were composed of aromatic and aliphatic compounds. The high UV_{254} and $Fl_{EX:254/EM:450}$ intensities are related to the components of DOC with aromatic structures and fluorescence characteristics. According to the Fig. 3.15, these DOC components were hardly biodegradable. On the other hand, the aliphatic components of DOC in F.2-3 were easily degraded after five days.

Previous studies were conducted SEC-OCD and SEC-UV_{254} to characterize the components of DOC in the surface waters (Frimmel and Abbt-Braun, 2011, Frimmel et al., 2002, Huber et al., 2011). Surface waters are composed of hardly biodegradable compounds. The DOC and UV_{254} chromatograms of a surface water (River Pfinz, Karlsruhe, Germany) show high DOC and UV_{254} responses in the retention time between 40 - 50 min (Huber et al., 2011), see Fig. 3.16a. Since the DOC and UV_{254} chromatograms of the surface water were in a good agreement with the chromatograms of the humic acid and fulvic acid standard substances derived from the International Humic Substances Society (IHSS), this fraction of DOC (derived from River Pfinz) was assigned to humic substances (Huber et al., 2011), see Fig. 3.16b.

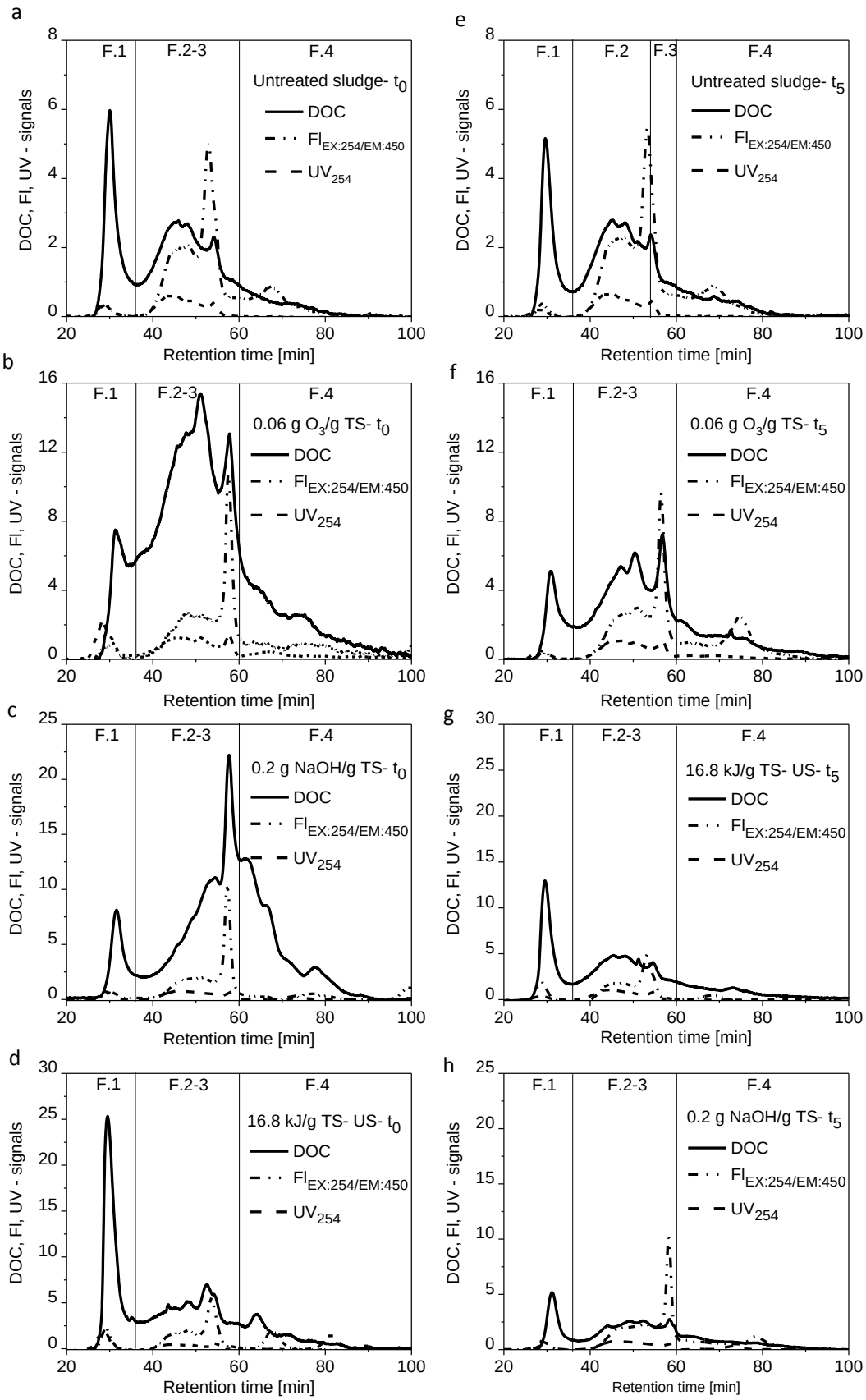


Fig. 3.15 DOC, UV_{254} and $Fl_{EX:254/EM:450}$ chromatograms during anaerobic digestion of the supernatant of the untreated sludge and sludge pretreated with 0.06 g O_3/g TS, 0.2 g NaOH/g TS and 16.8 kJ/g TS ultrasound a-d) at the beginning (t_0) and e-h) after five days (t_5) of the anaerobic digestion experiments.

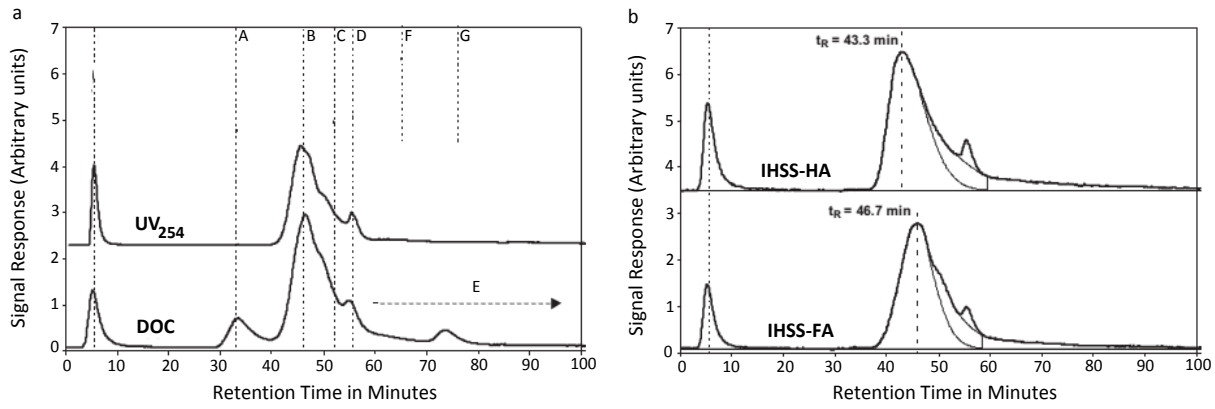


Fig. 3.16 DOC and UV_{254} chromatograms of a) a surface water (River Pfinz, Karlsruhe, Germany) and b) DOC chromatograms of humic acid (IHSS-HA) and fulvic acid (IHSS-FA) standard substances derived from the International Humic Substances Society (IHSS), taken and changed from Huber et al. (2011).

Brown water from Lake Hohloh (Black Forest, south of Germany) has high concentration of DOC and humic substances (Frimmel et al., 2002). The OC and UV_{254} chromatograms of brown water demonstrate high intensities approximately between 35 - 50 min retention time (Frimmel and Abbt-Braun, 2011), see Fig. 3.17. This organic carbon fraction was assigned to humic substances (Frimmel and Abbt-Braun, 2011).

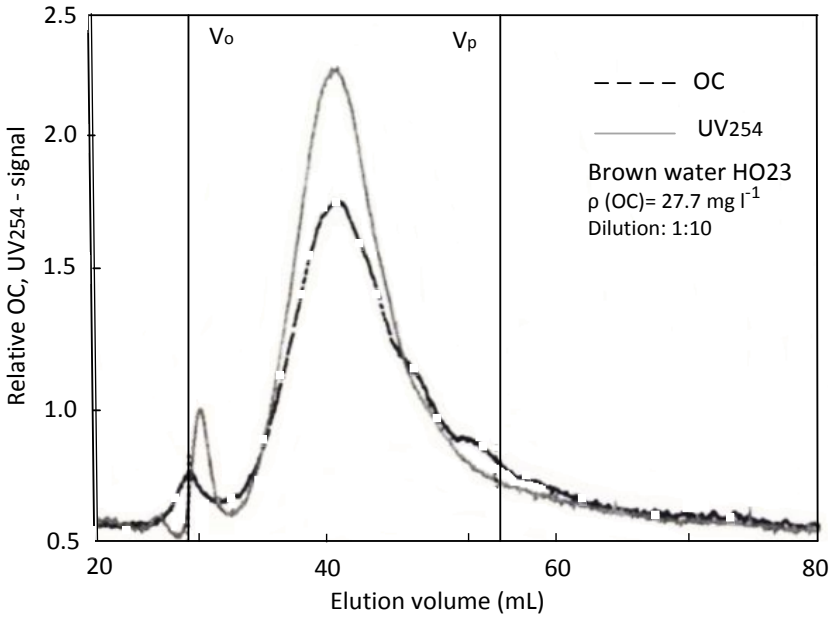


Fig. 3.17 OC and UV_{254} chromatograms of brown water from Lake Hohloh (Black Forest, south of Germany), HO23, taken from Frimmel and Abbt-Braun (2011).

According to the results of the mentioned studies, it can be proposed that the components of the DOC in F.2-3 with high UV_{254} and $Fl_{EX:254/EM:450}$ responses which were hardly biodegradable (Fig. 3.15) may be attributed to humic acid-like substances.

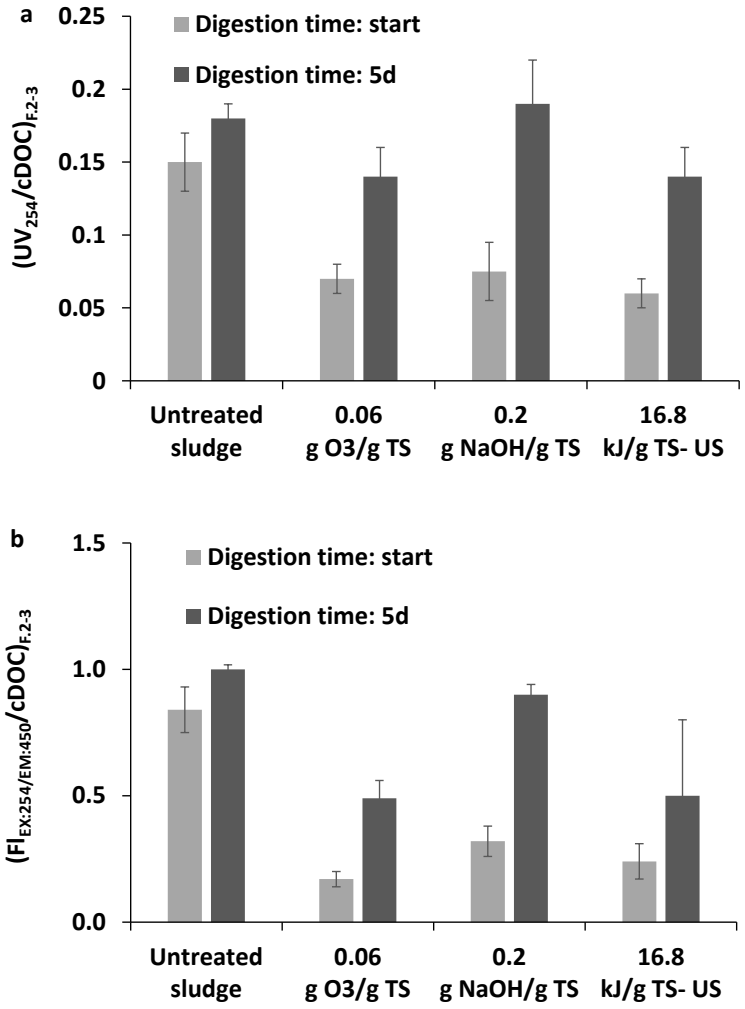


Fig. 3.18 Changes in the a) UV_{254} normalized to cDOC for the intermediate molecular size fraction (F.2-3), $(UV_{254}/cDOC)_{F.2\text{-}3}$, and b) changes in the $Fl_{EX:254/EM:450}$ normalized to cDOC in F.2-3, $(Fl_{EX:254/EM:450}/cDOC)_{F.2\text{-}3}$, during five days of anaerobic digestion. Samples were taken from the anaerobic reactors fed with the supernatant of untreated sludge and sludge pretreated with 0.06 g O_3/g TS, 0.2 g NaOH/g TS and 16.8 kJ/g TS ultrasound.

Based on the DOC, UV_{254} and $Fl_{EX:254/EM:450}$ chromatograms shown in Fig. 3.15 the UV_{254}/cDOC and $Fl_{EX:254/EM:450}$/cDOC ratios were calculated for the organic compounds in F.2-3. It can be seen in Fig. 3.18a that $(UV_{254}/cDOC)_{F.2\text{-}3}$ ratio at the start of the anaerobic digestion of the supernatant of the pretreated sludge was lower compared to its value for untreated sludge. The same trend was obtained for the $(Fl_{EX:254/EM:450}/cDOC)_{F.2\text{-}3}$ ratio at the start of the anaerobic digestion experiments (Fig. 3.18b). These results clearly show that the DOC of the pretreated sludge has different characters compared to the untreated sludge. The decrease in the $(UV_{254}/cDOC)_{F.2\text{-}3}$ and $(Fl_{EX:254/EM:450}/cDOC)_{F.2\text{-}3}$ ratios explain that the pretreated sludge has lower aromatic compounds which are related to the humic acid-like substances. The improvement in the methane production after sludge pretreatment can be attributed to

the changes in the characteristics of the released DOC. These changes are shown by the decrease of $(UV_{254}/cDOC)_{F.2\text{-}3}$ and $(Fl_{EX:254/EM:450}/cDOC)_{F.2\text{-}3}$.

After five days of anaerobic digestion $(UV_{254}/cDOC)_{F.2\text{-}3}$ and $(Fl_{EX:254/EM:450}/cDOC)_{F.2\text{-}3}$ ratios increased (Fig. 3.18a-b). According to Fig. 3.15 the DOC intensity in F.2-3 was decreased. However, the UV_{254} and $Fl_{EX:254/EM:450}$ intensities were not significantly changed. Because of that both ratios were increased. The increase in the ratios suggests the accumulation of hardly degradable compounds with intermediate molecular size. DOC in F.2-3 composed of different organic matter. The fractions of organic matter with aromatic structures, double bounds and fluorescence characteristics were not significantly affected by anaerobic bacteria during five days. These compounds were more resistant to anaerobic process compared to aliphatic compounds. It could be postulated that during anaerobic digestion, degradation of aliphatic compounds associated with the transformation of easily degradable substances to methane was dominant. Therefore, the anaerobic degradation of aliphatic structures might be responsible for the degradation of intermediate molecular compounds (F.2-3) after five days.

The biodegradability of DOC components during anaerobic digestion of the sludge was previously investigated by different analytical methods such as FT-IR and EEM fluorescence spectroscopy (Li et al., 2016, Li et al., 2014, Provenzano et al., 2014).

The FT-IR spectra of DOC during sludge anaerobic digestion process showed the reduction of aliphatic structures which indicates their high degradability (Li et al., 2014). On the other hand, the relative increase in the aromatic group derivative humic acid-like and polysaccharide-like substances was observed. These compounds were hardly degradable and were accumulated during anaerobic digestion process.

The EEM spectra of alkali-treated sludge during the anaerobic digestion process showed that the peak related to the humic acid-like substances intensified during anaerobic treatment (Li et al., 2016). Since humic acid-like substances components are highly resistant to anaerobic digestion, their accumulation inhibit the conversion of the organic matter to the biogas (Li et al., 2014, Provenzano et al., 2014).

In our study SEC-OCD, SEC-UV_{254} and SEC-$Fl_{EX:254/EM:450}$ were conducted to characterize the DOC during sludge anaerobic digestion. Compared to the results of the mentioned studies, during anaerobic digestion no changes in the UV_{254} and $Fl_{EX:254/EM:450}$ intensities related to the aromatic and humic acid-like substances were observed (Fig. 3.15). However, changes in the quality of the DOC chromatograms during anaerobic digestion imply that the aliphatic components of the DOC were easily degraded and the aromatic compounds were accumulated.

3.2.4 Sustainability of disintegrated sludge in denitrification process

The denitrification tests were done in batch vessels, using the same source of denitrification sludge, implying the same microbial community as inoculum and only the parameter that was changed was the carbon source. Therefore it should be possible to compare the impact of the different carbon sources on the nitrate removal rate. The supernatant of untreated sludge, sludge pretreated with ozone, sodium hydroxide and ultrasound and solution of sodium acetate were used as different carbon sources in the batch denitrification experiments. Fig. 3.19 represents the nitrate profiles for untreated sludge, pretreated sludge and sodium acetate.

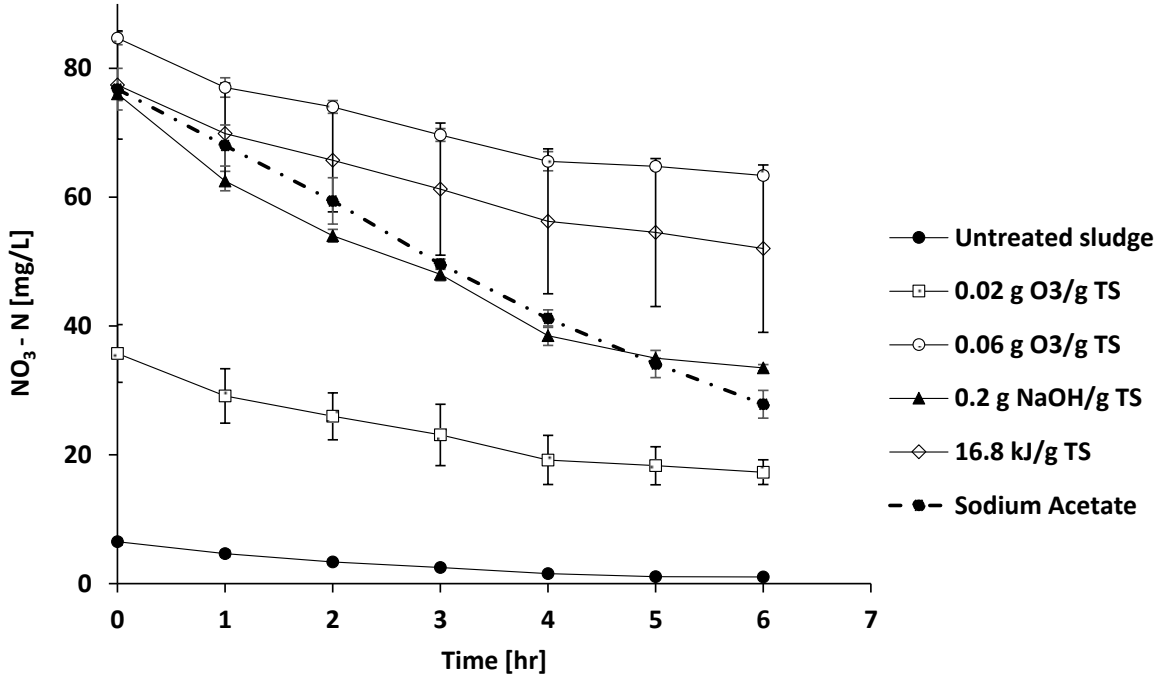


Fig. 3.19 Nitrate nitrogen concentration profile, during 6 hours of denitrification tests conducted with internal carbon sources; the supernatants of untreated sludge, ozonated sludge with 0.02 and 0.06 g O_3/g TS, sludge pretreated with 0.2 g NaOH/g TS and 16.8 kJ/g TS ultrasound specific energy, and an external carbon source; sodium acetate.

To assess the influence of carbon sources on the nitrate removal, carbon released after high sludge ozonation, sonication and sodium hydroxide pretreatment, was matched to approximately the same amount of SCOD. Moreover, nearly the same amount of nitrate in terms of KNO_3 was added to each vessel (Table 3.8). Nitrate removal in the vessels fed with supernatant of disintegrated sludge as an internal carbon source was compared to the nitrate removal in the sodium acetate-fed vessel as an external carbon source. The nitrate utilization rate (NUR) calculated for different carbon sources in three time intervals; 0-2h, 2-4h and 4-6h are illustrated in Table 3.8.

Table 3.8 Effect of sludge disintegration methods on the nitrate utilization rate expressed as g NO_3-$N_{removed}$/ (g TS.d), during 6 hours of batch denitrification tests.

$SCOD_{t0}/(NO_3\text{-}N)_{t0}$: 5.3 ± 0.6	$SCOD_{t0}$ [mg/L]	$(NO_3\text{-}N)_{t0}$ [mg/L]	g NO_3-$N_{removed}$/(g TS.d)		
			0 - 2 h	2 - 4 h	4 - 6 h
Untreated sludge	38 ± 7	7 ± 1	0.024 ± 0.0.004	0.014 ± 0.004	0.006 ± 0.001
0.02 g O_3/g TS	192 ± 7	36 ± 4	0.070 ± 0.014	0.050 ± 0.009	0.009 ± 0.015
0.06 g O_3/g TS	430 ± 6	86 ± 8	0.080 ± 0.030	0.060 ± 0.017	0.016 ± 0.005
16.8 kJ/g TS- US*	375 ± 15	78 ± 9	0.080 ± 0.005	0.070 ± 0.021	0.030 ± 0.011
0.2 g NaOH/g TS	390 ± 4	76 ± 1	0.150 ± 0.00	0.106 ± 0.003	0.034 ± 0.014
Sodium acetate	410 ± 2	77 ± 3	0.120 ± 0.002	0.125 ± 0.015	0.090 ± 0.024

$SCOD_{t0}$: initial soluble COD in the vessel at the start of the denitrification test

$(NO_3\text{-}N)_{t0}$: initial nitrate in the vessel at the start of the denitrification test

US*: Ultrasound

As it is shown in Table 3.8 the different carbon sources function very differently regarding the specific nitrate utilization rate. The supernatant of sludge pretreated with ozone, increased the NUR in the first and the second time intervals compared to untreated sludge. The rapid decrease in the nitrate concentrations during four hours of batch tests could be due to the utilization of easily biodegradable organic compounds released after sludge ozonation. In the third time interval, 4-6 h, NUR reduced to a very low value, reflecting the denitrification of slowly biodegradable organic carbon. It could be explained that once the easily biodegradable organic compounds has been depleted, the denitrification rate was reduced to the rate of utilization of compounds which could not be readily consumed by the microorganism. The present of slowly biodegradable compounds might be attributed to the chemical effect of sludge ozonation leading to the generation of products which could not be easily utilized by denitrifiers.

Nitrate utilization rate obtained in the vessel fed with the supernatant of sonicated sludge was approximately stable in the first and the second time interval, 0.07 – 0.08 g NO_3-$N_{removed}$/(g TS.d). It seems that sludge disintegration with ultrasound and ozone promoted the NUR in nearly the same range.

Nitrate profile obtained in the vessel fed with the supernatant of sludge pretreated with sodium hydroxide revealed three distinct phases (Fig. 3.19). Subsequently, the nitrate utilization rates were different in three interval times (Table 3.8). In the first two hours the NUR raised to 0.150 g NO_3-$N_{removed}$/ (g TS.d) which is remarkably high compared to untreated sludge and sludge treated with ozone and ultrasound. This increase in NUR could be due to the hydrolysis of large molecular

compounds to the smaller molecules through sludge treatment with sodium hydroxide. Therefore, microorganism could easily consumed the released readily biodegradable compounds, hence increased the NUR. The second rate of nitrate utilization raised to 0.106 g NO_3-$N_{removed}$/ (g TS.d) which is relatively higher than the rate obtained in ozonated and sonicated sludge, 0.050 and 0.07 g NO_3-$N_{removed}$/ (g TS.d), respectively. The hydrolysis rate of large molecular compounds to easier biodegradable compounds relatively limited the denitrification rate in ozonated and sonicated sludge. However, Sludge pretreated with sodium hydroxide speed up the denitrification rate as can be seen in Table 3.8. The decrease of the denitrification rate in the third phase (4 – 6 hr) might be due to the presence of hardly biodegradable organic compounds.

In this study sodium acetate was used as a typical readily biodegradable substrate for the denitrification test. As can be seen in Fig. 3.19 nitrate profile for sodium acetate was found to be a single linear phase and the nitrate concentration reduced with the rate of around 0.120 g NO_3-$N_{removed}$/(g TS.d) for 6 hours of denitrification test. The NURs obtained from three sludge disintegration methods were compared with the NUR of the sodium acetate used as an external carbon source in denitrification test (Table 3.8). The NUR comparison between different carbon sources produced as a result of sludge disintegration, suggests that the type of carbon solubilized after sludge pretreatment with sodium hydroxide, was more suitable for the application of denitrification process. The NUR obtained in the vessel fed with the supernatant of sodium hydroxide pretreated sludge during four hours of batch test, was approximately similar to the NUR obtained for sodium acetate which is a readily biodegradable organic compound. However, after four hours of denitrification test, nitrate was utilized at a very low rate in the vessel fed with sludge pretreated with sodium hydroxide compared to the rate obtained in the sodium acetate-fed vessel (Table 3.8). It might be due to depletion of readily biodegradable organic compounds released after sludge treatment with sodium hydroxide.

Fig. 3.20 shows a correlation between nitrate utilization rate during six hours of denitrification tests and methane production in the first day of anaerobic digestion experiments. It can be concluded that organic carbon produced after sludge disintegration with sodium hydroxide was easier consumed by both types of microorganisms, anaerobic bacteria and heterotrophic bacteria, compared to the organic carbon released after sludge sonication. On the other hand, higher methane production and higher NUR were obtained from sonicated sludge, compared to the values obtained from ozonated sludge at 0.02 g O_3/g TS. The lower methane production during anaerobic digestion of the supernatant of sludge ozonated at 0.06 g O_3/g TS compared to the corresponding value during anaerobic digestion of the supernatant of 0.02 g O_3/g TS ozonated sludge, can be attributed to the generation of by-products during higher sludge ozonation which are hardly biodegradable by anaerobic bacteria. The values of methane production [mL CH_4/g $cDOC_{t0}$] $_{t1}$ are available in Table 3.7 and were explained section 3.2.2.

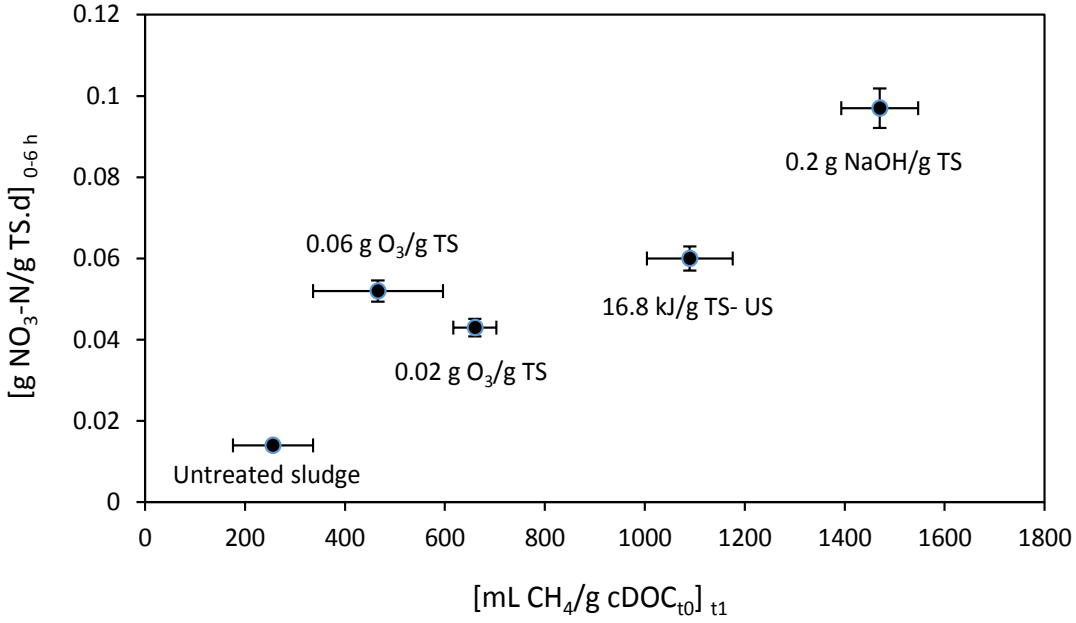


Fig. 3.20 Correlation between nitrate utilization rate, [g NO_3-$N_{removed}$ /(g TS.d)] $_{0\text{-}6\ h}$, during six hours of denitrification experiments and methane production, [mL CH_4/g $cDOC_{t0}$] $_{t1}$, after one day of anaerobic digestion experiments (t1), when supernatants of untreated and disintegrated sludge with 0.02 g O_3/g TS, 0.06 g O_3/g TS, 16.8 kJ/g TS ultrasound and 0.2 g NaOH/g TS were fed to the reactors as organic carbon sources.

3.3 Changes in the characteristic of DOC after sludge disintegration

Excess sludge consists of various organic compounds. Depending on the mechanism of disintegration the characteristics of the released organic compounds are changed, which cannot be easily determined only by chemical analyses. SEC-OCD, SEC-UV_{254} and SEC-$Fl_{EX:275/EM:335}$, coupled with three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy were conducted to provide more detailed information on the changes occurred in the characteristics of DOC released after sludge disintegration.

Fig. 3.21 demonstrates the UV_{254} chromatogram and the calculated UV_{254}/DOC chromatogram in the supernatant of untreated and disintegrated sludge with ozone, sodium hydroxide and ultrasound. The UV_{254}/DOC chromatogram obtained by normalizing the UV_{254} chromatogram to the DOC chromatogram (retention time: 26 – 100 min).

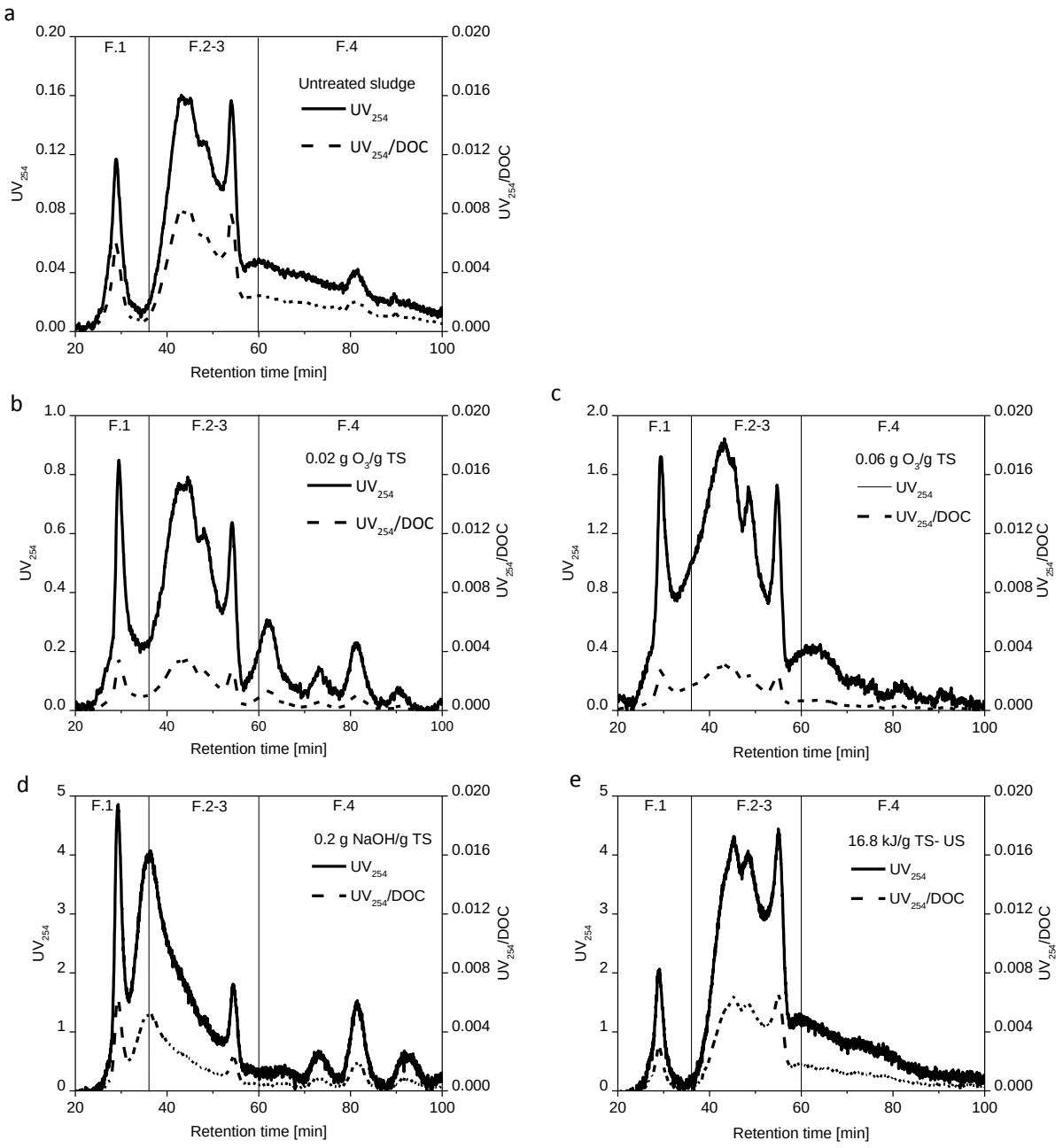


Fig. 3.21 UV_{254} and UV_{254}/DOC chromatograms for the supernatant of a) untreated sludge, b) sludge ozonated with 0.02 gO_3/g TS, c) sludge ozonated with 0.06 g O_3/g TS, d) sludge pretreated with 0.2 g NaOH/g TS and e) sludge sonicated with 16.8 kJ/g TS specific energy.

For the untreated sludge and sludge pretreated with ozone, sodium hydroxide and ultrasound, relatively high UV_{254} intensities were obtained in the large-intermediate molecular size fractions, F.1 and F.2-3 (Fig. 3.21). Comparison of the UV_{254} chromatograms of the untreated and pretreated sludge demonstrates the increase of the UV_{254} intensity after sludge disintegration. This implies that the large-intermediate molecules released after sludge disintegration might have aromatic and double bond structures. On the other hand, the UV_{254}/DOC intensity decreased after sludge disintegration, compared

to the untreated sludge. The decrease in UV_{254}/DOC was more severe after sludge ozonation (Fig. 3.21b-c) compared to the sludge pretreatment with sodium hydroxide and ultrasound (Fig. 3.21d-e).

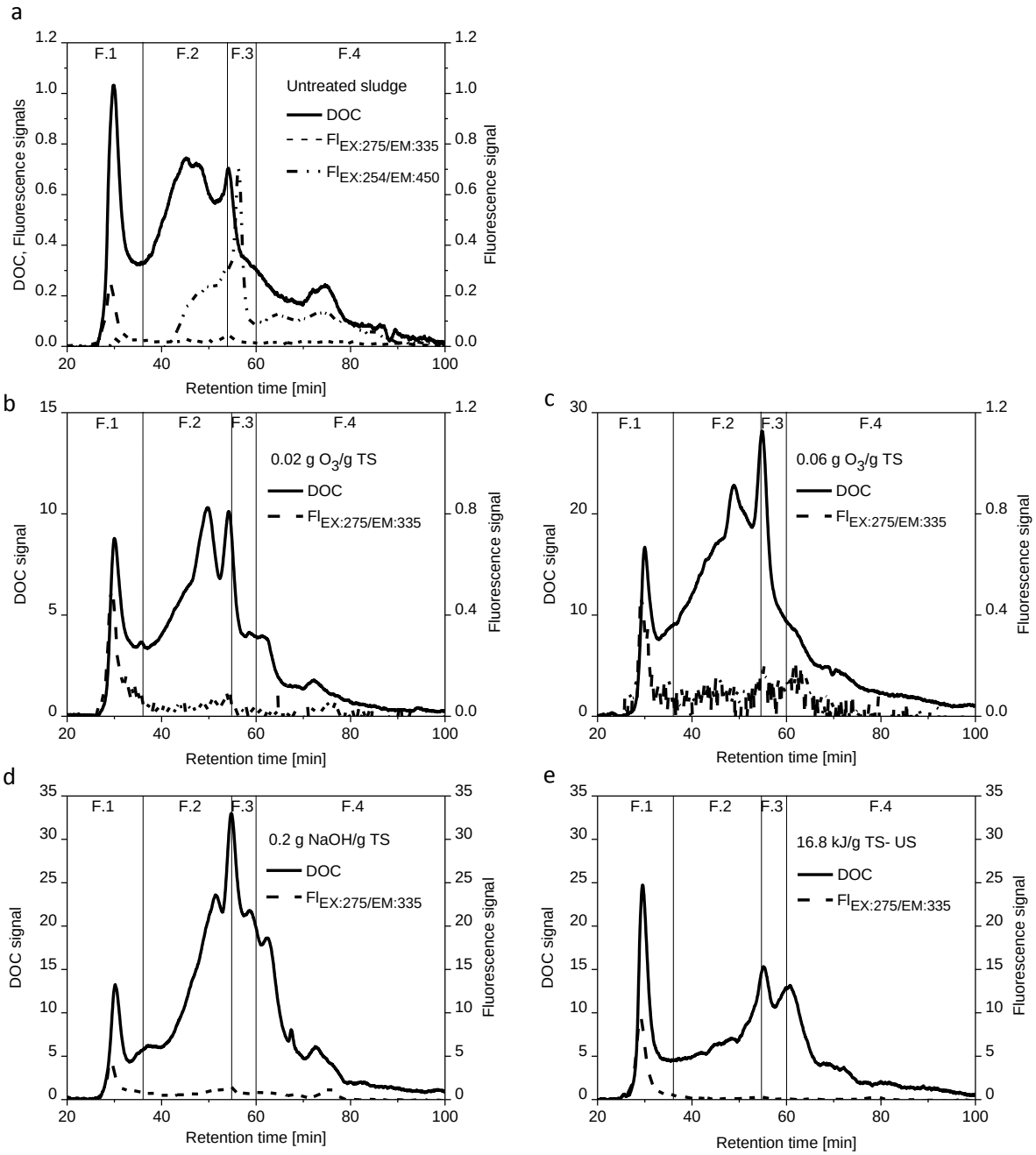
In addition to SEC-UV_{254}, the UV_{254} absorbance of the organic compounds in the supernatant of untreated and disintegrated sludge was measured with a spectrophotometer. This is a simple and fast technique to analyze the UV_{254} absorbance and based on that the SUVA can be calculated. SUVA exhibited a reduction from 2.2 L/(mg.m) for untreated sludge to about 0.6 L/(mg.m) after sludge ozonation, 1.2 after pretreatment with sodium hydroxide and 1.6 L/(mg.m) after sonication. The UV_{254}/DOC chromatogram and the SUVA values demonstrate that the aromatic contents of the organic compounds were decreased after sludge disintegration. In addition SEC-UV_{254} provides information on the molecular size of the aromatic compounds.

The significant decrease in SUVA after sludge ozonation can be explained by the direct reaction of ozone with double bounds and aromatic groups present in the sludge. Ozone reacts quickly with unsaturated compounds and double bound structures through Criegee mechanism (Atkinson and Arey, 2003, Waring and Wells, 2015). It also reacts fast and selectively with aromatic structural groups based on electrophilic reactions (Miao et al., 2015, Naumov and von Sonntag, 2010). Therefore, ozonating the sludge released large-intermediate molecules with aromatic and double bond structures in which some of them could be possibly destroyed due to the oxidation by ozone. It is also probable that sludge ozonation lose the carbon bounds of the large molecules and forms smaller molecules with lower aromaticity.

SEC in combination with an online fluorescence detector was conducted to analyze the fluorescence characteristic of the DOC before and after sludge disintegration. Based on the regional excitation-emission wavelengths which was introduced in section 1.4.3 (Fig. 1.6), the peak location for tryptophan protein-like substances with excitation wavelength of λ_{EX}= 275 nm and emission wavelengths of and λ_{EM}= 335 nm, and the peak location for humic acid-like substances with excitation wave length of λ_{EX}= 254 nm and emission wavelength of λ_{EM}= 450 nm were selected and applied to the online fluorescence detector.

For the supernatant of the untreated sludge $Fl_{EX:275/EM:335}$ chromatogram exhibits only one peak in the large molecular size fraction (F.1). By changing the excitation wave length to λ_{EX}= 254 nm and the emission wavelength to λ_{EM}= 450 nm the high $Fl_{EX:254/EM:450}$ intensity was obtained in the intermediate molecular size fractions, F.2 and F.3 (Fig. 3.22a). Therefore, the characteristics of the DOC compounds in F.1are quite different from the compounds in F.2 and F.3.

To study the characteristic of the DOC in F.1 before and after sludge disintegration, SEC-OCD and SEC-$Fl_{EX:275/EM:335}$ were performed. The $Fl_{EX:275/EM:335}$ intensity for the supernatant of the ozonated sludge slightly increased compared to the untreated sludge (Fig. 3.22b-c). Sludge pretreatment with sodium hydroxide and ultrasound result in a significant increase in the $Fl_{EX:275/EM:335}$ intensity in F.1



(Fig. 3.22d-e). The $Fl_{EX:275/EM:335}$ intensity of the disintegrated sludge shows only one peak in F.1. It suggests that the large molecular compounds released after sludge disintegration are probably composed of protein-like substances.

Fig. 3.22 DOC, $Fl_{EX:275/EM:335}$ and $Fl_{EX:254/EM:450}$ chromatograms a) for the supernatants of the untreated sludge. DOC and $Fl_{EX:275/EM:335}$ chromatograms for the supernatant of the sludge pretreated with b) 0.02 g O_3/g TS, c) 0.06 g O_3/g TS, d) 0.2 g NaOH/g TS and e) 16.8 kJ/g TS specific energy.

Based on the DOC and $Fl_{EX:275/EM:335}$ chromatograms the $Fl_{EX:275/EM:335}$/DOC ratio in the large molecular size fraction (F.1) of the supernatant of the untreated and disintegrated sludge was calculated and illustrated in Fig. 3.23. It can be seen that this ratio decreased severely after ozonating the sludge compared to untreated sludge. On the other hand, $Fl_{EX:275/EM:335}$/DOC ratio raised by pretreating the sludge with sodium hydroxide and ultrasound. The distinct differences between these three pretreatments might be related to the different mechanism that each pretreatment exerts on microorganism cells. The substantial decrease in UV_{254}/DOC and $Fl_{EX:275/EM:335}$/DOC ratios after sludge ozonation, can be attributed to the direct reaction of ozone with the large molecules that have aromatic and fluorescence characteristics. Bunning and Hempel (1996) have shown that intracellular proteins were released from cells and temporarily found in the sludge liquor but they were subsequently decomposed by consecutive reactions with ozone.

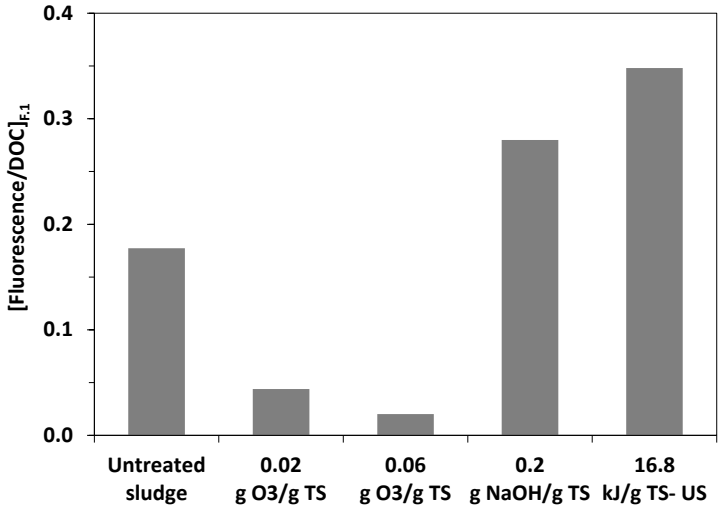


Fig. 3.23 $Fl_{EX:275/EM:335}$/DOC ratio of the large molecular size fraction (F.1) in the supernatant of untreated sludge and sludge pretreated with ozone, sodium hydroxide and ultrasound.

To verify the chromatographic results obtained from the SEC-$Fl_{EX:275/EM:335}$ (Fig. 3.22) EEM fluorescence spectra were derived for the supernatant of untreated and disintegrated sludge. The results are presented in Fig. 3.24. Two main peaks (peak A and peak B) at different regions were readily identified. Based on the regional excitation-emission wavelengths introduced by Chen et al. (2003), see Fig. 1.6, peaks detected in the region with excitation wavelength between 250 nm - 280 nm and emission wavelength shorter than 380 nm are associated with protein-like substances (peak A) and peaks in the region with excitation wavelength longer than 250 nm and emission wavelength longer than 380 nm are defined as humic acid-like substances (peak B). Peak A (EX:275/EM:335) and peak B (EX:336/EM: 438) were obvious in the supernatant of untreated and ozonated sludge (Fig. 3.24a-b-c). In the supernatant of sludge pretreatment with ultrasound and sodium hydroxide, peak A was readily detected, while fluorescence intensity of peak B was extremely lower than the fluorescence intensity of peak A (Fig. 3.24d-e).

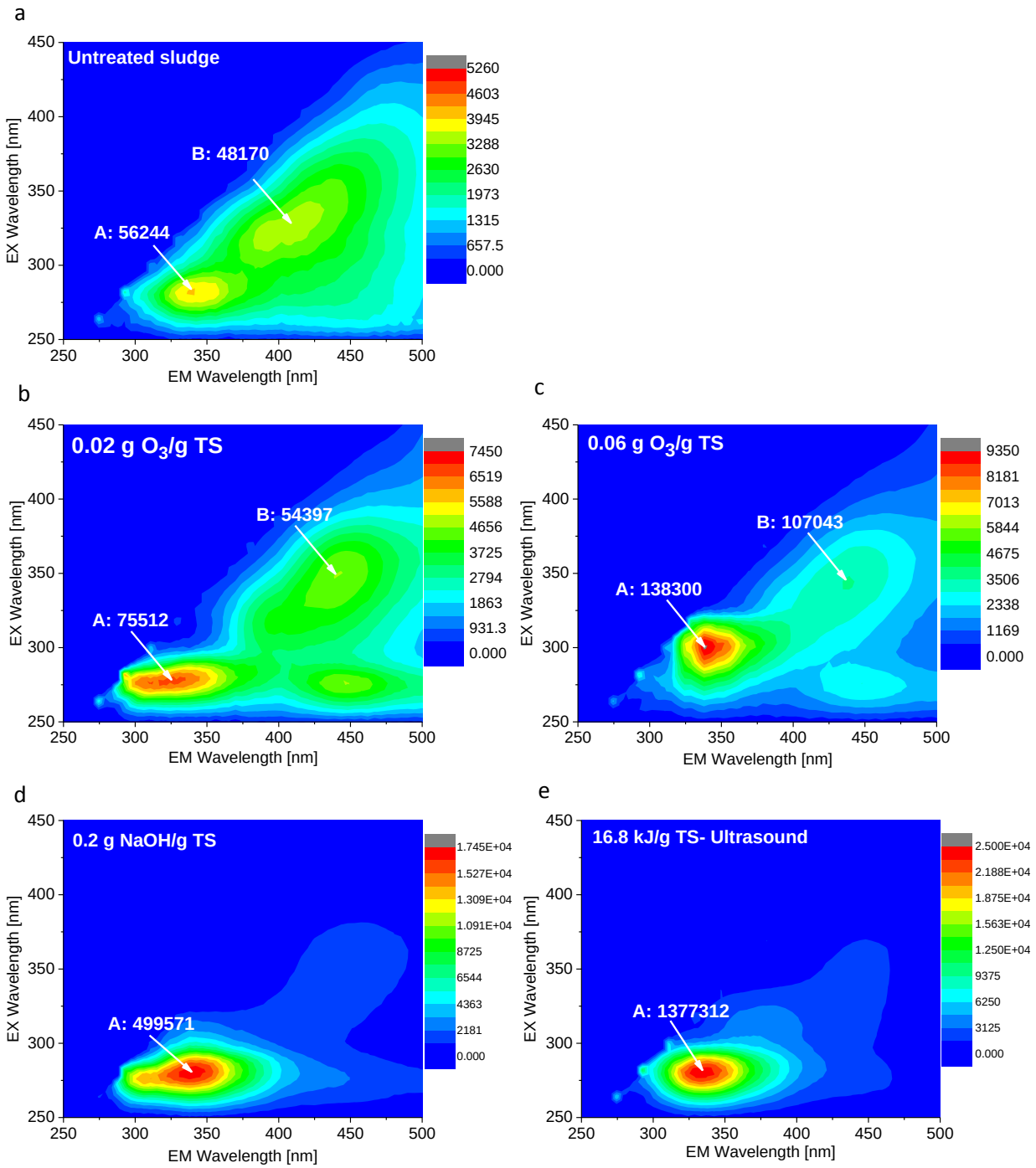


Fig. 3.24 Excitation-emission matrices (EEM) fluorescence spectra for the supernatant of a) untreated sludge, sludge pretreated with b) 0.02 g O_3/g TS, c) 0.06 g O_3/g TS, d) 0.2 g NaOH/g TS and e) 16.8 kJ/g TS ultrasound specific energy.

Table 3.9 Peak A (associated with protein-like substances) and peak B (associated with humic-acid like substances) intensities detected in the supernatant of untreated sludge and disintegrated sludge with ozone, sodium hydroxide and ultrasound. DOC was analyzed with thermal catalytic oxidation.

	DOC [mg/L]	A: Protein-like (EX:275/EM:335)	B: Humic acid-like (EX:336/EM:438)	A/B
Untreated sludge	15	56244	48170	1.2
0.02 g O_3/g TS	116	75512	54397	1.4
0.06 g O_3/g TS	318	138300	107043	1.3
0.2 g NaOH/g TS	260	499571	47153	11
16.8 kJ/g TS- US*	360	1377312	113700	12

US*: Ultrasound

It can be seen in Table 3.9 that peak A related to protein-like substances, slightly increased by ozonation compared to the untreated sludge. Although, approximately the same amount of DOC released after 0.06 g O_3/g TS, 0.2 g NaOH/g TS and 16.8 kJ/g TS ultrasound, the intensity of peak A was significantly higher after pretreatment with sodium hydroxide and ultrasound, compared to ozonation. Peak B associated with humic acid-like substances increased after sludge pretreatment with ozone and ultrasound. However the intensity of peak B was not significantly changed after sodium hydroxide pretreatment, compared to untreated sludge. It is demonstrated in Table 3.9 that by ozonating the sludge A/B ratio remained approximately the same as the ratio for untreated sludge, while it is remarkably high after pretreatment with sodium hydroxide and ultrasound. This suggests that the contents of protein-like substances evidently increased after sludge pretreatment with sodium hydroxide and ultrasound. The high fluorescence intensity at EX:275/EM:335 (peak A) observed in EEM fluorescence spectra of sludge pretreated with sodium hydroxide and ultrasound (Fig. 3.24d-e) is in agreement with the high $Fl_{EX:275/EM:335}$ intensity obtained by SEC-$Fl_{EX:275/EM:335}$ (Fig. 3.22d-e). The results imply that large organic carbon fraction (F.1) released after sodium hydroxide and ultrasound treatment, can be associated with protein-like substances.

Fluorescence components of the DOC released after sludge pretreatment with sodium hydroxide and ultrasound were characterized with EEM fluorescence spectroscopy (Tian et al., 2015a). Peaks of protein-like and humic acid-like substances were observed after both sludge pretreatment methods. Fluorescence intensity of protein-like substances was significantly raised after sodium hydroxide and ultrasound pretreatments. Whereas, fluorescence intensity of humic acid-like substances was obviously lower compared to that of protein-like substances, after both pretreatments. It was found that tryptophan protein-like substances were the main soluble component of the DOC with fluorescence characteristics after sludge sonication, while humic acid-like substances were the dominant compounds detected in the ozonated sludge (Yang et al., 2013). Increase in humic acid-like substances after ozonation could be

attributed to the increase of carboxylic, phenolic and alcoholic hydroxyl groups, due to the ozone oxidation (Liang et al., 2014, Peiris et al., 2011).

4 Summary and conclusions

In this study disintegration of excess sludge with ozone, sodium hydroxide and ultrasound were comprehensively investigated to understand the effects of different mechanisms on the molecular size distribution of the released organic compounds. SEC-OCD was conducted to separate organic compounds based on the molecular size. Substantial differences were obtained based on the methods of disintegration.

The relative cDOC distribution of the sludge pretreated with ozone demonstrated that low sludge ozonation (0.005 – 0.02 g O_3/g TS) primarily reacts with the sludge flocs resulting in the release of large molecular size compounds into the liquid phase. Moderate ozonation (0.06 – 0.08 g O_3/g TS) solubilized abundance of organic matter reflected in a remarkable increase in cDOC. Since the particle size distribution of sludge was not significantly changed during low and moderate ozonation, the significant increase in cDOC concentration at moderate ozonation can be attributed to the reaction of ozone with bacterial cells and thus release of intra-cellular substances. The relative cDOC distribution revealed that at moderate ozonation, ozone reacts with the released large molecules and slightly transformed them into the intermediate and smaller fractions, compared to low ozonation. Strong oxidation effect of ozone was more pronounced at high sludge ozonation (0.1 – 0.16 g O_3/g TS). A decrease of the large compounds and an increase of the intermediate substances, at high ozonation confirmed that the mineralization of the solubilized organic carbon was the predominant process compared to the transformation of the large molecules to the smaller ones.

The effect of different sodium hydroxide dosages and treatment durations on the solubilization of organic components were examined. Low sodium hydroxide dosage was more effective in the transformation of the large molecules into the smaller ones compared to the high sodium hydroxide dosage. Sludge pretreatment with 0.2 g NaOH/g TS for 2 hours was found to be the optimum dosage and treatment time for solubilizing large molecules into the sludge supernatant and hydrolyzing them into the smaller compounds.

Mechanical shear force produced by low ultrasound frequency of 20 kHz effectively disintegrated the sludge flocs and solubilized large-intermediate compounds. Due to low ultrasound frequency, conversion of large molecules to the smaller molecules which could be associated to the generation of hydroxyl radicals, was not predominant in this study.

To assess the biodegradability of the organic carbon released after sludge disintegration, batch anaerobic digestion experiments were conducted for five days. Supernatants of untreated sludge, sludge ozonated with 0.02 and 0.06 g O_3/g TS, sludge pretreated with 0.2 g NaOH/g TS and 16.8 kJ/g TS ultrasound were fed into the anaerobic digesters. Disintegrated sludge was able to provide more soluble organic carbon, thus enhanced hydrolysis and improved methane production. However, there were distinct differences in methane production depending on the method of disintegration.

Sludge disintegration with sodium hydroxide and ultrasound prior to anaerobic digestion increased methane production twice higher than ozonated sludge.

Changes in the relative cDOC distribution of the supernatant of untreated and disintegrated sludge during five days of anaerobic digestion experiments were investigated by SEC-OCD. The results illustrated that small components of DOC generated by sodium hydroxide was easily hydrolyzed and converted to methane. Large compounds solubilized by ultrasound were susceptible to be broken down and hydrolyzed by anaerobic bacteria, thus methane production highly improved after five days.

Intermediate molecular size compounds seemed to be hardly degradable. Characterization of these compounds with SEC-OCD, SEC-UV_{254} and SEC-$Fl_{EX:254/EM:450}$ revealed the possibility of the accumulation of aromatic compounds which are related to the humic acid-like substances.

The potential of disintegrated sludge to be used as an internal carbon source for biological removal of nitrate was investigated in batch denitrification experiments. The comparison of NURs for different carbon sources produced as a result of sludge disintegration, suggests that type of carbon solubilized after pretreatment with sodium hydroxide, was more suitable for the denitrification application, when compared to the sodium acetate commonly used as an external carbon source.

SEC-OCD and SEC-$Fl_{EX:275/EM:335}$ in combination with EEM fluorescence spectroscopy provided valuable information about the changes occurred in the fluorescence characteristics of organic compounds released after sludge disintegration. A significant decrease in $Fl_{EX:275/EM:335}$/DOC ration after sludge ozonation indicated that ozone oxidized the released protein-like substances. Oxdation of organic carbon such as proteins might be the reason for the lower efficiency of ozonation in enhancing methane production. On the other hand, $Fl_{EX:275/EM:335}$/DOC ratio highly increased after alkaline pretreatment. An increase in $Fl_{EX:275/EM:335}$/DOC ratio which is associated with the release of protein-like substances, in connection with a shift of the peaks of the DOC chromatogram towards higher retention time (production of small molecules) were the indicators for the substantial improvement in methane production. Mechanical shear forces produced at low ultrasound frequency, reacted with the sludge flocs and resulted in a substantial release of protein-like substances which was proved by the combination of SEC-$Fl_{EX:275/EM:335}$ and EEM fluorescence spectroscopy. These compounds were readily usable carbon sources for the anaerobic bacteria, thus enhanced methane production in the anaerobic digestion process.

5 Perspectives for further studies

5.1 Evaluation of energy consumption by sludge disintegration methods

In this study performance of sludge disintegration techniques were assessed in terms of DOC solubilization and methane production. Cost of energy consumed by sludge disintegration method is another important factor that should be consider to evaluate the applicability of the treatment technique in a full scale wastewater treatment plant. Methane as a valuable product of sludge anaerobic digestion which can be used as a source of energy to produce electricity. To evaluate whether the energy recovered from methane production is able to compensate the energy cost of sludge disintegration process, an energy balance can be carried out and based on that practical strategies should be applied to reduce the cost of energy. For instance, ultrasound and sodium hydroxide used in this study were recognized as the powerful sludge pretreatment methods which highly enhanced methane production in anaerobic digestion process. Nevertheless, considering an intensive energy consumption by ultrasound treatment its application can be limited in a full scale wastewater treatment plant. Therefore combination of ultrasound with sodium hydroxide or other treatment methods can be examined. The energy consumption of the individual treatments and their combination can be compared to the energy recovery from methane production.

5.2 Biological analysis of sludge floc structure and bacterial cell

In this study ozone, sodium hydroxide and ultrasound were used to disintegrate the excess sludge to nearly the same degree of solubilization. However, each disintegration method differently affect the integrity of the bacterial cell. Rapid and accurate technologies such as flow cytometry (FCM) and polymerase chain reaction - denaturing gradient gel electrophoresis (PCR-DGGE) has been successfully used to quantify the microbial parameters in the water samples. The application of FCM and PCR-DGGE to study cell permiabilization, cell damage and destruction of bacterial DNA will help to better understanding of the mechanism of sludge floc and cell disintegration. The level of energy consumption to damage the cells or completely disrupt them, can be evaluated. Regarding the cost of energy consumption, it can be investigated whether to reach the complete cell disruption is economic. It might be more beneficial to apply the level of energy in the range which causes sludge flocs disintegration. Applying high energy to achieve cell lyses, solubilize more intercellular organic compounds which could enhance anaerobic digestion process, but results in an excessive energy consumption.

5.3 Application of ozone as a sludge post-treatment technique

Application of ozone in the laboratory scale to disintegrate the excess sludge before anaerobic digestion was investigated in this study. Sludge disintegration with ozone reduced the amount of sludge solids and increased the bioavailability of soluble organic carbon for anaerobic bacteria. However, high amount of oxygen produced as a result of ozonation should not be neglected. Presence of oxygen in anaerobic digester is an inhibiter for the growth of several microorganism involve in the degradation process.

Considering this issue, sludge ozonation before anaerobic digester in a full scale wastewater treatment plant, may not be beneficial for the subsequent anaerobic digestion process. Therefore, it is suggested to implement the ozone generator after anaerobic digester and study the effects on sludge dewaterability, amount of total solids and oxidation of organic compounds which are hardly degraded by anaerobic bacteria.

5.4 Further studies for characterization of sludge components during anaerobic digestion

Digested sludge comprised of hardly biodegradable organic substances. Characterization of digested sludge components is important to find solution for enhancing the removal or degradation of hard to decompose compounds. In this study the aromaticity of these compounds and their fluorescence characteristic at a special excitation emission wavelength of λ_{EX}= 254 nm and λ_{EM}= 450 nm which generally refers to humic acid-like substances, were analyzed using SEC-OCD, SEC-$Fl_{EX: 254/EM: 450}$ and SEC-UV_{254}. To obtain more information on the characterization of digested sludge SEC-OCD, SEC-UV_{254} and SEC in combination with various fluorescence excitation and emission wavelengths coupled with EEM can be conducted. The fluorescence products such as protein-like substances and humic acid-like substances which remained in the digester can be detected by EEM. The excitation- emission wavelengths for these compounds obtained by EEM method can be applied to the fluorescence detector in the SEC system. The combination of DOC, UV and fluorescence chromatograms enable us to characterize the DOC fractions based on their molecular size.

Moreover, properties of EPS are the critical factors affecting the hydrolysis of sludge in the anaerobic digester. To well understand the sludge destruction mechanism during anaerobic digestion process, more studies are still required about the changes in the characteristics of EPS during anaerobic degradation. EPS contains aromatic structures and unsaturated compounds, it mainly comprises proteins, polysaccharides, humic substances. With the aid of spectroscopy tools such as FT-IR and the fluorescence EEM, the structural and functional characteristics of EPS during anaerobic digestion can be analyzed. In addition, combination of the spectroscopy technique and SEC-OCD with multiple online detectors provide valuable information about the fractions of organic carbon in EPS based on their molecular size.

5.5 Application of immobilized bacteria in anaerobic reactor

Use of various support media to immobilize anaerobic microorganism has been wildly investigated to improve anaerobic digestion performance (Lalov et al., 2001, Weiss et al., 2011). Anaerobic bacteria particularly methanogens which are immobilized on the carriers can be used as an inoculum when pretreated sludge is fed to the anaerobic reactor. Using immobilize bacteria to gather with providing more DOC due to sludge pretreatment might result in higher methane production compared to suspended bacteria. The inhibitory effect of compounds generated as a result of sludge ozonation and sodium hydroxide pretreatment with high dosage maybe reduced using immobilized anaerobic bacteria.

Polyurethane foam (PUF) showed promising results in adhering verity of anaerobic microorganism such as methanogens (Ribeiro et al., 2003). Therefore it is suggested to study the anaerobic biodegradability of disintegrated sludge using immobilized anaerobic bacteria as an inoculum in the anaerobic reactor.

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A Appendix

A.1 Methane production calculation

Table A.1 DOC concentrations (analyzed by thermal catalytic oxidation) in anaerobic reactors at the beginning (t0), after one day (t1) and after five days (t5), DOC removal and methane production after one day and five days of anaerobic digestion fed with the supernatant of untreated and pretreated. sludge

	DOC_{t0}	DOC_{t1}	DOC_{t5}	DOC $_{removal\ t0\text{-}t1}$	DOC $_{removal\ t0\text{-}t5}$	$(\frac{mL\ CH_4}{g\ DOC_{t0}})_{t1}$	$(\frac{mL\ CH_4}{g\ DOC_{t0}})_{t5}$
	mg/L	mg/L	mg/L	%	%		
untreated sludge	46±4	49±4	44±1	-	4	160±45	300±50
0.02 g O_3/g TS	125±30	80±25	60±10	36	52	430±100	660±250
0.06 g O_3/g TS	250±20	210±50	120±25	16	52	330±80	660±100
0.2 g NaOH/g TS	150±30	70±2	54±2	53	64	1170±5	1430±10
16.8 kJ/g TS	190±20	120±10	60±1	37	68	860±120	1440±270

Table A.2 soluble COD (SCOD) concentrations in anaerobic reactors at the beginning (t0), after one day (t1) and after five days (t5), SCOD removal and methane production after one day and five days of anaerobic digestion fed with the supernatant of untreated and pretreated sludge.

	$SCOD_{t0}$	$SCOD_{t1}$	$SCOD_{t5}$	SCOD $_{removal\ t0\text{-}t1}$	SCOD $_{removal\ t0\text{-}t5}$	$(\frac{mL\ CH_4}{g\ SCOD_{t0}})_{t1}$	$(\frac{mL\ CH_4}{g\ SCOD_{t0}})_{t5}$
	mg/L	mg/L	mg/L	%	%		
untreated sludge	120±20	140±30	130±10	-	-	60±20	105±10
0.02 g O_3/g TS	350±60	230±80	175±30	34	50	150±30	235±70
0.06 g O_3/g TS	720±85	590±13	320±56	18	56	115±30	225±20
0.2 g NaOH/g TS	515±10	242±10	177±40	53	66	333±56	404±60
16.8 kJ/g TS	792±80	448±80	234±50	43	70	208±30	350±70

Verification of the contribution from the co-authors

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The content of this paper has been included in Chapter 3

Contribution of Elham Fatoorehchi (First author):

- Project idea, experiment planning
- SEC-OCD data processing
- Methane production analysis
- Discussion on the results
- Writing of the manuscript

Contribution of Stephanie West (Second author)

- experiment planning
- Discussion on the results

Contribution of Gudrun Abbt-Braun (Third author)

- SEC-OCD data processing
- Discussion on the results

Contribution of Harald Horn (Last author)

- Discussion on the results

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