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UNIVERSITÉ DE MONTRÉAL

OZONATION OF PRIMARY AND DIGESTED SLUDGES TO ENHANCE METHANOGENESIS AT CHEMICALLY ENHANCED PRIMARY TREATMENT FACILITIES

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OZONATION OF PRIMARY AND DIGESTED SLUDGES TO ENHANCE METHANOGENESIS AT CHEMICALLY ENHANCED PRIMARY TREATMENT FACILITIES

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DEDICATION

To my family

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RÉSUMÉ

La digestion anaérobie est un processus lent qui requiert un long temps de rétention hydraulique et offre une efficacité de dégradation modérée qui requiert des digesteurs de grand volume et des coûts d'investissement relativement élevés. Le principal facteur limitant la digestion anaérobie est normalement l'hydrolyse de la matière particulaire. L'augmentation du taux d'hydrolyse permet d'augmenter la biodégradabilité de la matière particulaire et d'améliorer la performance des digesteurs anaérobies. Diverses techniques de traitement ont été étudiées pour augmenter le taux d'hydrolyse des boues en utilisant des procédés thermiques, chimiques, mécaniques et biologiques. L'ozonation est l'un des traitements chimiques préférés qui permet non seulement la réduction des boues mais qui est également efficace pour améliorer les processus de digestion anaérobie en modifiant les propriétés physico-chimiques et de biodégradabilité des boues.

Les études antérieures sur l'effet de l'ozone ont principalement porté sur les boues activées et peu d'informations sont disponibles sur l'effet de l'ozonation sur les boues primaires et les boues anaérobies digérées. Les mécanismes d'ozonation de ces types de boues demeurent toutefois mal connus. Une meilleure compréhension des mécanismes de ces procédés permettrait d'optimiser leur conception, application et optimisation des systèmes d'ozonation.

L'objectif général de ce projet était de maximiser la production de méthane dans une station d'épuration de traitement primaire chimique améliorée (CEPT) en ozonant des boues. Les objectifs spécifiques du présent rapport étaient de : 1) évaluer les effets de l'ozonation sur les propriétés physico-chimiques, la solubilisation, la minéralisation et la biodégradabilité des boues, 2) déterminer et d'évaluer l'impact et les mécanismes de l'ozonation des boues sur l'amélioration du potentiel de production de méthane et sa cinétique de production, et 3) évaluer et d'optimiser le transfert de masse d'ozone via un réacteur venturi à échelle laboratoire.

L'ozonation de boues a été étudiée en déterminant ses effets sur l'efficacité du transfert de masse, la production biochimique de méthane, l'activité microbienne et ses caractéristiques physico-chimiques, par example le fractionnement de la demande chimique en oxygène (DCO), minéralisation et solubilisation. L'ozonation des boues a été réalisée au moyen d'un réacteur venturi à échelle laboratoire. Le couplage de l'ozonation avec les digesteurs anaérobies a également été évalué par deux configurations de traitement : la pré-ozonation des boues primaires et la post-ozonation des boues digérées, chacune combinée à un digesteur anaérobie à l'échelle laboratoire.

Une évaluation technico-économique du traitement de l'ozone combinée à la digestion anaérobie a également été effectuée.

Les résultats ont montré que le réacteur venturi a été efficace pour augmenter l'efficacité de transfert d'ozone et contrôler l'accumulation de mousse pendant le traitement de la boue. À pression atmosphérique, l'ozonation des boues digérées a donné un rendement de transfert de masse d'ozone de 98% pour un rapport G/L <0,4, alors que pour des boues primaires, une pression de 103 kPa et un rapport G/L <0,2 ont été requis.

Une certaine oxydation de la matière organique et désintégration mécanique des boues ont été observées lors de l'ozonation. Les résultats de la distribution granulométrique ont indiqué que la réduction de la taille des particules lors du traitement des boues a été fortement influencée par le pompage et, dans une moindre mesure, par l'oxydation chimique par l'ozone. La friction mécanique exercée par le pompage des échantillons a provoqué la désintégration des boues mais n'a pas entraîné d'augmentation de la DCO soluble. L'augmentation de la solubilisation de la matière organique par l'ozonation semble avoir été causée par la désintégration partielle de la boue ainsi que le dommage à l'intégrité de la membrane cellulaire des bactéries présentes dans la boue anaérobie digérée. La matière organique a été relâchée sous forme de protéines et de polysaccharides en phase soluble, ce qui a amélioré la production de méthane pendant la digestion anaérobie. L'augmentation de la production de méthane peut non seulement être attribuée à la solubilisation, mais aussi à l'augmentation de la biodégradabilité des produits organiques produits lors de l'ozonation.

La biodégradabilité de la boue digérée anaérobie a augmenté au cours de l'ozonation. La DCO biodégradable des boues digérées anaérobies a augmenté de 2,5 à 3,9 g DCO/L pour une dose d'ozone de 90 mg O_3/g DCO (1.4 g O_3/L), ce qui représente une augmentation de la production de méthane de 55%. L'ozonation des boues primaires, toutefois, n'a pas entraîné d'augmentation de la DCO biodégradable. En raison de l'augmentation de la solubilisation et de la biodégradabilité des boues digérées, la dégradation anaérobie peut être améliorée, ce qui accroît le rendement de production de méthane et réduire le temps de digestion. Une surdose d'ozone, cependant, peut réduire le rendement de production en méthane en raison de la minéralisation de la matière organique solubilisée et de la réduction excessive de la viabilité de la biomasse anaérobie, ce qui pourrait avoir un impact négatif sur la stabilité des digesteurs anaérobies. L'action chimique de

l'ozone peut provoquer la minéralisation de la matière organique en CO_2, ce qui peut réduire la matière organique disponible et affecter négativement le potentiel de production de méthane de la boue ozonée. L'ozonation à doses jusqu'à environ 200 mg O_3 /g de DCO (3.0 g O_3/L), a résulté en une minéralisation atteignant 10 et 15% des boues primaires et des boues digérées anaérobies, respectivement.

La post-ozonation des boues anaérobies digérées a été jugée modérément efficace pour améliorer la production de méthane (+16%), l'enlèvement de la DCO, et le potentiel de déshydratation des boues par rapport au digesteur témoin, mais la pré-ozonation des boues primaires n'a pas été efficace pour améliorer les performances du digesteur anaérobie. L'augmentation de la production de méthane et la réduction de la production de boues pourraient permettre de réduire de 64% les coûts d'exploitation nécessaires de la post-ozonation des boues digérées anaérobies.

Sur la base de ces résultats, les investigations futures suivantes sont recommandées : a) optimisation du couplage de la digestion anaérobie avec l'ozonation (post-ozonation et pré-ozonation), en se concentrant sur l'optimisation des réacteurs anaérobies (p. ex. l'impact de la réduction du temps de rétention hydraulique, l'augmentation de la charge organique), b) bien qu'il soit bien établi que l'ozonation peut améliorer la biodégradabilité des matières organiques, on sait peu de choses sur la biodégradabilité des principaux produits de transformation individuels résultant de l'ozonation ainsi que sur leur toxicité éventuelle et c) étude de l'influence de l'ozonation sur la structure de la communauté microbienne et les principales voies de production de méthane de la digestion anaérobie du digesteur par utilisation d'une approche métagénomique.

ABSTRACT

Anaerobic digestion is a slow process with long hydraulic retention times and moderate degradation efficiencies which can result in large volume digesters and relatively high capital requirements. Usually, the main factor limiting anaerobic digestion is the hydrolysis of particulate matter. Improving anaerobic digestion through enhancing rate-limiting hydrolysis can increase the degradability leading to improved performance of the anaerobic digesters. A variety of treatment techniques have been studied to enhance sludge hydrolysis by using thermal, chemical, mechanical and other biological processes. Ozonation is one of the preferred chemical treatments, which not only permits sludge reduction, but is also considered to be effective in enhancing the anaerobic digestion processes by altering the physicochemical properties and biodegradability of sludge.

Past studies on the effect of ozone have mainly focused on activated sludge. There is limited information about the effect of ozonation on primary sludge and anaerobic digested sludge. The mechanisms involved in sludge ozonation are not well understood. A better understanding of the effect of ozone on primary sludge and anaerobic digested sludge could provide valuable information for the design, operation, and optimization of ozonation systems.

The general objective of this project was to maximize methane production in a chemically enhanced primary treatment (CEPT) facility by ozonating sludge. The specific objectives of this report were 1) to assess the effects of ozonation on physicochemical properties, solubilisation, mineralization, and biodegradability of ozonating sludge, 2) to determine and evaluate the impact and mechanisms of ozonation of sludge in the improvement of methane yield and methane production rates, and 3) to evaluate and optimize the ozone mass transfer in a lab-scale venturi reactor for the ozonation of primary sludge and anaerobic digested sludge.

Sludge ozonation was investigated by determining its effects on mass transfer efficiency, biochemical methane production, microbial activity and its physicochemical characteristics (e.g. COD fractionation, mineralization, and solubilisation). Sludge ozonation was performed by means of a lab scale-venturi loop reactor. The coupling of ozonation with anaerobic digesters was also evaluated in two process configurations, pre-ozonation of primary sludge and post-ozonation of digested sludge each combined with a semi-continuous lab-scale anaerobic digester. A technico-economical evaluation of ozone treatment combined with anaerobic digestion was also conducted.

Results showed that the venturi loop reactor was effective in increasing the ozone transfer efficiency and controlling foam accumulation during treatment. At atmospheric pressure, the ozonation of digested sludge resulted in an ozone mass transfer efficiency of 98% for a G/L ratio < 0.4, while for primary sludge a pressure of 103 kPa and a G/L ratio < 0.2 were required.

Oxidation of organic matter and mechanical sludge disintegration were observed during ozonation. The results from particle size distribution indicate that the reduction of particle sizes during the sludge treatment was greatly influenced by pumping and, to a lesser extent, by the chemical oxidation by ozone. Interestingly, the mechanical friction exerted by pumping of samples caused the disaggregation of sludge but did not result in an increase of soluble COD. Ozone treatment caused an increase in sludge solubilisation via partial disintegration of the sludge matrix and damage to the cell membrane integrity. Ozone treatment can disintegrate the sludge pellet and release organic matter as proteins and polysaccharides into the soluble phase, thereby, enhancing methane production during anaerobic digestion. The increase of methane production may not only be ascribed to solubilisation, but also is influenced by an increase in the biodegradability of organic products generated during ozonation.

Biodegradability of anaerobic digested sludge increased via ozonation. The biodegradable COD of anaerobic digested sludge increased from 2.5 to 3.9 g COD/L for an ozone dose of 90 mg O_3/g COD (1.4 g O_3/L), representing an increase of methane production of 55%; however, ozonation of primary sludge did not result in an increase in biodegradable COD. As a result of the increase in solubilisation and biodegradability of digested sludge, anaerobic degradation can be enhanced, improving methane yield and accelerating digestion times. An overdose of ozone can reduce the methane yield, due to the mineralization of the solubilized organic matter and the excessive reduction in viability of anaerobic biomass, which could have a negative impact on the stability of anaerobic digesters in a post-treatment configuration. The chemical action of ozone causes the oxidation of organic matter into CO_2, which can reduce the available organic matter and thus, negatively, affect the methane production potential. Ozonation at 200 mg O_3/g COD (3.0 g O_3/L) caused the TOC mineralization of primary sludge and anaerobic digested sludge up to 10 and 15%, respectively.

Post-ozonation of digested sludge was found to be moderately effective for improving methane production (+16%), COD removal efficiencies, and dewaterability of anaerobic digesters compared

to the control digester. However, pre-ozonation of primary sludge was not effective in enhancing the performance of the anaerobic digester. The increase in methane production and the reduction in sludge production reduced the operating costs by 64% required for post-ozonation of anaerobic digested sludge.

Based on these findings, recommendations for further investigation include a) optimization of the coupling of anaerobic digestion with ozone treatment (post-ozonation and pre-ozonation), by focusing on the optimization of anaerobic reactors (e.g. impact of reduction of retention times, increase of organic loading rates), b) although it was well established that ozonation can improve the biodegradability of organic matter, little is known about the biodegradability of the main individual transformation products resulting from ozonation as well as the its possible toxicity, and c) investigation of the influence of ozonation on the microbial community structure and the key methane-producing pathways of anaerobic digester digestion through applying a metagenomics approach.

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AD	Anaerobic digestion
AS	Activated sludge
B	Cumulative methane production
B_0	Methane production potential
$B_{0\text{-}exp}$	Ultimate cumulative methane yield
$B_{0\text{-}th}$	Theoretical methane yield
BCA	Bicinchoninic acid method
BMP	Biochemical methane potential
BOD	Biochemical oxygen demand
BSA	Bovine serum albumin
C_B	Colloidal biodegradable COD
CEPT	Chemically enhanced primary treatment
COD	Chemical oxygen demand
CS_P	Filterable phosphorus
CST	Capillary suction time
CS_{TKN}	Filterable TKN
CSTR	Continuous stirred-tank reactor
C_U	Colloidal unbiodegradable COD
C_{03}^*	Equilibrium ozone concentration in the liquid phase
D_{O2}	Molecular diffusivity of oxygen in water
D_{O3}	Molecular diffusivity of ozone in water
D_{OM}	Diffusion coefficient of organics matter in water
DS	Anaerobic digested sludge

E	Reaction factor
E&I	Electrical and Instrumentation
EEM	Excitation–emission matrix
E_i	Instantaneous enhancement factor
EPS	Extracellular polymeric substances
G/L ratio	Gas-to-liquid ratio
GC	Gas chromatograph
Ha	Hatta number
He	Henry's law constant
HRT	Hydraulic retention time
INF	Iodonitrotetrazolium formazan
k	Kinetic rate constant for ozone-organic matter reaction
kCAD	Thousand of Canadian dollars
k_L	Individual liquid-side mass transfer coefficient
k_La	Mass transfer coefficient
LB-EPS	Loosely bound EPS
LOX	Liquid oxygen
MBBR	Moving Bed Biofilm Reactor
MDA	Malondialdehyde
N	Nitrogen
$N_{0,O3}$	Actual flux of ozone
O&M	Operating and maintenance costs
o-PO_4	Orthophosphate
P	Phosphorus

PLCs	Programmable Logic Controls
P_{O3}	Ozone partial pressure of ozone
PS	Primary sludge
PSA	Pressure swing absorption
PSD	Particle size distribution
Q_G	Gas flowrate
Q_L	Liquid flow rate
$R_{G/R}$	Green/red fluorescence ratios
R_m	Maximum biogas production rate
ROS	Reactive oxygen species
S_B	Readily biodegradable COD
SEM	Scanning electron microscope
S_{HV}	Solubilisation of heavy metal
SRT	Solids retention time
STP	Standard temperature and pressure
S_U	Soluble unbiodegradable COD
SVI	Sludge volume index
TB-EPS	Tightly bound EPS
TKN	Total Kjeldahl nitrogen
TOC	Total organic carbon
TS	Total solids
TSS	Total suspended solids
TTF	Time to filter
VFA	Volatile fatty acids

VS	Volatile solids
VSA	Vacuum swing adsorption
WRRF	Water resource recovery facility
X_B	Particulate biodegradable COD
X_U	Particulate unbiodegradable COD
z	Stoichiometric ratio for the ozone-organic matter
θ	Temperature correction factor
λ	Lag time

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CHAPTER 1 INTRODUCTION

1.1 Background

Sludge is generated as a by-product of various physicochemical and biological processes in wastewater treatment. Sludge management is one of the major challenges in wastewater treatment due to economic, environmental and regulation factors of sludge despite the fact that it represents only about 1% of the influent wastewater flowrate (Foladori et al., 2010a). Treatment and disposal of sludge has been estimated to represent about 50% of the total operating cost of an activated sludge plant (Davis and Hall, 2004; Spellman, 1997). If handled properly, however, sludge can be a valuable resource for renewable energy production and a source of nutrients for agriculture.

In 2012, approximately 708 000 wet metric tons of municipal biosolids were produced in Quebec; 17% were landfilled, 49% were incinerated, and only 34% were beneficially used as fertilizer or soil amendments (Larose and Hébert, 2014). Biosolids refers to a wastewater sludge that meets the Quebec criteria for beneficial use. The relatively low rates of beneficial use for municipal biosolids in Quebec are mainly due to the low cost of landfilling and the presence of incinerators in the large cities (Larose and Hébert, 2014).

Anaerobic digestion has been widely adopted to treat sludge generated from industrial and municipal wastewaters. Anaerobic digestion provides several advantages over other methods of sludge treatment such as low sludge production, methane gas production that can be used as a source of energy, and generation of biosolids that contain nutrients that can be used as fertilizer (Turovskiy and Mathai, 2006). Despite these advantages, anaerobic digestion is considered a slow process with long hydraulic retention times (20 to 30 days), and moderate degradation efficiencies (30 to 50 %) which can result in large volume digesters and high capital requirements (Foladori et al., 2010a). Usually, the main factor limiting the anaerobic digestion of complex organic matter is the low rate of hydrolysis of particulate matter (Pavlostathis and Giraldo-Gomez, 1991). The acceleration of anaerobic digestion through enhancing the rate-limiting hydrolysis can increase the degradation rate and/or degradability leading to improved performance and capacity anaerobic digesters. For this reason, a variety of treatment techniques have been studied to enhance sludge destruction by using thermal, chemical, mechanical and biological processes. Among these

treatments, ozonation is one of the most effective methods to improve anaerobic biodegradation of sludge (Scheminski et al., 2000). Ozone is a strong oxidant and powerful disinfectant that can be used to disrupt the sludge flocs and cells releasing soluble substrates contained in biomass, accelerating hydrolysis and thus, enhancing the subsequent anaerobic digestion process (Weemaes et al., 2000).

There are 13 chemically enhanced primary treatment (CEPT) plants in the Province of Quebec, treating a total of about 3 600 000 m^3/d, representing 55% of the municipal wastewater treated (Fernandes and Tremblay, 2014). Effluent disinfection is typically achieved in Quebec with UV since there is a ban on the use of chlorination to avoid the discharge of potentially toxic chlorinated by-products in the receiving streams. The Montreal WRRF (average flowrate of 2 500 000 m^3/d) is currently installing an ozonation system to disinfect its effluent. A secondary benefit of this approach is that some emerging compounds are partially removed. The City of Repentigny is also considering this option to disinfect its effluent (25 000 m^3/d). Since an ozone production system would be available at the plant, there is an opportunity to test this system to determine if it can be used to also improve the efficiency of anaerobic digestion.

To enhance the methane production performance in a CEPT, the ozonation can be used on the primary sludge upstream of anaerobic digestion (pre-ozonation) or on the digested sludge in the recirculation loop of the anaerobic digester (post-ozonation). Ozone treatment aims to enhance the anaerobic digestion processes by altering the physicochemical properties and biodegradability of sludge. The impact of ozone on activated sludge has been studied but there are few studies on its impact on primary sludge and anaerobic digested sludge. A better understanding of the effect of ozone on primary and anaerobic digested sludge could provide valuable information for the design, operation and optimization of pre- and post-ozonation systems.

This research is part of the project entitled «High efficiency compact WRRF based on a highly loaded MBBR» headed by Professor Yves Comeau in collaboration with Veolia (Canada), EnviroSim Associates Ltd (Canada), the City of Repentigny and the Natural Sciences and Engineering Research Council of Canada (NSERC) through its Collaborative research and development (CRD) program.

1.2 Objectives

The general objective of this thesis is to maximize methane production in a chemically enhanced primary treatment (CEPT) facility by ozonating the sludge.

The specific objectives of this thesis are to:

- Assess the effects of ozonation on physicochemical properties, solubilisation, mineralization, and biodegradability of ozonating sludge.
- Determine and evaluate the impact and mechanisms of ozonation of sludge in the improvement of methane yield and methane production rate.
- Evaluate and optimize the ozone mass transfer in a lab scale venturi reactor for the ozonation of primary sludge and anaerobic digested sludge.

1.3 Original scientific hypotheses

1. The ozonation of primary sludge and anaerobic digested sludge causes the COD removal of sludge by means of two mechanisms: complete oxidation of organic matter into CO_2 and the partial oxidation of organic matter into intermediate products, triggering the solids reduction, and the increase of biodegradable and soluble organic matter.

Past studies on the effect of ozone have mainly focused on activated sludge and limited information about the effect of ozonation on primary sludge (PS) and anaerobic digested sludge (DS) is available. Some studies have investigated the effect of ozone on COD reduction, solubilisation of nutrients, and the impact on methane production potential, but the partial and total oxidation of organic matter as mechanism of COD reduction and the impact on the biodegradability of COD fractions of sludge samples have been not studied. This hypothesis will be verified by performing ozonation assays in both primary sludge and anaerobic digested sludge in an ozonation reactor at the laboratory scale. The ozonated and control sludges will be characterized by means of a physicochemical COD fractionation (soluble COD, colloidal COD and particulate COD) and biodegradability assays (biodegradable COD and unbiodegradable COD). The concentration of total organic carbon, total COD, and CO_2 in the off gas of the reactor will be also evaluated during the ozonation of sludge samples. The hypothesis will be rejected if both particulate and non-biodegradable COD are not significantly reduced during ozonation and if the fraction of soluble

and biodegradable COD are not significantly increased. This hypothesis will be also rejected if there is not a significant reduction in the concentration of total organic carbon or if the mass balance of carbon is unable to explain the reduction of organic carbon of the ozonated samples.

2. Ozonation results in the solubilisation of sludge mainly via partial disintegration of the sludge matrix and damage of the cell membrane, resulting in an increase in methane yield and in the acceleration of methane production rate of ozonated sludge.

The impact of ozonating digested sludge on its biological response and EPS physicochemical characteristics has not been reported. This hypothesis will be verified by performing ozonation experiments in an ozone reactor at the laboratory scale. The impact of ozonation will be evaluated by monitoring the methane production (biochemical methane potential), microbial activity (acetoclastic activity), viability (Baclight and INT-Dehydrogenase activity assay), and the production of reactive oxygen species (ROS) for ozone doses ranging from 0 to 200 mg O_3/g COD (0 to 3 g O_3/L). The anaerobic digested sludge matrix will be studied in terms of COD (COD removal and COD solubilisation) and the extracellular polymeric substances (EPS; soluble phase, loosely bound EPS, tightly bound EPS, and pellet remaining after chemical extraction). This hypothesis will be rejected if the methane yield and methane production rates does not increase significantly during ozonation, as well as if the solubilisation and EPS matrix are not affected significantly during this treatment.

3. Sludge ozonation is developed in a fast-kinetic regime (reaction occurring at the gas–liquid interface), which results in a high ozone mass transfer and an effective sludge treatment.

Most research has focused on the reaction kinetics of drinking water and synthetic wastewater with model pollutants, but there is limited information supporting the kinetics and mass transfer efficiency of ozonation on sludge treatment. The evaluation of ozone mass transfer on the treatment of primary sludge and anaerobic digested sludge will be performed in a venturi loop reactor at the laboratory scale. The ozonation will be analyzed regarding the impact of initial COD concentration and initial pH of sludge samples, and operational parameters such as gas-to-liquid ratio (G/L ratio), batch time, and pressure. The effect of these parameters will be analyzed in terms of ozone mass transfer efficiency, physical absorption gas-liquid (overall mass transfer coefficient), kinetic behavior, and ozonation performance (biodegradable COD, COD removal, and COD

solubilisation). Hatta number will be used to determine the regime of reaction. Dissolved ozone will also be measured. The hypothesis will be rejected if it is not possible to transfer more than 95% of ozone injected into the samples and the kinetic regime of sludge ozonation is not fast (Hatta number < 3 or if dissolved ozone is detected in liquid sample during ozonation).

CHAPTER 2 LITERATURE REVIEW

The literature review is divided into four main sections. The first section examines the characterization of organic matter, including chemical oxygen demand (COD) fractionation, extracellular polymeric substances (EPS) and biochemical methane potential (BMP). These methods were reviewed due to their importance on the development of this research and although there are many protocols, there is no general consensus for their determination. The second section covers the anaerobic digestion process, considering the general stages of methane production, as well as its main operational aspects. The third section is the review of various pretreatment processes used to enhance anaerobic digestion. Finally, the fourth section focuses on ozonation. This section presents a literature review concerning the influence of the reaction medium on the action mechanisms of ozone and its effects on ozonated sludge.

2.1 Characterization of organic matter

2.1.1 Fractionation of organic matter

The identification of sludge characteristics with regard to the organic content is useful to achieve a better understanding of the ozonation effect on sludge. The measurement of the COD is a method commonly used in the field of wastewater treatment to characterize the organic matter. COD consists of different forms of organic matter that require further differentiation in terms of their biodegradation and/or their physicochemical characteristics. COD fractionation is useful for consideration in relation to the design, modelling, operation, and optimization of treatment processes. COD fractionation is also useful from the standpoint of process kinetics and the evaluation of treatment performance. COD may be used as a direct parameter to yield the stoichiometric equivalent of carbonaceous substrate, with the provision that its biodegradable fraction is ascertained (Orhon et al., 1999). This fraction reflects the appropriate electron balance between substrate, biomass and the electron acceptor (Orhon et al., 1999).

A detailed characterization of organic matter can be achieved, dividing the total COD into fractions with different properties (Henze, 1992). Existing procedures for quantifying the COD fractions are based on physicochemical and biodegradable assays (Wentzel et al., 1999; Lu et al., 2010). In terms of physicochemical characterization of COD, the sludge can be classified in three major

components: soluble COD, colloidal COD and particulate COD. Likewise, each of these components can be subdivided into biodegradable and unbiodegradable fractions. A detailed fractionation of organic is presented in Figure 2.1.

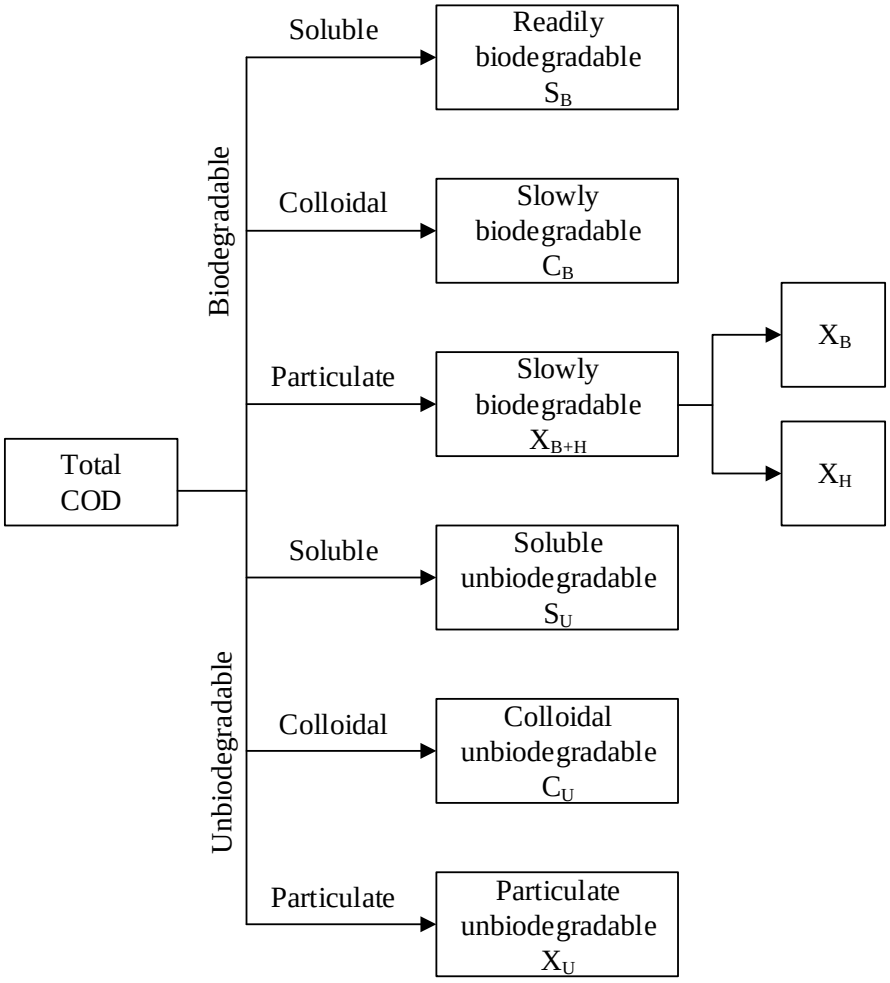

Figure 2.1: Detailed fractionation of total COD for wastewater (Based on Labelle, 2013)

The physicochemical characteristics of the compounds present in the wastewater vary considerably, resulting in a heterogeneous medium (Déléris, 2001). The soluble COD is presumably composed of soluble compounds, such as volatile fatty acids, simple carbohydrates, proteins, alcohols, amino acids, etc. that can be directly absorbed for synthesis. The colloidal COD has been reported to be composed of free bacteria, debris, fats, etc. while the particulate COD is comprised of cellulose fiber, lipid aggregates, flocs, macroproteins, etc. (Henze et al., 1987; Orhon et al., 1997; Déléris, 2001). The distribution of organic matter in the different COD fractions

depends on the operating conditions and the characteristics of the influent wastewater (Déléris, 2001).

The fractionation of total biodegradable COD was initially introduced by Dold et al. (1980) which identified two fractions: the readily biodegradable fraction which is considered soluble biodegradable (S_B) and the slowly biodegradable fraction which is considered particulate (X_B) and colloidal biodegradable matter (C_B). The soluble biodegradable fraction of COD has a direct effect on the biological kinetics and process performance. This fraction consists of relatively small biodegradable particles, which are easily transported across cell membranes and then they are quickly assimilated by the biomass, while the particulate biodegradable fractions (X_B) comprise larger particles and require extracellular breakdown prior to their transport into the cells for biodegradation; therefore, these are assimilated at a much slower rate. The soluble unbiodegradable fraction (S_U) embodies a variety of compounds which are dissolved, thus having access to the microbial cell interior, but cannot be biodegraded in a reasonable time due to their refractory nature. The unbiodegradable particulate fraction (X_U) is the particulate fraction which remains long enough that these materials are considered inert. The active heterotrophic biomass (X_{BH}) is involved in the biodegradation of organic matter (Wentzel et al., 1999; Metcalf & Eddy - AECOM, 2014).

Although several procedures to characterize the organic matter have been proposed, there is no standard method for the fractionation of COD and significant discrepancies can be found in the obtained values depending on the selected procedure (Mamais et al., 1993; Ruiz et al., 2014). The physicochemical methods are based on the assumption that COD fractions can be separated by filtration and/or flocculation processes and that the COD of the gained fractions are easily measurable by standard chemical methods (Mamais et al., 1993; Lu et al., 2010). Physical-chemical separation methods frequently used in wastewater characterization are: filtration (e.g. 0.04 µm, 0.1 µm, 0.45 µm, 1.2 µm), flocculation (e.g. $ZnSO_4$) and flocculation + filtration (Mamais et al., 1993; Randami et al., 2009; Lu et al., 2010). Flocculation and flocculation + filtration can give rapid results by effectively abating severe blocks occurring in direct filtration of raw wastewater. There is no consensus regarding the range of size of each physicochemical fraction, but based on the typical criteria used in the literature, it has been established for this research that the soluble COD is composed of the organic matter with nominal diameter below 0.1 µm (flocculation + 0.1 µm

filtration); colloidal COD is between 0.1 to 1.2 µm, and particulate COD is composed of organic matter above 1.2 µm.

The biodegradable characterization of COD fractions is based on the measurement of the biomass response during substrate degradation in either continuous flow or batch type experiments (Paztor et al., 2009). The measurement of biodegradable fractions can be performed indirectly through the oxygen uptake rate (OUR) as originally done by Ekana and Marais (1977) and further developed by other authors (Kappeler and Gujer, 1992). The experimental assessment of inert soluble and particulate COD of different wastewaters under aerobic and anaerobic conditions has been discussed previously in the literature. Methods for the characterization of biodegradable and unbiodegradable of each physicochemical COD fractions are summarized in Table 2.1.

Table 2.1: Methods for the determination of COD fractions used in wastewater (Based on Déléris, 2001).

Organic fraction	Methods	References
Soluble unbiodegradable (S_U)	Aeration > 10 days, residual soluble COD measured in a pilot (activated sludge), anaerobic degradation	Henze et al. (1987) Kappeler and Gujer (1992) Babuna et al. (1998)
Soluble biodegradable (S_B)	Measurement of soluble COD, respirometry measurement (OUR), anaerobic degradation.	Ekana and Marais (1977) Kappeler and Gujer (1992) Henze et al. (1992) Babuna et al. (1998)
Particulate unbiodegradable (X_U)	Respirometry and hydrolysis model, anaerobic degradation.	Babuna et al. (1998)
Particulate biodegradable (X_B)	Measurement of sludge production in a pilot (activated sludge), extended aeration assays (10 to 20 days), COD mass balance, anaerobic degradation.	Kappeler and Gujer (1992) Henze et al. (1992) Babuna et al. (1998)

An optimal strategy for sludge reduction must attack, solubilise or reduce the unbiodegradable fractions, converting them into soluble compounds or better still, biodegradable compounds, which are desirable when such treatments are coupled with a biological treatment process, such as

anaerobic digestion. A second strategy may point to a reduction in the net grown biomass of the biological process, but it must keep the active biomass high in order to ensure that the biological process remains efficient (Foladori, 2010).

Sludge characteristics vary according to the type of unit processes and its operating conditions. Wastewater sludge can be classified generally as primary and secondary. Primary sludge results from the capture of settleable suspended solids in the primary treatment process through sedimentation in primary settling tanks. Secondary sludge, also known as biological sludge, is produced by biological treatment processes such as activated sludge. After biological treatment, biodegradable components will be largely removed with the remaining organic matter consisting mostly of non-biodegradable particulate matter from the wastewater influent and from endogenous residues produced in biological unit processes (e.g. heterotrophic biomass, endogenous residues). While primary sludge contains a high fraction of particulate biodegradable matter, secondary sludge contains a higher fraction of heterotrophic biomass. Some treatment plants add chemicals to the primary settling or secondary biological processes to precipitate phosphorus or improve the removal of organic matter. With chemical addition, inorganic matter in primary and secondary sludge comes not only from the wastewater influent but also from the chemical precipitates.

2.1.2 Extracellular polymeric substances

In biological wastewater treatment, most of the microorganisms are present in the form of microbial aggregates, such as sludge flocs, biofilms, and granules (Nielsen and Jahn, 1999; Sheng et al., 2010). The sludge flocs are suspended microbial aggregates containing microorganisms and organic/inorganic compounds (Biggs and Lant, 2000). EPS and microbial cells inside the flocs are cross-linked forming a polymeric network which, with its pores and channels, is capable of adsorbing nutrients, minerals, pollutants and heavy metals (Brown and Lester, 1982; Guibaud et al., 1999). The EPS may determine the physicochemical, structural and functional properties of sludge flocs, which play a crucial role in flocculation, settling, and dewatering properties of the flocs (Niu et al., 2013). Furthermore, EPS are usually thought to protect the inner microorganisms from the harsh external environmental conditions such as exposure to chemicals, and also serve as carbon and energy reserves during starvation (Ma et al., 2013; Sheng et al., 2010).

EPS are present both outside of cells and in the interior of microbial aggregates (Sheng et al., 2010). They are composed of high-molecular-weight secretions from microorganisms, and the products of cellular lysis and hydrolysis of macromolecules (Nielsen and Jahn, 1999; Liu and Fang, 2003). EPS is a matrix rich in polymers, including mainly carbohydrates and proteins. In addition, humic substances, lipids, nucleic acids, uronic acids and some inorganic components have also been found in EPS from various matrixes (Frolund et al., 1996; Dignac et al., 1998; D'Abzac et al., 2010). The EPS composition can differ between activated sludge and anaerobic digested sludge. In activated sludge, the dominant component of EPS is carbohydrate. However, in general, anaerobic sludge tends to have higher concentrations of protein in their extracted polymers (Morgan et al., 1990).

EPS in sludge flocs have been classified as soluble EPS and bound EPS (Nielsen and Jahn, 1999). Bound EPS are closely bound with cells, while soluble EPS are weakly bound with cells or dissolved in the solution. Although the interaction between soluble EPS and cells is very weak, a previous study showed that soluble EPS also have a crucial effect on the microbial activity and surface characteristics of sludge (Sheng and Yu, 2007). In addition, the structure of bound EPS is generally depicted by a two-layer model. The inner layer consists of tightly bound EPS (TB-EPS), which has a certain shape and is bound tightly and it is stable at the cell surface. The outer layer which consists of loosely bound EPS (LB-EPS), however, is a loose and dispersible slime layer without an obvious edge (Nielsen and Jahn, 1999).

To study the composition and functions of the soluble-EPS, LB-EPS and TB-EPS in a sludge sample, the fractions need to be extracted separately. The amounts of extracted EPS vary widely as a function of sludge origin, methods of characterization, and also the extraction conditions (Comte et al., 2006). Generally, the soluble-EPS can be separated by centrifugation, with those remaining in the supernatant being soluble EPS and those forming microbial pellets being bound EPS (LB-EPS and TB-EPS). As the LB-EPS bound with cells loosely, a mild method (e.g. high-rate shear, heating at low temperatures, high speed centrifugation, or chemical extraction) should be chosen to avoid the inclusion of the TB-EPS. Subsequently, a harsh method (e.g., heating at high temperatures, sonication or chemical extraction methods) should be applied for the TB-EPS extraction (Sheng et al., 2010). Chemical extractions can include compounds such as formaldehyde and alkaline solutions for LB-EPS and TB-EPS extractions, respectively (Liu and Fang, 2002).

Although several EPS extraction methods have been reported, there is not a standard method. As a result, the comparison and interpretation of published results is difficult.

The efficacies of extracting EPS from aerobic, acidogenic and methanogenic sludges using EDTA, cation exchange resin and formaldehyde under various conditions were compared by Liu and Fang (2002). Results show that formaldehyde plus NaOH was most effective in extracting EPS for all sludges; only 1.1–1.2% of extracted material was DNA in the sludge samples were detected, suggesting the EPS extracted were not contaminated by intracellular substances. Formaldehyde could fix the cell, and thus prevent cell lysis, by reacting with amino, hydroxyl, carboxyl and sulfhydryl groups of proteins and nucleic acids of the cell membrane (Alcamo, 1997). The presence of NaOH increased the pH, resulting in the dissociation of acidic groups in EPS and the repulsion between the negative-charged EPS. This also increased the EPS solubility in water and thus, allowed more EPS to be extracted (Nielsen and Jahn, 1999).

The hydrolysis of EPS and/or cells together within the sludge flocs limits the rate and extent of biodegradation (Higgins and Novak, 1997). Since EPS, rather than cells, represent the major organic fraction determining flocs structure, integrity and strength, the disruption of EPS matrix could enhance the rate and extent of sludge biodegradation (Park and Novak, 2007). Considering previous studies of pretreatment of activated sludge (Yu et al., 2008), it is reasonable to propose that the ozone treatment of sludge could disintegrate its EPS matrix and release extracellular proteins, polysaccharides from inner layers of sludge flocs (pellet and TB-EPS), to outer layers (LB-EPS and soluble-EPS), increasing the contact and interaction among extracellular proteins, polysaccharides and enzymes that were originally embedded in the sludge flocs. This results in improved efficiency in anaerobic digestion. This consists in the use of centrifugation to separate the soluble EPS, followed by an extraction of remaining pellets with a solution of formaldehyde to obtain LB-EPS, and finally the new remaining pellet was extracted with a sodium hydroxide solution for measurement of TB-EPS (Liu and Fang 2002; Yu et al., 2008).

2.1.3 Biochemical methane potential

Biochemical methane potential (BMP) is a procedure developed to determine the ultimate methane production of a given organic substrate during its anaerobic decomposition. The BMP assay has

proved to be a relatively simple and reliable method which allows the determination of methane yield, rate of methane production, and the biodegradability of samples (Lay et al., 1996; Angelidaki et al., 2009; Raposo, 2012). The information provided by BMP is valuable for the evaluation of pretreatment on sludge samples and for the optimization of anaerobic digesters. Although the standardization of aerobic test methods has already reached an advanced stage, the BMP assay has not reached a consensus for carrying out its determination. Consequently, methane yields reported in the literature have limited comparability and cannot be precise because of possible differences in the experimental protocol used for the assay (Raposo, 2012). An overview of protocols and their main features is presented in Table 2.2.

Table 2.2: Description of experimental BMP procedures (Based on Raposo et al., 2012)

GMS	Physical operational conditions						Chemical operational conditions			ISR	Inoc	Reference
	Capacity (L)		Temp	Mixing		TD	Gas	Adj	MM	VS basis	C_0	
	TV	WV	°C	Type	Times	d		pH/Alk			g VS/L	
Manometer	1	0.5	35	man	1/day	30	He	na	na	1	2.1	El-Mashad and Zhang (2010)
Vol (liq-disp)	2	1.5	35	man	1/day	150	N_2	$NaHCO_3$	na	na	na	Lehtomäki et al. (2008)
Vol (syringe)	1	0.6	33	man	2/day	65	N_2	na	na	0.35	na	Mshandete et al. (2006)
Manometer	1	0.6	35-50	man	1/day	25	He	na	na	0.3	na	Liu et al. (2009)
Vol (liq-disp)	0.3	0.25	35	stirrer	40 rpm	7	N_2	$NaHCO_3$	Yes	0.5-3	15	Raposo et al. (2008)
Vol (liq-disp)	2	1	35	stirrer	na	30	nd	na	Yes	1.5	15	Pobeheim et al. (2010)
Vol (liq-disp)	na	5	35	stirrer	na	20	N_2	$NaHCO_3$	Yes	1-3	15	Raposo et al. (2006)
Manometer	0.25	0.12	35	man	2/day	30	Na_2+CO_2	na	na	0.5-2	na	Zeng et al. (2010)
Vol (liq-disp)	5	4	22	stirrer	na	120	N_2	NaOH	na	na	3	Lei et al. (2010)
Vol (liq-disp)	0.1	0.05	35	man	2/day	70	N_2	NaOH	na	0.5	8-10	Mahamat et al. (1989)
Vol (liq-disp)	0.5	0.3	37	shaker	70 rpm	32-85	Na_2+CO_2	$NaHCO_3$	na	0.65	4.9	Mshandete et al. (2005)
GC	0.28	0.1	35	na	na	60	Na_2+CO_2	na	Yes	3-4	na	Owens and Chynoweth (1993)
Vol (liq-disp)	1.18	0.6	35	man	2/day	10-72	N_2	HCl/NaOH	na	na	3.3	Zubr (1986)
Manometer	0.16	na	35	na	na	15-30	Na_2+CO_2	$NaHCO_3$	Yes	na	na	Shelton and Tiedje (1984)

VS: Volatile solids; Co: Concentration (g VS/L); GMS: gas measurement system; Vol: volumetric system; (liq-disp): liquid displacement; GC: gas chromatography; TV: Total volume; WV: working volume; Temp: Temperature; man: manually; TD: Test duration; Adj: Adjustment of pH and/or alkalinity; MM: Mineral medium; ISR: Inoculum substrate ratio; Inoc: inoculum.

The measurement of biogas volumes under test conditions have been performed mostly by two manometric methods: (a) Constant volume manometry, which measures a change in the pressure of a gas at constant volume and temperature, and (b) constant pressure manometry which measures a change in the volume of a gas at constant pressure and constant temperature. The methane production was measured following the protocol described in Appendix A, which is based on a manometric method.

The major disadvantage of the BMP test is the duration of the assays and the fact that it does not provide short-term results. The experimental methane yield can be used to calculate the level of anaerobic biodegradability under the defined test conditions in comparison with its theoretical value (Equation 2.1) (Raposo et al., 2012):

$$BD_{CH4}(\%) = 100 \cdot \frac{B_{0-exp}}{B_{0-th}} \quad (2.1)$$

where $B_{0\text{-}exp}$ (mL CH_4/g COD) is the ultimate cumulative methane yield, $B_{0\text{-}th}$ (mL CH_4/g COD) is the theoretical methane yield (350 mL /g COD). For the purposes of this research, the methane production was evaluated at the standard temperature and pressure (STP) of 0 °C and 1 atm.

When the anaerobic biodegradability of the organic material is calculated from the methane conversion efficiency, it can be considered that the main organic matter removed is converted into methane, but some defined amount of the organic matter is used for growth of the microorganisms and to maintain cellular metabolism. This amount cannot be measured directly but it can be estimated for higher accuracy. It is known from practical experience that about 5% of the organic matter removed is consumed in the generation of new microbial biomass (Scherer et al., 1990). This means that to find the real degree of biodegradation, the value obtained from the experimental data should be increased by the value of this cellular yield (Raposo et al., 2012).

The determination of the kinetic of the anaerobic digestion provides important information about the effect of the inhibitory compounds generated by the pretreatment on the biodegradability, and to determine if the hydrolysis is the limiting step (Krishania et al., 2013). There are several models of kinetic analysis of biogas production process; it depends on the types of substrate used for anaerobic digestion and the controlling step. The Gompertz model is well known among the available models for the kinetic behavior of the anaerobic digestion process considering inhibition.

The Gompertz equation (Equation 2.2) is used to estimate the kinetic parameters: biogas yield potential, duration of the lag phase, and maximum biogas production rate (Lay et al., 1996; Bolado-Rodriguez et al., 2016). The data obtained from the experiments may be used to fit and check the fitness of the modified Gompertz equation that describes the kinetics parameters for the pretreated substrates. It is possible to assume that the biogas production rate in batch conditions corresponds to specific growth rates of methanogenic bacteria in the anaerobic digester (Lay et al., 1996; Krishania et al., 2013).

Moreover, the parameters of the model can be calculated by minimizing the least squares difference between observed and predicted values.

$$B = B_0 \cdot \left[-\exp\left[\frac{R_m \cdot e}{B_0} (\lambda - t) + 1 \right] \right] \quad (2.2)$$

In this equation, B represents the cumulative methane production (mL CH_4/g COD) and t is the length of the assay (d). This model estimates the methane production potential B_0 (mL CH_4/g COD), the maximum biogas production rate R_m (mL CH_4/g COD·d), the exponential function *e* and the lag time λ (d).

2.2 Anaerobic digestion

Anaerobic digestion is a biological process in which organic material of a substrate is degraded by microorganisms in the absence of oxygen. The result of this degradation is a biogas consisting primarily of methane (50-85%), carbon dioxide (15-50%) and trace amounts of gases such as ammonia, hydrogen and hydrogen sulphide (Angelidaki et al., 2003). The conversion of organic matter involves several successive phases of chemical and biochemical reactions involving enzymes and a mixed culture of microorganisms. The phases involved in anaerobic digestion may be divided into: hydrolysis, acidogenesis, acetogenesis and methanogenesis as shown in Figure 2.2.

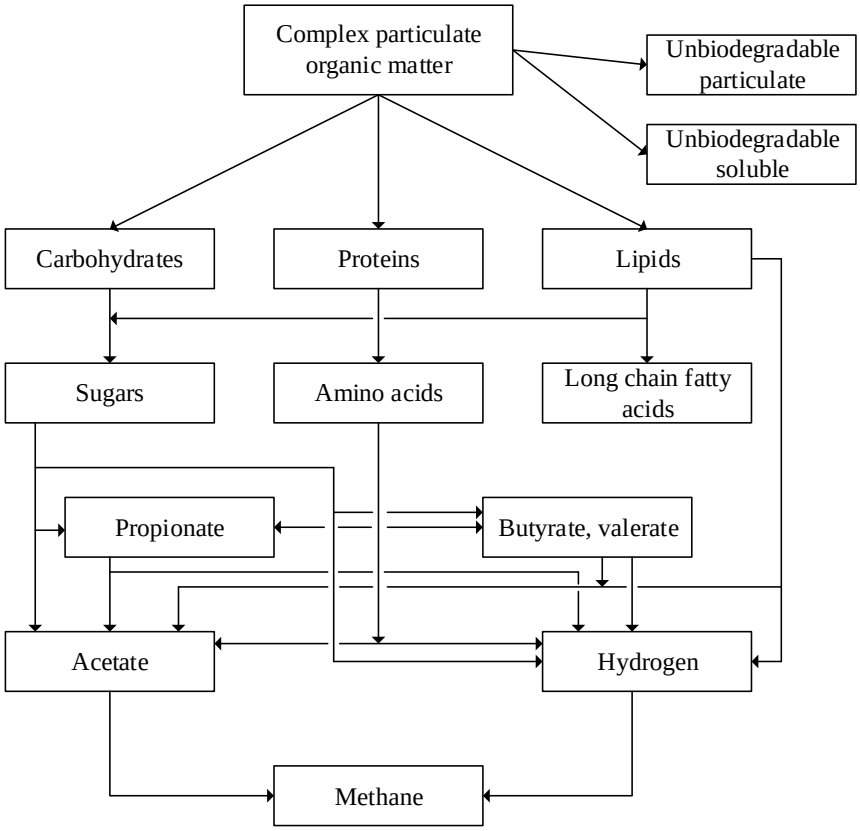

Figure 2.2: Simplified model of the anaerobic digestion process (modified from Batstone, 2002)

2.2.1.1 Hydrolysis

In the first phase of anaerobic digestion, hydrolysis degrades both insoluble organic material and high molecular weight compounds, such as lipids, polysaccharides and proteins, into simpler soluble components such as fatty acids, monosaccharides and amino acids, respectively (IWA, 2002; Mara and Horan, 2003; Pavlostathis and Giraldo-Gomez, 1991). This process is carried out by extracellular hydrolytic enzymes that are able to access large substrate molecules that are incapable of crossing the bacterial cell wall due to their size.

The process is catalysed by enzymes such as protease, lipase, cellulase, pectinase, amylase and chitinase, which are likely produced by hydrolytic genera such as *Clostridium, Peptococcus, Vibrio, Micrococcus* and *Bacillus* (Mara and Horan, 2003). The hydrolysis is normally rate-limiting of anaerobic digestion for substrates in particulate form (Vavilin et al., 1996; Pavlostathis and Giraldo-Gomez, 1991), such as primary and activated sludges that contain large amounts of solids

(particulate and colloidal wastes). The hydrolysis rate of protein is generally slower than the hydrolysis of polysaccharides (Demirbas and Balat, 2009).

2.2.1.2 Acidogenesis

The second phase, acidogenesis, includes the degradation of soluble sugars and amino acids, providing important substrates for acetogens and methanogens. VFA are produced by acidogenic bacteria along with NH_3, CO_2, H_2S and other by-products (Appels et al., 2008). The acidogenic stage includes many different fermentative genera and species, such as *Clostridium, Bacteroides, Ruminococcus, Butyribacterium, Propionibacterium, Eubacterium. Lactobacillus, Streptococcus, Pseudomonas, Desulfobacter, Micrococcus, Bacillus* and *Escherichia* (Gerardi, 2003). The facultative members of this group also help protect the oxygen-sensitive methanogens by consuming traces of oxygen that may enter in the feed (Mara and Horan, 2003).

2.2.1.3 Acetogenesis

The third stage is the acetogenesis, where the higher organic acids and alcohols produced by acidogenesis are further digested by acetogens to produce mainly acetic acid as well as CO_2 and H_2 (Appels et al., 2008), to be metabolized by the methanogens in the final stage of anaerobic digestion.

Two distinct groups of acetogenic bacteria can be distinguished on the basis of their metabolism (Mara and Horan, 2003). The first group, the obligate hydrogen-producing acetogens (OHPA), also called proton-reducing acetogens, produce acetic acid, carbon dioxide and hydrogen from the major fatty acid intermediates (propionate and butyrate), alcohols and other higher fatty acids (valerate, isovalerate stearate, palmitate and myristate via b-oxidation) (Mara and Horan, 2003). Only a limited number of OHPA species have been isolated and identified, namely, *Syntrophomonas wolfei* and *Syntrophobacter wolinii*, which oxidize butyrate and propionate, respectively. The second group of acetogenic bacteria are the homoacetogens, which are strictly anaerobic microorganisms catalyzing the formation of acetate from hydrogen and carbon dioxide. Homoacetogens are known in the genera *Acetobacterium*, *Acetoanaerobium*, *Acetogenium*, *Butribacterium*, *Clostridium* and *Pelobacter*. Homoacetogenic bacteria are also syntrophs because

they participate in the interspecies hydrogen transfer process which maintains the low hydrogen concentrations required by the OHPA (Mara and Horan, 2003).

2.2.1.4 Methanogenesis

Methanogenesis produces methane by two groups of methanogenic bacteria: the first group splits acetate into methane and carbon dioxide and the second group uses hydrogen as an electron donor and carbon dioxide as an acceptor to produce methane (Appels et al., 2008).

Methane-forming bacteria are some of the oldest bacteria and are grouped in the domain Archaebacteria. Methane-forming bacteria are oxygen-sensitive anaerobes and are found in habitats that are rich in degradable organic compounds (Gerardi, 2003).

According to Mara and Horan (2003) of the various end-products produced by acidforming bacteria, acetate is regarded as the most important precursor of methane production and the source of up to 70% of methane evolved in digesters. In spite of this fact, only two methanogenic genera contain species that are able to utilize acetate (acetoclastic), and these are *Methanosaeta* (formerly known as *Methanothrix*) and *Methanosarcina*. In addition to this acetoclastic activity, *Methanosarcia* spp. are also capable of using methanol, methylamines and sometimes H_2 and CO_2 as growth substrates, while *Methanosaeta* spp. are restricted to growth only on acetate. A significant quantity of the methane production within anaerobic digesters, up to 30% of the total, is produced by hydrogen-utilizing methanogenic bacteria. These methanogens reduce carbon dioxide, formate, methanol and methylamines, using the hydrogen produced fermentatively by the hydrolytic and acid-forming bacteria earlier in the digestion process.

2.2.2 Operational aspects

To prevent failure and low performance for anaerobic digestion, the operational parameters must be periodically and precisely monitored and maintained within their optimum ranges. The optimum conditions for anaerobic digestion operation are presented in Table 2.3.

Table 2.3: Optimum and extreme conditions for anaerobic conditions (adapted from Amani et al. 2010)

Parameter	Units	Optimum	Extreme
Volatile fatty acids	mg/L as AcOH	50–500	500–2000
Organic loading rate			
Mesophilic	kg VS m^{-3} d^{-1}	0.8–2.0	0.4–6.4
Thermophilic	kg VS m^{-3} d^{-1}	1.5–5.0	1.0–7.5
Temperature			
Mesophilic	°C	32–37	20–42
Thermophilic	°C	50–60	45–65
pH		6.8–7.4	6.3–7.9
Oxidation reduction potential	mV	–520 to –530	–490 to –550
COD:N:P ratio		1000:7:1	ND
High strength waste		350:7:1	ND
C:N		25:1	ND
Alkalinity	mg $CaCO_3$/L	1300-3000	1000-5000
Hydraulic retention time	days	12-18	7-30

2.2.2.1 Temperature

There are three major operating ranges nominally defined in anaerobic digestion: psychrophilic (4 to 15 °C), mesophilic (20 to 40 °C), and thermophilic (45 to 70 °C). The majority of full-scale applications and research effort have been concentrated on anaerobic digestion within the mesophilic or thermophilic temperature ranges, rather than psychrophilic conditions due to the lower microbial activity and biogas production rates. Temperature is one of the main factors affecting bacterial growth. Growth rates often increase with increasing temperature up to a certain limit of inhibition. Furthermore, temperature also influences such physical parameters as viscosity, surface tension and mass transfer properties (Angelidaki et al., 2003).

Optimal temperatures for mesophilic and thermophilic organisms are approximately 35 and 55 °C, respectively (Batstone et al., 2002). Digester performance oscillates near 42 °C, as this represents

the transition from mesophilic to thermophilic organisms (Gerardi, 2003). Thermophilic anaerobic digestion showed higher organic matter degradation and methane yield, when compared with mesophilic conditions (Moset et al., 2015). Although thermophilic operation is more advantageous compared to mesophilic operation, it requires additional energy to heat the digester (Fang and Chung, 1999). Application of thermophilic digestion is very limited due to poor process stability compared to mesophilic digestion (Kim et al., 2002).

Maintaining stable operating conditions is critical for process performance as major fluctuations in temperature have an adverse effect on methanogens (Appels et al., 2008; Turovskiy and Mathai, 2006). Sharp and frequent fluctuations in temperature affect almost all biological activity, especially methane-forming bacteria. Process failure can occur at temperature changes greater than 1 °C/d. Changes as small as 1–2 °C have significant adverse effects on process performance particularly when changes occur rapidly (< 2 hours). The bacteria become adversely affected by digester temperature variations; several days, or even weeks, may be required to restore a healthy population once again (Mara and Horan, 2003).

Despite the fact that the overall performance of the anaerobic digestion is strongly temperature dependent, this does not affect each of the stages of anaerobic digestion in the same way. The fluctuations in temperature may be advantageous to certain groups and disadvantageous to other groups (Gerardi, 2003). For example, a 10 °C temperature increase can stop methane production or methane-forming bacterial activity within 12 hours, while volatile acid production increases. The effect of temperature on hydrolysis of particulate and colloidal wastes is not very high. Hydrolytic bacteria are not as sensitive to temperature change as the acetate-forming bacteria and methane-forming bacteria.

2.2.2.2 pH and alkalinity

Anaerobic digestion is extremely sensitive to pH, and especially the methanogens that exhibit a characteristic sensitivity to extremes pH. Sufficient alkalinity is essential for proper pH control, as alkalinity serves as a buffer to prevent rapid changes in pH. Acceptable enzymatic activity of acid-forming bacteria occurs above pH 5.0, but for the methane-forming bacteria, this does not occur below pH 6.2 (Gerardi, 2003). The acceptable pH range for anaerobic digestion is between 6.8 to

7.2, with an optimum pH of 6.8, whereas the process may fail if the pH is lower than 6.1 or higher than 8.3 (Lay et al., 1997; Gerardi, 2003).

The pH in an anaerobic digester initially decreases with the production of volatile acids. However, as methane-forming bacteria consume the volatile acids and alkalinity is produced, the pH of the digester increases and then stabilizes, but under adverse environmental conditions, the buffering capacity of the system can be upset, eventually stopping methane production (Gerardi, 2003). One method for restoring the pH balance is to increase alkalinity by adding chemicals such as lime, anhydrous ammonia, sodium hydroxide, or sodium bicarbonate (Bitton, 2005). The optimum alkalinity for mesophilic anaerobic digestion is around 1300 to 3000 mg $CaCO_3$/L (Amani et al. 2010).

2.2.2.3 Solids and hydraulic retention times

The most important factor in sizing the anaerobic digester is that the bacteria be given sufficient time to reproduce and metabolize volatile solids. The key parameters in providing sufficient time are the solids retention time (SRT), which is the average time the solids are held in the digester, and the hydraulic retention time (HRT), which is the average time the liquid sludge is held in the digester (Turovskiy and Mathai, 2006). The SRT and the HRT are the same for a suspended-growth anaerobic digester that has no recycled solids. If recycled solids are incorporated in the operation of the digester, then the SRT and HRT may vary significantly (Gerardi, 2003). HRT is perhaps the most important operational condition affecting the conversion of volatile solids to gaseous products (Gerardi, 2003). A very short SRT could produce a loss of biomass from the reactor, while long SRT could prevent biomass washout and increase the stabilization and performance of the process. Consequently, the reactor volume will increase, as well as its capital costs (Kivaisi and Mtila, 1998; Gerardi, 2003).

2.2.2.4 Mixing

Anaerobic digestion comprises an inherent degree of mixing from the continuous rise of methane bubbles within the reactor; however, this natural mixing is usually considered to be the rate limiting efficient mass transfer (Mara and Horan, 2003). Mixing enhances the digestion process by

distributing bacteria, substrate, and nutrients throughout the digester as well as equalizing temperature (Gerardi, 2003).

The agitation rate plays an important role in the solubilisation of suspended organic material (Pinho et al., 2004). The COD degradation increases with the agitation rate due to the higher shear velocity of larger particles and major contact between the particulate organic matter and the extracellular enzymes. It was also reported that excessive mixing could actually lead to a reduction in reactor performance (Stafford et al., 1980). Mixing can be accomplished by using external pumped recirculation, internal gas mixing or mechanical mixing (Igoni et al., 2008).

2.2.2.5 Nutrients

Similar to other biological systems, to maintain optimum microbial activity as well as digester performance, the two major nutrients or macronutrients required for anaerobic microorganisms are nitrogen and phosphorous. Macronutrient requirements for anaerobic biological treatment processes are much lower than the requirements for aerobic biological treatment processes, which is due to lower cell yield compared with aerobic processes from the degradation of equal quantities of substrate (Gerardi, 2003).

Methane forming microorganisms also require several micronutrients in trace quantities such as iron, copper, zinc, nickel, cobalt, manganese, potassium, calcium, manganese, sodium, sulfur, molybdenum, vanadium (Gerardi, 2003; Speece, 2003).

2.3 Pretreatment

As stated in the previous section, the application of anaerobic digestion to sludge is often limited by its long retention time and its moderate organic matter removal efficiency. The limitation of anaerobic digestion of sludge is generally related to its hydrolysis stage (Weemaes and Verstraete, 1998; Vavilin et al., 1996). Considerable efforts have been spent to improve the performance of anaerobic digestion via the optimization of environmental conditions, reactor design and the substrate used (Appels et al., 2008; Carlsson et al., 2012). The pretreatment of substrates poses improvement opportunities as well as challenges for anaerobic digestion. A variety of pretreatments have been studied to enhance the biodegradability and solubilisation of sludge

substrate, which can reduce the rate-limiting hydrolysis stage of complex organic matter (Appels et al., 2008; Carrère et al., 2010). These treatments can alter the physicochemical properties of sludge treated, causing the increase of its biodegradability and its degradation rate. Improving these parameters allows for process intensification, and also faster kinetics; therefore, the performance and/or capacity of anaerobic reactors can be improved (Carrère et al., 2010).

Pretreatments that have been used to improve anaerobic digestion performance are based on different principles of operation and can thus be classified into the following categories: chemical, mechanical, thermal, and biological processes. Below, the principles governing these pretreatments, as well as their impact on the methane production of pre-treated sludge are discussed briefly.

2.3.1 Chemical treatment

According to the different principles of operation, the chemical treatments can be classified into acid, alkaline and oxidation process. The chemical treatment of sludge can be performed by using alkaline reagents, such as NaOH, KOH, CaO, $Mg(OH)_2$ or $Ca(OH)_2$ or acidic reagents, such as HCl or H_2SO_4. Some authors have combined the acid and alkaline addition with thermal treatment (Penaud et al., 1999; Chen et al., 2007; Rafique et al., 2010). The oxidation process consists of the use of ozone, hydroxide peroxide, chlorine or the combination of various oxidants (Foladori et al., 2010a). The most widely used chemical method is ozonation (Scheminski et al., 2000). Chemical pretreatment to enhance the anaerobic digestion treats the sludge to hydrolyse the cell walls and membranes and thus, increase the solubility of the organic matter contained within the cells (Appels et al., 2008).

The effect of pH from 4.0 to 11.0 on the hydrolysis and acidification of activated sludge was investigated by Chen et al. (2007). The performance of hydrolysis of activated sludge was influenced by pH. Acidic pH (4.0 and 5.0) and alkaline pH (9.0, 10.0 and 11.0) improved the solubilisation. Nevertheless, methane production was reduced in extreme pH conditions; thus, these results underline the fact that the pH of pretreated sludge needs to be neutralized before anaerobic biological treatment. Thermochemical treatment has a higher efficiency in sludge

solubilisation than the same chemical treatment performed at room temperature (Kim et al., 2003; Valo et al., 2004).

A summary of results obtained from the anaerobic digestion of sludge treated by chemical methods is shown in Table 2.4.

Table 2.4: Performances obtained in chemical pretreatments prior to anaerobic digestion

Substrate	Treatment conditions	Anaerobic digestion	Increase of CH_4 production	References
AS[a]	1.7 g KOH/L, pH=10, 130 °C, 60 min	CSTR, HRT=20 d, 35 °C	+75% 154 mL/g COD[c]	Valo et al. (2004)
AS SRT =7 d	pH=12 (NaOH), 160 °C, 16 min	Semi-continuous, HRT=15 days, 35 °C	+53% 144 mL/g COD[c]	Dogan and Sanin (2009)
AS+PS	0.1 g O_3/g COD	Batch, 30 d, 33 °C	+100% 110 mL/g COD[c]	Weemaes et al. (2000)
AS	0.1 g O_3/g COD	Batch, 30 d, 35 °C	+110% 82 mL/g COD[c]	Yeom et al. (2002)
AS+PS	0.1 g H_2O_2/g COD[d], 37 °C	CSTR, HRT=30 d, 37 °C	+20% 167 mL/COD[c,d]	Cacho-Rivero et al. (2006)
	0.1 g H_2O_2/g COD[d], 90 °C	CSTR, HRT=30 d, 37 °C	+7% 167 mL/COD[c,d]	Cacho-Rivero et al. (2006)

[a] AS: Activated sludge
[b] PS: Primary sludge
[c] Performance of anaerobic digestion without pretreatment.
[d] Values recalculated to COD basis.

2.3.2 Mechanical treatment

Mechanical treatment enhances sludge solubilisation by cell disintegration and disaggregation of sludge flocs, through the use of pressure, translational and rotational energy (Muller, 2000). While the disaggregation of flocs can be achieved using a relatively low energy input, the cell

disintegration requires a high specific energy input (Muller, 2000). Most frequently used technologies for sludge treatment include high pressure treatment, centrifugation, grinding and extrusion (Carlsson et al., 2012). Ultrasonic treatment is also included as a mechanical treatment.

Ultrasonic treatment includes a wide range of frequencies between 20 kHz and 10 MHz and energy consumption ranges from 1 000 to 16 000 kJ/kg TS (Tiehm et al., 2001; Carrère et al., 2010). The principle of ultrasonic treatment is associated with the mechanical action produced by cavitation at low frequencies starting from 20 kHz and the sonochemical action by the formation of radicals at frequencies higher than 200 kHz. In sludge treatment, low frequencies (20–40 kHz) are the most efficient. The mechanical phenomena of sludge sonication lead to sludge floc disintegration and microorganism lyses, according to the treatment time and power (Carrère et al., 2010).

Some studies reporting the effects of these mechanical methods on anaerobic digestion are summarised in Table 2.5.

Table 2.5: Performances obtained in mechanical pretreatments prior to anaerobic digestion

Substrate	Treatment conditions	Anaerobic digestion	Increase of CH_4 production	References
AS[a]	Homogeniser (Δ=300 bar)	CSTR, HRT= 10–15 d, 35 °C	+60% 81 mL/g COD[c]	Engelhart et al. (1999)
AS+PS[b]	Homogeniser (Δ=600 bar)	CSTR, HRT= 20 d, 35 °C	+18% 156 mL/g COD[c]	Barjenbruch and Kopplow (2003)
AS	Lysing-centrifuge 39 m^3/h, 3140 rpm	Continuous, HRT=40 d, 35 °C	+26% 209 mL/g COD[c]	Zabranska et al. (2006)
AS SRT=5-7 d	Grinding, db: 0.25mm *v*b: 10ms^{-1} 9min, 60 ◦C	Batch, 21 d, 35 °C	+10% 84 mL/g COD[c]	Baier and Schmidheiny (1997)
AS+PS	Ultrasonic 9 kHz, 200 W, 30 min	Batch, 11 d, 37 °C	+64% 131 mL/g COD[c]	Wang et al. (1999)
AS	Ultrasonic 20 kHz 0.33 W/mL, 20 min	Batch, 100 d, 37 °C	+104% 107 mL/g COD[c]	Chu et al. (2002)

[a] AS: Activated sludge
[b] PS: Primary sludge
[c] Performance of anaerobic digestion without pretreatment. Values recalculated to COD basis.

2.3.3 Thermal treatment

In thermal treatment, the sludge is subjected to moderate (< 100 °C) or high temperatures (150 °C to 200 °C) with contact times varying from minutes to hours (Kepp et al., 2000). Heat applied during thermal treatment disrupts the chemical bonds of the cell wall and membrane, thus solubilises the cell components (Appels et al., 2008). The input of thermal energy is mostly realized by heat exchangers or by the application of steam to the sludge (Neyens and Baeyens., 2003). A summary of the main results of thermal pretreatment of sludge prior to the anaerobic digestion is presented in Table 2.6.

Optimal conditions of operation have been reported to be between 170 to 175 °C for a contact time of 30 to 60 min. Thermal treatments increase the COD solubilisation and sludge biodegradability, allowing it to accelerate and to increase the methane production of anaerobic digesters (Bougrier et al., 2006; Bougrier et al., 2008). However, above its optimal temperature, biodegradability of sludge can decrease, affecting negatively the anaerobic digestion performance, which could be explained by the possible formation of refractory compounds linked to Maillard reactions (Valo et al., 2004). In this reaction, reduced sugars and amino-acids react with melanoidines, which are difficult to degrade or they are even inhibitory (Muller, 2001).

Table 2.6: Performances obtained in themal pretreatments prior to anaerobic digestion

Substrate	**Treatment conditions**	**Anaerobic digestion**	**Increase of CH_4 production**	**References**
AS[a]	175 °C, 30 min	CSTR, HRT=15 d, 35 °C	+62% 115 mL/g COD[c]	Haug et al. (1978)
PS[b]	175 °C, 30 min	CSTR, HRT=15 d, 35 °C	No influence 252 mL/g COD[c]	Haug et al. (1978)
AS+PS (1:1)	175 °C, 30 min	CSTR, HRT=15 d, 35 °C	+14% 205 mL/g COD[c]	Haug et al. (1978)
AS	170 °C, 60 min	CSTR, HRT=15 d, 35 °C	+100% 108 mL/g COD[c]	Li and Noike (1992)
AS	170 °C, 60 min	CSTR, HRT=15 d, 35 °C	+61% 88 mL/g COD[c]	Valo et al., (2004)
AS	170 °C, 30 min	Batch, 24 d, 35 °C	+51% 145 mL/g COD[c]	Bougrier et al. (2006)

[a] AS: Activated sludge
[b] PS: Primary sludge
[c] Performance of anaerobic digestion without pretreatment.

2.3.4 Biological treatment

Biological treatment aims to enhance the hydrolysis stage using an additional biological stage prior to the main digestion process or the addition of enzymes. The most common type is temperature phased anaerobic digestion, which uses a higher stage at either thermophilic (around 55 ◦C) or hyper-thermophilic (between 60 and 70 ◦C) conditions, anaerobic and aerobic (Carrère et al., 2010). The evaluation of thermophilic against mesophilic pretreatment (HRT of 2 days) prior to mesophilic anaerobic digestion has increased by 25% methane production and solids destruction (Ge et al., 2010).

Enzymatic compounds have been applied to enhance the anaerobic digestion in mesophilic and thermophilic conditions, by improving the hydrolysis stage prior to the acidogenesis. The hydrolytic enzymes added in anaerobic reactors at lab-scale have demonstrated the improvement of biodegradation and sludge reduction (Lagerkvist and Chen, 1993).

2.4 Ozonation

Ozone is a powerful oxidant and disinfectant, with an oxidation potential that is one of highest of the common oxidants used for drinking water and wastewater treatment. In principle, ozone should be able to oxidize inorganic substances to their highest stable oxidation states and organic compounds to carbon dioxide and water, but it is quite selective in its oxidation reactions (Glaze et al., 1987). Some physicochemical properties of ozone are compiled in Table 2.7.

Table 2.7: Physicochemical properties of ozone (Doré, 1989; von Sonntag and Von Gunten, 2012).

Properties	**Units**	**Value**
Molecular weight	Da	48
Specific gravity of gas (air = 1.0)	-	1.10
Melting point	°C	-192
Boiling point at 1 atm	°C	-183
Critical temperature	°C	-119
Critical pressure	kPa	5040
Solubility in water at 0 °C	v/v	0.0489
Henry constant at 20 °C	atm/M	100
Enthalpy of formation (from oxygen)	kJ/mol	142
Oxidation potential	V	2.07

Ozone is produced at industrial level by means of electrical discharges. Due to the high instability of the O_3 molecule, its production is realised at the point of use, by means of ozone generators fed with either air or pure oxygen (Rakness, 2005). The global reaction of ozone production is endothermic :

$$3\,O_2 \rightleftharpoons 2\,O_3 \qquad (2.3)$$

The theoretical specific energy requirement to produce ozone is 0.820 kWh/kg O_3; however, the actual energy requirement is much higher due to generation inefficiencies (Rakness, 2005). The energy requirement for ozone production has been estimated between 12 to 15 kWh/kg O_3 for ozone including oxygen production, transport and destruction (Sonntag and Gunten, 2012).

Ozone is mainly used for the disinfection of natural waters for drinking and for the oxidation of specific contaminants (Rakness, 2005). But additionally, it has an extensive use in the domain of wastewater treatment. Although the main objective of ozonation in wastewater treatment is

disinfection after the secondary biological treatment, it also plays a variety of other roles, mainly to improve the efficiency of other unit operations such as coagulation-flocculation-sedimentation or carbon filtration, to remove biologically refractory or toxic compounds in order to improve biological units; or to reduce the amount of sludge generated in these latter systems (Beltran, 2003). Ozonation is the oxidation treatment most widely used for sludge reduction (Carrère et al., 2010).

2.4.1 Ozone generation system

Ozone water treatment systems have four basic components: a gas feed system, an ozone generator, an ozone contactor, and an off-gas destruction system. The gas feed system provides a clean, dry source of oxygen to the generator. The ozone contactor transfers the ozone-rich gas into the water to be treated, and provides contact time for the reactions. The final process step, off-gas destruction, is required as ozone is toxic in the concentrations present in the off-gas.

2.4.1.1 Gas feed systems

Ozone feed systems are classified as using air, high purity oxygen or a mixture of the two. High purity oxygen can be purchased and stored as a liquid (LOX), or it can be generated on-site through either a cryogenic process, with vacuum swing adsorption (VSA), or with pressure swing adsorption (PSA) (U.S. EPA, 1999). Cryogenic generation of oxygen consists of the separation of oxygen from nitrogen based on the boiling/condensation points at different pressures (Langlais et al., 1991). Cryogenic systems are feasible for large installations, generally within the range of 20 to 20,000 tons/day of oxygen production. Pressure swing adsorption is a process whereby a special molecular sieve is used under pressure to selectively remove nitrogen, carbon dioxide, water vapor, and hydrocarbons from air, producing an oxygen rich feed gas (80–95 percent O_2). The components used in pressure swing adsorption systems are similar to high pressure air feed systems in that both use pressure swing molecular absorption equipment. Low pressure air feed systems use a heat reactivated desiccant dryer (U.S. EPA, 1999). The product gas usually contains approximately 90 to 95% of oxygen, 5% of argon, and a small amount of nitrogen (Langlais et al., 1991). Liquid oxygen feed systems are relatively simple, consisting of a storage tank or tanks, evaporators to convert the liquid to a gas, filters to remove impurities, and pressure regulators to limit the gas pressure to the ozone generators. Air feed systems for ozone generators are fairly complicated as

the air should be properly conditioned to prevent damage to the generator. Air should be clean and dry, with a maximum dew point of -60º C (-80º F) and free of contaminants (Langlais et al., 1991; U.S. EPA, 1999).

Air preparation systems typically consist of air compressors, filters, dryers, and pressure regulators. A comparison of the advantages and disadvantages of each gas feed system is presented in Table 2.8.

Table 2.8: Comparison of air and high purity oxygen feed systems (U.S. EPA, 1999)

Source	Advantages	Disadvantages
Air	• Commonly used equipment, • Proven technology, • Suitable for small and large systems	• More energy consumed per ozone volume produced, • Extensive gas handling equipment required, • Low ozone concentration
Oxygen (general)	• Higher ozone concentrations, • Approximately doubles ozone concentration for same generator, • Suitable for small and large systems	• Safety concerns, • Oxygen resistant materials required.
LOX	• Less equipment required • Simple to operate and maintain • Suitable for small and intermediate systems • Can store excess oxygen to meet peak demands	• Variable LOX costs, • Storage of oxygen onsite (safety concerns), • Loss of LOX in storage when not in use.
Cryogenic Oxygen generation	• Equipment similar to air preparation systems • Feasible for large systems • Can store excess oxygen to meet peak demands	• More complex than LOX, • Extensive gas handling equipment required, • Capital intensive, • Complex systems to operate and maintain.

2.4.1.2 Ozone generators

The energy requirement for the production of ozone can be provided by high voltage electrical discharges in a stream of oxygen, water electrolysis, photolysis of oxygen by UV irradiation at wavelengths less than 185 nm or by radiolytic of oxygen by ionizing radiation (Gottschalk et al., 2009). However, the electrical discharge is the only technique that allows the production of ozone at an industrial level (Baig and Pierre, 2010).

In electrical - discharge ozone generators, ozone is produced using energy from electrons in an electrical field between the two electrodes. The electrodes are separated by a space or gap containing a gas. A discharge of electrons from one of the electrodes ionizes the gas (Figure 2.3). The ionization is limited to a small region around the electrode and produces a collection of electrons, ions, radicals and neutral or excited molecules called a plasma, in the case of an ozone generator, a nonthermal plasma. The ions generated function as the charge carriers to the other electrode. When one of the electrons in this plasma collides with an oxygen molecule, it transfers part of its energy to the oxygen, causing it to dissociate into monoatomic, reactive atoms. These collide with other oxygen molecules. Overall, in a complex reaction mechanism, some of the oxygen atoms form ozone, while others recombine to molecular oxygen (Gottschalk et al., 2009). However, the conversion yields are relatively low, about 1 to 4% when the ozone generator is fed with air, while it can reach about 6 to 16% when the generator is fed with pure oxygen (Rakness, 2005).

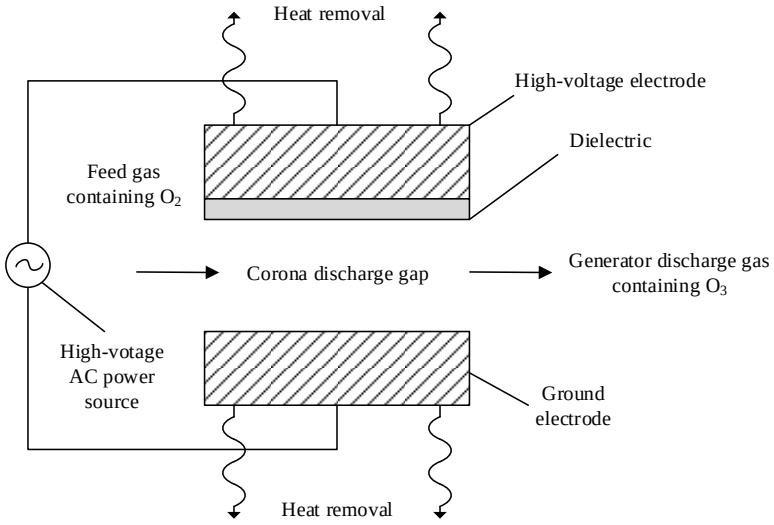

Figure 2.3: Schematic detail of generation of ozone (Metcalf & Eddy - AECOM, 2014).

2.4.1.3 Ozone contactor

Once ozone gas is transferred into water, the ozone reacts with the organic and inorganic constituents. Ozone not transferred into the processed water during contact is released from the contactor as off-gas. Common ozone dissolution methods include: Bubble diffuser contactors, injectors, and turbine mixers.

The bubble diffuser contactor offers the advantages of no additional energy requirements, high ozone transfer rates, process flexibility, operational simplicity, and no moving parts. Bubble diffuser contactors are typically constructed with 5.5 to 6.7 meters water depths to achieve 85 to 95% ozone transfer efficiency. Since all the ozone is not transferred into the water, the contactor chambers are covered to contain the off-gas. Bubble diffuser contactors use ceramic or stainless steel diffusers that are either rod-type or disc-type to generate bubbles (Renner et al., 1988).

The injectors transfer the ozone into the water stream under negative pressure, which is generated in a Venturi section, pulling the ozone into the water stream. In many cases, a sidestream of the total flow is pumped at a higher pressure to increase the available vacuum for ozone injection. After ozone is injected into this sidestream, the sidestream containing all the added ozone is combined with the remainder of the plant flow under high turbulence to enhance dispersion of

ozone into the water. The gas to liquid ratio is a key parameter used in the design of injector contacting systems. This ratio should be less than 0.067 cfm/gpm to optimize ozone transfer efficiency (Langlais et al., 1991). Meeting this criterion typically requires relatively low ozone dosages and ozone gas concentrations greater than 6 percent by weight (DeMers and Renner, 1992).

Turbine mixers are also used to feed ozone gas into a contactor and mix the ozone with the water in the contactor. Ozone transfer efficiency for turbine mixers can be in excess of 90%. However, the power required to achieve this efficiency is 2.2 to 2.7 kW-hr of energy per lb of ozone transferred (Dimitriou, 1990).

2.4.1.4 Off-gas destruction systems

For water treatment, the concentration of ozone in the off-gas from a contactor is usually well above the fatal concentration. In this system, the off-gas is collected and the ozone converted back to oxygen prior to release to the atmosphere. Ozone is readily destroyed at high temperatures (> 350 °C or by a catalyst operating above 100 °C) to prevent moisture buildup.

2.4.2 Reaction of ozone in water

Mechanisms of reaction in dilute aqueous medium with inorganic or organic compounds have been extensively studied (Doré, 1989). Ozone dissolved in water can react directly via its molecular form or via indirect reaction, producing free radical species as a consequence of the decomposition of ozone (Figure 2.4).

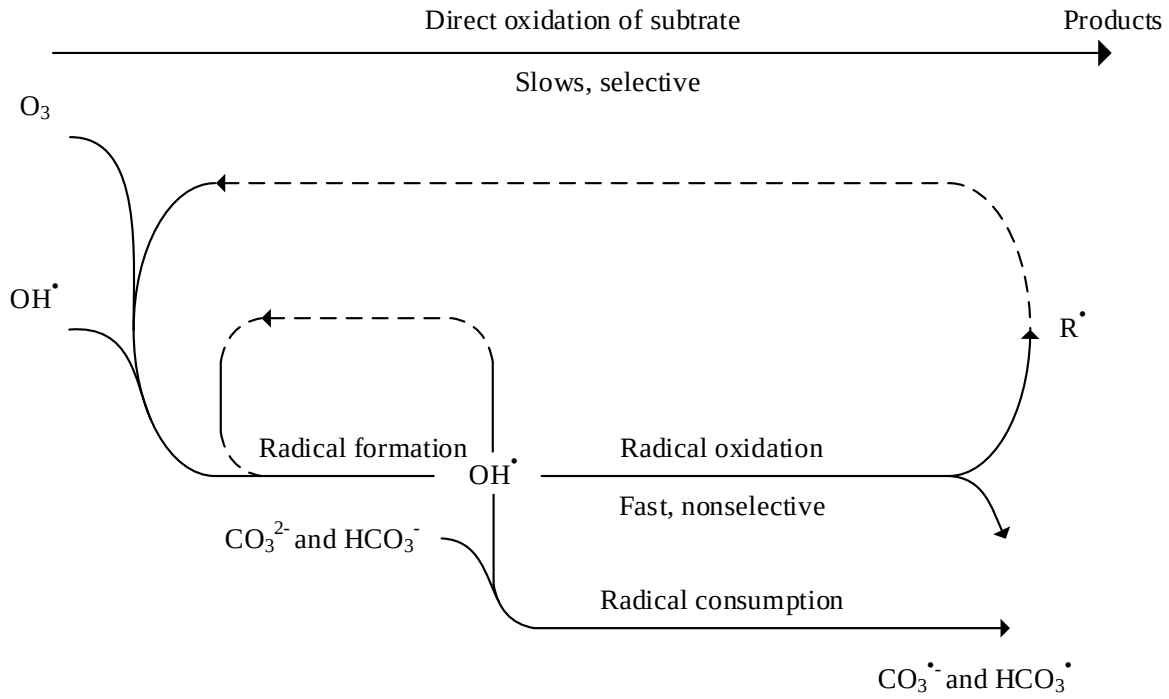

Figure 2.4: Reaction pathways for ozone (Aieta et al., 1988).

Direct reactions involving molecular ozone are very selective; ozone reacts very rapidly with some species, but very slowly with other species. However, the OH radical is non-selective in its behavior, reacting rapidly with a large number of species, but the concentration of OH radicals under normal ozonation conditions is relatively small (U.S. EPA, 1999).

2.4.2.1 Direct reactions of molecular ozone with organic matter

The direct oxidation of organic components by ozone is a selective reaction with slow reaction rate constants, typically being in the range of ($k_D = 1.0 - 10^6\ M^{-1}s^{-1}$) (Gottschalk et al., 2009). Due to the electronic configuration of ozone, it has different reactions in water. These reactions can be divided into three categories (Doré, 1989):

- Cycloaddition: The ozone reacts as a dipole leads to 1-3 dipolar cycloaddition of unsaturated bonds to form a primary ozonide, which decomposes to form aldehydes and/or ketones, and hydrogen peroxide.
- Electrophilic agent: This mechanism leads to localized attacks on sites of high electron densities. Ozone reacts with compounds such as aromatic hydrocarbons or amines. The

presence of electron donating groups facilitates this response. The final compounds obtained are unsaturated aliphatics, which can then undergo a cycloaddition (dipole action).

- Nucleophile agent: Ozone can also react with amines on the free electron pair of the nitrogen atom (at basic pH) or by insertion at a bond alpha to the nitrogen.

2.4.2.2 Indirect reaction of ozone

The indirect reaction pathway involves the production of radicals, which are molecules that have an unpaired electron. Most radicals are highly unstable and immediately undergo a reaction with another molecule in order to obtain the missing electron (Gottschalk et al., 2009). The ozone radical chain mechanism can be divided into three different steps, the initiation, chain propagation, and termination. The first step is the decay of ozone, accelerated by initiators, for example, OH^-, to form secondary oxidants such as hydroxyl radicals ($OH^\bullet$). They react nonselectively and immediately ($k = 10^8 – 10^{10}\ M^{-1}s^{-1}$) with target molecules (Gottschalk et al., 2009). This reaction pathway is favored by an alkaline medium, by the presence of easily oxidizable solute and by the presence of radical initiators, such as ultraviolet light, certain metal cations or hydrogen peroxide (Doré, 1989).

Numerous studies have been developed to clarify the mechanism of ozone decomposition. The mechanism of Staehelin, Hoigné, and Buhler is generally accepted as the mechanism of ozone in water (Table 2.9), although when the pH is high, the mechanism of Tomiyasu, Fukutomi, and Gordon is considered the most representative (Table 2.10) (Beltran, 2003).

Table 2.9: Ozone decomposition mechanism in pure water according Staehelin, Hoigné, and Bühler (Beltran, 2003).

Reaction	**Rate constant**
Initiation Reaction	
$O_3 + OH^- \rightarrow HO_2^\bullet + O_2^{-\bullet}$	70 $M^{-1}s^{-1}$
Propagation Reactions	
$HO_2^\bullet \rightarrow O_2^{-\bullet} + H^+$	$7.9 \cdot 10^5$ s^{-1}
$O_2\text{-}^\bullet + H^+ \rightarrow HO_2^\bullet$	$5 \cdot 10^{10}$ $M^{-1}s^{-1}$
$O_3 + O_2^{-\bullet} \rightarrow O_3^{-\bullet} + O_2$	$1.6 \cdot 10^9$ $M^{-1}s^{-1}$
$O_3^- + H^+ \rightarrow HO_3^\bullet$	$5.2 \cdot 10^{10}$ $M^{-1}s^{-1}$
$HO_3^\bullet \rightarrow O_3^{-\bullet} + H^+$	$3.3 \cdot 10^2$ s^{-1}
$HO_3^\bullet \rightarrow HO^\bullet + O_2$	$1.1 \cdot 10^5$ s^{-1}
$O_3 + OH^\bullet \rightarrow HO_4^\bullet$	2 $M^{-1}s^{-1}$
$HO_4^\bullet \rightarrow HO_2^\bullet + O_2$	$2.8 \cdot 10^4$ s^{-1}
Termination Reactions	
$HO_4^\bullet + HO_4^\bullet \rightarrow H_2O_2^\bullet + 2O_3$	5 $M^{-1}s^{-1}$
$HO_4^\bullet + HO_3^\bullet \rightarrow H_2O_2^\bullet + O_2 + O_3$	$5 \cdot 10^9$ $M^{-1}s^{-1}$

Table 2.10: Ozone decomposition mechanism in pure water at alkaline conditions according to Tomiyasu, Fukutomi, and Gordon (Beltran, 2003).

Reaction	**Rate constant**
Initiation Reaction	
$O_3 + OH^- \rightarrow HO_2^\bullet + O_2^{-\bullet}$	40 $M^{-1}s^{-1}$
$O_3 + HO_2\text{-} \rightarrow HO_2^\bullet + O_3\text{-}^\bullet$	$2.2 \cdot 10^6$ $M^{-1}s^{-1}$
Propagation Reactions	
$HO_2^\bullet \rightarrow O_2^{-\bullet} + H^+$	$7.9 \cdot 10^5$ s^{-1}
$O_2^{-\bullet} + H^+ \rightarrow HO_2^\bullet$	$5 \cdot 10^{10}$ $M^{-1}s^{-1}$
$O_3 + O_2^{-\bullet} \rightarrow O_3^{-\bullet} + O_2$	$1.6 \cdot 10^9$ $M^{-1}s^{-1}$
$O_3^- + H_2O \rightarrow HO^\bullet + O_2 + OH^-$	20 – 30 $M^{-1}s^{-1}$
$O_3^- + HO^\bullet \rightarrow HO_2^\bullet + O_2^{-\bullet}$	$6 \cdot 10^9$ $M^{-1}s^{-1}$
$O_3 + HO^\bullet \rightarrow HO_2^\bullet + O$	$3 \cdot 10^9$ $M^{-1}s^{-1}$
$HO_2^- + H^+ \rightarrow H_2O_2$	$5 \cdot 10^{10}$ $M^{-1}s^{-1}$
$H_2O_2 \rightarrow HO_2^- + H^+$	0.25 s^{-1}
Termination Reactions	
$O_3 + HO^\bullet \rightarrow O_3 + OH^-$	$2.5 \cdot 10^9$ $M^{-1}s^{-1}$
$HO^\bullet + CO_3^{-2} \rightarrow OH^- + CO_3^{-\bullet}$	$4.2 \cdot 10^8$ $M^{-1}s^{-1}$
$CO_3^{-\bullet} + O_3 \rightarrow O_2 + CO_2 + O_2^{-\bullet}$	n.d.

All the free radicals do not have the same affinity for organic matter. The hydroxyl radical ($HO^\bullet$) is extremely active, superoxide radical ($O_2^\bullet$) is moderately active on organic matter, while the ozone radicals ($O_3^\bullet$) and hydroperoxides (HO_2) are not very active (Doré, 1989).

Most of the available data in the literature are focused on the study of the chemical reactions of ozone and its interaction with isolated molecules in an aqueous medium simple. However, these data are not applicable in the case of the use of ozone in complex media containing high concentrations of organic matter and dissolved salts, as in the case of activated sludge (Déléris, 2001).

Thus, the decay of ozone initiated by the hydroxide ion leads to a chain reaction, producing fast - reacting and nonselective OH – radicals. The $OH^{\bullet}$ reacts with the target molecule at the position with the highest electron density due to its electrophilic properties. Many substances may initiate, promote or terminate the chain reaction. Bicarbonate and carbonate play an important role as scavengers of $OH^{\bullet}$ radicals in natural systems. The reaction rate constants are relatively low but their concentration range in natural systems is comparatively high, so that this reaction cannot be ignored. Since many water constituents can influence the chemical oxidation reaction, the composition of the water to be treated should be evaluated carefully. For example, the presence of scavengers such as carbonate, or ozone – consuming compounds such as reduced metal species, natural organic matter or other organics can drastically affect the required ozone dose (Gottschalk, 2009).

2.4.3 Reaction of ozone in a complex medium

Most of the studies of ozone are largely based on the chemistry of ozone in simple aqueous media. However, the reactions of ozone in a complex medium are kinetically more complex and can be altered by several factors such as the pH, the nature and the concentration of the oxidizable organic matter and the ozone dosage.

When ozone is applied to wastewater or sludge there will likely be numerous parallel ozone reactions, depending on the complexity of this medium. If the presence of initiators, promoters, and inhibitors is of great importance in the treatment of natural water, the unknown nature and concentration of these compounds and others that directly react with ozone constitute the main problem in this field of ozonation of wastewater (Beltran, 2003). Compounds with specific functional groups such as aromatic rings, unsaturated hydrocarbons are prone to ozone attack while other compounds such as saturated hydrocarbons, alcohols and aldehydes can be considered resistant to ozone attack. In these cases, the indirect reactions can play an important role, although this will also depend on the concentration of fast ozone-reacting compounds (kinetic regime) and hydroxyl radicals, the way they are generated, inhibiting substances and pH of water (Beltran, 2003). Knowledge of the composition of the medium is fundamental to predicting ozone reactivity and potential applications.

The ozonation of wastewater is a multiple series-parallel system of ozone reactions. For the establishment of the kinetic regime of ozone absorption has been recommended to assume that the ozone would react with the matter in water through the following irreversible second-order reaction, using COD concentration as global parameter to represent the content of organic matter (Beltran 2003):

$$O_3 + z{\cdot}COD \xrightarrow{k} \text{Products} \tag{2.4}$$

If the kinetic regime is slow, it is also assumed that ozone decomposes into free radicals that react with the organic matter through the following reaction:

$$OH\bullet + COD \xrightarrow{k'} \text{Products} \tag{2.5}$$

However, a high concentration of pollutants would suggest a high reactivity ozone, which is an indication of fast kinetic regime and ozone direct reactions, and low concentration usually means low ozone reactivity and, hence, a factor that favours the development of ozone indirect reactions (Beltran, 2003). Therefore, the high concentration of pollutants in the wastewater sludge could suggest the predominance of direct reactions. In addition, this type of medium does not have the characteristics that can promote the initiation step of the radical reactions and the presence of free radical scavengers in high concentration (organic matter and salts) limits or eliminates the propagation phase radical reactions (Déléris, 2001).

The steps for studying the kinetics of the direct wastewater ozonation are similar to those for single compounds. The first step is to establish the kinetic regime of ozone absorption because this will allow the ozone absorption rate law to be fixed. This can be done by determining the experimental reaction factor, E (Equation 2.6), defined as the ratio between the actual flux of ozone per the maximum flux corresponding to the physical absorption (Déléris et al., 2000; Paul and Debellefontaine, 2007).

$$E = \frac{\text{actual flux of ozone}}{\text{maximum flux due to physical absorption}} = \frac{N_{A,O3}}{k_L a \cdot C_{03}^*} \tag{2.6}$$

The actual flux of ozone ($N_{0,O3}$) is defined as the ozone effectively transferred to the liquid and it is quantified considering the gas flow rate and the ozone concentrations at the inlet and outlet of ozone reactor. The maximum flux is calculated multiplying the equilibrium ozone concentration in the liquid phase (C_{03}^{*}) and the overall mass transfer coefficient (k_La). k_La of ozone must be obtained experimentally. It can be determined indirectly by means of physical absorption of oxygen, considering the effect of diffusivities of both compounds in water (Equation 2.7). The ozone concentration at the gas-water interface can be expressed as a function of the ozone partial pressure according to Henry's law (Equation 2.8).

$$k_L a_{O3} = k_L a_{O2} \sqrt{\left(\frac{D_{O2}}{D_{O3}}\right)} \tag{2.7}$$

$$C_{03}^{*} = \frac{P_{O3}}{He} \tag{2.8}$$

where D_{O2} is the diffusivity of oxygen in water, D_{O3} is the diffusivity of ozone in water, He is the Henry's law constant, and P_{O3} ozone partial pressure within the reactor.

The reaction factor E can be defined as the number of times the maximum physical absorption rate increases due to the chemical reaction, but this definition has only physical meaning when the kinetic regime is fast or moderate. However, the values of E can be lower than unity (the cases of slow kinetic regime or some others with the moderate regime), although they have no practical use (Beltran, 2003). This factor characterizes the importance of the chemical reaction in relation to the diffusion processes. E can reach up to 10 depending on the type of reactor and on the conditions, leading to an effective film thickness that does not exceed just a few micrometers (Paul et al., 2007).

Various kinetic regimes that allow the determination of parameters are presented in Table 2.11. The kinetic regime depends on the relative importance of chemical and mass-transfer rate steps. This relationship can be established by calculating the dimensionless Hatta numbers and the instantaneous reaction factor, the latter need only when the reactions are fast or instantaneous. The Hatta number is also unknown since parameters such as the reaction rate constant have to be determined. Thus, the kinetic study starts from the assumption that at the experimental conditions to be applied the kinetic regime is known, and, then, the absorption rate law (Beltran, 2003). This

means that some conditions referring to the Hatta number have to be confirmed once the rate constant and/or individual liquid phase mass-transfer coefficient are known. In order to ensure that the hypothesis is solid, some preliminary experiments can be done to classify the kinetic regime as fast or slow, where the concentration of dissolved ozone is the key parameter to follow. Thus, the absence of dissolved ozone is definitive proof of a fast or instantaneous regime while the opposite situation indicates a slow kinetic regime (Beltran, 2003).

Table 2.11: Absorption rate law equations for different kinetic regimes of ozonation (Beltran, 2003).

Kinetic regime	**Kinetic equation**	**Conditions and parameter to determine**
Very slow	$N_{O3} = K_L a \cdot (C^*_{O3} - C_{O3})$ $N_{O3} = \frac{dC_{O3}}{dt} + \sum_i r_i$	Ha < 0.02, $C_{O3} \neq 0$ Rate constant
Diffusional	$N_{O3} = K_L a \cdot C^*_{O3}$	0.02 < Ha < 0.3, $C_{O3} = 0$ Mass transfer coefficient
Fast	$N_{O3} = K_L a \cdot \frac{Ha}{\tanh Ha}$	Ha > 3, $C_{O3} = 0$ Rate constant or mass transfer coefficients
Fast pseudo first-order	$N_{O3} = a \cdot C^*_{O3} \sqrt{k_D D_{O3} C_M}$	3 < Ha < $E_i/2$, $C_{O3} = 0$ Rate constant or specific interfacial area
Instantaneous	$N_{O3} = K_L a \cdot C^*_{O3} E_i$	Ha > nE_i, $C_{O3} = 0$ Mass transfer coefficients

The absence of dissolved ozone, reaction factors higher than unity, and Hatta numbers higher than 3 characterize the fast kinetic regime. Conditions regarding the reaction factor and the dissolved ozone concentration can be checked to conclude that the kinetic regime is fast. In the ozonation of any wastewater, the slow kinetic regime could be checked for the presence of ozone, reaction

factors approximately equal to or lower than unity, and Hatta numbers much lower than 0.3. In this kinetic regime, it is likely that the indirect reaction of ozone competes with the direct reaction (Beltran, 2003).

As shown in Figure 2.5, slow kinetic regimes occur when the ozone chemical reactions result in lowering the concentration of dissolved ozone in the bulk liquid only, thereby, increasing the driving force for ozone mass transfer from the gas phase into the liquid phase, and the ozone mass transfer may shift to the fast or instantaneous kinetic regime. Unlike the slow kinetic regime, dissolved ozone is completely depleted within the liquid film beside the gas-liquid interface.

The apparent rate of ozone mass transfer may even exceed the maximum rate of physical gas-liquid mass transfer because of steeper dissolved ozone concentration profiles (Zhou and Smith, 2000).

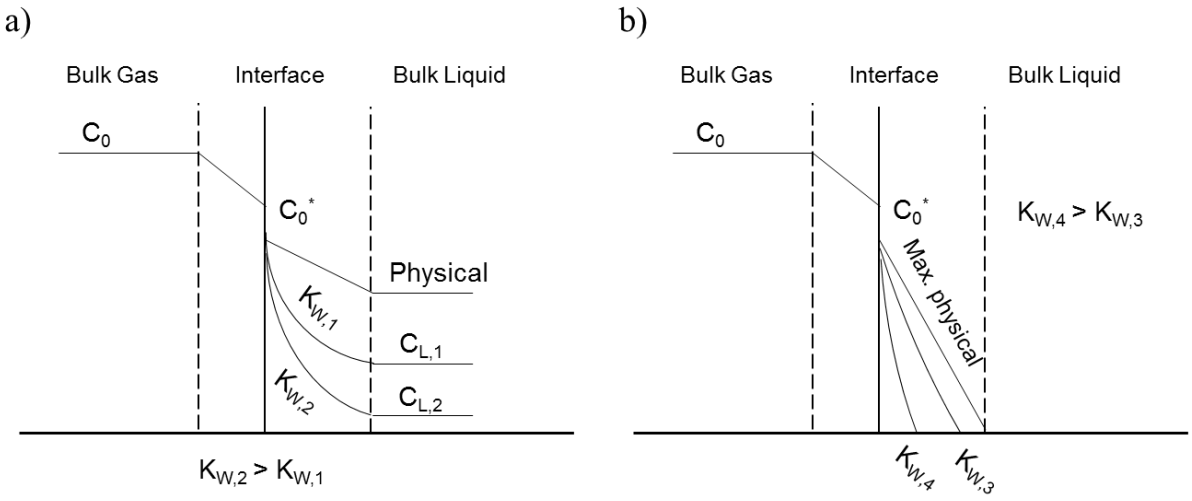

Figure 2.5: Ozone mass transfer schemes in water and wastewater treatment. a) Slow reaction, b) fast or instantaneous reaction (Zhou and Smith, 2000).

In general, low off-gas concentrations of ozone were observed in several ozonation reactors. This could be due to the fact that in the initial chemical reactions of ozone transferred to the liquid with the various dissolved organics are fast enough to be completely depleted at the gas-liquid interface (El Din and Smith, 2001). As a consequence, a total absence of dissolved ozone within the bulk liquid is often observed and a fast kinetic regime or an instantaneous-reaction kinetic regime occurs, where the apparent rate of ozone mass transfer can exceed the maximum rate of physical gas-liquid mass transfer.

The reaction between ozone and sludge occurs only near the gas-liquid interface, a liquid film having thickness to the order of μm (El-Din and Smith, 2001). Without any enhancement by chemical reactions, the thickness of the liquid film is δ (Equation 2.9), and the ozone concentration decreases from Co* to 0. In the presence of organic compounds leading to an enhancement of the transfer, the ozone concentration reaches 0 concentration in the bulk liquid at thickness δE, which is smaller than δ.

$$\delta_E = \frac{\delta}{E} \tag{2.9}$$

According to the literature, a second-order kinetics for the reactions between ozone and organic compounds can be assumed (Beltran, 2013). Therefore, the chemical reaction rate for the disappearance of ozone can be expressed as:

$$\mathrm{r_{O3}} = \mathrm{k} \cdot C_{\mathrm{COD}} \cdot C_{\mathrm{O3}} \tag{2.10}$$

In the case of an ozone batch reactor, the following COD mass balance can be elaborated:

$$\frac{\mathrm{d\ COD}}{dt} = -\mathrm{r}_{COD} \tag{2.11}$$

Equation 2.11 can also be expressed as a function of chemical reaction rate for the disappearance of ozone:

$$\frac{\mathrm{d\ COD}}{dt} = -\frac{1}{z}\, r_{O3} \tag{2.12}$$

In a process like the sludge ozonation, where no free dissolved ozone could be detected in the liquid solution, the ozone reactions can take place in the fast-kinetic regime. Therefore, for this case, the film theory proposes that E = Ha (Beltran, 2003). For a second-order kinetics, Hatta number and enhancement factor can be evaluated by the following equations:

$$\mathrm{Ha} = \frac{1}{k_L}\sqrt{k\ \mathrm{D_{O3}\ C_{COD}}} \tag{2.13}$$

$$E = 1 + \frac{D_{COD}}{D_{O3}} \frac{z\,DCO}{C^*_{O3}} \quad (2.14)$$

This pseudo-m-order regime of absorption is accomplished when the following criterion is fullfilled:

$$3 < Ha < E/2 \quad (2.15)$$

On the basis of Equations 2.6, 2.12, 2.13 and 2.15, it can be written

$$-\frac{dCOD}{dt} = \frac{a \cdot C^*_{O3}}{z} \sqrt{k \cdot D_{O3} \cdot COD} \quad (2.16)$$

where a is the specific interfacial area (m^{-1}), C^*_{O3} is the equilibrium ozone concentration (mol O_3/L), k is the kinetic rate constant for ozone-organic matter reaction ($L \cdot mol^{-1} \cdot s^{-1}$), D_{O3} is the ozone diffusivity in liquid phase (m^2/s), z is the stoichiometric ratio for the ozone-organic matter reaction (mol O_3/mol O_2), and COD is the chemical oxygen demand concentration (mol O_2/L).

After rearranging and integrating the Equation 2.16, Equation 2.17 is obtained:

$$\sqrt{COD_0} - \sqrt{COD} = k' \cdot t \quad (2.17)$$

With k' being equal to:

$$k' = \frac{a \cdot C^*_{O3}}{2 \cdot z} \sqrt{k \cdot D_{O3}} \quad (2.18)$$

According to Equation 2.17, a plot of the first term vs. time should lead to a straight line whose slope is k', and from Equation 2.18 the kinetic rate constant k can be deduced.

2.4.4 Action of ozone on sludge

This section presents an overview of the impact of ozone treatment on sludge characteristics. It has been evaluated in terms of the impact of ozone treatment by focusing on the mineralization, solubilisation, biodegradability, biomass activity, settling properties and dewatering conditions.

2.4.4.1 Mineralization

The monitoring of COD and total organic carbon (TOC) concentrations during ozonation of sludge has made it possible to assess the oxidant effect of ozone on organic matter. Stoichiometrically, 48 g of ozone can decompose 16 g COD (Chu et al., 2009). However, the detected mineralization is generally lower than this value (Goel et al., 2003; Chu et al., 2009). Several authors have observed the reduction of total COD during the ozonation of activated sludge which could be caused in part by the complete oxidation of organic matter into carbon dioxide and water (mineralization) (Weemaes et al., 2000; Deleris et al., 2009).

A summary of the main results obtained in the literature regarding the action of ozone on COD reduction is presented in Figure 2.6. As can be seen, the percent of COD removal increases as the ozone dose increases. Efficiency values depended on the properties of the sludge and the operating conditions (Chu et al., 2009).

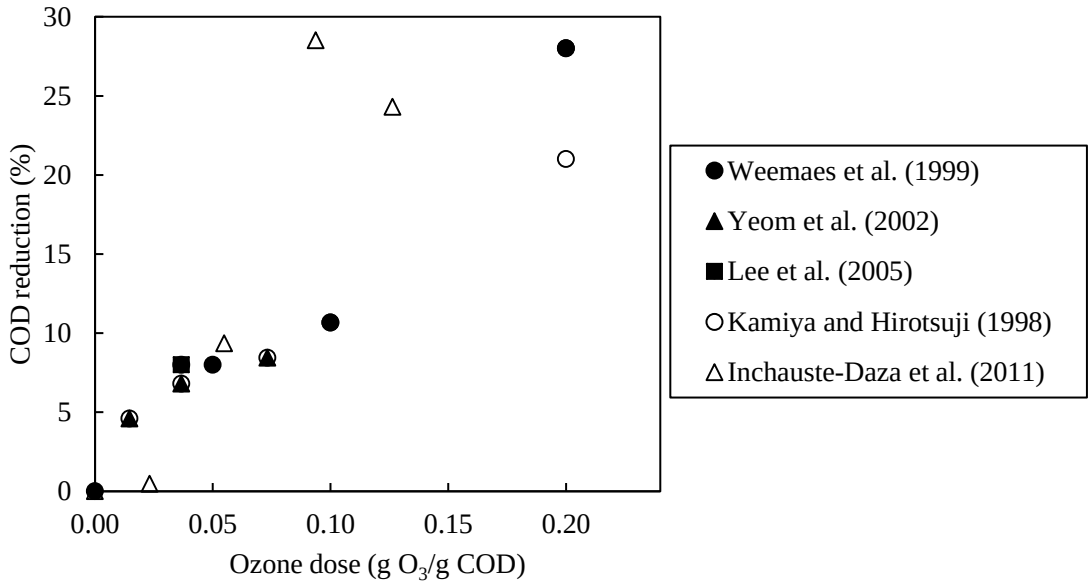

Figure 2.6: COD reduction during ozonation of activated sludge.

The reduction of TOC and the increase of CO_2 in the stripping gas have been observed during ozonation of activated sludge; thus, this could partially validate the mineralizing effect of ozonation on organic matter (Weemaes et al., 2000; Deleris et al., 2009). However, the reduction of COD has been higher than the reported decrease of TOC (Weemaes et al., 2000), suggesting the

mineralization of organic matter does not explain entirely the COD removal during ozonation of sludge; it could be attributed to the partial oxidation of organic compounds into intermediate products. The monitoring of TOC and COD during the ozonation of different phenolic wastewaters has demonstrated that the COD drop is caused by mineralization and partial oxidation, where high ozone dosages increase the mineralization (Carbajo et al., 2007). However, partial oxidation has not been considered for sludge ozonation and additional experiments are needed to validate its contribution to the COD drop.

The ozonation of sludge can reduce drastically the amount of sludge. It has been reported that ozonation can mineralize up to 90% of activated sludge (Délélis, 2009), but to obtain these performances the required ozone doses are not economically feasible. Thus, the coupling of ozonation with biological units is a more efficient solution for sludge reduction at the source, but results in high costs when compared to conventional sludge treatment and disposal trains (Bohler and Siegrist 2004; Nagare et al. 2008; Labelle et al., 2013). To optimize the cost of a combined process, the biological step should be maximized and the chemical step minimized (Bougrier et al., 2007). Various full-scale applications of sludge ozonation for sludge reduction are presented in Table 2.12, where 40–100% of sludge reduction was achieved as a result of these applications.

Table 2.12: Full-scale application of ozonation for sludge treatment (based on Chu et al., 2009).

Capacity	**Wastewater type**	**Ozonation**	**Sludge reduction (%)**	**Source**
Activated sludge process with partial ozonation of returned sludge				
12700 m^3/d	Industrial	0.05-0.1 g O_3/g TSS	40	Vergine et al. (2007)
22000 m^3 BOD/d	Chemical	0.056 g O_3/g TS	45	Wolff and Hurren, (2006)
Anaerobic digestion with ozonation				
Reactor 1125 m^3	Municipal	24 kg O_3/d, 22-44 m^3/d	70	Yasui et al. (2005)
25 m^3/d, 17000 p.e.	Municipal	0.03-0.06 g O_3/g TSS	56	Winter (2002)

2.4.4.2 Solubilisation

To obtain a better understanding of the action of ozone, solubilisation of organic matter needs to be evaluated. The solubilisation can be evaluated in terms of soluble COD and also by measuring nitrogen and phosphorus release. Ozonation is expected to generate soluble organic matter by the disintegration of solids and the oxidation of organic polymers (Foladori et al., 2010a). A summary of the main results obtained from the literature regarding the action of ozone on COD solubilisation is presented in Figure 2.7.

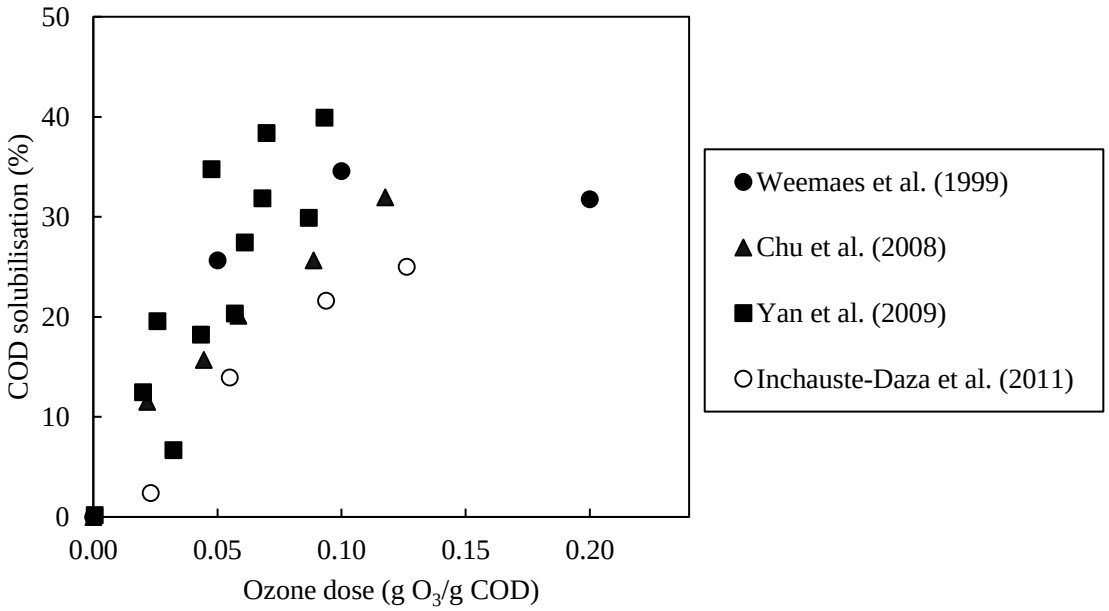

Figure 2.7: COD solubilisation during ozonation of activated sludge.

As can be observed in Figure 2.7, the ozonation increases the COD solubilisation of activated sludge. It has been reported that the sludge solubilisation increases linearly at the initial stage with the increase of ozone dosage, but high ozone doses do not significantly increase the soluble COD (Weemaes et al., 2000), probably caused by the mineralization of organic matter. The reported results show large variability, the solubilisation increased from 20 to 40% for ozone doses of 0.1 g O_3/ COD. The same ozone dosages result in different efficacy levels in the various investigations because several factors can affect the ozonation process (Foladori et al., 2010a), such as:

- Size of sludge floc which influences the floc surface and the diffusion of ozone,

- Presence and concentration of soluble organic compounds which react with ozone,
- Efficiency of ozone transfer and the hydrodynamic of the ozonation reactor.

For certain conditions, ozone can react first with the soluble organic matter and later, attacks the particulate fraction, despite the differences of reactivity of each fraction: the soluble fraction has a screening effect on the particulate matter attack by ozone (Cesbron et al., 2003). The competition for ozone between soluble and particulate matter during activated sludge ozonation has been described as a non-classical competition phenomenon (Cesbron et al., 2003). Even at low solubilisation levels, hydroxyl radicals react quickly with solubilised compounds which act as scavengers of particulate solids (Foladori et al., 2010a).

Sludge contains various types of microorganisms that can release a wide range of soluble substrates following their destruction during the ozonation process. These soluble substrates may then affect the efficiency of the ozonation process. Therefore, examining the relationship between the biological response and the formation of soluble substrates capable of acting as scavengers at certain ozone dosages is essential for understanding the sludge ozonation process (Yan et al., 2009). The production of soluble nitrogen and phosphorus compounds can be used as indicators of ozonation efficiency (He et al., 2006; Chu et al., 2008; Manterola et al., 2008).

With the breakup of the cell wall, the release of proteins, carbohydrates, nitrogen and phosphorus compounds, as the major components of microorganisms, are released into the soluble phase (Chu et al. 2008). The solubilisation of nitrogen and phosphorus obtained by previous studies is presented in Figure 2.8. The solubilisation of these compounds is highly variable, probably due to the type and characteristics of sludge samples. During ozonation, nitrogen and phosphorus are solubilized proportionally to the soluble COD (Camacho et al., 2005; Chu et al., 2008). Due to the release of nitrogen and phosphorus compounds in the soluble phase, special care should be taken if the solution of sludge was used as carbon source, because the solution of organic nitrogen and phosphorus may be an additional burden for the subsequent biological system (Zhao et al., 2007).

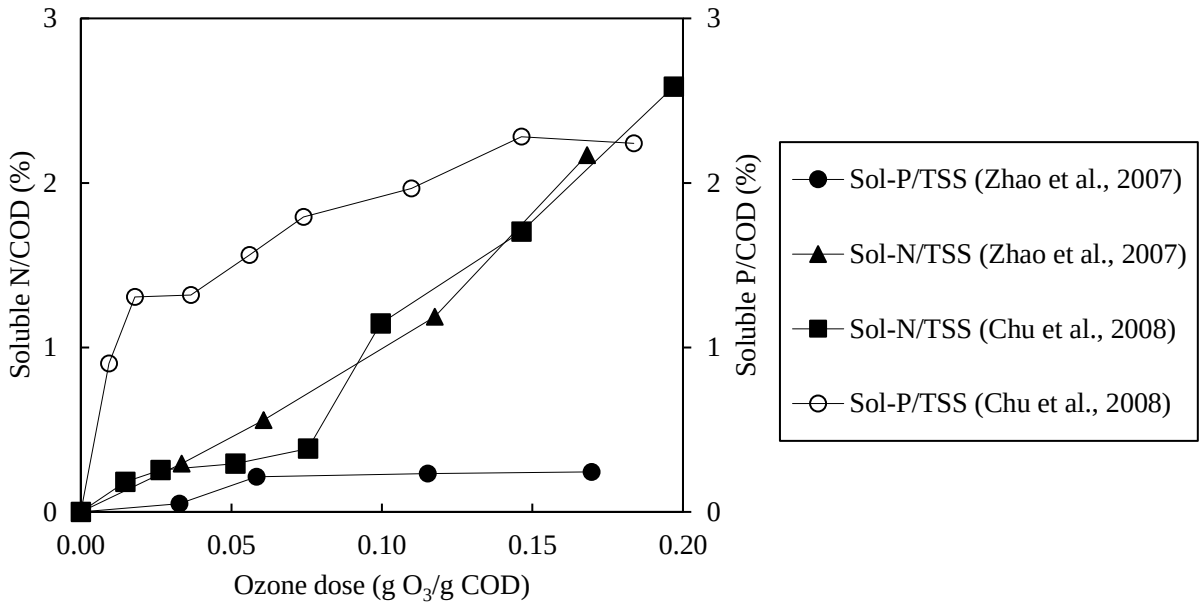

Figure 2.8: Effect of ozonation on nitrogen and phosphorus solubilisation of activated sludge (Based on Foladori, 2010).

2.4.4.3 Biodegradability

Ozone treatment is effective in partially solubilized sludge solids, leading to the subsequent improvement in anaerobic biodegradability and methane production (Battimelli et al., 2003, Goel et al., 2003; Yasui et al., 2005; Weemaes et al., 2000). A comparison of some studies monitoring the increase of methane in anaerobic digestion is presented in Figure 2.9.

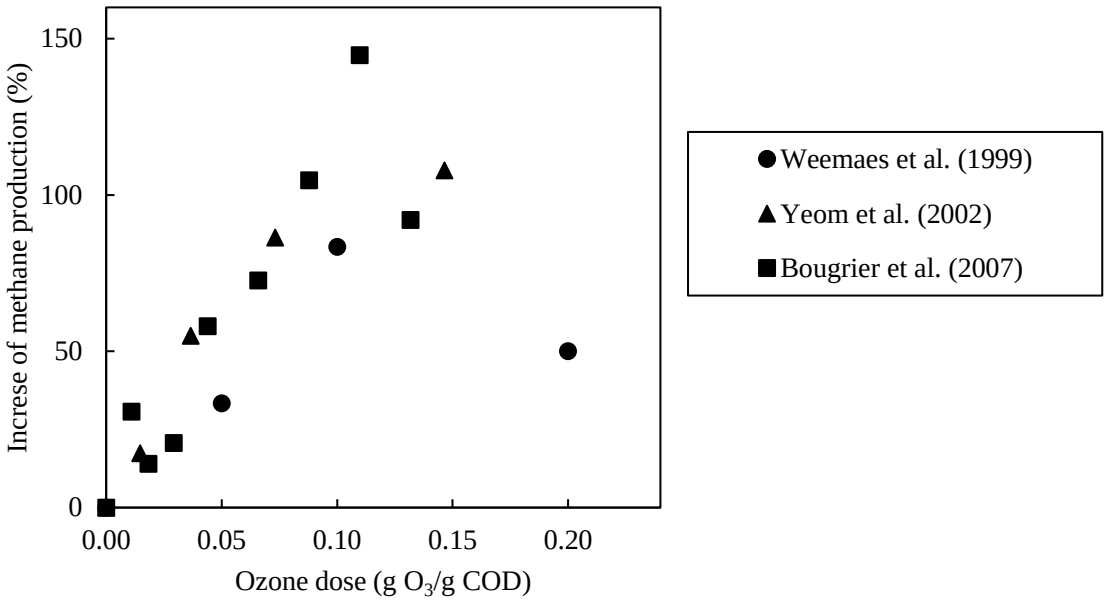

Figure 2.9: Increase of methane production during ozonation of sludge.

Yeom et al. (2002) showed that the methane and total gas production rates increased with ozone dosage up to 0.15 g O3/g COD. Further increases in ozone dosage did not increase the biodegradation. The methane production or biodegradation consists of two phases; initial rapid biodegradation is followed by slower, but steady degradation. Interestingly, both the initial and the second phase biodegradation rates do not change much with ozone dosage. Most of the differences in gas and methane production between the raw sludge and the treated sludge can be attributed to the differences in the duration of the initial fast degradation phase (Yeom et al., 2002). An overdose of ozone can reduce the methane production as has been observed by Weemaes et al. (2000), probably due to the mineralization of organic matter and the lower solubilisation rate.

It is reasonable to suppose that the enhancement in methane production is influenced by the solubilisation of slowly biodegradable matter and the increase of biodegradability of unbiodegradable organic products. As a result of the increase in solubilisation and biodegradability, anaerobic degradation can be enhanced, improving the methane yield and accelerating the digestion time.

2.4.4.4 Activity of the biomass

The biomass activity may be modified by the action of ozone. Ozone penetrates into the microorganisms, increases the osmosis of cell membranes, damages the uniformity of the cell walls, and releases the intracellular components, proteins, and DNA, into the soluble phase, causing the permeabilization of cells and loss of culturability (Komanapalli and Lau, 1996, Zhang et al., 2009). The results presented in the disinfection of wastewater show that ozone can cause the death and lysis of microorganisms and can also lead to increased energy requirements needed to repair the damage in the active biomass (Déléris, 2001). In the context of the coupling between a biological process and ozone treatment, the kinetics of death of microorganisms induced by ozonation of sludge must be taken into account to determine the conditions of application that will allow the development of an active biomass concentration sufficient to ensure the biological treatment.

In the field of wastewater treatment, the effect of ozone has been evaluated mainly for the removal of a target pathogen and also to evaluate its impact on activated sludge activity. The impact of ozonation on the reduction of pathogens depends on the dose and the target pathogen. Park et al. (2008) observed that a dose of about 0.1 g O_3/g TS is capable of inactivating fecal coliforms, while a dose of 0.2 to 0.4 g O_3 / g TS is capable of inactivating the *Strepcoccus* spp. and *Salmonella spp.* It has been demonstrated on activated sludge that the inactivation of microorganisms increases with the ozone doses, but higher amounts of ozone are required due to the presence of organic compounds and the formation of protective flocs (Salhi, 2003). Carballa et al. (2007) studied the effect of ozonation and anaerobic digestion on the inactivation of pathogens (*T. coliforms*, *E. coli*, *F. streptococcus*, *C. Perfringens*, *Salmonella* spp., and *Shigella* spp.). All pathogens were removed significantly (> 85%) during the ozonation, excluding *F. streptococcus* (about 63%) and *Salmonella* spp. *Salmonella* spp were inactivated during anaerobic digestion. Total coliforms and E. coli were removed in the digesters, with a removal efficiency greater than 98% and 90%, respectively.

It has been reported that the relative activity (aerobic respirometry) and viability (Baclight) of active biomass in diluted activated sludge decrease linearly with the ozone dose, reaching a reduction up to 50% at 8 mg O_3/g COD (Labelle et al., 2013). Higher ozone doses, however, are

required to decrease the activity of biomass from concentrated sludge. Zhao et al. (2007) reported a decrease of 50% for an ozone dose 80 mg O_3/g VSS. The ozone action on active biomass can be ascribed to decay rather than to an increase in energy requirements for maintenance or reactivation (Labelle et al., 2013). The impact of ozonation on activity and viability of anaerobic digested sludge has been not determined; thus, more studies are required to obtain a better understanding of the mechanisms of sludge ozonation and its impact on anaerobic digesters.

2.4.4.5 Sludge settling properties and dewatering conditions

The settleability and water content of sludge have been reported to be improved with the increase of ozone dose (Zhao et al., 2007). It has been reported a reduction of SV30% and SVI increasing the ozone dose, with an optimal dose in the range of 0.02–0.06 g O_3/g TSS, the SV30% decreased from 74 to 39 quickly and the SVI also decreased from 110 to 70, above which they decreased gently (Zhao et al., 2007). During ozonation, the sludge flocs become rounder and more compact which also improves the settling properties (Wolff and Hurren, 2006).

Sludge filterability quantified by capillary suction time (CST) is deteriorated by ozone treatment (Weemaes et al., 2000; Zhao et al. 2007). Zhao et al. (2007) shows that the CST changes with the increase of ozone dose, from which it can be seen that the filterability of sludge only slightly deteriorated when the ozone dose was less than 0.04 g O_3/g TSS. Above the dose, the value of CST increased significantly from 17 s of the original sludge to 950 s. But, when the ozone dose was more than 0.2 g O_3/g TSS, the CST value decreased from 950 s to 870 s. The reason may be found in the particle size distribution. With the increase of ozone dose, more and more small particles appeared which could provide a large surface and thus adsorbed a greater amount of water (Zhao et al., 2007). A significant increase in the polymer demand for dewatering is observed after ozonation of sludge at very high ozone dosages up to 0.5 g O_3/g TSS (Scheminski et al., 2000).

For systems combining a biological wastewater process and ozonation, the negative impact of ozonation on dewaterability is minimal (Chu et al., 2009). It has been reported that the average CST of sludge from a combined SBR and ozonation system increased slightly from 5.9 s to 6.2 s (Dytczak et al., 2006).

CHAPTER 3 THESIS ORGANIZATION

This thesis consists of relevant articles and is divided into 9 Chapters and 4 Appendices. The approach and main contributions of each chapter are described below.

- **Chapter 1** is the Introduction chapter. It presents background information, explaining the global context of this thesis, as well as the problem that has motivated the development of this research. In addition, the objectives and the original scientific hypotheses are presented.
- **Chapter 2** presents a Literature review on the main methods used to characterize the organic matter, the anaerobic digestion process, and the main pretreatment processes used for enhancing its performance, focusing on ozone treatment.
- **Chapter 3** presents the approach of the research and the general organization of the thesis.
- **Chapter 4** presents the results of an evaluation and optimization of the ozonation of sludge as a method to improve anaerobic digestion performance in a chemically enhanced primary treatment facility. The impact of ozonation on primary and anaerobic digested sludge, was determined in terms of changes in sludge and supernatant characteristics. It was possible to determine the impact of ozone on physicochemical and biodegradable COD fractionation, solids reduction, mineralization, partial oxidation, size distribution, foaming potential, volatile fatty acids (VFAs), alkalinity, heavy metal solubilisation, and the solubilisation of nitrogen and phosphorus compounds. The coupling of ozonation with anaerobic digesters was also evaluated in a two process configuration: pre-ozonation of primary sludge and post-ozonation of digested sludge both combined with semi-continuous lab-scale anaerobic digesters. This chapter is presented as a scientific article.
- **Chapter 5** presents an evaluation of the impact of ozonation on the methane yield and methane production rate in batch tests, and determines the microbial response of ozonated sludge by monitoring the microbial cell integrity, the metabolism behaviour (key enzyme), the acetoclastic methane activity and the intracellular reactive oxygen species (ROS) formed for various ozone dosages. Furthermore, the extracellular polymeric substances (EPS) are evaluated in terms of proteins and polysaccharides present in soluble-EPS, loosely bound EPS (LB-EPS), and tightly bound EPS (TB-EPS). A potential mechanism of

improving the methane production is proposed to consider the biological response of ozonated sludge and the disintegration of sludge EPS matrix. This chapter is presented as a scientific article.

- **Chapter 6** presents an evaluation and optimization of ozone mass transfer during the treatment of primary sludge and anaerobic digested sludge samples in a venturi loop reactor at the laboratory scale. The ozonation is analyzed regarding the impact of initial COD concentration and initial pH of sludge samples, and operational parameters such as gas-to-liquid ratio (G/L ratio), batch time, and pressure. The effect of these parameters is analyzed in terms of ozone mass transfer efficiency, physical absorption gas-liquid (overall mass transfer coefficient), kinetic behavior, and ozonation performance (biodegradable COD, COD removal, and COD solubilisation). This chapter is presented as a scientific article.
- **Chapter 7** presents an investigation of the technical and economical performances of the anaerobic digestion of sludge subjected to ozonation. Considering the capital and operating cost, as well as the benefits of the effect of ozone on sludge reduction and methane production.
- **Chapter 8** presents a general discussion of the three articles constituting Chapters 4 to 6 and the supplementary information of Chapter 7.
- **Chapter 9** presents the major conclusions of this research and provides Recommendations for future research based on the findings of this study.
- **Appendix A** presents the main protocols of the methods used in this research. **Appendix B** presents the ozone generator and reactor. **Appendix C** presents supplemental information of Articles 1, 2 and 3. **Appendix D** presents a comparison of the results obtained from the ozonation of sludge of a CEPT facility with its impacts (methane yield, production rate, solubilisation and COD reduction) on sludge coming from a conventional activated sludge WRRF (water resource recovery facility).

CHAPTER 4 ARTICLE 1 - OZONATION OF PRIMARY SLUDGE AND DIGESTED SLUDGE TO INCREASE METHANE PRODUCTION IN A CHEMICALLY ENHANCED PRIMARY TREATMENT FACILITY

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ABSTRACT

The purpose of this research was the investigation of the ozonation of sludge as a method to improve anaerobic digestion performance in a chemically enhanced primary treatment facility. Batch tests were conducted to evaluate the effect of ozonation on the physicochemical characteristics of both primary and digested sludge. Then, the performance of semi-continuous anaerobic digesters in combination with ozone treatment was investigated (pre-ozonation and post-ozonation). Ozonation of primary sludge did not increase the soluble COD nor the biodegradable COD, but resulted in the mineralization of a fraction of the organic matter into CO_2. However, the ozonation of anaerobic digested sludge resulted in an increase in soluble COD and biodegradable COD and in a small level of mineralization at the dose of 90 mg O_3/g COD. Pre-ozonation of primary sludge was not effective in enhancing the performance of the anaerobic digester. The coupling of ozonation and anaerobic digestion by means of the post-ozonation of digested sludge was found to be effective in improving methane production (+16%), for COD removal efficiency and for the dewaterability of anaerobic digesters compared to the control digester.

Keywords: Ozone; anaerobic digestion; digested sludge; primary sludge; methane production

4.1 Introduction

Physicochemical and biological processes in wastewater treatment result in the generation of a large amount of sludge. Sludge treatment and disposal represent a major factor in the design and operation of water resource recovery facilities (WRRFs) (Erden et al., 2010). Increasing sludge production, costs of sludge treatment and disposal, and stringent regulations, have resulted in the development of new strategies to reduce sludge production (Wei et al., 2003). Anaerobic digestion is commonly used for sludge reduction and methane production; furthermore, its performance can be improved with mechanical, chemical, thermal and other biological methods (Weemaes et al., 2000).

Chemical oxidation with ozone is one of the preferred chemical treatments, which not only permits sludge reduction, but is also considered to be effective in enhancing methane production via the oxidation and solubilisation of sludge (Weemaes et al., 2000). To enhance the methane production of a chemically enhanced primary treatment (CEPT) facility, ozonation can be applied to primary sludge upstream of the anaerobic digester (pre-ozonation) or in the recirculation loop of the anaerobic digester (post-ozonation). Ozone treatment targets the enhancement of anaerobic digestion processes by altering the physicochemical properties and biodegradability of sludge (Weemaes et al., 2000, Meng et al., 2015). Past studies on the effect of ozone have mainly focused on activated sludge. Limited information about the effect of ozonation on primary sludge (PS) and anaerobic digested sludge (DS) is available. Some studies have investigated the effect of ozone on COD reduction, solubilisation of nutrients, and the impact on methane production potential (Weemaes et al., 2000; Manterola et al., 2008; Meng et al., 2015), but the partial and total oxidation of organic matter as a mechanism of COD reduction and the impact on the biodegradability of COD fractions of sludge samples have been not studied. A better understanding of the effect of ozone on PS and DS will provide valuable information for the design, operation and optimization of pre- and post-ozonation systems for a CEPT facility.

The main objective of this study was the evaluation of the ozonation of sludge as a method to increase the methane production of anaerobic digestion in a CEPT facility. This study first analyzes the impact of ozonation on PS and DS, in terms of changes in sludge and supernatant physicochemical characteristics at varying ozone doses. Second, the coupling of ozonation with anaerobic digesters was evaluated in two process configurations, pre-ozonation of PS and post-

ozonation of DS each combined with semi-continuous lab-scale anaerobic digesters. The effects of ozone dose on methane production, COD removal efficiency, sludge settling properties and dewatering conditions were evaluated.

4.2 Material and methods

4.2.1 Sludge ozonation

Sludge samples were collected from the Repentigny WRRF in Quebec. The plant treats an average flow of 25 000 m^3/d by a CEPT process. The PS evacuated from the settling tanks are treated by mesophilic anaerobic digestion (35 °C) with a hydraulic retention time of 19 days. PS and DS were collected from settling tanks and anaerobic digesters, respectively. Samples were sieved through a 5 mm sieve to remove large debris and stored at 4 °C until use.

Sludge ozonation was accomplished in a 3.8 L column operated in a closed-loop (Appendix B). Ozone gas was injected into the reactor by means of a venturi injector (Model 484X, Mazzei, USA), while the sludge was recirculated with a peristaltic pump at 6 L/min. Ozone was generated from pure oxygen by an ozone generator (Model Peak 2X, Pinnacle, USA) producing 6 L STP/min at 12% by weight. The ozone concentration in the feed gas was measured with an ultraviolet (UV) ozone meter (BMT 964, BMT Messtechnik GmbH, Germany), whereas the amount of ozone in the off-gas was measured with the standard iodometric method (Rakness, 2005). The highest ozone doses required to operate the closed-loop system for a period of 16 and 8.4 min for PS and DS, respectively. Under these conditions, average ozone transfer efficiency was calculated as 68 and 73%, respectively.

4.2.2 Analytical methods

Total solids (TS) and volatile solids (VS) were analysed according to Standard Methods (APHA et al., 2012). Samples were analyzed for total Kjeldahl nitrogen (TKN), orthophosphate (o-PO_4) and total phosphorus (TP) by the QuickChem Method 8500 (Lachat Instruments, USA). Ammonia (was measured by the AmVer™ Salicylate Test 'N Tube™ Method (Hach Method 10031). Nitrate and nitrite were analyzed by Chromotropic Acid Test 'N Tube Method (Hach Method 10020) and USEPA Diazotization Method (HACH Method 10207), respectively. Volatile fatty acids (VFAs) and alkalinity were measured by the titration method based on Lutzhoft et al. (2014). The

ozonation experiments were performed in triplicate for ozone doses between 0 to 220 mg O_3/g COD.

Heavy metals in DS were measured using an inductively coupled plasma optical emission spectrometer (Agilent 7700x, Agilent Technologies, Germany) with sludge and filtered samples (S-Pak 0.45 µm filter, Millipore, USA) being acidified with a solution of hydrochloric acid and nitric acid before measurement. Solubilisation of heavy metals (S_{HV}), was used to represent the release of heavy metal during ozonation (Wan et al., 2014). This was calculated by Eq. (4.1):

$$S_{HV} = (C_{sD0} - C_{sO3})/C_{T0} \quad (4.1)$$

where C_{T0} is the concentration of heavy metals in the sludge before ozonation, C_{sD0} is the concentration of heavy metals in the filtered sample before ozonation and C_{sO3} is the concentration of heavy metals in the filtered sample after ozonation. S_{HV} was calculated for each measured heavy metal as well as for the total amount heavy metals.

The particle size distribution (PSD) was measured by laser granulometry (Mastersizer 3000, Malvern Instruments Ltd., U.K.). The type of particle was considered to be opaque (Fraunhofer approximation) as is recommended for sludge samples (Govoreanu, 2004). The particle distributions were expressed in volume equivalent particles in a range of 0.01 to 3500 µm.

4.2.3 COD fractionation

Chemical oxygen demand (COD) characterization of ozonated samples and controls was performed by a physicochemical separation method and a biodegradability assay, based on Wentzel et al. (1999) and Lu et al. (2010). A control was tested to evaluate the effect of treatment without ozone injection. COD were measured by using HACH methods (HACH Reactor Digestion Method 8000). The physicochemical COD characterization of sludge was classified into three major components: soluble COD (S_{COD}), colloidal COD (C_{COD}) and particulate COD (X_{COD}). Likewise, each of these components was subdivided into biodegradable and non-biodegradable fractions.

Initially, the samples were centrifuged at 2000 g for 2 minutes to remove very large particles (>> 1.2 µm). After centrifugation, the remaining suspension was filtered using Whatman GF (1.2 µm) filters. Then, a portion of the filtered suspension was flocculated with 1 g/L $ZnSO_4$ solution and

the suspension was filtered using 0.10 µm filters (Supor®-100 membrane filter, PALL Life Sciences, USA), based on the method described by Mamais et al. (1993). S_{COD} was measured on the 0.1 filtered samples, the colloidal + soluble COD (CS_{COD}) was defined as the obtained from the 1.2 µm-filtered samples and the total COD was measured on the samples before the initial centrifugation. C_{COD} was determined from the difference between CS_{COD} and S_{COD}. X_{COD} was calculated from the difference between the total COD and CS_{COD}.

Biochemical methane potential (BMP) tests were carried out in triplicate to study the anaerobic biodegradability of samples according to the method of Saha et al. (2011) and Raposo et al. (2011). The biodegradability of the resulting fractions was presented in terms of biodegradable COD and non-biodegradable COD. Batch tests were performed under mesophilic conditions (at 35 °C) in 160 mL glass bottles. The sludge from the mesophilic anaerobic digester from the Repentigny WRRF was used as inoculum for tests. Then, samples from flocculation + filtration, 1.2 µm-filtered sample and total samples were submitted to BMP assays. Biodegradable COD of samples was calculated indirectly from the theoretical methane yield of 350 mL STP CH_4/g COD, considering the conversion of CH_4 to COD. A gas manometer (model DG25, Ashcroft, USA) was used to measure the biogas production. The methane gas content was measured with a gas chromatograph (model GC-456, Bruker, USA) equipped with a thermal conductivity detector (150 °C).

4.2.4 Mineralization and partial oxidation of COD

Total COD, total organic carbon (TOC), TS, and the CO_2 in the off gas of the ozone reactor were analysed to monitor the effect of ozonation on sludge. Before ozonation of sludge samples, the headspace of the ozone reactor was purged with nitrogen gas to avoid any interference with the CO_2 present in the headspace of the reactor. TOC was analyzed with a Total Organic Carbon Analyzer (Dohrmann DC 190, Rosemount Analytical, USA), and the CO_2 in the gas was measured by gas chromatography (model GC-456, Bruker, USA). The following equations were used to determine the percentage of COD decrease triggered by partial oxidation ($\mu_{CODpartox}$, Eq. 4.3) and mineralization of organic matter (μ_{CODmin}, Eq. 4.4) (Carbajo et al., 2007):

$$COD_{partox} = TOC_i \cdot (COD_0/TOC_0) - COD_i \quad (4.2)$$

$$\mu_{CODpartox} = 100 \cdot COD_{partox}/(COD_0 - COD_i) \quad (4.3)$$

$$\mu_{CODmin} = 100 - \mu_{CODpartox} \quad (4.4)$$

where COD_0 and TOC_0 are the total COD and TOC of sample before ozonation; COD_i and TOC_i are the total COD and TOC of sample after ozone treatment.

4.2.5 Evaluation of foaming potential

The foaming potential of samples was determined based on the method of Kougias et al. (2013). The foam formation tendency was evaluated by adding a 50 mL sample to a cylinder that was aerated at an air flow rate of 100 mL/min during 5 min. After the aeration period, the volume of the foam formed was recorded. The foam stability was estimated by stopping the air supply and measuring the remaining foam after 30 min. The foam tendency was defined as the foam volume after aeration (mL) per flow rate of air (mL/min) and the foam stability was defined as the foam volume remaining in the cylinder, 30 min after aeration (mL) per foam volume after aeration (mL). Measurements of foam potential were conducted in duplicate.

4.2.6 Coupling of sludge ozonation and anaerobic digestion

Coupling sludge ozonation with anaerobic digestion was performed in two process configurations. The first one was pre-ozonation of PS in combination with anaerobic digestion while the second configuration was post-ozonation of DS. For each configuration, an anaerobic digester was operated in parallel with a control digester not receiving ozonated sludge.

The lab-scale anaerobic digesters consisted of cylindrical PVC tanks (9.0 L) equipped with a magnetic stirrer. The digesters were operated in semi-batch mode, with manual sampling and feeding. The organic loading rate and the hydraulic retention time were controlled at 1.3 kg $VS \cdot m^{-3} \cdot d^{-1}$ and 19 days, respectively. The temperature and pH of digesters were maintained at 35 °C and 7.0, respectively. Biogas production was measured by a respirometry system operated in anaerobic mode (AER-200, Challenge Technology, USA). Biogas production was periodically collected and its methane content was measured by using a gas chromatograph (model GC-456, Bruker, USA). VS, pH and biogas production were monitored daily.

The start-up of anaerobic digesters was carried out using DS from the Repentigny WRRF. Throughout the course of the start-up and ozone experiments, the digesters were fed with PS. DS samples were collected every 1 to 2 weeks from Repentigny WRRF. The samples were adjusted to 40 g COD/L with distilled water and then stored at 4 °C until use. The reactors were fed with PS at an average flowrate of 420 mL/d.

After stabilization of VS in the sludge effluent, the digesters were operated in a pre-ozonation mode. For this configuration, the first digester was fed with ozonated PS at ozone doses of 5, 25 and 75 mg O_3/g COD, while the second digester was fed with PS without ozone treatment (control digester). Afterwards, both digesters were again stabilized with unozonated PS. Then, the digesters were operated in a post ozonation configuration. Both digesters were fed with PS, but a fraction of DS was withdrawn as the same sample was fed to the digester after the ozone treatment. The recycling rate ($mL \cdot d^{-1}/mL \cdot d^{-1}$) was defined as the ratio between the ozonated flow rate and the influent flow. For each scenario, the ozone dose was applied in the range needed to produce the maximum impact on increasing the biodegradability at recycling rates between 0 and 1.2. The ozonation of sludge was performed two to three times per week and the ozonated sludge was stored at 4 °C.

For each experiment, the digesters were operated until VS stabilization, which took a minimum duration of 1 month, and then the samples of DS, biogas and PS were collected during three consecutive days for analysis. Dewatering and sludge settling properties were also evaluated by means of capillary suction time (CST) (304B CST-meter, Triton Electronics, UK) time to filter (TTF) and sludge volume index (SVI) (APHA et al. (2012).

4.3 Results and discussions

4.3.1 Effect of ozonation on primary sludge and anaerobic digested sludge

4.3.1.1 COD fractionation

The effect of ozonation was evaluated based on the physicochemical and biodegradable characterization of COD. COD fractionation of PS and DS is presented in Figures 4.1a and 4.1b, respectively. More detailed information is presented in Tables C.1 and C.2 (Appendix C). Prior to ozonation, the predominant COD fractions in the PS and DS were particulate COD (95 and 88%,

respectively), whereas the colloidal and soluble fractions were very small. The non-biodegradable fractions of the PS and DS were 38 and 83%, respectively. This large difference of biodegradability between these the two sludges is attributed to the effect of anaerobic digestion degrading a large part of the biodegradable fraction. Ozonation of PS did not significantly increase the soluble COD, and the impact on its biodegradable and non-biodegradable soluble fractions was low. However, ozonation of DS resulted in an increase in sludge solubilization. The observed solubilisation was lower than in previous studies reporting ozonation of activated sludge (Bougrier et al., 2007), which could be caused by the difference in the nature and composition of the organic matter of these samples. The mechanical effect of pumping, evaluated by means of control, showed no significant impact on the solubilisation of organic matter.

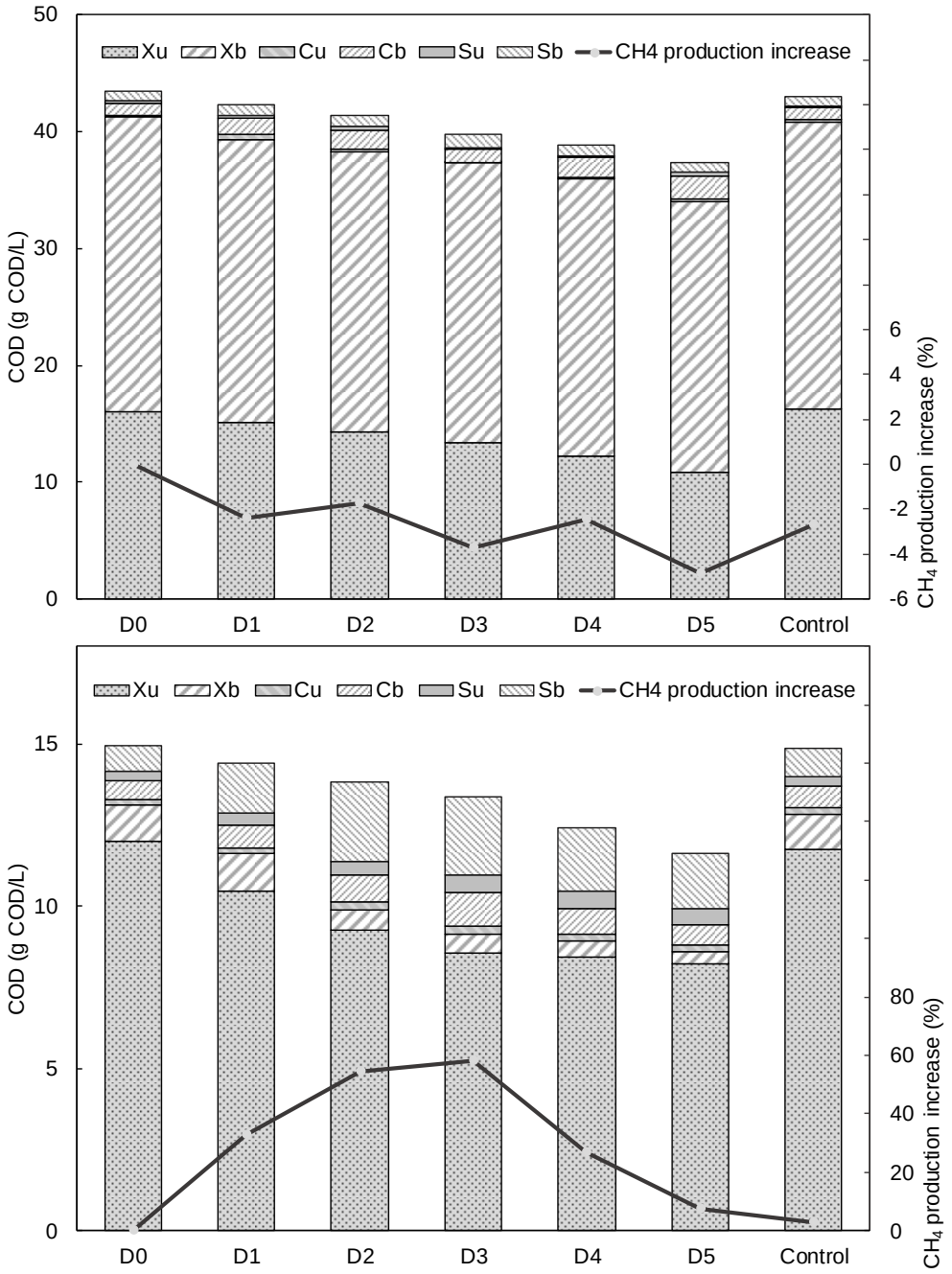

Figure 4.1: Effect of ozonation on COD fractionation of (a) primary sludge (D0 = 0; D1 = 10; D2 = 30; D3 = 50; D4 = 140; D5 = 220 mg O_3/g COD) and (b) anaerobic digested sludge (D0 = 0; D1 = 50; D2 = 90; D3 = 140; D4 = 1740; D5 = 210 mg O_3/g COD). Particulate unbiodegradable COD (X_u); particulate biodegradable COD (X_b); colloidal unbiodegradable COD (C_u); colloidal biodegradable COD (C_b); Soluble unbiodegradable COD (S_u); Soluble biodegradable COD (S_b).

Ozonation is expected to generate soluble organic matter by the oxidation of organic polymers and the release of intracellular compounds due to the damage and lysis of bacteria (Manterola et al., 2008; Meng et al., 2015). The higher release of soluble COD resulting from the ozonation of DS rather than PS can be caused by the higher content of microorganisms in the DS. High biodegradable colloidal COD was also obtained by increasing the ozone dose of PS and DS (Figure 4.1a and 4.1b). However, particulate COD decreased.

The biodegradable COD of DS increased from 2.5 to 3.9 g COD/L for an ozone dose of 90 mg O_3/g COD, representing an increase of methane production of 55% (Figure 4.1b). A similar effect was observed at 140 mg O_3/g COD, but the methane production was not significantly increased compared with the ozone dose of 90 mg O_3/g COD ($p < 0.01$). Its non-biodegradable fraction, however, was reduced from 12.4 to 8.9 g/L at 140 mg O_3/g COD ($p < 0.01$) . The increase of biodegradable COD was lower than the decrease of non-biodegradable COD, which could be ascribed to the mineralization to CO_2 of a fraction of the organic matter, as further discussed below.

4.3.1.2 Mineralization and partial oxidation

Total COD, TOC, $CO_{2(g)}$ and total solids were determined during the ozonation of PS and DS to clarify the impact of ozone oxidation on sludge organic matter (Table 4.1). An increase in ozone dose resulted in a decrease in COD and TOC concentration. The treatment of PS with an ozone dose of 220 mg O_3/g COD achieved a COD decrease of 14% and a TOC decrease of 10%. Similarly, for the treatment of DS at an ozone dose of 210 mg O_3/g COD, the COD and TOC decreased by 22 and 14%, respectively.

Table 4.1: Sludge characteristics before and after ozonation

Sample	Dose	COD	TOC	TS	Carbon mass balance		
					TOC loss	CO_2 gas	Balance
	mg O_3/g COD	g COD/L	g C/L	g/L	mg C	mg C	%
PS	0	43.5	10.0	42.9	0	0	
	10	42.3	10.0	42.8	49	57	118
	30	41.4	9.9	42.5	228	164	72
	50	39.8	9.6	42.0	551	303	55
	140	38.9	9.3	41.2	401	340	85
	220	37.3	9.0	40.2	521	427	82
DS	0	14.9	4.2	18.3	0	0	
	50	14.4	4.2	18.2	32	33	102
	90	13.8	4.2	18.1	76	76	99
	140	13.4	4.0	17.7	283	171	60
	170	12.4	3.9	17.3	245	211	86
	210	11.6	3.6	16.8	357	271	76

The partial oxidation and mineralization efficacy was evaluated at different ozone doses (Figure 4.2a). A high partial oxidation efficiency was achieved for ozone doses below 30 mg O_3/g COD and 90 mg O_3/g COD for PS and DS, respectively. This indicates that for low ozone doses, the COD decrease is not only caused by the mineralization of organic matter but also by the partial oxidation of organic matter into intermediate products. The partial oxidation efficiency was lower for high ozone doses, indicating that a higher fraction of COD decrease is due to the complete oxidation of organic matter into carbon dioxide and water. For the highest ozone doses, the percentage of COD decrease triggered by mineralization was 74 and 67% for PS and DS, respectively.

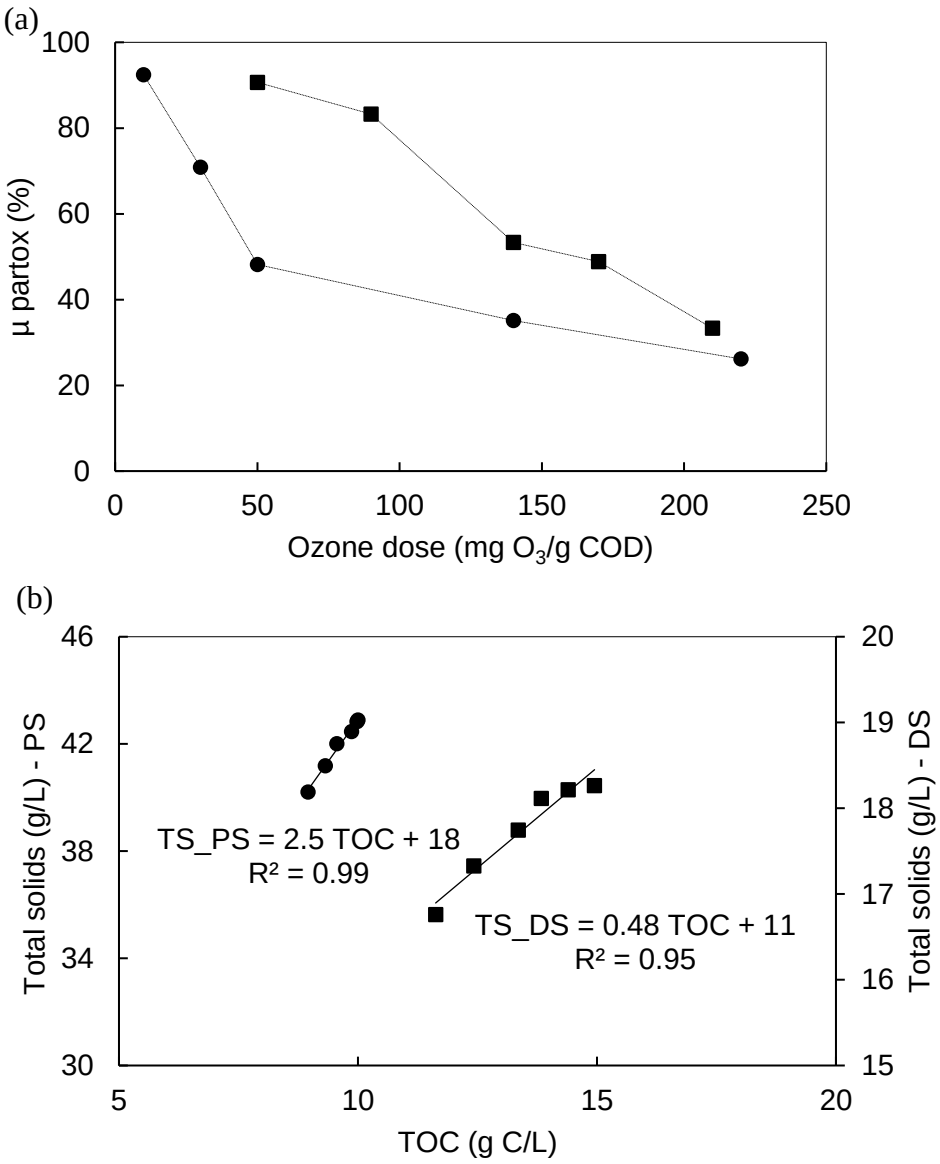

Figure 4.2: (a) Effect of ozone dose on the partial oxidation efficiency. (b) Correlation between total solids and TOC. (●) Primary sludge; (■) Anaerobic digested sludge.

For the ozonation of DS, the maximum biodegradability coincides with a low mineralization of organic matter. The biodegradability of samples was reduced for higher ozone doses (90 mg O_3/g COD), probably caused by the increased mineralization of organic matter. The low performance of ozone to increase the biodegradability of PS may be related to the high mineralization observed with low ozone doses.

High ozone doses decreased significantly the concentration of TOC confirming that the decrease of COD during ozonation is caused in part by the mineralization of organic matter. Furthermore, the increase of CO_2 in the off gas of the ozone reactor and the carbon mass balances further supports this conclusion (Table 4.1). The decrease in TOC during ozonation is consistent with previous studies on the ozonation of activated sludge which suggests that mineralization was the main mechanism of COD decrease (Déléris et al., 2000; Weemaes et al., 2000). However, our study indicates that the COD decrease not only results from mineralization but also from partial organic matter oxidation, especially for low ozone doses.

The sludge evaluation based on TS has shown a slight decrease reaching up to 6 and 8% for ozonation of PS and DS, respectively (Table 4.1). TS and TOC concentrations are strongly correlated following ozonation (Figure 4.2b), indicating that the mineralization of organic matter is the main mechanism of sludge mass reduction during ozone treatment. It has been reported that the ozone is able to oxidize most of the organic matter contained in a sludge, but the complete mineralization of sludge requires unrealistically high ozone doses (Déléris et al., 2000).

During ozonation, the effect of solubilisation of organic matter appears to be most important at medium ozone doses, whereas mineralization of organic matter requires high ozone doses. The main impact of ozonation on DS was the increase of biodegradable COD and soluble COD, as well as the mineralization of organic matter. These parameters could allow the increase in performance and/or capacity of anaerobic digesters, due to the improved degradation of organic matter and the increased methane production.

4.3.1.3 Particle size distribution

The effect of ozone treatment on the particle size of PS and DS is shown in Figures 4.3, C.1 and C.2 (Appendix C). The particle size distribution (PSD) indicated that ozone treatment causes the formation of smaller particles, as confirmed by the decrease in the mean particle sizes (D_v50) for both sludge samples (Figures 4.3a and 4.3b). The ozone treatment of PS resulted in a decrease of D_v50 up to 59% while its control was reduced up to 47%. A similar behavior was observed for ozonated DS and its control with a reduction of D_v50 up to 49 and 39%, respectively.

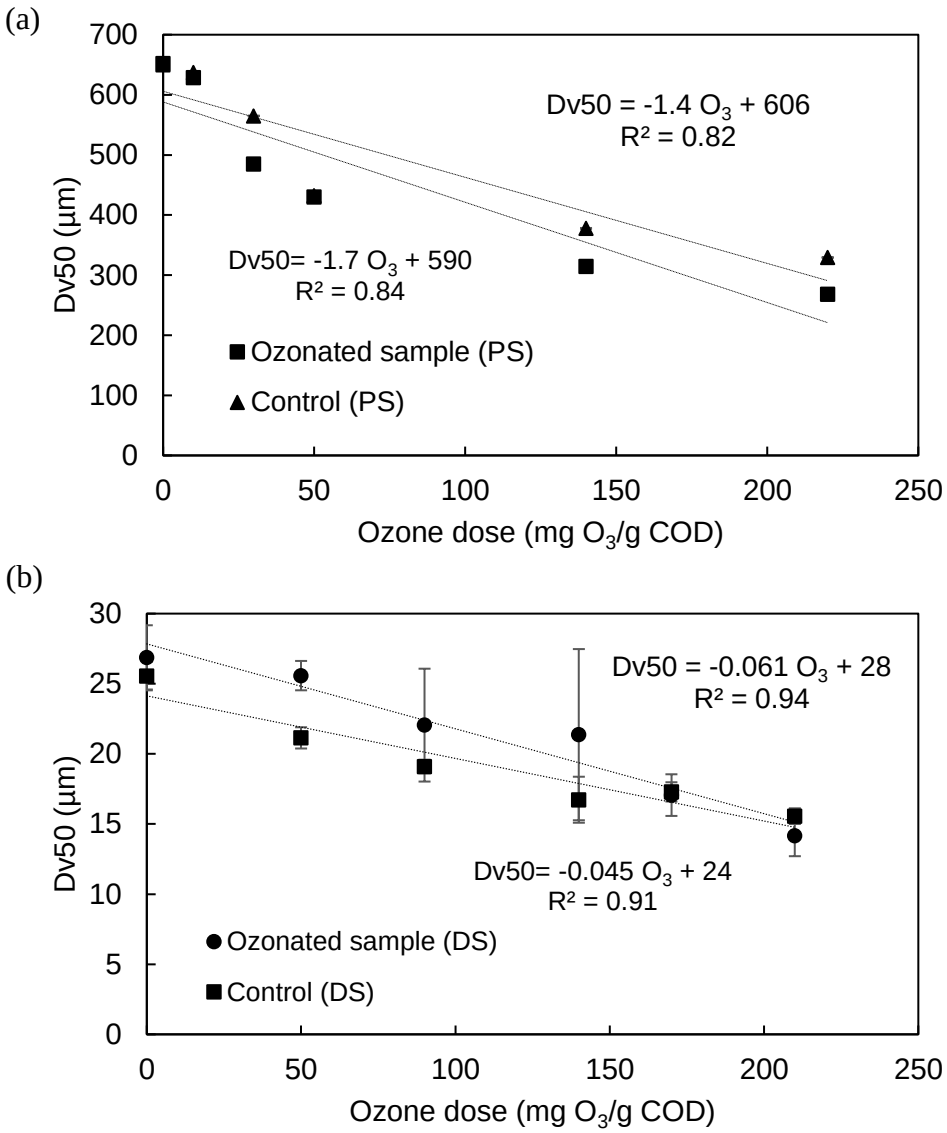

Figure 4.3: Effect of ozone dose on particle size (median Dv50) of (a) primary sludge and (b) anaerobic digested sludge.

During ozone treatment, the samples were subjected to the oxidizing effect of ozone (and free radicals) and the mechanical effect of pumping due to sludge recirculation. Therefore, these results indicate that the reduction of particle sizes during the treatment was greatly influenced by the pumping of sludge and, to a lesser extent, by the action of ozone. The mechanical friction exerted by pumping and recirculation of samples in the ozone reactor likely caused the disaggregation of sludge, a process that has been reported in several mechanical methods using a relatively low energy input (Müller, 2000). It has not been possible to verify the effect of ozone on soluble

molecules using the laser granulometer due to the limitations of the device for measurement as well as the inaccuracies related to the use of Fraunhofer diffraction theory for very small colloids (Govoreanu, 2004). However, as discussed previously, the soluble COD was not increased through the pumping and recirculation of sludge samples (controls); thus, these results suggest that the disaggregation of particles mainly affected the size distribution of larger particles. The solubilisation by cell disintegration requires a large amount of mechanical energy (Müller, 2000). Ozone oxidation causes cell disintegration, releasing intracellular compounds from the microorganisms present in digested sludge, thus, increasing the soluble matter, such as the COD fractionation assays that have been shown for DS.

4.3.2 Effect of ozone on solubilisation of nitrogen and phosphorus

The effect of different ozone doses on nitrogen and phosphorus compounds was evaluated in terms of filterable TKN (CS_{TKN}), ammonia, nitrite, nitrate, filterable phosphorus (CS_P), and orthophosphates.

During ozonation, CS_{TKN} of PS and DS increased significantly from 180 to 200 mg N/L (11%) and from 430 to 570 mg/L (33%), respectively (>200 mg O_3/g COD) (Figure 4.4a). Ozone doses above 50 mg O_3/COD reduced significantly the concentration of ammonia in the PS, reaching a maximum decrease of 40% at 140 mg O_3/g COD, but it was slightly increased at higher ozone doses. Although the ozonation of DS showed an initial decrease in ammonia, its concentration was increased up to 19% ($p = 0.04$) at 210 mg O_3/g COD (Figure 4.4b). The increase in ammonia during the ozonation of DS can be related to the hydrolysis of proteins from the solubilized organic matter (Bougrier et al., 2007; Manterola et al., 2008). Nitrate concentration increased during ozonation (Figure 4.4c) but nitrite was initially oxidized. (Figure 4.4d).

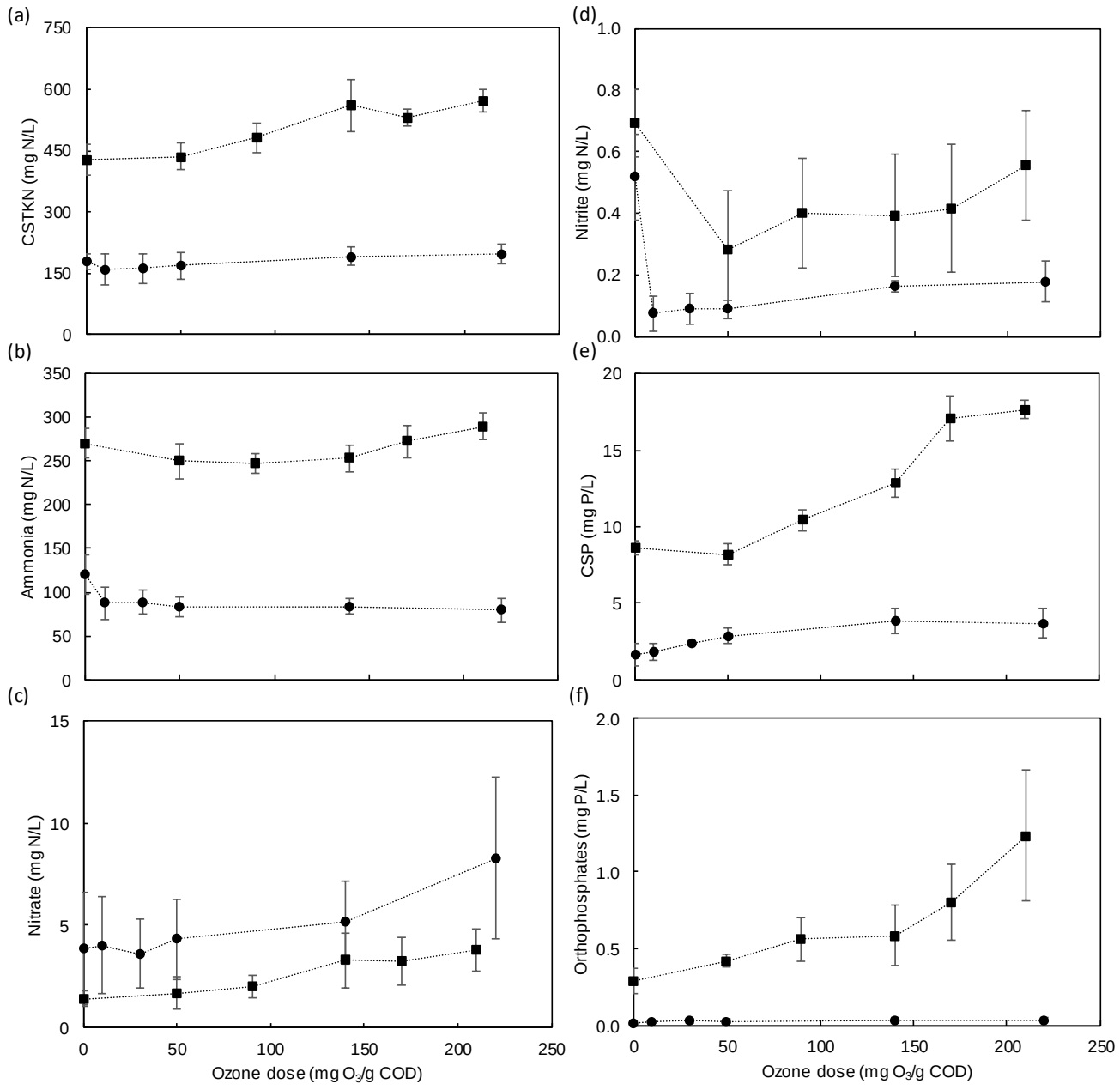

Figure 4.4: Effect of sludge ozonation on nitrogen and phosphorus compounds: (a) filterable TKN, (b) ammonia, (c) nitrate, (d) nitrite, (e) filterable phosphorus and (f) orthophosphates. (●) Primary sludge; (■) Anaerobic digested sludge.

Phosphorus was also solubilized by ozonation. An increase in orthophosphate and total phosphorus in the soluble phase was observed for both sludge samples (Figure 4.4e and 4.4f). This increase in organics and nutrients in the soluble phase can be attributed to the lysis of extracellular polymeric substance of sludge flocs and of sludge cells (Meng et al., 2015). Ozonation of DS resulted in a

rapid increase of organic carbon (soluble COD) and nutrients (nitrogen and phosphorus) in solution.

4.3.3 Effect of ozone on alkalinity, VFAs and pH

The alkalinity of the ozonated PS was reduced from 850 to 460 mg $CaCO_3$/L at an ozone dose of 220 mg O_3/g COD, while the alkalinity of the DS was reduced from 1670 to 660 mg $CaCO_3$/L at an ozone dose of 210 mg O_3/g COD (Figure 4.5a). While the concentration of VFAs of the DS gradually increased during ozonation, the concentration of VFAs of PS decreased, which is consistent with its low solubilisation and high mineralization (Figure 4.5b).

The pH of PS was decreased from 7.1 to 5.2 as ozone doses increased from 0 to 220 mg O_3/g COD as illustrated in Figure 4.5c. The pH of the DS, however, was decreased from 7.4 to 6.9 at ozone doses from 0 to 210 mg O_3/g COD (Figure 4.5c). The decrease in sludge pH and alkalinity may be due to the production acids compounds, such as carboxylic acids and VFAs, caused by the oxidation of organic matter (Bougrier, 2005; Weemaes et al., 2000).

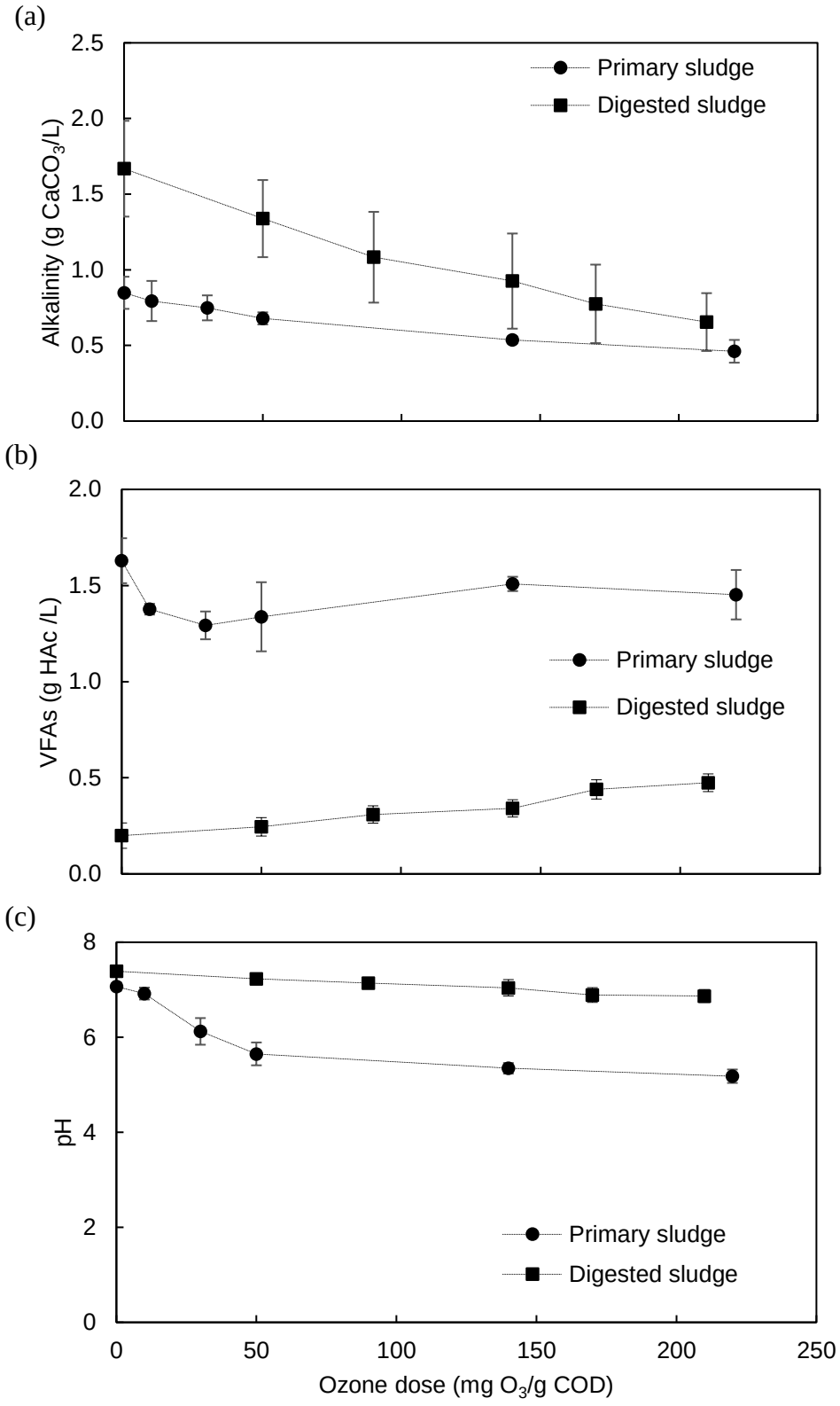

Figure 4.5: Effect of ozonation on (a) alkalinity, (b) VFAs and (c) pH.

4.3.4 Heavy metals solubilisation

The changes in heavy metal concentrations during sludge ozonation of DS are presented in Table 4.2. Sludge showed a high content of Fe due to the use of $Fe_2(SO_4)_3$ as a coagulant for the CEPT process of the Repentigny WRRF. The results showed that heavy metals in sludge were released into solution at an ozone dose of 210 mg O_3/g COD. At this dose, the solubilisation of COD increased to 8.1%, while the solubilisation of total heavy metals only increased 1.0%. Molybdenum and nickel were the heavy metals that were released the most, reaching a solubilisation up to 67 and 22%, respectively. Although the content of iron was high in the sludge, its solubilisation was very low (<1.0%). Iron solubilisation might have been inhibited by the precipitation of $Fe(OH)_3$ caused by the ozone oxidation of dissolved iron (Fe^{+2}).

Table 4.2: Heavy metal solubilisation after sludge ozonation (DS; 210 mg O_3/g COD).

Metals	Sludge (µg/L)	Supernatant (µg/L)		Solubilisation (%)
		Control	Ozonated	
As	32	5.0	5.2	0.63
Cd	5.4	<0.20	0.23	0.6-4.3
Co	95	7.3	13	6.0
Cr	250	2.7	3.2	0.20
Cu	2300	13	34	0.91
Fe	1 900 000	3500	21 000	0.92
Mo	37	8.3	33	67
Ni	260	34	91	22
Pb	130	4	6.8	2.2
Se	<24	2	4.3	10-54
Zn	3800	16	38	0.58

It has been reported that ozonation can release heavy metals from activated sludge due to the decrease in pH which facilitates its mobilization from the particulate matter to the supernatant (Park et al., 2008). Therefore, a possible reason for the low observed impact on the solubilisation of heavy metals during this study could be the low impact of ozone on the pH of DS.

4.3.5 Foaming potential for ozonated samples

The foaming properties of PS and DS at different ozone doses are presented in Figure 4.6. Ozonation did not significantly increase the foaming tendency of PS. Otherwise, the foaming tendency of DS before ozonation was approximately 0.1 mL foam·mL air^{-1}·min^{-1}, but after ozonation, it increased significantly to 7.7 mL foam·mL air^{-1}·min^{-1}, thus, representing an increase of 77 times in foam volume. The foams produced by ozonated PS or DS were not stable and collapsed in less than 10 min once the air supply was stopped. These results are in agreement with the experimental observations that during the ozonation of DS, the foam increased as the operation time increased, consuming the reactor space, but that no significant loss of foam was detected. During ozonation of PS, there was no observed foam accumulation. Apparently, the internal recycle loop of sludge used during ozonation allowed the foaming to be reduced by the mechanical breaking of foam.

The impact of ozonation on foam development has been attributed to the increase of concentrations of surface active agents in sludge supernatant, such as VFAs, proteins, and lipids, which have been recognized as foam-forming agents (Ganidi et al., 2009). The excessive accumulation of foam can complicate the control of a process by consuming reactor space and making inoperative the whole ozonation process (Janknecht et al., 2001). Strategies for enhancing foam reduction could include the dosing of a foam inhibitor (Ganidi et al., 2009).

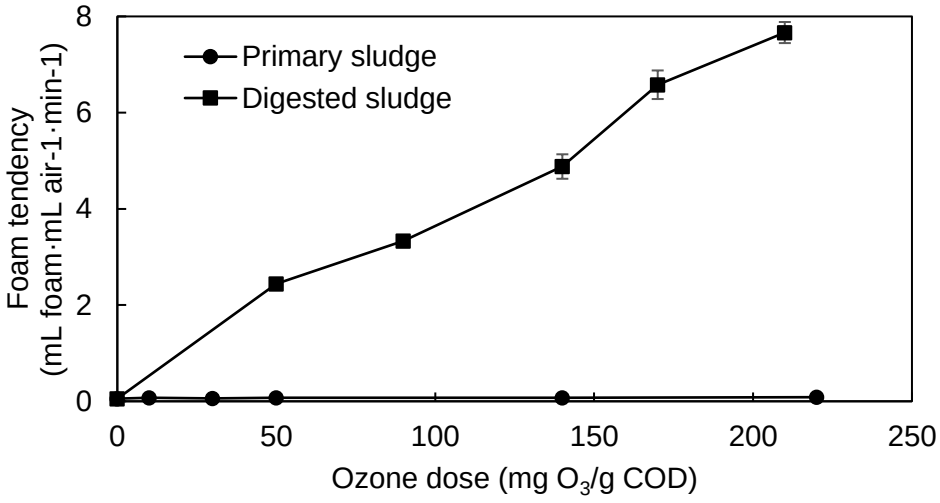

Figure 4.6: Evaluation of foam tendency during ozonation of sludge samples.

4.3.6 Ozone treatment combined with anaerobic digestion

The evaluation of the performance of pre-ozonation showed that the effect of ozonation does not significantly improve the methane production of PS for ozone doses between 0 to 75 mg O_3/g COD (Figure 4.7a). This coincides with the previous results from the COD fractionation of ozonated PS, which showed a limited effect on biodegradability and solubilisation. Higher ozone doses were not evaluated considering the results obtained during the semi-continuous assays, as well as the previous COD fractionation assays that showed an increased mineralization of sludge at higher doses of ozone, and therefore, a probable decrease of anaerobic digester performance.

In terms of the combination of ozonation with an anaerobic digester by means of post-ozonation of DS, ozonation was found to be effective in increasing COD removal leading to subsequent improvements in methane production (Figure 4.7b). The highest methane production was achieved for an ozonated recycling rate of 1.2. The specific methane production increased from 189 to 218 mL N CH_4/g COD fed (+16%, p = 0.00) and the COD removal efficiency was increased from 51 to 59% (p=0.00) with respect to the control digester. A higher recycling rate reduced the enhancement of anaerobic digestion performance, probably due to the increased biomass lysis caused by ozone compared to the growth rate of anaerobic biomass.

The post-ozonation of DS was the most effective configuration to operate with the anaerobic digesters, while the changes due to the pre-ozonation of PS were low. These results are consistent with anaerobic biodegradability tests performed in batch, in which the ozonation of DS produced a more pronounced increase of biodegradability than ozonation of PS. The increase in methane production depends on the initial biodegradability of the sludge with a greater effect on sludge containing a high fraction of non-biodegradable organic matter (Carrère et al., 2010).

A technico-economical evaluation has shown that the sludge ozonation requires greater operating and maintenance costs than the additional benefits from enhanced methane production; the post-ozonation requires approximately 0.15 USD/ kg COD, but these costs are reduced by 30 % due to the additional methane production and sludge reduction (Supplementary information, C.2). Full-scale application of ozone is an expensive alternative for improving anaerobic digester performance. However, a WRRF with available ozone for effluent disinfection could use the excess ozone capacity to improve anaerobic digester performance during winter, considering that the ozonation systems are expected only to operate at 100% capacity under the max flow and the

disinfection requirements during this season are lower. This approach minimizes the capital expenditures, makes ozonation add flexibility for plant operation, as well as enhances the digester performance during this period of year. This alternative could be of interest for chemically enhanced primary treatment plants.

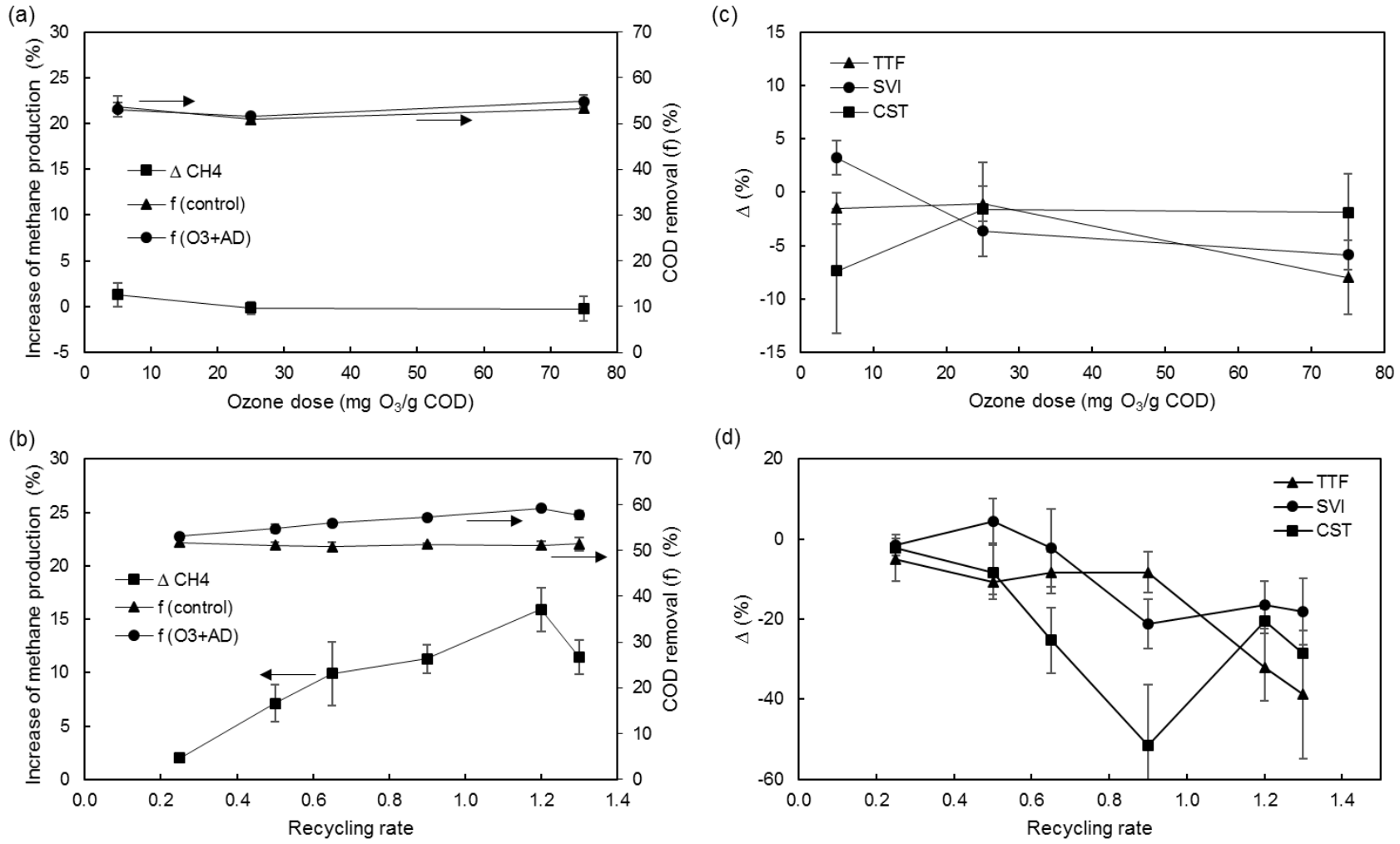

Figure 4.7: Effect of ozonation and anaerobic digestion on methane production, COD removal and change of dewaterability (a, c) pre-ozonation of PS configuration, (b, d) post-ozonation of DS configuration. Recycling rate = ozonated flow rate/influent flow.

4.3.7 Evaluation of digested sludge dewaterability

The effect of ozone treatment combined with anaerobic digestion on sludge filterability and settleability is presented in Figures 4.7c and 4.7d. The pre-ozonation configuration has no significant effect on sludge filterability, in terms of CST and TTF compared with the control digester ($p > 0.18$). Likewise, for this configuration, settleability, measured as SVI, did not significantly change ($p > 0.07$).

Several studies have shown that ozonation deteriorates sludge filterability, which is heavily influenced by the increase of soluble COD (Scheminski et al., 2000; Weemaes et al., 2000). Therefore, the low solubilisation of COD caused by the ozonation of PS could explain the low impact of pre-ozonation on sludge dewaterability.

The post-ozonation configuration improved dewatering characteristics of sludge compared to the control digester: for a recycling rate of 1.2, the CST and SVI were decreased by 20% ($p < 0.01$), and 17% ($p < 0.01$) respectively while the decrease of TTF was not significant ($p = 0.30$). The discrepancy between the TFF and the other indicators can be explained by the high imprecision of measurement methods; however, the trends show an improvement in dewaterability. These results agree in part with those reported in the literature, in which anaerobic digestion was shown to neutralize the negative effect of ozonation on sludge dewaterability (Foladori et al., 2010; Weemaes et al., 2000). However, these results showed a larger effect than expected, possibly due to the high COD solubilisation observed during DS ozonation, and the high biodegradation of solubilized COD during the anaerobic digestion. These results suggest that the post-ozonation configuration could effectively reduce the energy and reagents consumption required for the dewatering process.

4.4 Conclusions

Based on the results of this study, the following conclusions can be drawn:

- Ozonation of primary sludge did not result in an increase in soluble COD while the ozonation of anaerobic digested sludge did, resulting in an increase from 1.1 to 2.9 g COD/L at an ozone dose of 140 mg O_3/g COD.
- Biodegradable COD of primary sludge did not increase following ozonation. However, biodegradable COD of anaerobic digested sludge was increased from 2.5 to 3.9 g COD/L

for ozone doses up to 90 mg O_3/g COD, representing an increase in methane production of 55%.

- Ozonation caused the TOC mineralization of primary sludge and anaerobic digested sludge by 10 and 15%, respectively.
- Post-ozonation of digested sludge was found to be effective for improving methane production (+16%), COD removal efficiencies, and dewaterability of anaerobic digesters compared to the control digester. However, the pre-ozonation of primary sludge was not effective in enhancing the performance of the anaerobic digester.

The above findings provide a better understanding of the impact of ozone treatment in the anaerobic digestion of a chemically enhanced primary treatment.

Acknowledgements

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CHAPTER 5 ARTICLE 2 - EFFECT OF OZONATION ON ANAEROBIC DIGESTION SLUDGE ACTIVITY AND VIABILITY

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ABSTRACT

The effect of ozonation of anaerobic digested sludge on methane production was studied as a means of increasing the capacity of municipal anaerobic digesters. Ozone doses ranging from 0 to 192 mg O_3/g sludge COD were evaluated in batch tests with a bench scale ozonation unit. Ozonation initially, and temporarily, reduced biomass viability and acetoclastic methanogenic activity, resulting in an initial lag phase ranging from 0.8 to 10 days. Following this lag phase, ozonation enhanced methane production with an optimal methane yield attained at 86 mg O_3/g COD. Under these conditions, the yield of methane and the rate of its formation were 52% and 95% higher, respectively, than those factors measured without ozonation. A required optimal ozone dose could be feasible to improve the anaerobic digestion performance by increasing the methane production potential with a minimum impact on microbial activity; thus, an optimal ozone dose would enable an increase in the capacity of anaerobic digesters.

*Keywords: A*naerobic digestion, sludge, ozone, extracellular polymeric substances, mechanisms.

5.1 Introduction

Anaerobic digestion (AD) of primary and secondary sludge is commonly used for sludge reduction, stabilization and energy recovery at municipal water resource recovery facilities (WRRFs) (Appels et al., 2008). Sludge consists of a polymeric network of organic and inorganic compounds; however, its actual composition depends on the source of the sludge (Sheng et al., 2010). The presence of these chemicals, including extracellular polymeric substances (EPS), e.g., polysaccharides, proteins, and lipids, strongly influence the hydrolysis of sludge during anaerobic digestion (Sheng et al., 2010). The hydrolysis of sludge requires long hydraulic retention times (20 to 30 days), leading to moderate degradation efficiencies (30 to 50%) and translating into large volume digesters and high capital expenditures (Foladori et al., 2010a).

Usually, the main factor limiting anaerobic digestion is the hydrolysis of particulate matter. Improving anaerobic digestion through enhancing rate-limiting hydrolysis can increase degradability leading to improve anaerobic digestion performance (Appels et al., 2008). A variety of treatment techniques have been studied to enhance sludge hydrolysis by using thermal, chemical, mechanical and other biological processes (Appels et al., 2008). One of the preferred treatments is ozonation, which permits sludge reduction and is effective in enhancing methane production via the oxidation and solubilization of sludge (Weemaes et al., 2000). Ozonation of activated sludge prior to anaerobic digestion (pre-ozonation) effectively enhances its anaerobic biodegradability, but ozonation is not effective with primary sludge (Carrère et al., 2010). Alternatively, the ozonation of digested sludge in the recirculation loop of the anaerobic digester (post-ozonation) has been shown to produce a significant increase in methane production (Battimelli et al., 2003).

Past studies on the effect of ozone have mainly focused on activated sludge but limited information about the effect of ozonation on anaerobic digested sludge is available. The effect of ozonation differs due to the nature and composition of different sludge samples. The evaluation of the biological response of anaerobic digested sludge to ozonation by monitoring the microbial cell integrity, the metabolism (key enzyme), the acetoclastic methane activity and the production of intracellular reactive oxygen species (ROS) has not been reported. In addition, the changes in the distribution pattern of proteins and polysaccharides among different sludge layers (soluble EPS, bound EPS, pellet) will provide an original and valuable information to understand the potential

mechanisms for improving anaerobic biodegradability through ozonation. A better understanding of the mechanisms of sludge ozonation and its impact on methane production and biological responses will allow for better operational control and design of an anaerobic digestion process integrated with post-ozonation.

The objective of this study was to evaluate the effect of ozonation on the methane production of anaerobic digested sludge, including the mechanisms involved in this process. The specific objectives were to evaluate the impact of ozonation on the methane yield and methane production rate in batch tests, and to evaluate the microbial response of ozonated sludge for various ozone dosages.

5.2 Methods

5.2.1 Sludge ozonation

Anaerobic digested sludge was obtained from the Repentigny WRRF (Quebec), which treats 25 000 m^3/d using a chemically enhanced primary treatment (CEPT) process and stabilizes the sludge in a completely mixed mesophilic (35 °C) anaerobic digester with a hydraulic retention time of 19 days. The collected sludge was passed through a 5 mm sieve to remove large debris and was then stored at 4 °C until further use.

Ozone was generated by a pure oxygen ozone generator (Peak 2X, Pinnacle, USA). Ozonation of digested sludge was performed in a batch reactor. The gas flow rate was 6 L STP/min with an ozone mass concentration of approximately 12% by weight. The transferred ozone dose (mg/L) was calculated from the difference between the mass of ozone transferred (mass fed to the reactor minus the mass in the off gas) divided by the volume of sludge. Ozone dosages were normalized as mg O_3/mg COD by dividing the transferred ozone dosage by the initial total COD content of the sample.

Sludge ozonation was conducted on 2.2-L volumes of digested sludge fed into a 3.8 L column and operated at room temperature. Using a peristaltic pump operating at a flowrate of 6 L/min, the sludge was recirculated through a Venturi (484X, Mazzei, USA) into which ozone was injected continuously. Higher ozone dosages required longer recirculation time. The contact time ranged from 0.0 to 6.1 minutes for ozone doses between 0 to 192 mg O_3/g COD. Sludge samples were

periodically collected during the operation of the ozonation system. Additionally, a control was prepared to evaluate the effect of treatment without ozone injection.

5.2.2 Analytical methods

5.2.2.1 Ozone measurements

The inlet ozone concentration was measured using an ultraviolet ozone meter (BMT 964, BMT Messtechnik GmbH, Germany) while ozone in the off gas was measured using the standard KI method (Rakness, 2005). Dissolved ozone was not measured; it was considered negligible as it was never detected during preliminary tests.

5.2.2.2 EPS extraction and quantification

EPS were extracted from the control and ozonated samples based on the method of EPS extraction of Liu and Fang (2002) and Yu et al. (2008). First, 15 mL of the sample was centrifuged at 2 000 g for 15 min at 4 °C. The supernatant was collected and filtered (S-Pak 0.45 µm filter, Millipore, USA) to measure soluble EPS. The sludge pellet was re-suspended to its original volume using a phosphate buffer saline (PBS) solution supplemented with 90 µL of formaldehyde (36.5% v/v), then incubated at 4 °C for 1 hour under agitation. The suspension was centrifuged at 5 000 g for 15 min at 4 °C and the supernatant was collected and filtered (0.45 µm) for measuring the loosely bound EPS (LB-EPS). The remaining sludge pellet was re-suspended with a PBS solution to its original volume and incubated for 3 hours at 4 °C after the addition of 6 mL of a 1 M NaOH solution. The suspension was then centrifuged at 12 000 g for 15 min at 4 °C, the decanted supernatant contained the tightly bound EPS fraction (TB-EPS). The residual sludge pellet was re-suspended with a PBS solution to its original volume (pellet fraction).

Proteins and polysaccharides were then measured in the samples before extraction and in soluble EPS, LB-EPS, TB-EPS and pellet fraction. The protein content in the samples was determined using the bicinchoninic acid (BAC) method (Pierce© BCA Protein Assay Kit, Thermo Scientific, USA) with bovine serum albumin (BSA) as the standard. The polysaccharide content of the extracts was analyzed using the phenol-sulfuric acid method with glucose as a standard. Proteins and polysaccharides were measured using a microplate reader (Synergy-HT, BioTek, USA). Excitation-emission matrix (EEM) fluorescence spectra were obtained from the extracts using

luminescence spectrometry (RF-5301pc, Shimadzu, Japan). Samples for EEM analysis were diluted to a final COD of 30 mg COD/L with Milli-Q water. The EEM spectra were collected with the scanning emission spectra (Em) from 220 to 550 nm at 1 nm intervals by varying the excitation wavelengths (Ex) from 220 to 400 nm at 10 nm sampling intervals. Excitation and emission slits were set to 5 nm.

5.2.2.3 Biochemical methane potential

Methane yield and acetoclastic activity were evaluated by measuring the biochemical methane potential (BMP) in 160 mL serological bottles incubated at 35 °C based on Saha et al. (2011). A gas manometer (DG25, Ashcroft, USA) was used to measure the biogas production and the methane gas content was quantified with a gas chromatograph (GC-456, Bruker, USA) equipped with a thermal conductivity detector (150 °C). The modified Gompertz model was applied to the cumulative methane production data to determine the maximum methane production rate in the samples (Lay et al., 1996). Methane yield was evaluated without substrate addition, and the acetoclastic activity test was fed with a sodium acetate solution. The methane production was evaluated at the standard temperature and pressure (STP) of 0 °C and 1 atm.

5.2.2.4 Characterization of biological response

Bacterial viability of anaerobic sludge was evaluated using the Live/Dead *Baclight* bacterial viability kit (Molecular Probes, Invitrogen, Kit L13152) and the microplate reader (Synergy-HT, BioTek, USA) using the modified protocol of Chen et al. (2012). The fluorescence intensity of the stained bacterial suspensions (F_{cell}) was determined at an excitation of 488 nm and detection at 635 nm (red) and 530 nm (green), for red-fluorescent nucleic acid stain propidium iodide (PI) and green-fluorescent nucleic acid stain SYTO 9, respectively. The green/red fluorescence ratios ($R_{G/R}$) were used to compare the bacterial inactivation triggered by different doses of ozone. Different proportions of fresh sludge (optimal viable cells) and positive control, inactivated cells with alcohol treatment (2-propanol, 70%), were used as standards. The viability calibration curve was obtained using a linear regression of the green/red fluorescence ratio ($R_{G/R}$) vs the percentage of viable cells.

The dehydrogenase activity was quantified using the protocol described by Von Mersi and Schinner (1991). The technique uses soluble and colorless 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-

phenyl-2H-tetrazolium chloride (INT) reduction to the red insoluble iodonitrotetrazolium formazan (INF) as a tracer of active bacterial electron transport systems (Caravelli et al., 2004). Briefly, triplicate sludge samples (0.5 g) were spiked with 0.75 mL of TRIS buffer (1 M; pH 7.0) and 1 mL of 0.5% INT solution (9.88 mM) and were slightly mixed using a vortex for 30 seconds. After a 2-hour incubation at 40 °C in the dark, the intracellular INF crystals were extracted with a 5 mL ethanol/N,N-dimethylformamide solution (1/1 v/v) and incubated for 1 h at 40 °C in the dark. The concentration of developed formazan in the retained supernatant of sludge was determined using a UV/vis spectrophotometer at 464 nm using the extraction solution, and ethanol/N,N-dimethylformamide solution (1/1 v/v) as reference blank. INT-electron transport system activity was calculated using the modified equation proposed by Yin et al. (2005) (equation 5.1)

$$\text{INT–ETSA} = D_{464} \cdot V / k_i \cdot W \cdot t \qquad (5.1)$$

where INT-ETSA is the INT-electron transport system activity (mg INTF/g biomass/h), D_{464} is the absorbance of the supernatant at 464 nm; V is volume of solvent (mL), k_i is the slope of standard curve of absorbance at 485 nm vs INTF concentration (O.D. mL/mg INTF), W is the weight of biomass (g) and t is the incubation time (h).

ROS was determined using an established fluorescence assay (You et al., 2015). The sludge samples were rinsed three times with a 0.1 M phosphate buffer (pH 7.4) and the pellets were re-suspended in 0.1 M phosphate buffer containing 50 µM dichlorodihydrofluorescein diacetate (H2DCF-DA, Molecular Probes, Invitrogen). The resulting mixture was incubated at 25 ± 1 °C in the dark for 30 min. The generated fluorescent fluorescein DCF was measured using a microplate reader (Synergy-HT, BioTek, USA) at an excitation wavelength of 488 nm and an emission wavelength of 525 nm.

5.2.2.5 Other analytical methods

Chemical oxygen demand (COD) was measured using the HACH method (HACH Reactor Digestion Method 8000). Soluble COD was determined on centrifuged (10 000 g, 10 min) and filtered (S-Pak 0.45 µm filter, Millipore, USA) samples.

The morphologies of blank and ozonated sludge were visualized using a scanning electron microscope (SEM, JEOL JSM7600F). The sample preparation procedure was adapted from Sheng et al. (2011). Sludge sample preparation included the fixation with 2.5% glutaraldehyde in phosphate buffer for 30 min, followed by serial ethanol dehydration. The gold-coated samples were observed using a high-resolution SEM equipped with a field emission gun at a resolution of 1.4 nm at 1 kV and an accelerating voltage of 0.1 to 30 kV.

5.2.3 Statistical analysis

Anaerobic biodegradability tests and EPS extraction were conducted in duplicate, 3D-EEM tests without replication and other analyses were conducted in triplicate. Student's t-test was used to compare the quantitative variables considering a p value < 0.05 to be statistically significant. A nonlinear optimization by least-squares procedure was applied to calculate the maximum methane production by the Modified Gompertz model (Lay et al., 1996).

5.3 Results and discussion

5.3.1 Effect of ozonation on COD solubilisation and mineralization

The impact of ozonation on total COD was shown in Figure 5.1A. During ozonation, the total COD was reduced from 15.0 to 12.3 g COD/L, which was a decrease of approximately 18% at 192 mg O_3/g COD. The decrease of COD by ozonation could be attributed mostly to the complete oxidation of a portion of the organic compounds to CO_2 and water (mineralization); this hypothesis is based on previous studies for ozonation of activated sludge that reported a decrease of total organic carbon (TOC) similar to the reduction of COD and an increase of CO_2 in the residual gas of ozone reactor (Weemaes et al., 2000; Déléris, 2001).

Soluble COD increased significantly from 1.13 to 3.31 g COD/L (157 mg O_3/g COD) during ozonation, representing a solubilization of 15.7% (Figure 5.1A). Higher ozone doses resulted in an apparent decrease in the solubilized COD, possibly due to increased mineralization. Solubilization effects observed in this study are consistent with the study of Weemaes et al. (2000), who reported a 29% increase in COD solubilization of sludge exposed to 200 mg O_3/g COD. A comparison of the efficiency of sludge solubilization and mineralization in different studies is difficult since the performance depends on several factors including ozone injection conditions,

ozone dosage and sludge characteristics (Foladori et al., 2010a). No significant solubilization and COD decrease were observed in the control.

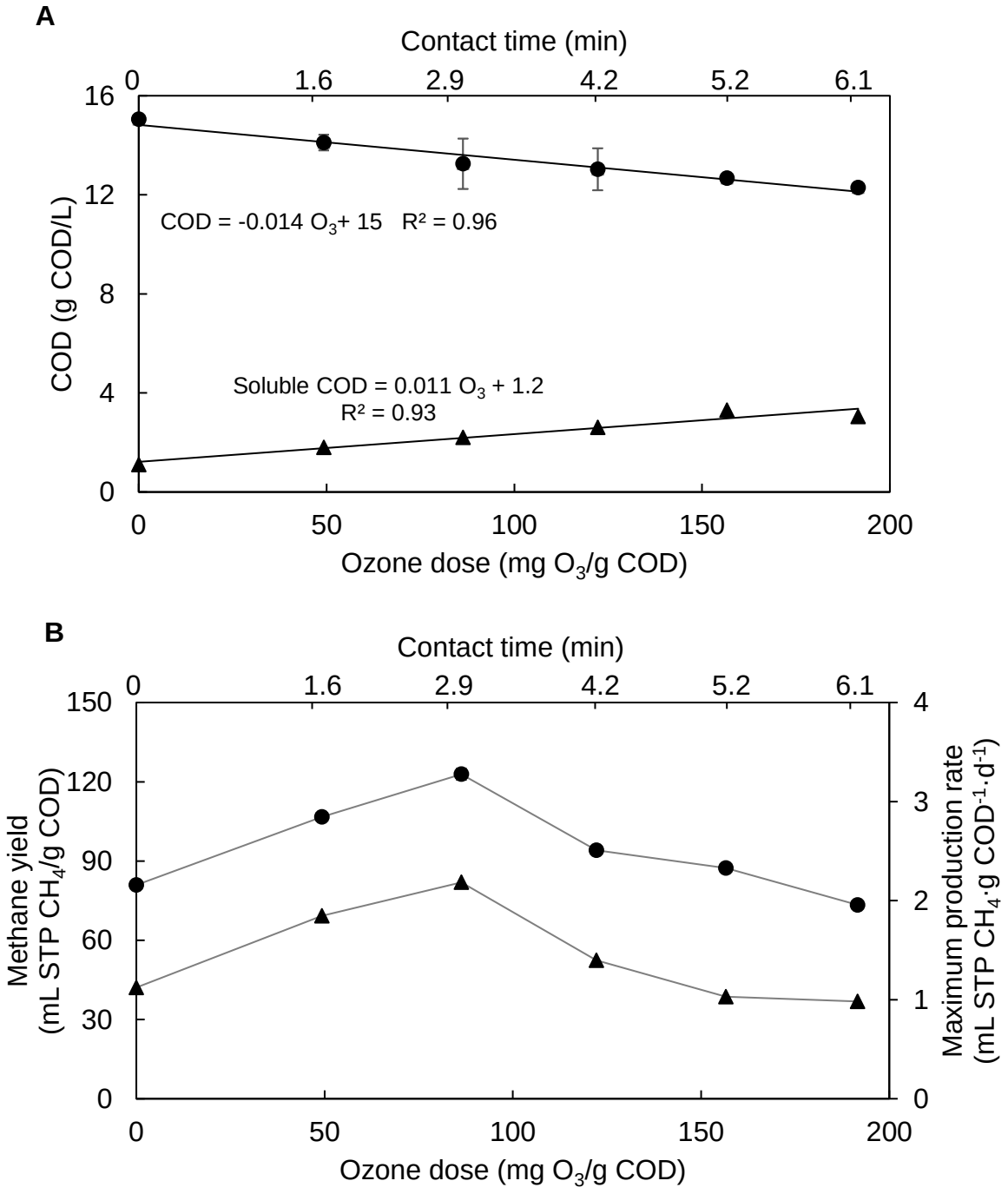

Figure 5.1: Effect of ozone dose and contact time on COD and methane production: (A) total COD (●) and (▲) soluble COD; (B) Methane yield of ozonated sludge (●) and Gompertz maximum production rate (▲).

5.3.2 Effect of ozonation on methane production

The efficiency of ozonation on methane yield was evaluated in BMP assays using ozonated sludges and controls (Figure 5.1B). Ozonation led to a significant increase in methane production and reached a maximum yield of 123 mL STP CH_4/g COD for an ozone dose of 86 mg O_3/g COD. In the absence of ozone, methane production did not exceed 81 mL STP CH_4/g COD. The composition of the biogas was not impacted significantly during ozonation. The average composition of the biogas in both ozonated sludges and controls was 71.3%, 28.6, and 0.05% for CH_4, CO_2 and H_2, respectively. These experimental findings demonstrated that ozonation could increase methane production. Interestingly, using doses of ozone higher than 86 mg O_3/g COD reduced the improvement in methane production. Similar behavior was reported by Weemaes et al. (2000), who found an optimal methane production for an ozone dose of 100 mg O_3/g COD (80%) but also that a higher ozone dose reduced the positive effect on methane production (30%) for activated sludge mixed with primary sludge.

The maximum methane production rate of samples was determined by fitting the cumulative methane production data to the modified Gompertz model (Lay et al., 1996). A good agreement between the experimental data and the modified Gompertz model (R^2>0.95) was obtained. The maximum methane production rate was 2.2 mL STP $CH_4 \cdot g\ COD^{-1} \cdot d^{-1}$ for an ozone dose of 86 mg O_3/g COD, representing an increase of 94.5% relative to the untreated sludge (Figure 5.1B). Ozone doses between 122 to 192 mg O_3/g COD did not significantly change the maximum methane production rate compared to the untreated sample. The maximum methane production rates of the current study are low compared to Weemaes et al. (2000). These authors observed a methane production rate of 4.3 mL STP $CH_4 \cdot g\ COD^{-1} \cdot d^{-1}$ for untreated sludge, while for the optimal ozone dose, the production rate was 9.1 mL STP $CH_4 \cdot g\ COD^{-1} \cdot d^{-1}$. This difference may be due to the type of sludge used. Digested sludge has a low biodegradability since the anaerobic digester has already removed readily biodegradable matter.

Ozonation can induce the release of soluble substances into the aqueous phase, this phenomenon increases the accessibility of compounds to microorganisms, and therefore, improves the anaerobic biodegradability of ozonated samples. The maximum ozone dose tested (192 mg O_3/g COD) reduced methane yield and the methane production rate, probably due to the complete oxidation of solubilized matter caused by the mineralization. Therefore, mineralization should be minimized,

while organic matter solubilization should be maximised to enhance methane production (Weemaes et al. 2000; Carballa et al., 2007).

5.3.3 Effect of ozonation on EPS

The effect of ozonation on the protein and polysaccharide content from different extracted EPS fractions and pellets of anaerobic digested sludge is shown in Figure 5.2A. For the un-ozonated sludge, the total content of proteins and polysaccharides were 6.6 and 1.8 g/L, respectively, with almost 85% of both polymer substances found in the pellet remaining after centrifugation, while the bound EPS and soluble EPS accounted for only 8.6% and 6.2%, respectively. The ratio of proteins and polysaccharides of extracted EPS (soluble EPS and bound EPS) was 1.84, compared with the reported ratios of 1.1 to 2.8 for digested sludge (Morgan et al., 1990).

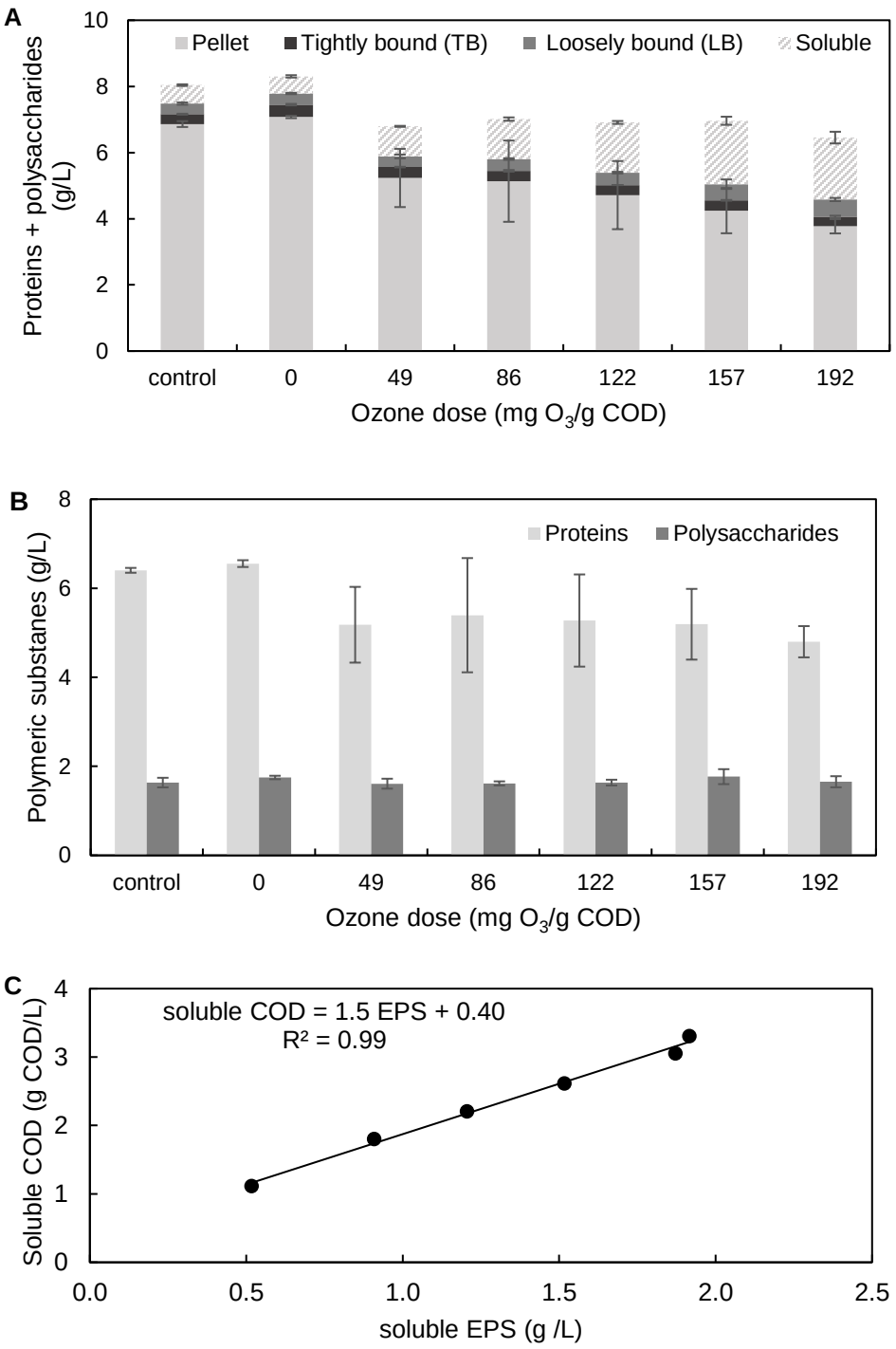

Figure 5.2: (A) Determination and distribution of EPS (proteins and polysaccharides) in extracted EPS fractions and pellet of digested sludge for an ozone dose between 0 to 192 mg O_3/g COD, (B) effect of ozonation on protein and polysaccharide content, and (C) correlation between soluble EPS and soluble COD.

A non-significant change in protein concentration was observed for ozone doses between 0 and 157 mg O_3/g COD (Figure 5.2B). However, the protein content was reduced by 27% for an ozone dose of 192 mg O_3/g COD. Oxidation can cause structural modification of proteins ranging from fragmentation of the polypeptide backbone to aggregation by cross-linking between amino acid residues (Davies, 2005). Furthermore, ozone can oxidize amino acid residues, such as cysteine, tryptophan and tyrosine (Cataldo, 2003; Meng et al., 2016), and these residues should usually be quantified by the BCA method (Wiechelman et al., 1988). However, the by-products of oxidation could not be quantified as proteins.

As for polysaccharides, no significant decrease in content was noted for doses up to 192 mg O_3/g COD (Figure 5.2B). Polysaccharides were reported to react weakly with ozone (Bablon et al., 1991). This result is expected knowing that proteins have more reactive functional groups (-NH_2, -SH, -COOH, amide linkages) than polysaccharides (mostly –OH and ether linkages). Ozonation of β-D-glycosidic linkages in polysaccharides leads to selective depolymerisation into short chain polysaccharides and oligosaccharides (Wang et al., 1999). Using the phenol-sulfuric acid method, these oligosaccharides will be detected as polysaccharides, thereby, the total sugar content will remain constant.

For the pellet residues, measured amounts of proteins and polysaccharides were significantly reduced during ozonation from 7.1 to 3.8 g/L at 192 mg O_3/g COD. The total content of proteins and polysaccharides decreased from 8.3 to 6.5 g/L using an ozone dose of 192 mg O_3/g COD. The TB-EPS, LB-EPS and soluble-EPS content of the sludge changed significantly upon exposure to ozone compared to the non ozonated sample. TB-EPS decreased from 0.37 to 0.29 g/L for an ozone dose of 192 mg O_3/g COD whereas the amount of LB-EPS and soluble-EPS increased linearly from 0.34 to 0.52 g/L (R^2 = 0.71) and 0.52 to 1.9 g/L (R^2 = 0.98), respectively.

Ozonation was found to have a significant effect on the distribution of proteins and polysaccharides in various fractions of the digested sludge. Initially, 85% of proteins and polysaccharides were concentrated in the pellet fraction, but after ozonation 59% remained in the pellet (192 mg O_3/g COD). On the other hand, proteins and polysaccharides in the soluble fraction increased from 6.2 to 29% after ozonation (192 mg O_3/g COD).

During ozonation, the concentration of EPS in the soluble layer increased, while the amount of proteins and polysaccharides from the pellet was reduced as the ozone dose was increased

suggesting that ozonation causes the release of EPS from the inner layer to the outer layer. Protein release to the soluble phase was higher than it was for polysaccharides. The increase in EPS content in the soluble layer correlated with the COD solubilization (Figure 5.2C). These results suggest that ozonation disintegrates sludge flocs and releases COD, proteins and polysaccharides from the pellet into the soluble phase. The control showed that the mechanical friction of the pump did not cause any significant effect on the protein and polysaccharide content and its distribution in the different fractions.

Three-dimensional EEM spectroscopy was applied to characterize the EPS extracted from untreated and treated sludge (192 mg O_3/g COD). Peaks at four different locations were identified according to the literature (Chen et al., 2003). The fluorescence peak positions and fluorescence intensity of the different EPS fractions are detailed in Table 5.1 and Figure C.3 (Appendix C). The peaks were associated with the presence of aromatic amino acids, e.g., tryptophan in proteins (peak A), fulvic acid-like (peak B), soluble microbial by-products-like (peak C) and humic acid-like (peak D). The EEM intensities of peaks tended to decrease after ozonation. Intensity reduction of the fluorescence peaks can be an indication of oxidation and the removal of some of the molecular functionalities responsible for fluorescence. Although protein content increased in soluble EPS and LB-EPS, tryptophan and tyrosine are susceptible to oxidation by ozone, thus, reducing the intensity of fluorescence peaks A and C Figure C.3 (Appendix C).

Table 5.1: Impact of ozonation on peak intensities of the fluorescence spectra for soluble EPS, LB-EPS, and TB-EPS fractions of anaerobic digested sludge (A = tryptophan, B = fulvic acid-like, C = soluble microbial by-products-like, and D=humic acid-like)

EPS fractions	Ozone dose mg O_3/g COD	Peak intensities A	B	C	D
Soluble	0	340	1000	270	880
	192	200	540	140	720
LB-EPS	0	220	440	200	300
	192	67	180	140	180
TB-EPS	0	860	970	910	570
	192	490	590	650	430

5.3.4 Observations of samples by scanning electron microscopy

SEM observations revealed a distinct difference in the morphology of the control and the ozone treated sludge floc (Figure C.4, Appendix C). The untreated sludge samples consisted of smooth, dense and integrated structures, with embedded cells in the sludge matrix. As the ozone dose increased, more irregular porous and rough surface structures were observed in the treated samples. Surface deformation and sludge floc disaggregation were observed in sludge samples treated with a dose higher than 86 mg O_3/g COD. The morphology modification of sludge agrees with the alteration of sludge properties, such as for EPS, which was confirmed by the release of soluble proteins.

5.3.5 Effect of ozonation on viability, enzymatic activity, ROS production and acetoclastic activity of anaerobic sludge

5.3.5.1 Viability and dehydrogenase activity assay

Modified microbial activity of anaerobic sludge following ozonation was characterized by the determination of the biomass viability and the dehydrogenase activity (Figure 5.3A). The primary ozone dose of 49 mg O_3/g COD inhibited the relative viability of cells by 57%. Ozone treatment between 49 and 122 mg O_3/g COD significantly tailed off for the viable biomass with intact membrane, coupled with a higher ratio of inactivated cells. The ozone treatment at doses higher than 157 mg O_3/g COD resulted in significant lysis of biomass with a relative viability of less than 5%. Therefore, significant inactivation of active biomass was observed by ozonation at all tested doses. Membrane integrity defines the potential metabolic activity of the intact cells; therefore, cells with damaged membranes can be classified as permeabilized/dead cells (Foladori et al., 2010b). The influence of ozonation on bacterial viability consists of progressive degradation initiated with the physical alteration of membrane permeability and cell integrity, followed by the lysis reaction (Thanomsub et al., 2002). The bacterial cell membrane is comprised dominantly of lipids with abundant C=C double bonds as well as proteins (Winter et al., 2008; Arts et al., 2015). Ozone is a strong electrophile and thus, can easily react with unsaturated lipids via their nucleophilic –C=C– functionality leading to cellular membrane decomposition and the release of cellular components, including EPS. It has been reported that oxidation of C=C double bonds in lipids forms malondialdehyde (MDA) (Han et al., 2016), causing decomposition of the cellular membranes and, resulting in cell disruption and subsequent leakage of cellular contents (Foladori et al., 2010b).

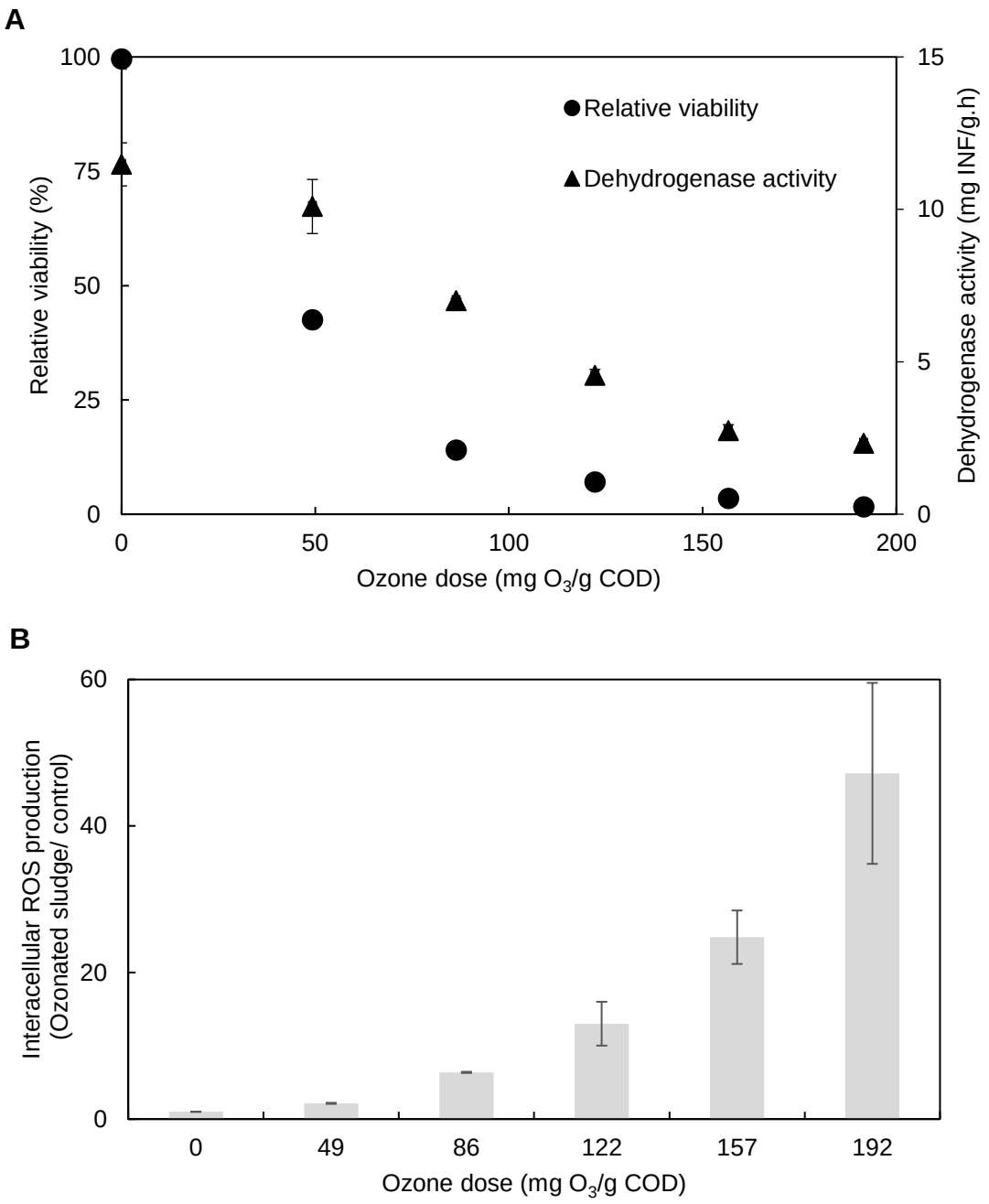

Figure 5.3: Effect of ozone dose on (A) relative viability, dehydrogenase activity, and (B) intracellular ROS production.

5.3.5.2 Intracellular ROS production

Ozonation induced ROS in treated sludge for each ozone dose (Figure 5.3B). Intracellular ROS increased upon an increase in ozone dosage. The ROS concentration was 46 times higher than the control at the highest ozone concentration of 192 mg O_3/g COD. The phenolic and olefinic groups and proteins in the lipid bilayers of the bacterial cell wall were the primary oxidative sites leading to the formation of ROS, such as hydroxyl radicals ($OH^{\cdot}$), peroxides ($RCOO^{\cdot}$) and superoxide radical anions ($O\text{-}O^{\cdot -}$) (Pryor et al., 1991). Subsequent reactions of ROS with cellular components, such as lipids, proteins and nucleic acids, leading to cell disruption and decomposition and causing the release of intracellular components (Baier et al., 2005). Thus, the significantly higher intracellular ROS above 86 mg O_3/g COD confirms the potential of oxidative stress to trigger cell membrane damage and enzyme inhibition for ozonated sludge.

5.3.5.3 Acetoclastic methane activity

The acetoclastic methanogenic activity of sludge was used to determine the effect of ozonation on the anaerobic biodegradability of sludge. The acetoclastic methanogenic yield of control and ozonated sludge are illustrated in Figure 5.4A. Acetoclastic activity after short-term exposure to ozone showed a lag phase, which increased as the ozone dose increased. The initial inhibition of acetoclastic activity was consistent with the significant decrease of dehydrogenase enzymatic activity and loss of intact viable cells measured at the beginning of experiment. Similarly, the complete inhibition of respiratory activity of activated sludge has been reported at 100 mg O_3/g TSS (Chu et al., 2008). However, approximately 95% of the theoretical methane production (350 mL STD CH_4/g COD) was achieved in the samples over 14 days, despite the presence of a lag phase of 0.8 to 10 days in the initiation of activity for all ozonated sludge. Furthermore, the dehydrogenase activity of sludge increased during the incubation (192 mg O_3/g COD) (Figure 5.4B). The cell membrane disintegration, alteration of permeability and interaction of membrane proteins and lipids with ozone can inhibit the acetoclastic activity of sludge. The extension of the activity test, up to 80 days, demonstrated the recovery of microbial activity of ozonated sludge due to the potential recovery of the bacterial community.

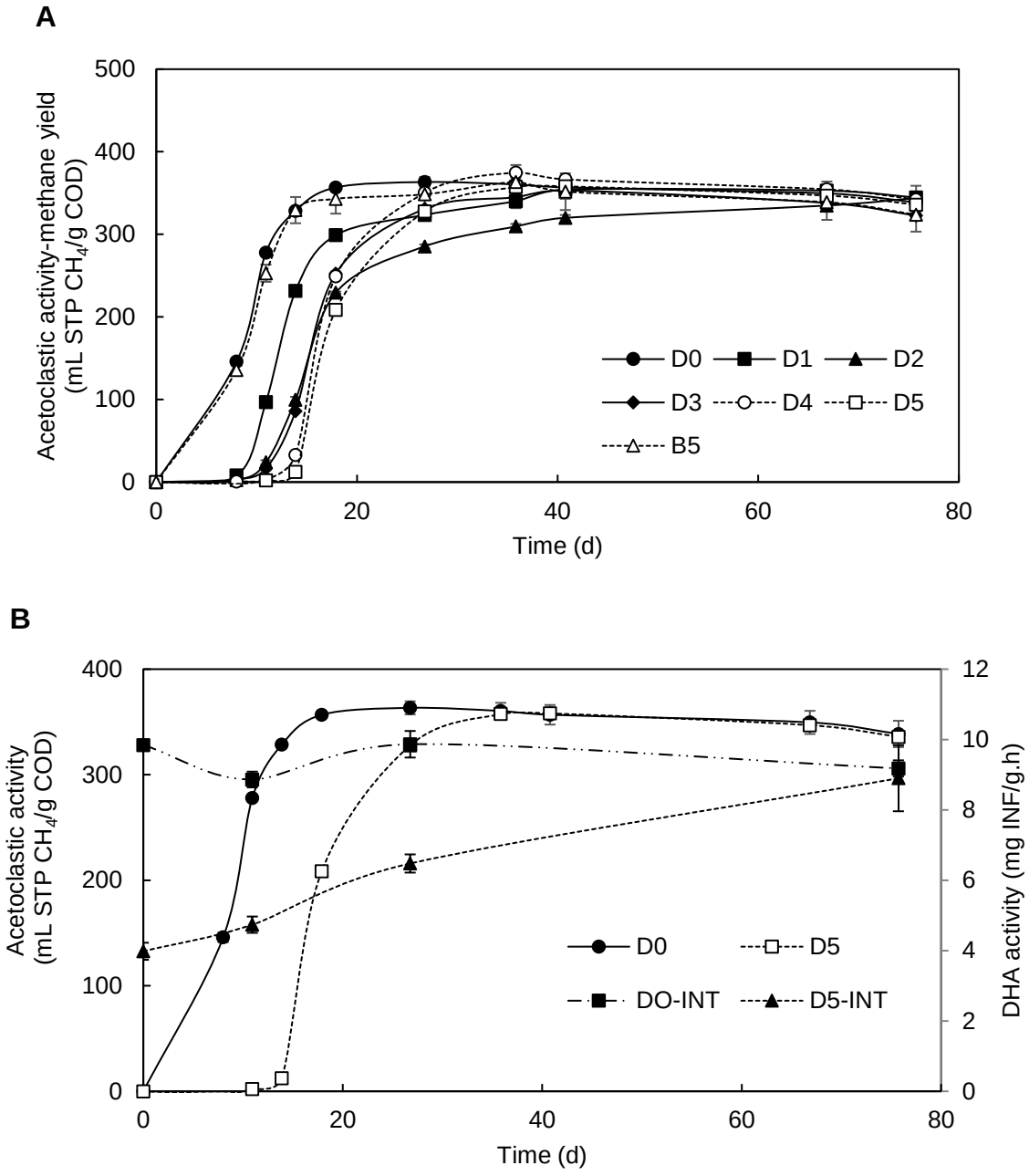

Figure 5.4: (A) Impact of exposure to ozone on acetoclastic methanogenic activity of anaerobic sludge and (B) comparison of dehydrogenase activities and acetoclastic activity for D0 and D5 (D0=0 mg O_3/g COD, D1=49 mg O_3/g COD, D2=86 mg O_3/g COD, D3=122 mg O_3/g COD, D4=157 mg O_3/g COD, D5=192 mg O_3/g COD, B5= control).

5.3.6 Potential mechanisms of improving of anaerobic biodegradability

Ozonation was shown to increase the solubilization of sludge mainly via partial disintegration/solubilization of the sludge matrix and damage to the cell membrane integrity. Ozonation can disintegrate the sludge matrix and release COD, proteins and polysaccharides from the pellet into the soluble phase, thereby promoting the enhancement of methane production during anaerobic digestion. Furthermore, the reduction in the viability of the sample suggests that the broken cells can release intracellular matter into the solution. The enhancement in methane production may not only be ascribed to solubilization but also influenced by the increase of the biodegradability of organic products generated during ozonation, e.g., the products of oxidation by ozone of olefins and aromatic compounds are more biodegradable than their parent compounds (Hübner et al., 2015). As a result of the increase in solubilization and biodegradability, anaerobic degradation can be enhanced, improving methane yield and accelerating digestion time. An overdose of ozone can reduce the methane production potential, probably due to the potential mineralization of the solubilization matter. Additionally, an overdose of ozone can minimize the viability of anaerobic biomass and enzymatic activity which could have a negative impact on the stability of anaerobic digesters in a post-treatment configuration.

An energy analysis has shown that the sludge ozonation requires a greater amount of energy consumed for ozone generation than the additional energy recovered from enhanced methane production; the sludge ozonation requires approximately 1.0 kWh/kg COD (hyp.: 12 kwh/kg O_3) while the additional energy recovered has been estimated to be 0.4 kWh/kg COD at 86 mg O_3/g COD (hyp.: increase of methane production from 86 to 123 mL STP CH_4/g COD and 10 kWh/m^3 CH_4). However, this approach does not consider the positive impact of sludge mineralization on the operational costs. Operating expenditures would be increased by ozone generation but reduced by the increase in methane production and the decrease of sludge handling costs.

5.4 Conclusions

The effect of ozonation on anaerobic digested sludge and its impact on microbial response were evaluated by monitoring methane production, EPS, microbial activity, viability and ROS. The EPS matrix was impacted by ozonation, resulting in the release of COD, proteins and polysaccharides into the soluble phase. Ozonation, initially and temporarily, reduced biomass viability and activity, but following this lag phase, ozonation enhanced methane production. The optimized ozone dose of 86 mg O_3/g COD increased the methane yield up to 52% and the methane production rate up to 95%. Therefore, ozonation could be used to increase the capacity of anaerobic digesters.

Acknowledgements

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CHAPTER 6 ARTICLE 3 - MASS TRANSFER AND KINETICS OF OZONATION OF PRIMARY AND DIGESTED SLUDGES FROM A CHEMICALLY ENHANCED PRIMARY TREATMENT FACILITY

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ABSTRACT

The purpose of this research was to investigate and optimize ozone mass transfer for the treatment of primary and anaerobic digested sludge by means of a lab-scale venturi loop reactor. The effect of reactor operating conditions and the sludge sample characteristics on mass transfer and ozonation performance was determined. A fast absorption regime with a second-order reaction was obtained for the reaction of ozone with the organic matter of both sludge samples. The kinetic rates increased with an increase in the initial organic matter concentration but the pH had minimal effect. The venturi loop reactor was effective in increasing ozone mass transfer efficiency. At atmospheric pressure and temperature of 22 °C, the ozonation of anaerobic digested sludge resulted in an ozone mass transfer efficiency of 98% for a G/L ratio < 0.4. A pressure of 103 kPa and a G/L ratio < 0.2 were required, however, for the effective ozonation of primary sludge (96%). Operating conditions and organic matter content affected the ozone dose effectively transferred which impacted ozonation performance (solubilisation, COD removal and biodegradable COD content).

Keywords: kinetics, mass transfer, ozonation, sludge, venturi injector.

6.1 Introduction

Ozonation has been used mainly in wastewater treatment for disinfection of effluents and for the oxidation of specific contaminants (Rakness, 2005). It has also been used for improving the efficiency of biological units such as anaerobic digesters to increase methane production and reduce sludge generation (Appels et al., 2008). Ozone can disrupt sludge flocs and cells which leads to the release of soluble substrates, the acceleration of hydrolysis and the enhancement of subsequent anaerobic digestion processes (Weemaes et al., 2000). The required ozone dosages for the treatment of sludge are very high compared to typical applications, requiring doses of 0.1 to 0.2 g O_3/g COD in order to enhance anaerobic digestion performance (Weemaes et al., 2000; Bougrier et al., 2007). Therefore, it is essential to utilize ozone efficiently due to the high production costs and the high ozone doses required for sludge treatment.

The ozonation process consists of gas absorption with a chemical reaction in which the total reaction rate can be affected by both the reaction kinetics and mass transfer (Beltran, 2003). The rate of ozone mass transfer depends on several factors such as the characteristics of the aqueous system (e.g. composition, concentrations), the hydrodynamic conditions of the gas–liquid contactor, the kinetics of ozone decay in water, and the number and size of the ozone bubbles produced (Shin et al., 1999; Rosal et al., 2006). Understanding the mass transfer behavior and kinetics will result in improved reactor designs, reactor operation, and modelling tools, which are all important to maximize efficiency and minimize costs. To date, most research on this topic has focused on the reaction kinetics of drinking water and synthetic wastewater with model pollutants (Beltran, 2003) and there is limited information supporting the ozonation kinetics and mass transfer during sludge treatment. Ozonation of sludge is complex due to the presence of a large variety of compounds of unknown nature and concentration, which can negatively impact the efficiency of ozone transfer and its effect on sludge treatment.

Proper selection and design of the contact equipment is essential for the efficient use of ozone in water-ozone systems (Beltran et al., 2001). Various types of dissolution processes, mostly bubble diffusers or venturi type injectors, have been used for transferring ozone to aqueous systems. A dissolution system using venturi-type injectors achieves higher ozone transfer efficiency and requires lower maintenance than conventional fine bubble diffusion (Jackson et al., 2011). In venturi injectors, the ozone is transferred into a water stream under negative pressure, which is

generated in a venturi section, thereby, pulling the ozone into the water stream. The use of a venturi loop reactor may provide an effective and low cost approach for transferring ozone during sludge treatment. The intense mixing occurring in this device may also favor the break-up of aggregates which could potentially lead to improved oxidation. On the other hand, venturi injection could also lead to excessive foaming, an important issue for the control of an industrial process.

In this study, a venturi loop reactor with sludge recirculation was investigated for the effective mass transfer of ozone on the treatment of primary sludge and anaerobic digested sludge produced by a chemically enhanced primary treatment (CEPT) facility. Due to the very high ozone dosages required to oxidize sludge samples, several passes through the venture reactor were needed to achieve the target transferred ozone dosages. Ozonation performance was analyzed in terms of its impact on sludge characteristics (initial COD concentration, pH), and operational parameters such as gas-to-liquid ratio (G/L ratio), batch time, and pressure. Besides the evaluation of kinetic behavior, the effect of these operational parameters on ozone mass transfer efficiency and their impact on ozonation performance was also studied in terms of biodegradable COD, COD removal, and COD solubilisation. Finally, foaming was also monitored of the two types of sludge tested.

6.2 Materials and methods

6.2.1 Sludge ozonation

Sludge samples were obtained from the Repentigny water resource recovery facility (WRRF) in Quebec, which treats 25 000 m^3/d using a CEPT process. Primary sludge and anaerobic digested sludge were collected from settling tanks and mesophilic anaerobic digesters (35 °C), respectively. The collected samples were sieved at 5 mm to remove large debris and then stored at 4 °C until further use. The main characteristics of the primary sludge and the anaerobic digested sludge are summarized in Table 6.1. The effect of initial COD concentration on sludge ozonation was tested through dilutions of these samples with tap water.

Table 6.1: General characteristics of the primary sludge and the anaerobic digested sludge before ozonation.

Parameters	Units	Primary sludge	Digested sludge
COD	g COD/L	40.1	15.4
Soluble COD	g COD/L	1.0	1.2
VS	g/L	24	9.9
Alkalinity	g $CaCO_3$/L	1.0	1.8
pH	-	7.1	7.3

Sludge ozonation was conducted in a 3.8 L column-batch reactor. A peristaltic pump was used to recirculate the sludge which was mixed with ozone gas through a venturi injector (484X, Mazzei, USA). The ozone was generated using a pure oxygen ozone generator (Peak 2X, Pinnacle, USA). Inlet ozone concentration was measured using an on-line ultraviolet ozone meter (BMT 964, BMT Messtechnik GmbH, Germany), while ozone in the off gas was measured using the standard KI method (Rakness, 2005). Residual ozone was analyzed according to the Indigo method (Bader and Hoigné, 1981) on the supernatant of sludge samples which had been centrifuged at 10 000 g for 2 min. In this study, the ozone dose (mg O_3/mg COD) was determined from the mass of ozone fed to the reactor normalized by dividing the initial total COD content of the sample. Ozonation experiments for the evaluation of mass transfer and kinetics were performed in duplicate. To assess the effect of operating conditions on ozone mass transfer, the gas and liquid flow rates were adjusted to meet the required experimental conditions. The ozone gas flow rate was produced at a fixed value of 6 L STP/min with an ozone concentration of 12% by weight. A variable fraction of this flowrate was adjusted with a needle valve and a rotameter prior to the injection in the venturi while the excess gas was sent to an ozone destructor. The experimental setup for the ozone treatment is shown in Figure 6.1.

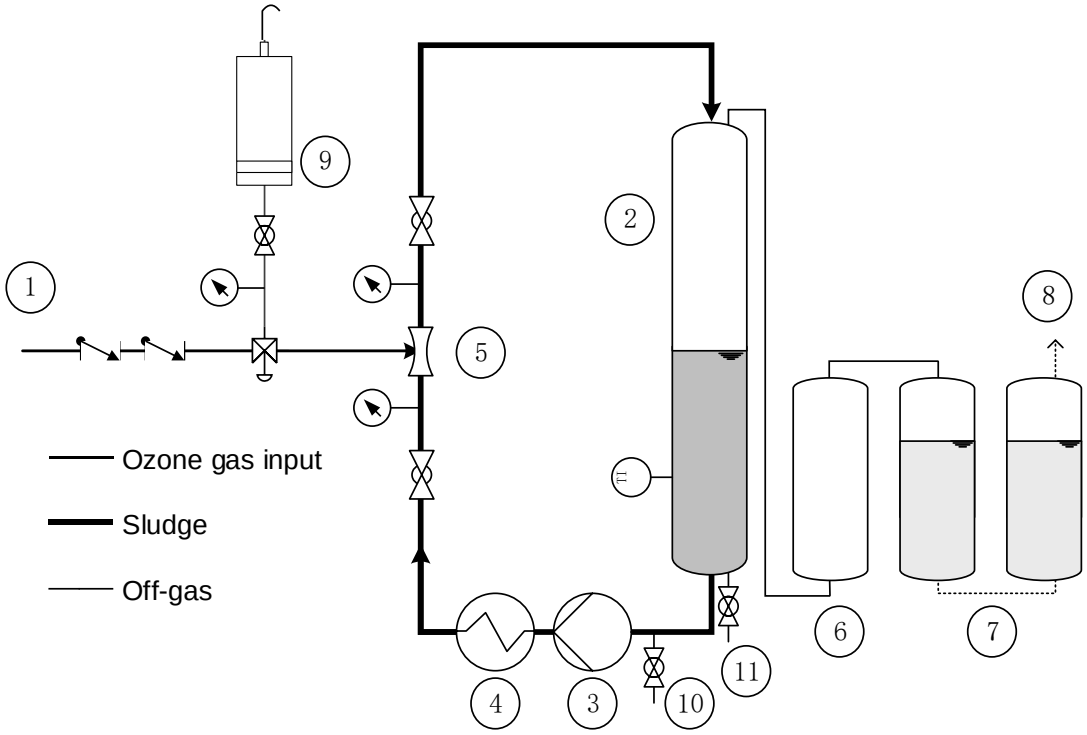

Figure 6.1: Experimental setup used for ozonation of sludge. (1) oxygen and ozone generator, (2) column vessel, (3) peristaltic pump, (4) cooling water, (5) venturi injector, (6) foam trap, (7) KI traps, (8) gas vent, (9) ozone destructor, (10) feed valve and (11) sampling valve.

6.2.2 Mass transfer experiments

Ozone mass transfer was described using the physical mass transfer coefficient (k_La). The k_La coefficients were measured using the same experimental setup described previously, with the addition of a dissolved oxygen probe that was placed 0.10 m above the bottom of the column vessel (LDO dissolved oxygen probe, HQ40d, HACH, USA). The ozone-based k_La coefficient (k_La_{O3}) was calculated indirectly by the measurement of the k_La coefficient for oxygen gas (k_La_{O2}) in clear water and sludge samples. First, the samples were recirculated in the system and the dissolved oxygen was removed from the reactor with nitrogen until the dissolved oxygen concentration fell below 0.5 mg O_2/L. The nitrogen flow was then stopped by keeping recirculation constant, and once the dissolved oxygen attained a steady state, a constant flow rate of air was injected into the samples until saturation with oxygen.

The collected data was analyzed to calculate the k_La coefficients by plotting the dissolved oxygen against the aeration time according to equation 6.1:

$$\ln\left(\frac{C^{*}-C_L}{C^{*}-C_0}\right) = -k_L a \cdot t \qquad (6.1)$$

where C* is the saturated concentration of oxygen in the liquid sample (mg O_2/L), C_L is the concentration of dissolved oxygen in the sample at time t (mg O_2/L), C_0 is the initial concentration of dissolved oxygen (mg O_2/L), and t is the aeration time (s).

The k_La_{O3} coefficient was calculated by using the k_La_{O2} coefficient by applying the following relationship:

$$\frac{k_L a_{O_2}}{k_L a_{O_3}} = \left(\frac{D_{O_2}}{D_{O_3}}\right)^{0.5} \qquad (6.2)$$

where D_{O3} and D_{O2} are the molecular diffusivities of ozone and oxygen gases, respectively, in water (1.76×10^{-9} and 2.50×10^{-9} m^2/s, respectively).

The k_La_{O3} coefficient were obtained at 20 °C by applying the equation 6.3:

$$k_L a_{20°C} = k_L a_T \cdot \theta^{(20-T)} \qquad (6.3)$$

where k_La_T is the k_La coefficient at temperature T, $k_La_{20°C}$ is the k_La coefficient at 20 °C, and θ is a temperature correction factor. The factor θ was determined experimentally for temperature ranging from 10 to 26 °C by using a nonlinear least square regression method.

6.2.3 Ozonation kinetics

To develop the kinetic study, the kinetic regime of ozone absorption must be established. In accordance with the literature on the ozonation of wastewater, a fast absorption regime with a second-order reaction can be initially assumed to calculate the kinetic parameters (Beltran et al., 2001; Lan et al., 2008).

For these conditions, equation 6.4 can be formulated:

$$-\frac{dCOD}{dt} = \frac{a \cdot C^{*}_{O3}}{z}\sqrt{k \cdot D_{O3} \cdot COD} \quad (6.4)$$

where a is the specific interfacial area (m^{-1}), C^{*}_{O3} is the equilibrium ozone concentration (mol O_3/L), k is the kinetic rate constant for ozone-organic matter reaction ($L \cdot mol^{-1} \cdot s^{-1}$), D_{O3} is the ozone diffusivity in liquid phase (m^2/s), z is the stoichiometric ratio for the ozone-organic matter reaction (mol O_3/mol O_2), and COD is the chemical oxygen demand concentration (mol O_2/L).

A detailed description of assumptions leading to equation 6.4 can be found in Beltran et al. (2001). After rearranging and integrating the equation 6.4, equation 6.5 is obtained:

$$\sqrt{COD_0} - \sqrt{COD} = k' \cdot t \quad (6.5)$$

With k' being equal to:

$$k' = \frac{a \cdot C^{*}_{O3}}{2 \cdot z}\sqrt{k \cdot D_{O3}} \quad (6.6)$$

where k_{O3} is the second-order kinetic rate constant for the ozone reaction. The individual liquid-side mass transfer coefficient (k_L) was calculated from Calderbank's equation (Froment and Bischoff, 1979). The specific interfacial area (a) was obtained by dividing k_La by k_L. C^{*}_{O3} was calculated from Henry's law constants, which were taken from Beltran et al. (1995) while the stoichiometric coefficient (z) was obtained experimentally according to the ozone consumption per COD removal for each condition evaluated.

The reaction kinetic coefficients k_{O3} were determined from the measurement of the COD concentrations in the liquid samples during the ozonation for each of the conditions tested. For the estimation of these kinetic coefficients, it was required to determine experimentally the constant k' by means of the slope obtained from the plot of the first term of equation 6.5 vs. ozonation time. The kinetic rate constants were then deduced from equation 6.6.

Once the kinetic rate constants were obtained, the kinetic regime of ozone absorption was determined to verify if the previous assumptions were fulfilled according to the equation 6.7 (Beltran, 2003). For this purpose, it was necessary to evaluate the Hatta number (Ha) (equation

6.8) and the instantaneous enhancement factor, E_i (equation 6.9). The Hatta number indicates the relative importance of the chemical reaction rate versus the physical absorption rate.

$$3 < \mathrm{Ha} < E_i/2 \tag{6.7}$$

$$\mathrm{Ha} = \frac{1}{k_L}\sqrt{k \cdot D_{O3} \cdot COD_0} \tag{6.8}$$

$$E_i = 1 + \frac{D_{OM}}{D_{O3}} \frac{z \cdot COD_0}{C^*_{O3}} \tag{6.9}$$

D_{OM} is the diffusivity of the dissolved organic matter in water. A value of $5 \cdot 10^{-10}$ m^2/s was used as recommended by Beltran (2003).

6.2.4 Analytical methods

The chemical oxygen demand (COD) was measured using a HACH method (HACH Reactor Digestion Method 8000). The sludge samples were centrifuged at 10 000 g for 10 min, and filtered through at 0.45 µm filter (S-Pak, Millipore, USA). Then, soluble COD and alkalinity were analyzed on the filtered samples. Alkalinity and volatile solids (VS) were analysed according to Standard Methods (APHA et al., 2012). These analyses were performed in triplicate.

Biochemical methane potential (BMP) tests were carried out in triplicate to study the anaerobic biodegradability of samples based on the method of Raposo et al. (2011) and Saha et al. (2011). Batch tests were performed under mesophilic conditions (at 35 °C) in 160 mL glass bottles. The sludge from the mesophilic anaerobic digester from the Repentigny WRRF was used as the inoculum for the tests. The biodegradable COD of samples was calculated indirectly from the theoretical methane yield of 350 mL STP CH_4/g COD, considering the conversion of CH_4 to COD. A gas manometer (model DG25, Ashcroft, USA) was used to measure biogas production while the methane gas content was measured with a gas chromatograph (model GC-456, Bruker, USA) equipped with a thermal conductivity detector (150 °C).

6.3 Results and discussions

6.3.1 Volumetric mass transfer coefficient

The volumetric mass-transfer coefficient achieved in the venturi loop reactor was found to be dependent on gas flowrate (Q_G), liquid flow rate (Q_L) and volume of sample (V). For design purposes, these parameters where grouped in terms of gas-liquid flow rates ratio (G/L ratio = Q_G/Q_L) and batch time ($t = V/Q_L$). Attempts were made to correlate the volumetric mass-transfer coefficient obtained under different operating conditions (Table C.3, Appendix C). The following empirical correlation was found to describe k_La:

$$k_L a_{O3} = \beta \cdot (G/L)^{\gamma} \cdot (t)^{\delta} \tag{6.10}$$

where k_La, Q_G, Q_L, and V are expressed in min^{-1}, L/min, L/min, and mL, respectively. Due to the variability of the water temperature, the measured k_La's were corrected for the temperature effect (Equation 6.3) to obtain the corresponding k_La's at 20 °C. A correction factor θ of 1.03 (R^2=0.94) was determined experimentally (Figure 6.2b).

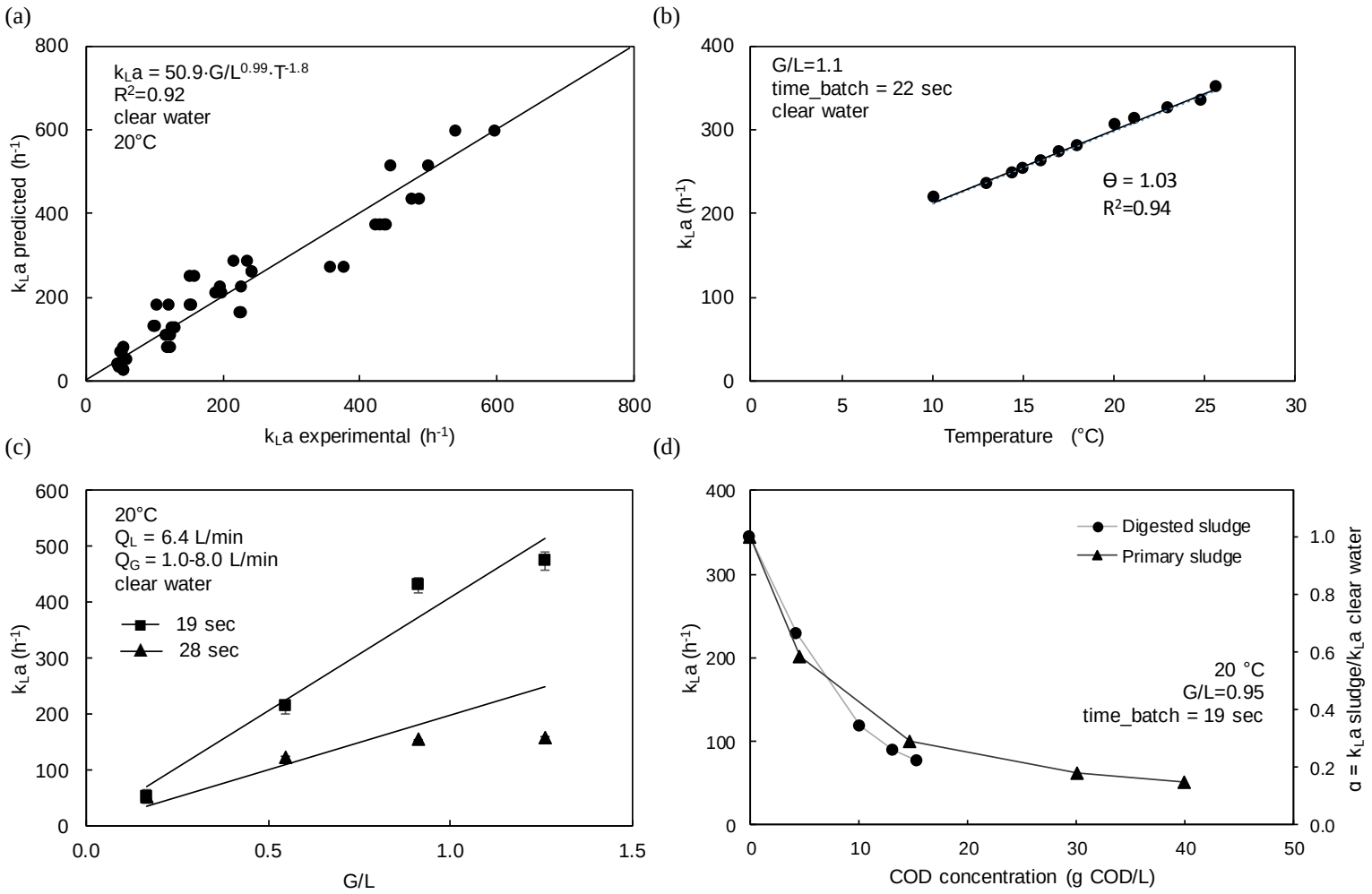

Figure 6.2: Effect of operating conditions and sludge characteristics on k_La (a) Parity plot showing the distribution of experimental versus predicted values of the k_La_{O3} coefficient, (b) Determination of temperature correction factor Θ, (c) Effect of gas-to-liquid flow ratio and batch time on the k_La_{O3} coefficient, (d) Effect of COD concentration on k_La_{O3} and alpha correction factor.

The non-linear regression analysis predicted the constants β, γ, and δ to be 51, 0.99 and -1.8, respectively, with a R^2 value of 0.92. Good agreement between the model predictions and the experimental data was achieved (Figure 6.2a). It was found that the physical mass transfer coefficient varied from 46 to 600 h^{-1} for the range of experimental conditions tested.

The effects of the G/L ratio was evaluated for a batch time of 19 and 28 seconds (Figure 6.2c). Under these conditions, the increase of G/L ratio increased significantly the coefficient k_La. On the contrary the increase of batch time decreased significantly the coefficient k_La. In both cases, the empirical model provided an acceptable description of the experimental data (R^2 = 0.95 and 0.78, respectively). The impact of increasing the G/L ratio was more pronounced at low batch time (low volume of sample for a constant liquid flow rate). For any given pumping rate of liquid, the k_La values increased due to the greater impact of turbulence as the gas flow rate increased (Rosso et al., 2006). The increase of gas flow rate has been reported to improve the specific interfacial area (a), and, therefore, to increase the coefficient k_La (Fadavi and Chisti, 2005).

The effects of COD concentration on k_La and the alpha correction factor were also evaluated (Figure 6.2d). Similar values were observed for both sludge samples, where the coefficient k_La and alpha correction factor were decreased significantly by the increase of COD concentration (organic matter). The alpha factor decreased from a value of 1.00 for clean water down to 0.15 for undiluted primary sludge and 0.22 for undiluted anaerobic digested sludge. The changes in the mass transfer coefficient can be due to the presence of constituents such as surfactants, dissolved solids and suspended solids that can affect the bubble shape and size, resulting in a lower gas transfer efficiency (Metcalf & Eddy - AECOM, 2014). Surfactant accumulation increases the rigidity of the gas-liquid interface and reduces internal gas circulation and overall transfer rate (Rosso & Stenstrom, 2006). These results are in agreement with the alpha factors reported in the literature, which are between 0.2 and greater than 1.0 for wastewater (ASCE & WEF, 1988).

6.3.2 Effect of operating conditions on ozone mass transfer efficiency

The effects of G/L ratio and injector outlet pressure on ozone mass tranfer efficiency are presented in Figure 6.3. The results were obtained using different G/L ratios and two injector outlet pressures. At atmospheric pressure and 22 °C, the ozonation of primary sludge had an ozone mass transfer

efficiency of 89% for a G/L ratio of 0.2, however, its efficiency decreased to 63% for a G/L ratio of 1.0 (Figure 6.3a). Higher ozone mass transfer efficiencies were obtained at 103 kPa in comparison to those at atmospheric conditions. For example, the ozonation performed under a G/L ratio of 0.2 reached an ozone mass transfer efficiency of 96% for an injector outlet pressure of 103 kPa, while at atmospheric pressure, it was 89%.

The anaerobic digested sludge showed high mass transfer efficiencies at low G/L ratios at atmospheric pressure as well as at 103 kPa. Under atmospheric conditions, the ozone mass transfer efficiency was approximately 98% for a G/L ratio between 0.2 to 0.4, while higher values reduced its efficiency to 72% for a G/L ratio of 1.0 (Figure 6.3b). Due to the high efficiencies obtained for anaerobic digested sludge at atmospheric pressure, a pressure of 103 kPa had a negligible impact on the transfer efficiency for G/L ratios compared to those at atmospheric conditions ($p>0.06$), but once G/L was greater than 0.4, the increase in pressure significantly improved the transfer efficiency compared with the test performed at atmospheric pressure ($p<0.04$).

Lower ozone mass transfer efficiencies were obtained for sludge samples with higher initial COD concentrations. The ozone mass transfer efficiency varied from 84 to 63% for primary sludge (5 vs 40 g COD/L), while it decreased from 86 to 72% for anaerobic digested sludge (4 vs 15 g COD/L). Accordingly, higher amounts of ozone were required to reach the same ozone doses effectively transferred for higher COD concentrations. The effect of initial pH was also evaluated in terms of ozone mass transfer efficiency. An increase of pH from 4 to 11 resulted in a slight increase in mass transfer efficiency varying from 63 to 66% for primary sludge, and from 72 to 74% for anaerobic digested sludge, respectively (Figure 6.3d). The impact of initial COD and pH will be analyzed in more detail in section 6.3.3.

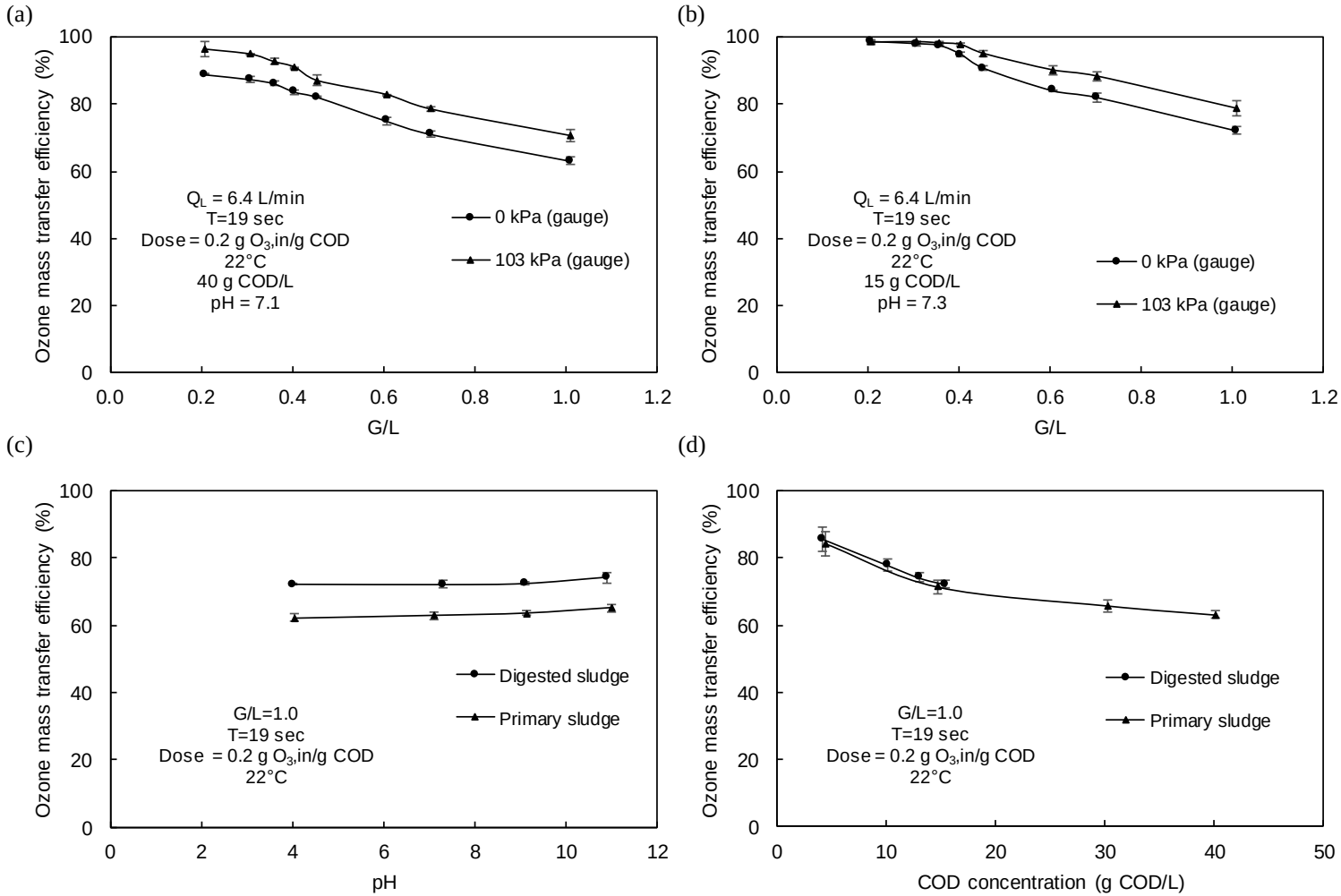

Figure 6.3: Determination of effect of G/L ratio on ozone mass transfer efficiency (a) primary sludge, (b) anaerobic digested sludge. Influence of initial pH (c) and initial COD (d) on ozone mass transfer efficiency.

6.3.3 Impact of ozonation on sludge treatment and kinetics

The impact of initial COD concentration and pH on the increase of biodegradable COD (bCOD), COD removal and COD solubilisation following ozonation of primary sludge and anaerobic digested sludge is illustrated in Figure 6.4. Kinetic rate constants for ozone-COD reaction were also investigated.

6.3.4 Effect of initial COD concentration

The effects of ozone mass transfer efficiency on biodegradable COD formation, COD removal, and COD solubilisation was evaluated for different initial COD concentrations, at constant pH, temperature and ozone dose applied.

The results show that ozonation increased the biodegradable COD concentration of anaerobic digested sludge, whereas it decreased slightly that of primary sludge (Figure 6.4a and 6.4c). The ozonation of high concentrated-digested sludge was determined to be more effective in enhancing biodegradable COD than samples with lower COD content for a fixed ozone dose of 200 mg O_3/g COD (710 mg O_3/g C). In this case, the biodegradable COD increased by 25 and 54% for an initial COD of 4 and 15 g COD/L, respectively. The ozonation of primary sludge resulted in a marginal reduction of bCOD, reaching -4 to -2% for an initial COD of 5 and 40 g COD/L, respectively, for a constant ozone dose of 200 mg O_3/g COD (870 mg O_3/g C).

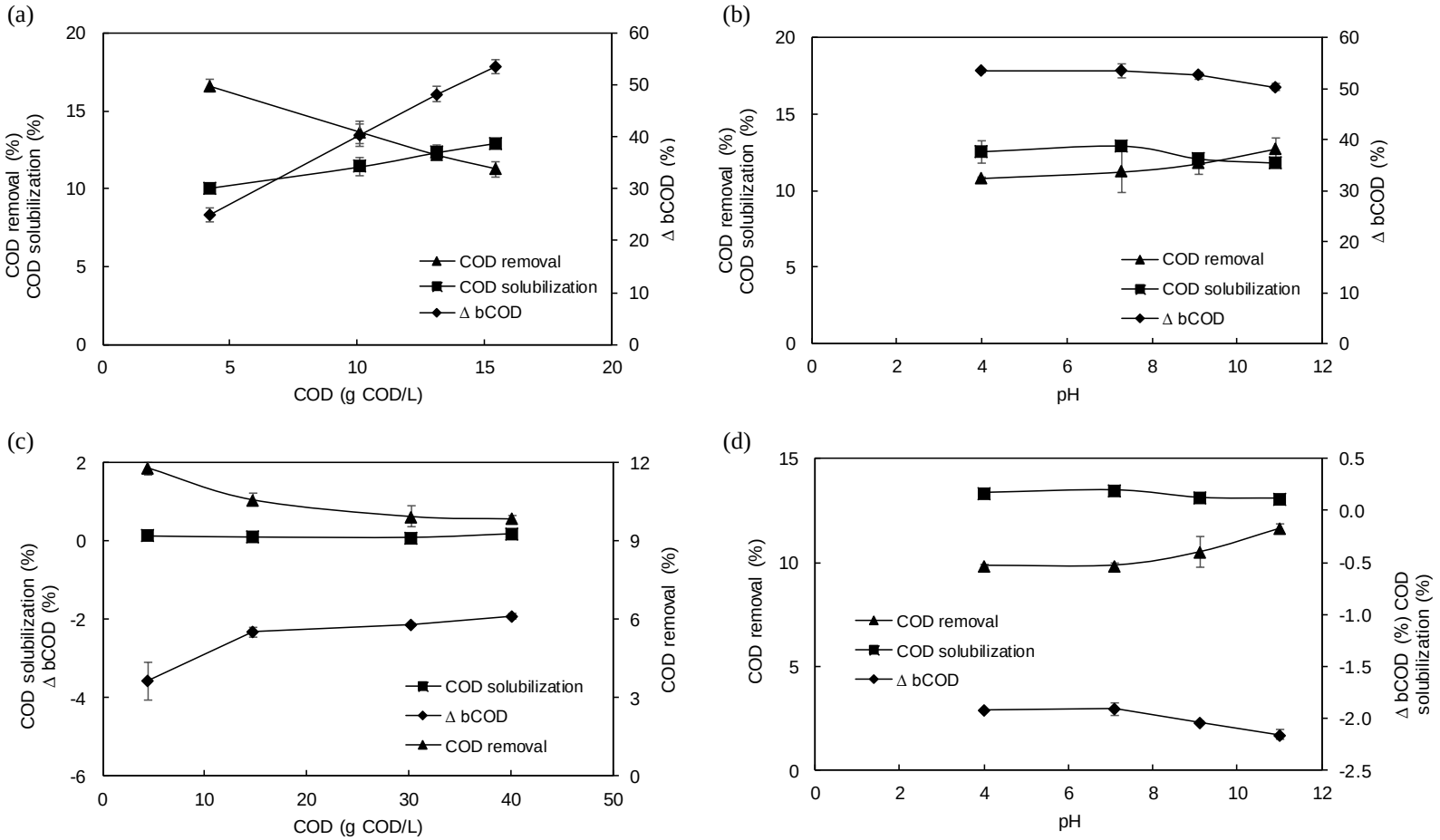

Figure 6.4: Impact of ozonation on sludge treatment performance. Effect of initial COD and pH on the ozonation of anaerobic digested sludge (a,b) and primary sludge (c,d). G/L ratio = 1.0, batch time = 19 sec, Temperature 22 °C and dose = 0.2 g O_3/g COD.

Ozonation increased the COD removal (11-17%) and COD solubilisation (10-13%) of anaerobic digested sludge. A high impact on COD removal was also observed during the ozonation of primary sludge (10-12%) but its effect on COD solubilisation was very low (<0.2%). The ozonation of diluted sludge samples resulted in greater COD removal than undiluted samples but the COD solubilisation was reduced. As previously discussed in section 6.3.2, diluted samples were more effective for transferring ozone than concentrated samples and therefore, the reduction of bCOD and soluble COD of diluted samples can be attributed to the higher transferred ozone dose. It has been reported that the ozone increases soluble and biodegradable COD for an optimal ozone dose, but a very high dose can increase mineralization, decreasing the availability of soluble and biodegradable organic matter (Weemaes et al., 2000). For a fixed dose of 200 mg O_3/g COD, the ozone effectively transferred ranged from 170 to 125 mg O_3/g COD and from 170 to 140 mg O_3/g COD for primary sludge (5 to 40 g COD/L) and digested sludge (4 to 15 g COD/L), respectively.

An increase in COD concentration led to a significant increase on the rate constant (Table 6.2). The COD rate constant for the ozonation of primary sludge varied from 1.2×10^2 to 5.7×10^2 $L \cdot mol^{-1} \cdot s^{-1}$ for an initial COD concentration of 5 to 40 g COD/L, respectively. While the COD rate constant of ozonation of digested sludge increased from 1.0×10^2 to 3.9×10^2 $L \cdot mol^{-1} \cdot s^{-1}$ for an initial COD concentration of 4 to 15 g COD/L, respectively.

Table 6.2: Influence of initial COD concentration on volumetric mass transfer coefficient, kinetic rate constants, Hatta numbers (Ha) and instantaneous enhancement factors in the ozonation of primary sludge (PS) and anaerobic digested sludge (DS).

Sample	COD_0	k_La	k	Ha	Ei/2
	g COD/L	min^{-1}	10^2 L·mol^{-1}·s^{-1}		
PS	40.1	39	5.7	30	295
	30.2	48	4.9	24	231
	14.7	77	3.2	13	102
	4.5	155	1.2	4.5	28
DS	15.4	59	3.9	15	117
	13.1	68	3.5	13	95
	10.1	91	2.6	10	71
	4.2	175	1.0	4.1	29

Dissolved ozone was never detected in the liquid phase of sludge samples. The absence of dissolved ozone, according to the film theory concept, suggests that all ozone reacted in the diffusion film, a situation that could validate that, at least at the preliminary stage, the regime of the reaction was fast (Charpentier, 1981).

As mentioned previously, the Hatta number and the instantaneous enhancement factor must be calculated to corroborate that the kinetic regime follows a fast regime and that the equations used to determine the kinetics constants (section 6.2.3) are valid for the tested scenarios. The Hatta number and instantaneous enhancement factor values obtained for primary sludge and anaerobic digested sludge at different initial COD concentrations are shown in Table 6.2. It can be observed that condition (6.7) is fulfilled in all the experiments, corroborating that the previously assumed absorption-reaction process is fast and second-order with respect to ozone. The Hatta number indicates the relative importance of the chemical reaction rate vs the physical absorption rate. Therefore, as the Hatta numbers were in the range of 4-30, ozonation of sludge samples is strongly influenced by chemical reactions. It should be noted, however, that despite the increase of initial COD concentration resulteing in higher values of reaction rate coefficients, the ozone mass transfer was reduced (section 6.3.2), decreasing the performance of ozonation on COD removal, probably due to the limitation of physical absorption observed in samples with high COD concentrations (section 6.2.1). Organic matter content may impact the efficiency of ozone treatment; therefore, its monitoring is recommended in full scale applications for an effectively application of ozone, controlling costs and the performance of treatment. Operating conditions as G/L ratio and pressure can be controlled for optimize ozone mass transfer depending of sludge characteristics.

6.3.5 Effect of initial pH

Ozonation was examined to determine the effect of pH on ozone treatment at fixed initial COD concentration, temperature and ozonation time (Figure 6.4b and 6.4d). The differences in COD removal efficiencies between low and high pH conditions were marginal for sludge samples. The COD removal efficiencies at pH 4 or 9 were not significantly different ($p>0.13$) compared with samples treated at neutral pH (~7.0). However, a slight increase of COD removal was observed at pH 11 ($p<0.05$). On the other hand, biodegradable COD formation and COD solubilisation were not significantly impacted by ozonation performed at pH from 4 to 11. A kinetic study of COD removal for primary sludge and anaerobic digested sludge as a function of initial pH was also

investigated (Table 6.3). The second-order reaction rate constant of COD removal varied between $5.6 \cdot \times 10^2$ to $6.0 \cdot \times 10^2$ $L \cdot mol^{-1} \cdot s^{-1}$ and 3.9 to 4.1×10^2 $L \cdot mol^{-1} \cdot s^{-1}$ (pHs 4 to 11), for primary sludge and anaerobic digested sludge, respectively. Generally, in water treatment an increase in pH leads to accelerated decay of dissolved ozone and higher free radical activity. However, in our case, the low impact of the effect of pH is most likely related to the fact that the ozone action on sludge results from the direct action of molecular ozone in the liquid/gas film. In addition, both sludge types had significant alkalinities which provided an important free radical scavenging potential (Beltran, 2003; Gottschalk et al., 2009). Finally, these results are in agreement with those obtained in a previous study where the evaluation of pH effect (pH 4 to 9) of ozone performance for COD removal did not show any influence for a buffered domestic wastewater (Beltran, 2003).

Table 6.3: Influence of initial pH on kinetic rate constants, Hatta numbers (Ha) and instantaneous enhancement factors (Ei) in the ozonation of primary sludge (PS) and anaerobic digested sludge (DS).

Sample	**pH**	**k**	**Ha**	**Ei/2**
		10^2 $L \cdot mol^{-1} \cdot s^{-1}$		
PS	4.0	5.6	30	291
	7.1	5.7	30	295
	9.1	6.0	31	304
	11	6.0	30	302
DS	4.0	3.9	15	119
	7.3	3.9	15	117
	9.1	3.8	15	115
	11	4.1	16	115

6.3.6 Foam production monitoring

Foaming production was effectively controlled during ozonation performed using the venturi loop reactor. Despite the fact that ozonation of anaerobic digested sludge resulted in high foam production, a low, or zero, foam loss was detected in all tested conditions. High foaming potential of anaerobic digested sludge could be attributed to the increase of foam-forming agents as VFA, proteins and lipids solubilized during ozonation. On the other hand, ozonation of primary sludge resulted in low foaming production, coinciding with its low solubilisation. Apparently, the

pumping and internal recycle loop of sludge used during ozonation allowed the reduction of foam by mechanical breaking.

6.4 Conclusions

The effect of sludge characteristics and operating conditions on ozone mass transfer efficiency and ozonation performance were evaluated by means of a laboratory scale-venturi loop reactor. Based on these results, the following conclusions can be drawn:

- k_La and alpha correction factor depend on the COD concentration of samples. The alpha factor diminished from a value of 1.00 for clean water down to 0.15 for undiluted primary sludge and 0.22 for undiluted anaerobic digested sludge.
- A fast absorption regime with a second-order reaction was observed for the reaction of ozone with COD for both sludge samples. The kinetics increased with increasing the initial COD concentration; however, a low impact of pH was observed, suggesting a marginal role of free radicals for COD removal.
- The ozonation of digested sludge resulted in significant COD solubilisation (10-13%) and biodegradable COD formation (25-54%); however, a reduction in biodegradable COD formation (2-4 %) and low COD solubilisation was obtained for primary sludge. A high COD removal was obtained for both sludge samples (10-17%).
- The venturi loop reactor was effective in increasing ozone mass transfer efficiency. It was found that the ozone mass transfer efficiency is dependent on the gas-liquid ratio and operating pressure. At atmospheric pressure and 22 °C, the ozonation of digested sludge resulted in an ozone mass transfer efficiency of 98% for a G/L ratio < 0.4. However, a pressure of 103 kPa and a G/L ratio < 0.2 were required for the effective ozone transfer on primary sludge (96%).

Acknowledgements

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CHAPTER 7 TECHNICO-ECONOMICAL ANALYSIS

7.1 Introduction

This chapter presents a technico-economical analysis of a full-scale application of sludge ozonation at the Repentigny WRRF via pre- or post-anaerobic digestion treatment. These configurations were evaluated separately for a chemically enhanced primary treatment (CEPT), which corresponds to the actual treatment train of Repentigny WRRF (Figure 7.1), and for a CEPT integrated with a moving bed biofilm reactor (MBBR) as secondary treatment (Figure 7.2). This second train was evaluated, because starting in 2022, Repentigny will have to meet a 25 mg/L $CBOD_5$ effluent limit to meet the requirements of the "Règlement sur les ouvrages municipaux d'assainissement des eaux usées" (MDDELCC, 2016).

The different treatment trains evaluated are presented in Table 7.1. Results and assumptions obtained from the technical evaluation of ozone treatment will form the basis for the cost estimation of the full-scale application. The results and methodology for performing the technical and economic evaluations of ozone treatment are presented below.

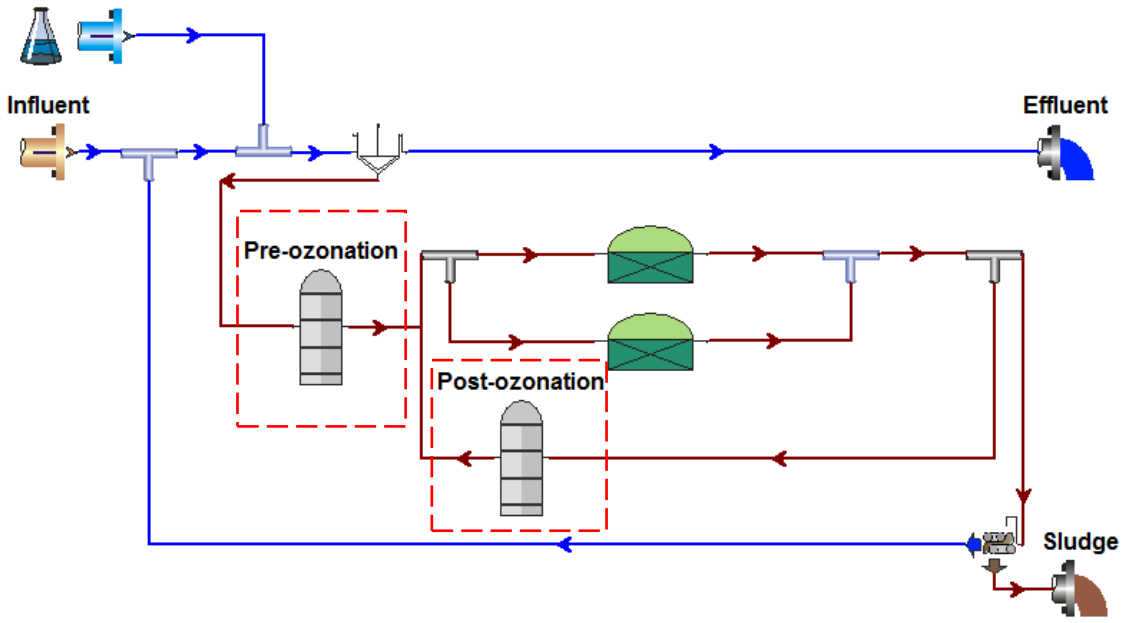

Figure 7.1: Schematic of the ozonation system in a CEPT facility. The dashed lines represent the ozone operating configuration to be evaluated.

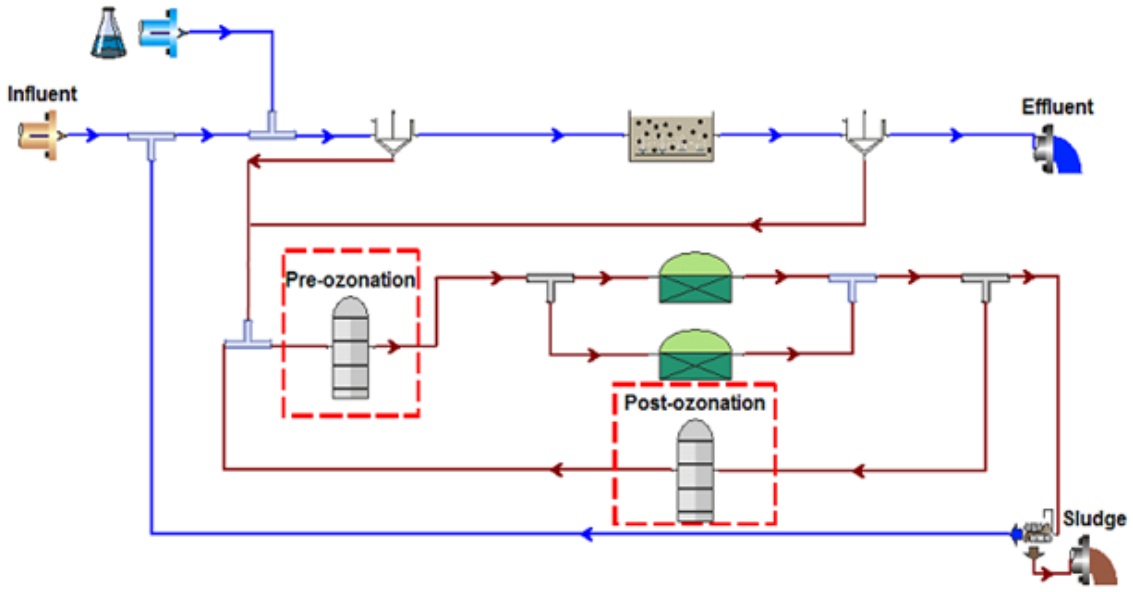

Figure 7.2: Schematic of the ozonation system in a CEPT facility with a MBBR as secondary treatment. The dashed lines represent the ozone operating configuration to be evaluated.

Table 7.1: Summary of treatment trains for the technico-economical evaluation.

ID	Description	Process unit[1]					
		PC[2]	**MBBR**[3]	**SC**[2]	**PreO$_3$**[4]	**PostO$_3$**[4]	**AD**[5, 6]
A-1	CEPT	1					2
A-2	CEPT + Pre-Ozonation	1			1		2
A-3	CEPT + Post-Ozonation	1				1	2
B1-1	CEPT + MBBR	1	1	1			2
B1-2	CEPT + MBBR + Pre-Ozonation	1	1	1	1		2
B1-3	CEPT + MBBR + Post-Ozonation	1	1	1		1	2
B2-1	CEPT + MBBR	1	1	1			3
B2-2	CEPT + MBBR + Pre-Ozonation	1	1	1	1		3
B2-3	CEPT + MBBR + Post-Ozonation	1	1	1		1	3

[1]PC = primary clarifier, SC = secondary clarifier, PreO$_3$ = Preozonation, PostO$_3$ = Post ozonation, and AD = Anaerobic digesters.
[2]Separation process efficiency: PC= 99.5%, SC=85.0%.
[3]MBBR: HRT = 30 min, 2 mg O_2/L, media filling fraction 50%, specific area 600 m^2/m^3.
[4] Thermal hydrolysis unit. Primary sludge = 75 mg O_3/g COD; anaerobic digested sludge ozone dose = 90 mg O_3/g COD; secondary sludge ozone dose = 100 mg O_3/g COD.
[5]V (A, B1) = 1215 m^3 x 2 digesters; temperature = 35 °C.
[6]V (B2) = 1215 m^3 x 2 digesters plus one 1710 m^3 digester; temperature = 35 °C.

7.2 Technical evaluation

The technical evaluation of ozonation was performed using Biowin 4.1. The performance of each process was evaluated in terms of methane, sludge production and effluent BOD_5 concentration. Simulations of each scenario were carried out based on the annual average influent characteristics of the Repentigny WRRF for 2013 to 2015 (Table 7.2).

Table 7.2: Average influent characteristics of Repentigny WRRF (2013-2015).

Description	Units	Value
Flowrate	m^3/d	26000
COD	mg COD/L	280
BOD	mg O_2/L	133
TSS	mg/L	181
pH	-	7.5

CEPT simulation (A-1) was calibrated based on the average biogas production and BOD_5 concentration of effluent of Repentigny WRRF (2013-2015). Once the A1 treatment train was calibrated, pre-ozonation (A-2) and post-ozonation (A-3) were simulated through a thermal hydrolysis unit calibrated for the experimental data presented in Chapter 4. Another simulation was performed for a combined CEPT-MBBR process to determine its impact on methane and sludge production for a facility with 2 and 3 anaerobic digesters. For this simulation, the MBBR was considered to operate continuously in aerobic conditions (2 mg O_2/L) with a hydraulic retention time of 30 min. The effects of pre-ozonation (B1-2 and B2-2) were estimated based on the experimental results obtained for ozonation of a secondary sludge (Appendix D).

The performance of each treatment train is presented in Table 7.3. From these results, it is possible to verify that the existing treatment train of Repentigny WRRF (CEPT, A-1) is insufficient to meet the BOD requirements in the effluent for the 2022 (MDDELCC, 2016). The addition of MBBR as a secondary treatment resulted in higher BOD removal, thus, allowing the plant to reach the target 25 mg BOD_5/L in the effluent. The impact of methane production is highly dependent on the operating conditions of the secondary clarifier due to the increase in the sludge withdrawal flowrate, there could be an adverse effect on the performance of the anaerobic digesters through a decrease in hydraulic retention times. CEPT-MBBR (B1) treatment trains resulted in a lower amounts of methane production than reference train (A-1) due to the two available anaerobic digesters worked under more restricted operational conditions. The addition of a third anaerobic digester (B2) increases the methane production as well as provides higher robustness to the entire

system. CEPT-MBBR (B2-1) increased by 9 % the methane production compared with the scenario without secondary treatment.

The performance of anaerobic digesters improved for post-ozonation configurations, resulting in an increase of methane production of 15% for A-3 and 18% for B2-3 compared to the reference scenario A-1. Pre-ozonation configuration A-2, however, had a slight impact on methane production compared to the reference scenario A-1. Pre-ozonation B2-2 resulted in an increase of methane production by 12% compared with the reference scenario but this increase was highly influenced by the addition of third anaerobic digester and, to a lesser extent, by the ozonation of the secondary sludge.

Ozonation can improve the performance of installed anaerobic digesters, reducing sludge production and moderately increasing methane production when it is operated under a post-ozonation configuration (scenarios A3 and B2-3). Despite the low impact of pre-ozonation on methane production, simulations showed a possible increase in the performance of anaerobic digesters through the increase of the organic loading rate (6%) or the reduction of the HRT from 19 to 18 days, without a deterioration of the current performance of anaerobic digesters.

Table 7.3: Performance of simulated scenarios.

Scenarios	**Ozone consumed**	**Methane production**	**CH_4 increase wrt A-1**	**Sludge production**	**Sludge prod. reduction wrt A-1**	**BOD_5 effluent**
	kg O_3/d	m^3 N/d	%	dry tons/d	%	mg O_2/L
CEPT (A-1)	-	1360	0	3.8	0	35
CEPT+preO_3 (A-2)	440	1360	0	3.5	8	35
CEPT+postO_3 (A-3)	180	1570	15	3.4	11	35
CEPT+MBBR (B1-1)	-	1110	-18	4.6	-20	18
CEPT+MBBR+PreO_3 (B1-2)	480	1140	-16	4.2	-10	18
CEPT+MBBR+PostO_3 (B1-3)	220	1340	-1	4.0	-6	18
CEPT+MBBR (B2-1)	-	1480	9	4.0	-5	18
CEPT+MBBR+PreO_3 (B2-2)	480	1520	12	3.6	6	18
CEPT+MBBR+PostO_3 (B2-3)	170	1600	18	3.7	3	18

7.3 Economical evaluation

Capital costs, operating and maintenance costs (O&M), and benefits were estimated separately for the full-scale implementation of ozone for different scenarios of treatment (Table 7.1). Calculations were estimated according to the U.S.EPA (2006) approach using the results obtained from laboratory scale experiments. All costs are expressed in CAD (2015). ENR Building Costs index and BLS CPI Inflation factors were used to update the costs (2015).

7.3.1 Capital costs

The economical evaluation was performed for three scenarios:

1. Annual ozone application (12 months/year) without an existing ozonation system. Capital costs for a facility without an ozone generation system. Capital costs include ozone generation system, piping, pumping, ozone process E&I, and contactor reactor.

2. Annual ozone application (12 months/year) with an existing ozonation system. Facility with a surplus of ozone available for sludge ozonation purposes. Capital costs do not include an ozone generation system.

3. Seasonal ozone application with an existing ozonation system. Ozone application during 3 months/year (winter) for a facility with an ozone generation system.

The costs of building an ozone system were based on the treatment trains presented in Figures 7.1 and 7.2. For capital costs, the major investments were the ozone generator and the injection system. Costs for pumps, pipes, residual ozone destructors, compressor, control systems, instruments, cooling system, installation and electricity were also included in the costs of a full-scale implementation. All cost estimations were based on U.S.EPA (2006). Indirect costs for the ozone system (e.g. housing, land) were not considered.

Ozone generation costs includes those for the ozone generator, ozone dissolution system (venturi injectors) and ambient air ozone monitors. The costs include all equipment necessary to generate oxygen on-site using pressure swing absorption (PSA). PSA requires feed gas equipment such as an air compressor, air chiller and air dryer. The contact tank to mix the ozone and the sludge (HRT of 15 seconds) is a stainless-steel reactor for which the cost was estimated based on McGraw-Hill (2016).

In-plant pumping costs include pumps, piping and valves and all related electrical and instrumentation costs. Sludge pumping costs were based on Qasim (1998). The cost of stainless steel piping, including valves and duct work, was considered to represent 25% of the cost of the ozone generation system. The cost of electrical and instrumentation equipment: cabling, motor control centers, programmable logic controls (PLCs), additional ozone analyzers, flow meter communications and alarm systems were considered to represent an additional 20% of the cost of the ozone generation system. A summary of capital costs for the implementation of sludge ozonation is presented in Table 7.4.

Table 7.4: Capital cost calculations for pre- and post-ozonation.

Description	Units[1]	Scenarios[4]					
		A-2	A-3	B1-2	B1-3	B2-2	B2-3
Stainless pipes, valves, ductwork	kCAD	770	390	830	440	820	370
Ozone process E&I	kCAD	610	310	660	350	660	290
Pumping	kCAD	94	110	160	150	160	150
Total capital costs[2]	kCAD	1480	810	1660	940	1640	810
Ozone generation system	kCAD	3070	1530	3320	1750	3270	1450
Total capital costs[3]	kCAD	4550	2340	4970	2690	4910	2260

[1] kCAD: thousand of Canadian dollars.

[2] Scenarios 2-3: WRRF with an existing ozonation system.

[3] Scenario 1: WRRF without an ozonation system.

[4] Only capital costs for sludge ozonation were included. Capital cost of scenarios A1, B1-1 and B2-1 = 0 kCAD.

7.3.2 O&M costs

O&M costs include electricity consumption and parts replacement costs but not labor costs. The electricity costs were estimated based on the electricity consumption for oxygen production by the PSA for ozone generation and for sludge pumping. The following assumptions were considered to estimate O&M costs:

- The electricity consumption of oxygen production was assumed to be 15 kWd/ton O_2 (Rakness, 2005).
- The electricity consumption of ozone generator was assumed to be 9.7 kWh/kg O_3, based on the information provided by Pinnacle Ozone Solutions, LLC (Smith, personal communication, October 14th, 2015). An ozone concentration of 12% was assumed.
- The power requirement due to sludge pumping was calculated for venturi injectors with a differential pressure of 7 psig and a G/L ratio of 0.4. A pump efficiency of 0.6 was assumed.
- Electricity price of 0.086 CAD/kWh was assumed (average value for 2013-2015, Annual Report-Repentigny WRRF).
- Costs of parts replacement were estimated to be 2% of the ozone generation system costs.

Based on the previous assumptions, the specific energy demand of the ozone generation system (PSA, ozone generation and sludge pumping) was 13 kWh/kg O_3 for all scenarios, which is in the range found in the literature (Sonntag and Gunten, 2012). Electricity consumption calculations and annual O&M costs are presented in Table 7.5 and 7.6, respectively.

Table 7.5: Electricity consumption calculations for oxygen production, pumping and ozone generation.

Description	**Units**	**Scenarios**					
		A-2	**A-3**	**B1-2**	**B1-3**	**B2-2**	**B2-3**
Oxygen production							
Power requirement	kWd/ton O_2	15	15	15	15	15	15
Power requirement	kWh/d	1350	560	1480	670	1460	520
Sludge pumping							
ΔP injector	psi	7	7	7	7	7	7
Number of venturi	-	19	7	12	7	13	5
Flow capacity (q)	m^3/d	130	150	220	210	220	210
Hydraulic power	kW	1.36	0.60	1.46	0.81	1.59	0.58
Power requirement	kWh/d	54	24	59	32	63	23
Ozone production							
Ozone requirement	kg O_3/d	450	190	490	220	490	170
Power requirement	kWh/d	4370	1810	4790	2170	4720	1670
Electricity consumption							
Daily	kWh/d	5800	2400	6300	2900	6200	2200
Specific	kWh/kg O_3	12.8	12.8	12.8	12.8	12.8	12.8

Table 7.6: Summary of annual O&M costs for ozone treatment.

Description	Units	Scenarios[1]					
		A-2	**A-3**	**B1-2**	**B1-2**	**B2-2**	**B2-3**
Parts replacement	kCAD	61.4	30.7	66.3	34.9	65.5	29.0
Electricity	kCAD	181	75	199	90.1	196	69.3
O&M costs	kCAD	243	106	265	125	261	98

[1] Only O&M costs for sludge ozonation were included. O&M cost of scenarios A-1, B1-1 and B2-1 = 0 kCAD.

7.3.3 Expected benefits

The expected benefits were calculated based on the reduction in costs resulting from reduced sludge production and increased methane production, which has an impact on sludge handling and natural gas consumption costs. The following assumptions were made for the calculation of expected benefits:

- Natural gas price of 0.53 CAD/m^3 (average unit cost for the Repentigny WRRF, 2013-2015).
- Sludge handling cost of 59 CAD/mt. Sludge disposal includes transportation and valorization (average unit cost for the Repentigny WRRF, 2013-2015).

The expected benefits calculations and net benefits are presented in Tables 7.7 and 7.8, respectively. The expected benefits reduced the annual O&M costs from 30 to 40% for post-ozonation scenarios.

Table 7.7: Expected benefits calculations.

Description	Units	Scenarios					
		A-2	**A-3**	**B1-2**	**B1-2**	**B2-1**	**B2-2**
Increase in methane production by ozonation	%	0.3	15	-16.3	-1	12	18
Cost reduction (natural gas consumption)	kCAD/year	0.8	40.4	-42.9	-2.7	30.4	46.2
Sludge reduction percent	%	7.9	10.8	-10.0	-5.5	5.8	3.4
Cost reduction (sludge disposal)	kCAD/year	19.7	26.9	-25.0	-13.8	14.5	8.5
Benefits	kCAD/year	20.5	67.4	-67.9	-16.5	44.8	54.8

Table 7.8: Annual expected benefits and annual O&M costs for ozone treatment.

Description	Units	Scenarios					
		A2	A3	B1-2	B1-2	B2-2	B2-3
O&M costs	kCAD	243	106	265	125	261	98
Benefits	kCAD	20.5	67	-67.9	-16.5	44.8	55
Net O&M costs	kCAD	222	38	333	141	217	43

7.3.4 Discussion

The mesophilic anaerobic digestion was combined with sludge ozonation to maximize methane production in a CEPT facility with, and without secondary treatment. The experimental results and simulations indicated that the post-ozonation of anaerobic digested sludge is a moderately efficient method for increasing methane production and sludge reduction, however, the addition of a secondary treatment requires a third anaerobic digester. CEPT train with post-ozonation of anaerobic digested sludge resulted in an increase of 15% of methane production compared to the train without ozone application. According to technico-economical evaluation of current operating configuration of Repentigny WRRF, the addition of post-ozonation to CEPT train is the most favorable investment (Table 7.9), resulting in a present value of 1300 k CAD for an annual application of ozone, excluding capital costs of ozone generator. Post-ozonation also improved the performance of treatment trains with MBBR and an extra anaerobic digester, resulting in an improved performance (+18% methane production) (Table 7.3) and lower investment costs compared to pre-ozonation (Table 7.9).

Annual application of ozone is an expensive alternative due to its high O&M costs, while its seasonal application is an interesting alternative to minimize O&M costs. Winter is the season during which takes place 50% of the annual consumption of natural gas of Repentigny WRRF (Figure 7.3). Thus, it is desired that anaerobic digesters consume less external natural gas for heating during winter. In this context, ozone application during winter can be an efficient operating alternative to reduce O&M costs. The seasonal post-ozonation of digested sludge for CEPT train resulted in a present value of 940 kCAD, excluding capital costs of ozone generators.

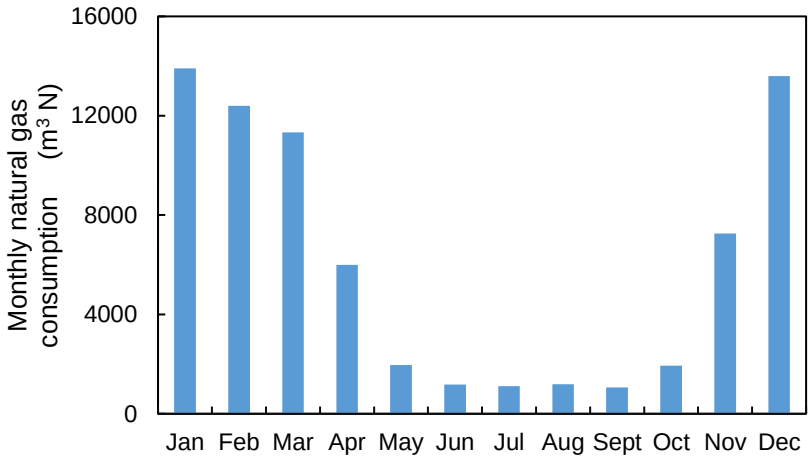

Figure 7.3: Average monthly natural gas consumption - Repentigny WRRF (2013-2015)

Table 7.9: Summary of costs for sludge ozonation.

Description	Units	Scenarios					
		A-2	**A-3**	**B1-2**	**B1-2**	**B2-2**	**B2-3**
Capital costs included and annual ozone application (12 months/year):							
Capital costs	kCAD	4550	2340	4970	2690	4910	2260
Annual O&M costs	kCAD	222	38	333	141	217	43
Net Present Value[1]	kCAD	7570	2860	9490	4610	7860	2850
Capital costs not included and annual ozone application (12 months/year):							
Capital costs	kCAD	1479	807	1657	943	1639	808
Annual O&M costs	kCAD	222	38	333	141	217	43
Net Present Value[1]	kCAD	4500	1330	6180	2870	4580	1400
Capital costs not included and seasonal ozone application (3 months/year, winter)							
Capital costs	kCAD	1480	810	1660	940	1640	810
Annual O&M costs	kCAD	56	10	83	35	54	11
Net Present Value[1]	kCAD	2240	938	2790	1420	2370	956

[1] Present value of annuity 13.59 (20 years, interest rate 4%)

7.3.5 Sensitivity analysis

A sensitivity analysis of net benefits was performed as a function of price of sludge handling, natural gas, and electricity due to their high impact on operating costs of ozone production (electricity consumption) and benefits (reduction of costs for sludge handling and natural gas consumption). These factors were evaluated separately for a ±50% change of reference values used for economical evaluation of post-ozonation integrated with the current treatment train of Repentigny WRRF (scenario A-3).

The sensitivity analysis of the sludge handling price is presented in Figure 7.3. The cost of sludge handling was derived for a reference cost of 59 CAD/tm, ranging from 29 to 88 CAD/tm (-50 and 50% change, respectively). The increase in the sludge handling cost resulted in higher net benefits, due to the increase of expected benefits. Despite the increase in benefits, scenario A-3 did not achieve positive net benefits by increasing the sludge disposal price by 50%.

The sensitivity analysis of natural gas price is illustrated in Figure 7.4. Similarly, as was seen in the sludge handling price, an increase in natural gas prices resulted in more profitable scenarios. The net benefits of scenario A-3 were increased by increasing the natural gas price but it was not possible to achieve positive net benefits.

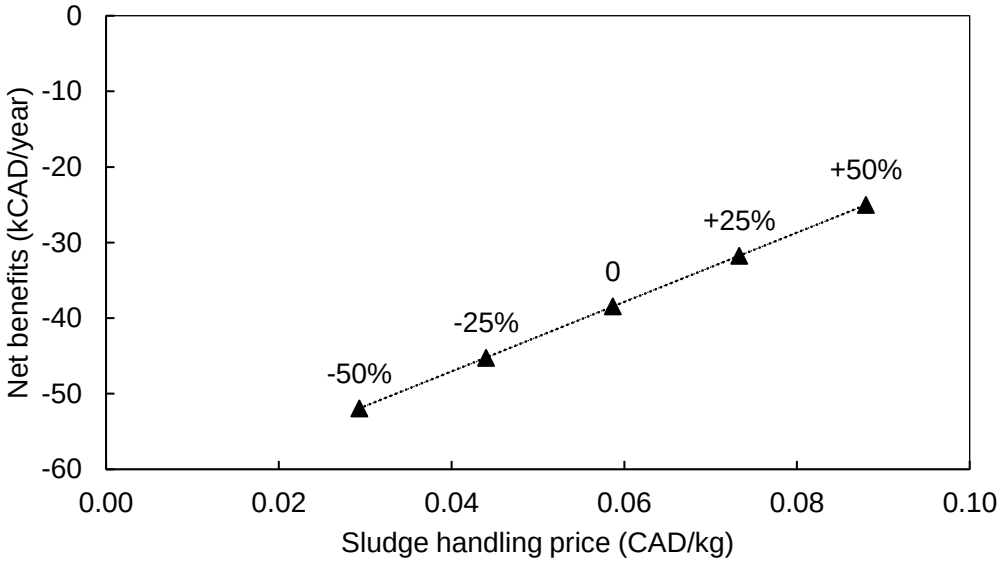

Figure 7.4: Net benefits for ±50% variation in sludge handling price (scenario A-3).

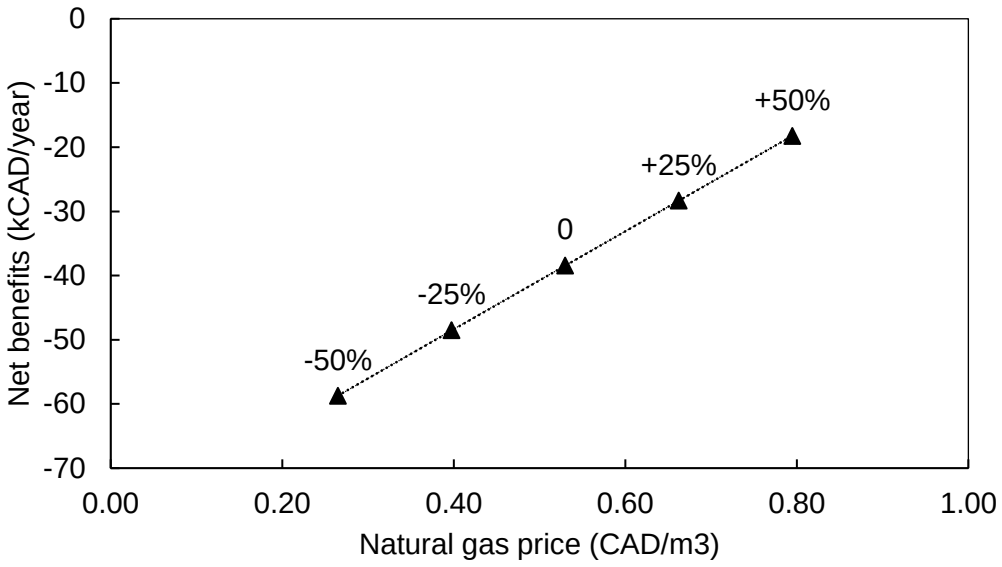

Figure 7.5: Net benefits as a function of natural gas price (scenario A-3).

The reduction in electricity prices reduced the operating costs, and as a consequence, the net benefits increased. A reduction of 50% in the price of electricity resulted in a high increase in net benefits, thus, achieving the best scenario in terms of balance of benefits versus operating costs (Figure 7.6). Ozone treatment results in high operating costs, which are partly offset by a reduction in operating costs due to a decrease in natural gas consumption and a reduction in sludge production. Nevertheless, the operating costs of ozone treatment is highly influenced by electricity prices.

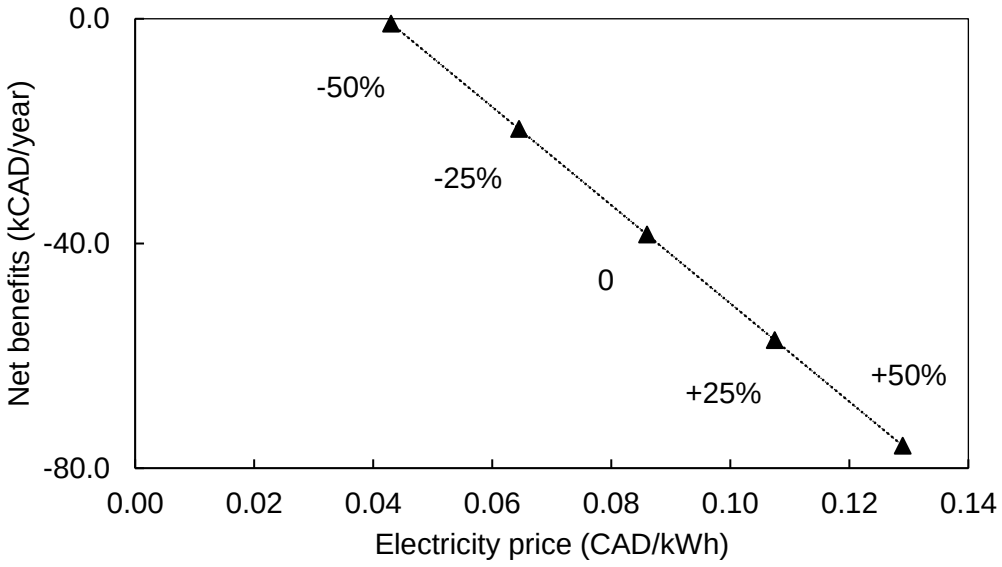

Figure 7.6: Net benefits for ±50% variation in electricity price (scenario A-3).

CHAPTER 8 GENERAL DISCUSSION

This chapter highlights the main findings from this research project. The overall objective was to maximize methane production in a CEPT facility by sludge ozonation. This thesis consists of three main topics. The first topic focused on the impact of ozonation on primary and anaerobic digested sludge in terms of the physicochemical changes and biodegradability of sludge. The second topic focused on the impact of ozonation on methane production and extracellular polymeric substances and the determination of the microbial response of ozonated anaerobic digested sludge. The third topic provided an evaluation and optimization of ozone gas transfer during the treatment of primary and anaerobic digested sludge samples in a venturi loop reactor at the laboratory scale. Accordingly, this thesis was presented in three scientific articles. An additional chapter was added for the technico-economical evaluation of ozone treatment for post-treatment of anaerobic digestion at the full WRRF of Repentigny. Based on these results and the current literature, the ozone mass transfer and ozonation mechanisms are discussed below. Recommendations will be covered as part of the conclusions and recommendations chapter.

8.1 Ozone mass transfer

One of main challenges in transferring ozone in sludge samples has been the accumulation of foam during the ozonation of anaerobic digested sludge. The excessive accumulation of foam during sludge ozonation can complicate the process control by consuming reactor space and making the whole process inoperative. The impact of ozonation on foam development has been attributed to the increase in concentration of surface active agents in the sludge supernatant, such as VFAs and proteins, which are recognized as foam-forming agents. Ozonation experiments confirmed the increase of VFAs and proteins in the sludge supernatant as well as the high foaming potential of anaerobic digested sludge. Primary sludge ozonation; however, resulted in low VFA and protein solubilisation, as well as in a low foaming potential.

Initially, a conventional bubble contactor was tested for ozone transfer but the high accumulation of foam resulted in the consumption of reactor space and the loss of organic matter during ozonation, preventing the use of high ozone doses. The dilution of sludge samples (at 1 g COD/L) and high agitation allowed more efficient control of the foam production.

Due to the limitations of the bubble contactor, a venturi loop reactor was then used for the ozonation of samples, resulting in a more effective system for controlling foam production, and at the same time, allowing for the treatment of concentrated sludge samples at high ozone doses. Despite the increase in the volume of foam during the ozonation of anaerobic digested sludge, its control was more effective than observed for the bubble contactor, allowing zero or low loss of foam at 200 mg O_3/g COD. Apparently, the internal recycle loop of sludge used during ozonation allowed the foaming to be reduced by the mechanical breaking of the foam. During the ozonation of primary sludge, foam accumulation was not observed coinciding with the foaming potential experiments.

The ozonation process consists of gas absorption with a chemical reaction in which the total reaction rate is affected by both the reaction kinetics and the physical mass transfer. A fast absorption regime with a second-order reaction was obtained for the reaction of ozone with organic matter for primary and anaerobic digested sludges. According to the film theory concept, a fast regime implies that all ozone reacts in the diffusion film, a situation that was validated by the absence of dissolved ozone in the liquid phase of sludge samples. A fast regime also indicates ozonation of sludge samples is strongly influenced by chemical reactions. It should be noted, however, that despite an increase of initial COD concentration resulting in higher values of reaction rate coefficients, the ozone mass transfer was reduced, decreasing the performance of ozonation (e.g. COD removal). This is probably a limitation of the physical absorption observed in samples with high organic matter content. A low impact of pH was observed, suggesting a marginal role of free radicals for COD removal.

8.2 Sludge ozonation mechanisms

The oxidation of organic matter and the mechanical disintegration of sludge were observed during ozonation. These results from PSD indicate that the reduction of particle sizes during the sludge treatment was greatly influenced by pumping and, to a lesser extent, by the chemical oxidation by ozone. Interestingly, the mechanical friction exerted by pumping of samples caused the disaggregation of sludge as SEM observations for controls confirmed. However, control tests conducted without ozone injection did not result in an increase of soluble COD. Despite the low impact on COD solubilisation, the disaggregation of sludge by the mechanical action of pumping could improve the contact between ozone and organic matter contained in sludge flocs. EPS experiments confirmed the low impact of pumping on organic matter solubilisation.

Solubilisation of sludge increased mainly via partial disintegration of the sludge matrix and damage to the cell membrane. Ozonation can disintegrate the sludge pellet and release organic matter as proteins and polysaccharides into the soluble phase, thereby, enhancing methane production during anaerobic digestion. The reduction in viability of the sample suggests that the broken cells can release intracellular matter into the solution. The enhancement in methane production may not only be ascribed to solubilisation but may also be influenced by the increase in the biodegradability of organic products generated during ozonation.

Biodegradability of anaerobic digested sludge increased via ozonation. The biodegradable COD of anaerobic digested sludge increased from 2.5 to 3.9 g COD/L for an ozone dose of 90 mg O_3/g COD, representing an increase of methane production of 55%. Ozonation of primary sludge, however, did not result in the increase of biodegradable COD. As a result of the increase in solubilisation and biodegradability of digested sludge, anaerobic degradation can be enhanced, improving methane yield and accelerating digestion times. An overdose of ozone can reduce the methane yield, due to the mineralization of the solubilized organic matter. An overdose of ozone can also increase the lag phase of methane production due to the excessive reduction in viability of anaerobic biomass, which could have a negative impact on the stability of anaerobic digesters in a post-treatment configuration (ozonation of anaerobic digested sludge).

High ozone doses significantly decreased the concentration of TOC confirming that the decrease of COD during ozonation is caused in part by the mineralization of organic matter. The decrease in TOC during ozonation is consistent with previous studies on ozonation of activated sludge which suggested mineralization as the main mechanism of COD reduction. This study, however, shows that the COD decrease is not only resulting from organic matter mineralization but it is also caused by its partial oxidation. The results suggest that the mineralization of organic matter is the main mechanism of sludge mass reduction during ozone treatment.

The effect of solubilisation of organic matter appears to be most important at medium ozone doses, whereas mineralization of organic matter requires high ozone doses. The main impact of ozonation on digested sludge was the increase of biodegradable COD and soluble COD as well as the mineralization of organic matter. These parameters could allow an increase in performance and/or capacity of anaerobic digesters, due to the improved degradation of organic matter and the increased methane production.

Ozonation of anaerobic digested sludge resulted in an increase in methane yield, confirming that the addition of an ozone treatment makes it possible to increase the sludge biodegradability and demonstrate the possible synergy between ozonation and the anaerobic digestion process. Nevertheless, the experimental post-ozonation configuration resulted in moderate performance in terms of methane production and sludge reduction. A lower impact than expected, resulted in high operating costs and lower benefits. Preliminary tests were performed to evaluate the performance of sludge ozonation from a conventional activated sludge system. These results showed a high efficiency in increasing methane production of activated sludge, with a similar impact to that observed for primary sludge and anaerobic digested sludge of CEPT process.

8.3 Effects induced by different pretreatments on sludge characteristics

An estimation of X_{COD} characteristics of wastewater sludge is presented in Figure 8.1. While primary sludge contains a high fraction of particulate biodegradable matter, activated sludge and anaerobic digested sludge contain a higher fraction of heterotrophic biomass. After anaerobic digestion of primary and activated sludge, biodegradable components are largely removed with the remaining organic matter consisting mostly of non-biodegradable particulate matter from the wastewater influent and from endogenous residues produced in biological unit processes. The impact of ozonation and other pretreatments may differ due to changes in the composition of treated sludge.

An overview of the effects induced by different pretreatments on sludge characteristics has been evaluated preliminary in Table 8.2. Pretreatments tend to enhance the biodegradability of anaerobic digested sludge and activated sludge; however, some pretreatments have side effects that counteract their positive effects, as for example, the generation of recalcitrant compounds by high-temperature thermal pretreatments. The pretreatment effects are intertwined with sludge characteristics and pretreatment mechanisms. Primary sludge a substrate inherently biodegradable may not need pretreatment, whereas other substrates, such as those containing high levels of heterotrophic biomass and unbiodegradable organic matter (e.g. activated sludge, anaerobic digested sludge), are more amendable to pretreatment for enhancing biodegradability. Therefore, optimization of pretreatment techniques to sludge characteristics remains a challenge.

Table 8.1: Estimation of X_{COD} characteristics of wastewater sludge[1].

Influent	PS	AS	PS+AS	DS
X_B (%)	76	2.1	50	0.58
X_H (%)	3.2	47	19	13
$X_{U,Inf}$ (%)	21	24	22	52
X_E (%)	0.00	24	8.5	30
X_{VSS} (kg/d)	1190	720	1900	820

[1] Based on Biowin 4.1-WAS and primary digestion simulation. Influent: Flow = 10 000 m^3/d, 500 mg COD/L; activated sludge: SRT =19 d, HRT=3.1 d; anaerobic digestion: HRT=19 d, VSS destruction =57%.

Table 8.2: Overview of the effects induced by different pretreatments on X_{COD}.

Pretreatments	X_B	X_H	X_U	X_E
Chemical				
Oxidation	+ [a,c]	+ [a,c,i]	+ [a,h]	+ [a,i]
Acid	0 [l]	+ [c]	0 [l]	0 [l]
Alkaline	0/+ [b]	+ [c]	0/+ [b]	0/+ [b]
Mechanical				
Ultrasonication	+ [b,d,g]	+ [c]	+ [k]	0 [k]
Others	0/+ [b]	0/+ [c]	0 [j]	0 [j]
Thermal				
>100 °C	-/+ [b,f]	+ [c,e]	-/+ [e,h]	- [f]

+ = positive effect, 0 = low effect, - = negative effect. [a] This study, [b] Carlsson et al. (2012), [c] Foladori et al. (2010), [d] Carrère et al. (2008), [e] Eskicioglu et al. (2008), [f] Dwyer et al. (2008), [g] Chu et al. (2002), [h] Brugrier et al. (2006), [i] Labelle et al. (2013), [j] Baier and Schmidheiny (1997), [k] Neis et al. (2000), [l] Devlin et al. (2011).

CHAPTER 9 CONCLUSION AND RECOMMENDATIONS

This research project sought to maximize methane production in a chemically enhanced primary treatment facility by ozonating sludge being either fed or produced by an anaerobic digester. The study of sludge ozonation was performed using a venturi loop reactor at laboratory scale. Ozonation was investigated by monitoring its effects on mass transfer efficiency, biochemical methane production, microbial activity and physicochemical characteristics (e.g. COD fractionation, EPS, and solubilisation). The coupling of ozonation with anaerobic digesters was also evaluated in two process configurations, pre-ozonation of primary sludge and post-ozonation of digested sludge each combined with a semi-continuous lab-scale anaerobic digester. A technico-economical evaluation of ozone treatment combined with the anaerobic digestion was also conducted.

9.1 Conclusion

The following conclusions were drawn from this research.

- Anaerobic digested sludge ozonated at a dose of 90 mg O_3/g COD resulted in an increase of 2.5 to 3.9 g COD/L of biodegradable COD, representing an increase of methane production of 55%. This ozone dose resulted in the highest effect on methane yield and sludge reduction, confirming that the addition of ozone treatment can be used to increase sludge biodegradability in synergy with an anaerobic digestion process. Ozonation of anaerobic digested sludge at a dose of 140 mg O_3/g COD resulted in a soluble COD concentration from 1.1 to 2.9 g COD/L and at a dose of 200 mg O_3/g COD, resulted in a soluble TKN increase from 430 to 570 mg N/L and in a soluble phosphorus increase from 8 to 18 mg P/L.
- Ozonation of primary sludge at a dose of 220 mg O_3/g COD resulted in a low impact on biodegradable COD (-4.8%), in a small increase in soluble TKN from 180 to 200 mg N/L, and in a soluble phosphorus increase from 2 to 4 mg P/L.
- A venturi loop reactor was effective in increasing the ozone transfer efficiency and controlling foam accumulation during treatment. At atmospheric pressure, the ozonation of digested sludge resulted in an ozone mass transfer efficiency of 98% for a G/L ratio < 0.4. However, a pressure of 103 kPa (gauge) and a G/L ratio < 0.2 were required for the effective ozonation of primary sludge (96%).

- The methane potential of ozonated sludge depends on organic matter solubilisation as well as an increase in biodegradable organic matter. Organic matter solubilisation was affected by the disintegration of the sludge matrix as well as the release of intracellular matter from the broken cells triggered by the chemical oxidation. The chemical action of ozone also resulted in the mineralization of organic matter into CO_2, which can reduce the organic matter available and thus, affect negatively the methane production potential. Ozonation caused the TOC mineralization of primary sludge and anaerobic digested sludge up to 10% (TOC_0 = 10 g C/L) and 15% (TOC_0 = 4.2 g C/L), respectively, for ozone doses up to approximately 200 mg O_3/g COD.
- An 86% inhibition of viable cells was obtained for an ozone dose of approximately 90 mg O_3/g COD. This inhibition was, however, temporary with an initial lag phase of 8 days. Following this lag phase, the viability and activity were recovered, resulting in an increase of methane yield of ozonated samples.
- The pH of primary sludge decreased from 7.1 to 5.2 as ozone doses increased from 0 to 220 mg O_3/g COD while the pH of the anaerobic digested sludge decreased from 7.4 to 6.9 at ozone doses from 0 to 210 mg O_3/g COD. Similarly, for these ozone doses, the alkalinity was reduced during ozonation, resulting in reductions of 46 and 60% for primary sludge and anaerobic digested sludge, respectively.
- Post-ozonation of digested sludge was found to be moderately effective for improving methane production (from 189 to 218 mL N CH_4/g COD, a +16% increase), COD removal efficiency and dewaterability of digested sludge compared to the control digester. Pre-ozonation of primary sludge, however, was not effective in enhancing the performance of the anaerobic digester.
- Post-ozonation of anaerobic digested sludge can be an efficient operating alternative to increase methane production for a CEPT process during winter. Seasonal ozone treatment required a capital cost of 810 kCAD, excluding capital costs of ozone generators, with an operating cost of 40 kCAD per year, considering the expected benefits of reduction in sludge handling and natural gas consumption.

9.2 Recommendations

Based on these findings, recommendations for further investigation include:

- Optimization of the coupling of anaerobic digestion with ozone treatment (post-ozonation), by focusing in the optimization of anaerobic reactors (e.g. impact of reduction of retention times, increase of loading rates).
- Characterization of biodegradability of individual transformation products (biodegradable and unbiodegradable) resulting from ozonation considering that it is known that ozonation can improve the biodegradability of organic matter.
- Investigation of influence of ozonation on the microbial community structure and the key methane-producing pathways of anaerobic digester digestion through applying metagenomics approach could be done.
- Investigation of effect of adding hydrogen peroxide (H_2O_2) to primary and anaerobic digested sludge. The goal with adding H_2O_2 to the wastewater after a certain ozone dosage is to enhance the transformation of O_3 to $OH^{\bullet}$ in the aqueous phase. Even though H_2O_2 has an oxidation potential comparable to O_3, a larger quantity of radicals is produced for the same concentration of oxidant in the presence of H_2O_2 compared to O_3 used alone.
- Evaluation of ozonation to enhance methane production by testing various types of wastes (e.g. industrial wastewaters and food wastes).

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APPENDICES

APPENDIX A – Protocols

A.1 Biochemical methane potential (BMP) test

A.1.1 Object:

This protocol concerns the determination of methane production of sludge samples by means of a manometric method.

A.1.2 Materials:

- 160 mL glass bottles
- Digital manometer Ashcroft 30 psi D625 connected to a needle
- Incubator (35 °C)
- Gastight syringe, exetainter vials (or foil gas sampling bags)
- Glucose or acetate solution, anaerobic digested sludge (inoculum)
- Gas chromatograph (GC-456, Bruker, USA) equipped with a thermal conductivity detector (150 °C).

A.1.3 Methodology:

- Characterize the sludge samples (e.g. COD, VSS, pH, nitrogen and phosphorus)
- Add anaerobic digested sludge (screened under 5 mm) into each bottle (suggested final concentration: 5.0 g VSS/L) (inoculum)
- Add sludge samples (substrates). The ratio inoculum/substrate (IRS) must be greater than 2 g COD/g COD. Ideally, at least 1 g COD of substrate can be added. The final volume should ideally represent 75 to 80% of the reactor volume
- Prepare controls with distilled water and inoculum
- Prepare positives controls with glucose (or acetate) solution and inoculum
- Prepare each assay in triplicate
- Purge bottles with N_2, immediately before to close the bottles with the caps.
- Manually shake the bottles before incubation. A manual shake of bottles should be done routinely

- Routinely the overpressure generated by biogas must be measured by a manometer. Before each measurement, the bottles must be cooled to room temperature. Gas samples can be sampled for GC analysis. After each measurement, the overpressure must be purged with a needle
- The duration of the BMP assay is determined for each substrate, and the test is finished when the cumulative biogas curve reaches the plateau phase, usually after 30 days
- The ideal gas equation can be used for calculation of the biogas production at normal or standard conditions
- For each BMP assay, COD mass balances need to be determined for the COD concentrations of samples at the start and at the end of BMP assays, including methane gas production expressed in terms of COD (theoretical conversion factor = 350 mL N CH_4/g COD). The validation was considered acceptable for an accuracy of COD mass balances greater than 95% and a final pH between 6.6 and 7.6.

A.2 Iodometric method for the determination of ozone in gas

A.2.1 Object:

The present method concerns the determination of ozone in gas phase (based on Rakness, 2005).

A.2.2 Materials and reagents:

- Unbuffered KI: Potassium iodide stock regent (2%): 20 g KI/L
- Sulfuric acid solution: 2N H_2SO_4
- Sodium thiosulfate ($Na_2S_2O_3$) solution: 0.1 mol/L
- Potassium iodate (KIO_3).

A.2.3 Methodology:

a) Standardization of titrant:

- Weigh 0.5 g of potassium iodate and dilute to 250 mL in a 250-mL Erlenmeyer flask, add 25 mL of this solution to a 125-mL Erlenmeyer flask. Add 0.5 g KI, 50 mL of waster, and 2 mL of 1 N sulfuric acid. The iodine formed is titrated with the approximately sodium thiosulfate titrant until the yellow color is almost gone. The normality of $Na_2S_2O_3$ titrant = 2/$Na_2S_2O_3$ mL consumed.

b) Determination of ozone in gas:

- Add KI solution to each gas-washing column. Minimum 2 traps.
- Bubble ozone gas through the gas-washing columns.
- After bubbling has stopped, transfer the liquid from gas-washing columns to Enlenmeyer flasks. Then, add about 10 mL 2N H_2SO_4 per 400 mL of KI sample.
- Resulting solution is titrated with a standardized sodium thiosulfate solution.
- Ozone mass (g O_3) = 24 x volume of thiosulfate in L x Normality of thiosulfate

  Ozone concentration in gas (O_3/L) = Ozone mass/volume of sample.

This section presents the experimental setup used for sludge ozonation.

APPENDIX B – Ozone generation system

This section presents the experimental setup used for sludge ozonation.

Figure B.1: Front view of skid/enclosure of ozone generator (Peak 2X, Pinnacle).

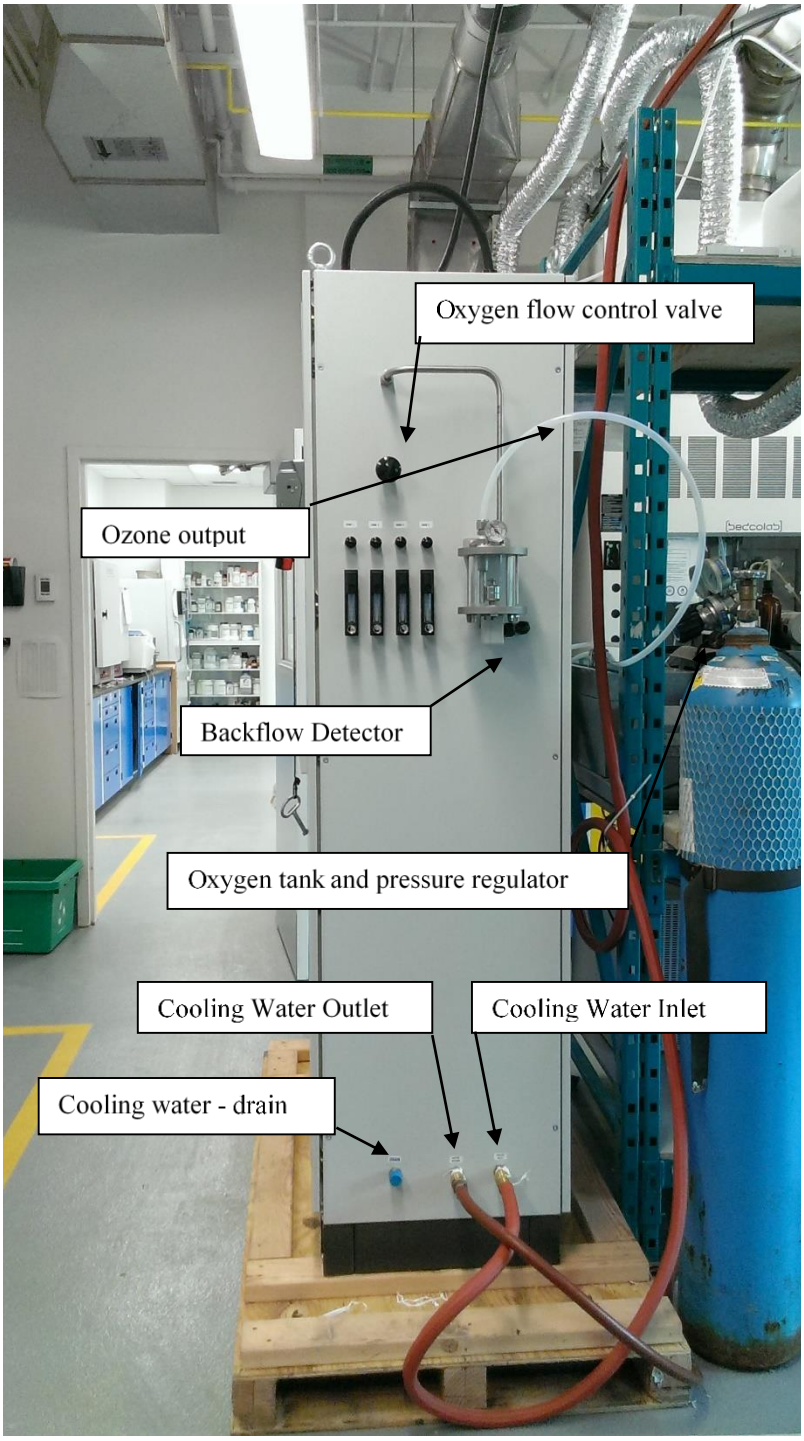

Figure B.2: Left view of skid/enclosure of ozone generator (Peak 2X, Pinnacle).

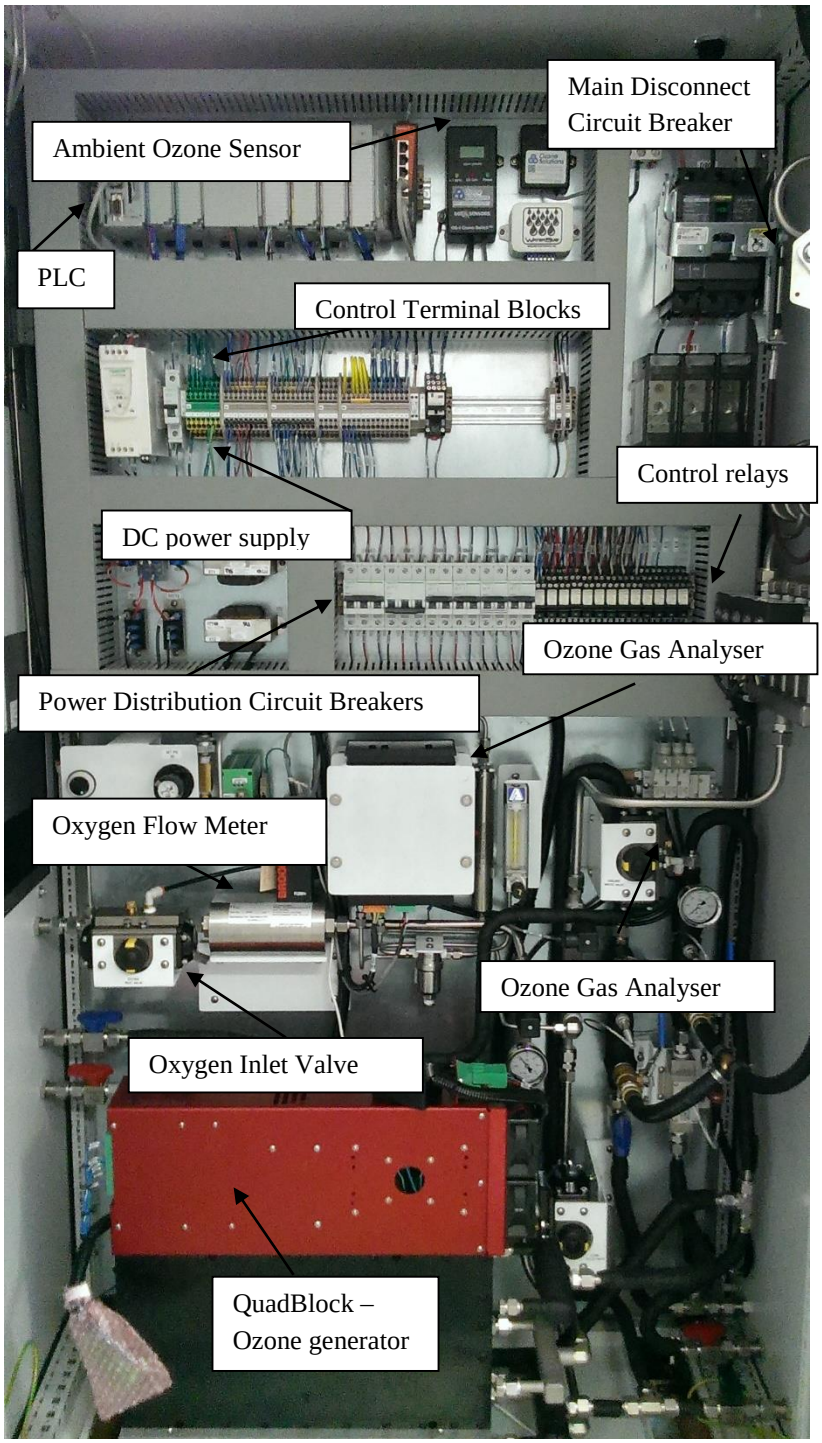

Figure B.3: Internal view of ozone generator (Peak 2X, Pinnacle).

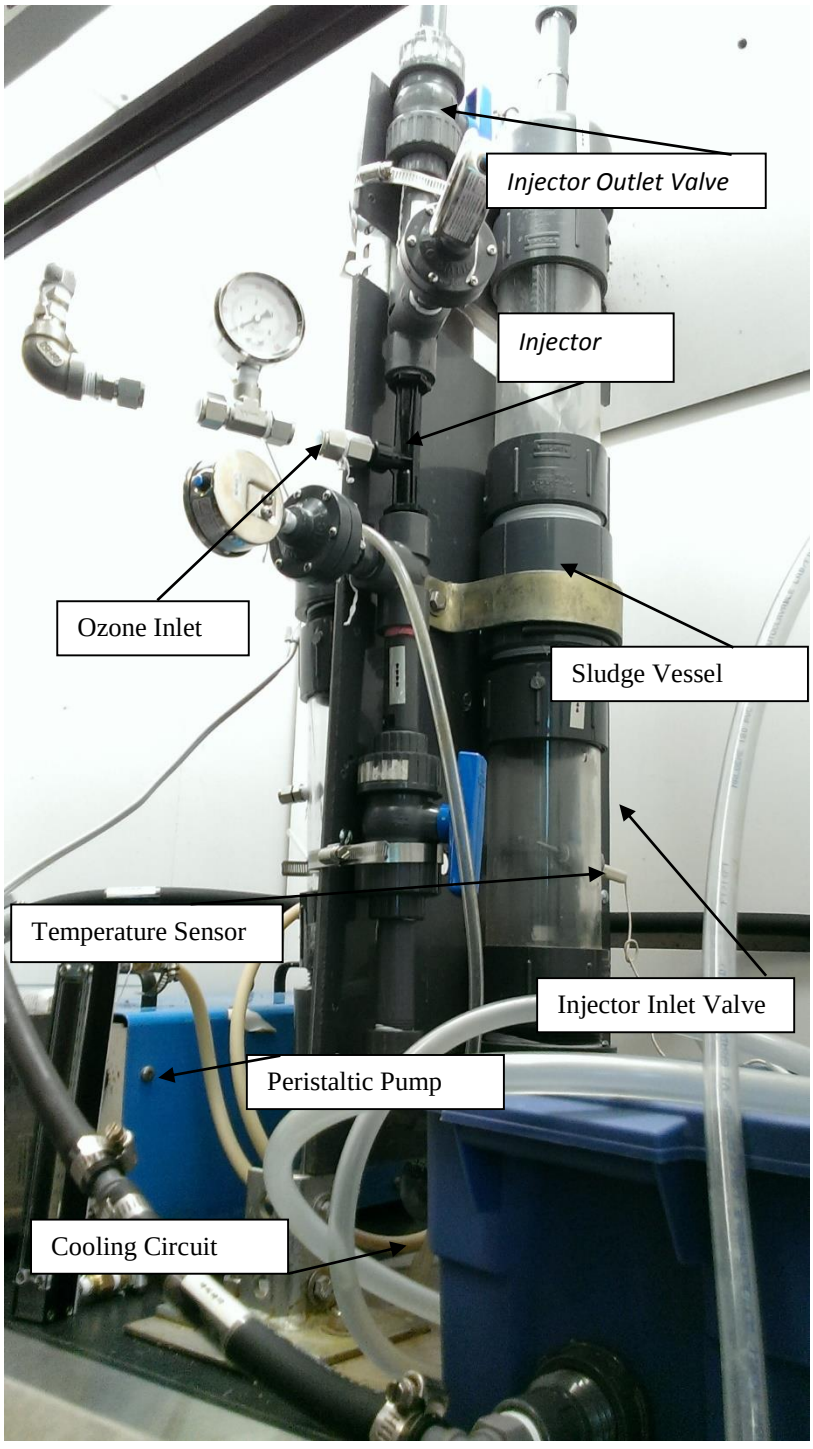

Figure B.4: Lab-scale venturi loop reactor.

APPENDIX C – Supplementary information

C.1 Supplementary information Articles 1, 2, and 3.

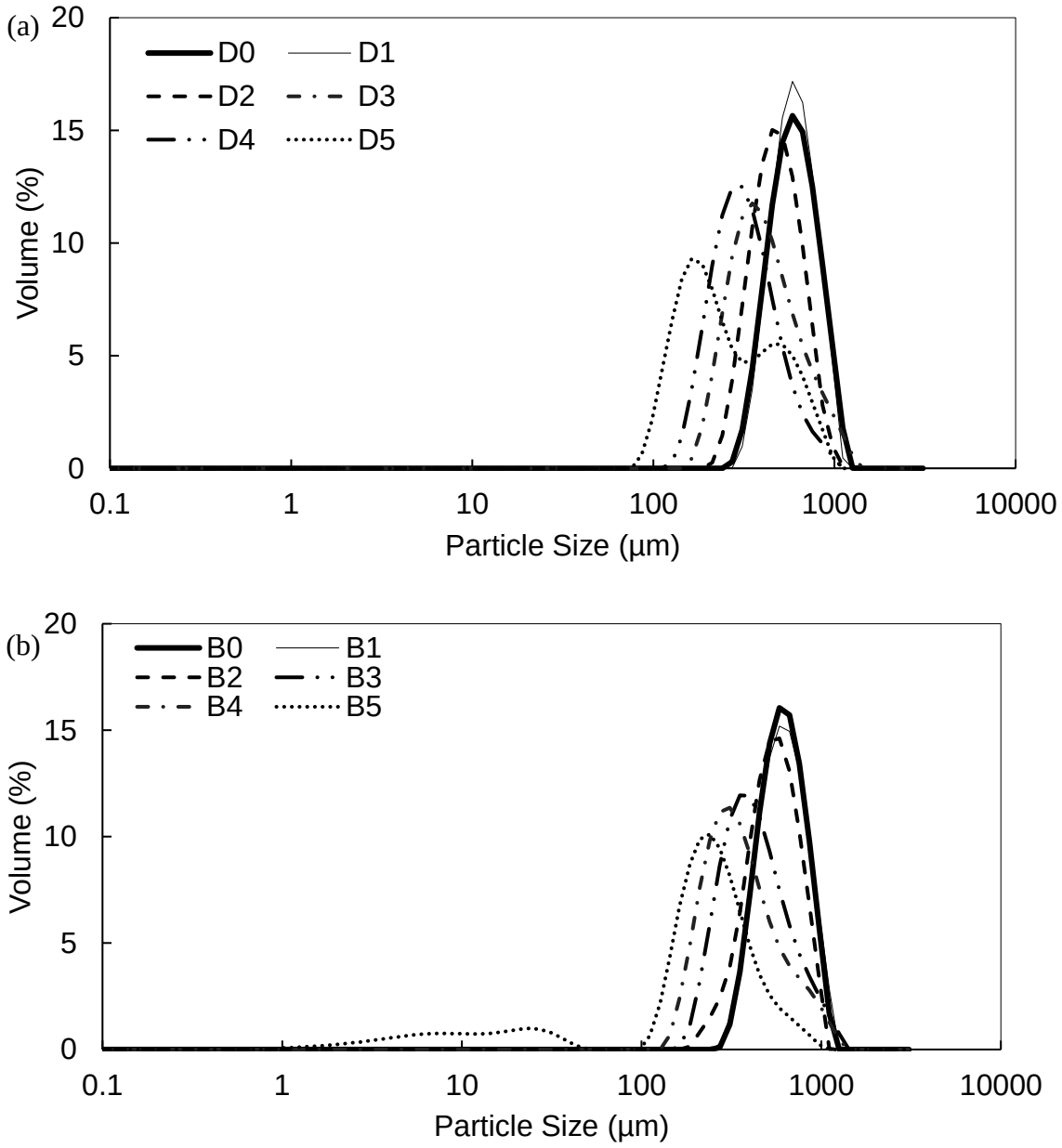

Figure C.1: Size distribution changes during ozonation of primary sludge. (a) Ozonated samples; (b) controls (D0 = 0; D1 = 10; D2 = 30; D3 = 50; D4 = 140; D5 = 220 mg O_3/g COD, B0-5 = controls for each ozone dose).

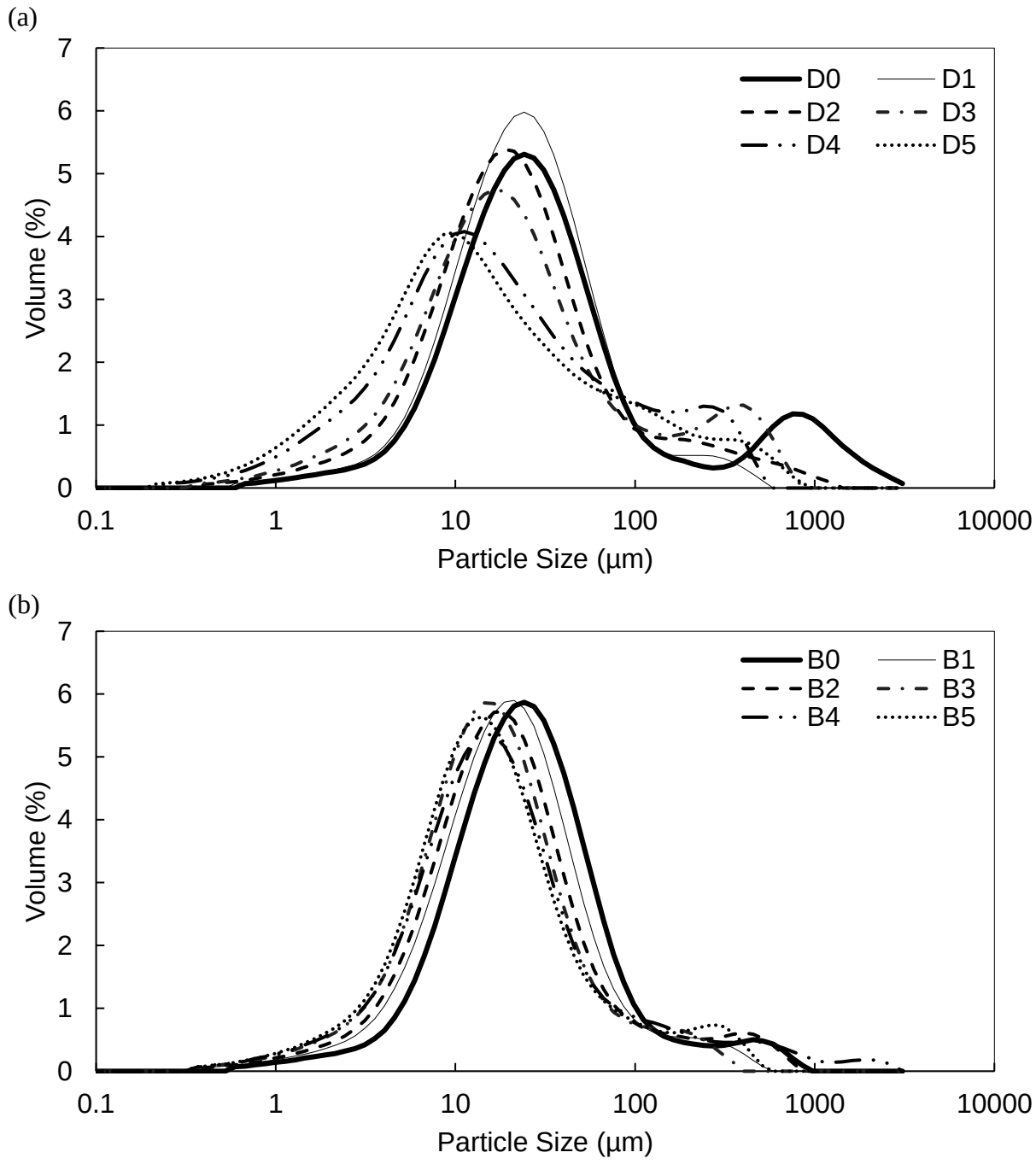

Figure C.2: Size distribution changes during ozonation of anaerobic digested sludge. (a) Ozonated samples; (b) controls (D0 = 0; D1 = 50; D2 = 90; D3 = 140; D4 = 1740; D5 = 210 mg O_3/g COD, B0-5 = controls for each corresponding ozone dose).

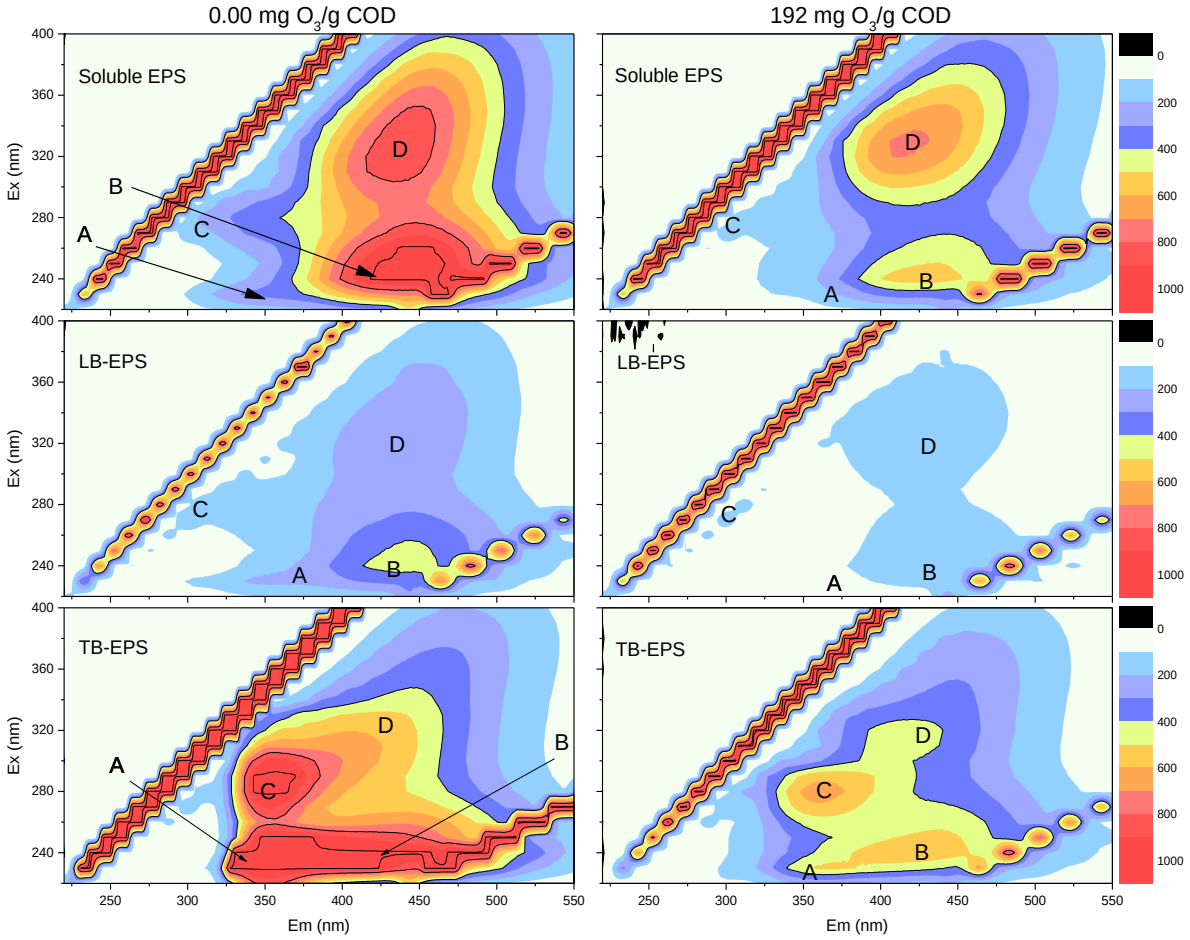

Figure C.3: EEM spectra of the extracted EPS fractions for untreated and treated sludge (192 mg O_3/g COD).

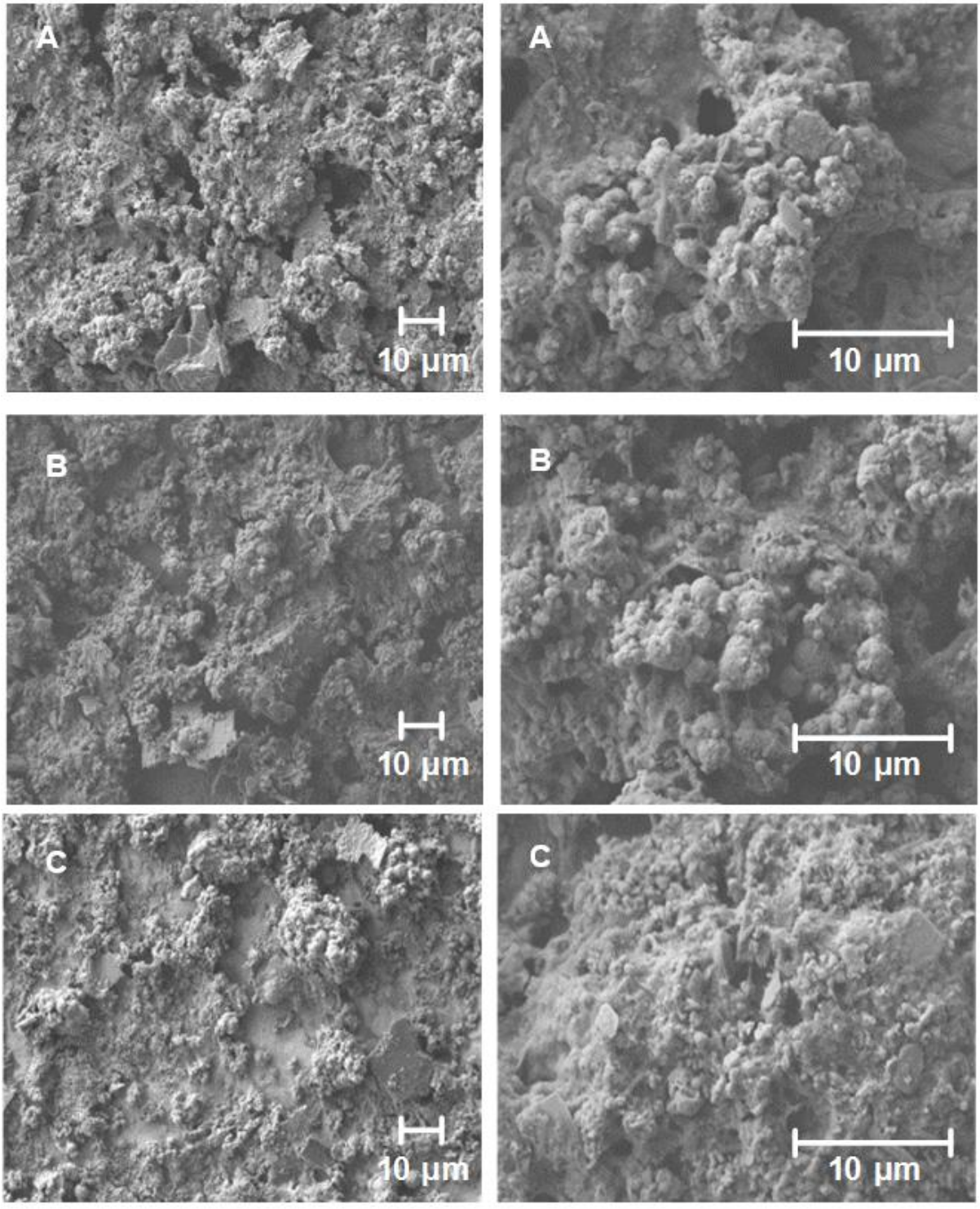

Figure C.4: Scanning electron micrographs imaging of anaerobic sludge exposed to 0 mg O_3/g COD (A), 86 mg O_3/g COD (B), and 192 mg O_3/g COD (C).

Table C.1: Effect of ozonation on COD fractionation of primary sludge (D0 = 0; D1 = 10; D2 = 30; D3 = 50; D4 = 140; D5 = 220 mg O_3/g COD).

Sample	bCOD (g COD/L)			nbCOD (g COD/L)		
	Xb	Cb	Sb	Xu	Cu	Su
D0	25.2	1.05	0.85	16.1	0.10	0.18
D1	24.2	1.39	0.88	15.1	0.42	0.23
D2	24.0	1.67	1.00	14.3	0.20	0.28
D3	23.9	1.14	1.09	13.4	0.06	0.14
D4	23.8	1.71	0.91	12.2	0.08	0.16
D5	23.1	1.97	0.78	10.9	0.25	0.33
Control	24.5	1.07	0.81	16.3	0.15	0.16

Table C.2 : Effect of ozonation on COD fractionation of anaerobic digested sludge (D0 = 0; D1 = 50; D2 = 90; D3 = 140; D4 = 1740; D5 = 210 mg O_3/g COD).

Sample	bCOD (g COD/L)			nbCOD (g COD/L)		
	Xb	Cb	Sb	Xu	Cu	Su
D0	1.10	0.60	0.82	12.0	0.18	0.25
D1	1.16	0.68	1.52	10.4	0.19	0.41
D2	0.62	0.82	2.46	9.3	0.23	0.44
D3	0.57	1.02	2.39	8.6	0.26	0.56
D4	0.46	0.77	1.96	8.4	0.23	0.54
D5	0.39	0.63	1.69	8.2	0.21	0.51
Control	1.05	0.67	0.87	11.8	0.22	0.28

Table C.3: Summary of operating conditions during volumetric mass transfer experiments.

Experiment number	**Volume L**	**Q_{gas} L STD/min**	**Q_{liquid} L/min**
1	3.0	1.0	4.3
2	3.0	1.0	6.4
3	3.0	1.0	7.6
4	3.0	3.5	4.3
5	3.0	3.5	6.4
6	3.0	3.5	7.6
7	3.0	5.8	4.3
8	3.0	5.8	6.4
9	3.0	5.8	7.6
10	3.0	8.0	4.3
11	3.0	8.0	6.4
12	3.0	8.0	7.6
13	2.0	1.0	4.3
14	2.0	1.0	6.4
15	2.0	1.0	7.6
16	2.0	3.5	4.3
17	2.0	3.5	6.4
18	2.0	3.5	7.6
19	2.0	5.8	4.3
20	2.0	5.8	6.4
21	2.0	5.8	7.6
22	2.0	8.0	4.3
23	2.0	8.0	6.4
24	2.0	8.0	7.6

C.2 Technico-economical analysis Article 1.

This section presents a technico-economical analysis of a full-scale application of digested sludge post-ozonation. This configuration was evaluated for a chemically enhanced primary treatment (CEPT) plant (Table C.4 and Table C.5):

Table C.4: Average influent characteristics.

Description	Units	Value
Flowrate	m^3/d	26000
COD	mg COD/L	280
BOD	mg O_2/L	133
TSS	mg/L	181
pH	-	7.5

Table C.5: Sludge production.

Description	Units	Value
PS concentration	g COD/L	46.0
DS concentration	g COD/L	13.2
Sludge flow rate	m^3/d	128
HRT	days	19

The sludge ozonation characteristics are presented in Table C.6. Calculations were estimated based on the results obtained from laboratory scale experiments (section 4.3.6). Operating and maintenance costs (O&M) include electricity consumption and parts replacement costs but not labor costs (Table C.7). The electricity costs were estimated based on the electricity consumption for oxygen production by the PSA (pressure swing adsorption) for ozone generation and for sludge pumping. Therefore, the specific energy demand of the ozone generation system (PSA, ozone generation and sludge pumping) was 13 kWh/kg O_3 (electricity cost 0.1 USD/kWh).

Table C.6: Sludge ozonation characteristics.

Description	Units	Value
Recycling rate	-	1.2
Ozone dose	mg O_3/g COD	90
Ozone mass transfer	%	98
Ozone production	kg O_3/d	183

Costs of parts replacement were estimated to be 1.3% of the ozone generation system costs (ozone generation, PSA, pumps, valves, etc.). The capital cost was assumed to be 9800 USD·kg $O_3 \cdot d^{-1}$.

The expected benefits were calculated based on the reduction in costs resulting from reduced sludge production and increased methane production, which has an impact on sludge handling and natural gas consumption costs. The following assumptions were made for the calculation of expected benefits: natural gas price of 0.14 USD/m^3 (16 % increase methane production); sludge handling cost of 45 USD/ton (11% reduction of sludge production).

Table C.7: Performance of post-ozonation.

Scenarios	**Ozone consumed**	**Methane production**	**CH_4 increase wrt CEPT**	**Sludge production**	**Sludge prod. reduction wrt CEPT**
	kg O_3/d	m^3 N/d	%	dry tons/d	%
CEPT	-	1360	0	3.8	0
CEPT+postO_3	183	1580	16	3.4	11

The expected benefits calculations and net O&M costs are presented in Table C.8. The expected benefits reduced the annual O&M costs from 30 % for sludge post-ozonation.

Table C.8 : Summary of annual O&M costs for ozone treatment.

Description	Units	Value
Parts replacement	USD/year	23300
Electricity	USD/year	86800
O&M costs	USD/year	110100
Benefits	USD/year	32000
Net O&M costs	USD/year	78100

APPENDIX D – Ozonation of sludge from conventional activated sludge system

The aim of this appendix is to compare the effect of ozonation of sludge coming from an activated sludge system with the results previously presented for a CEPT process (Chapter 4). Sludge ozonation was evaluated in terms of COD removal, COD solubilisation and methane yield and methane production rate. The methods used were previously described in Chapter 4. Primary sludge, activated sludge and anaerobic digested sludge were collected from a full scale WRRF (Quebec) operated under a conventional activated sludge system.

The results of this evaluation show that COD removal increases as the ozone dose increases (Figure D.1), reaching 19 to 29% of COD removal for the highest ozone doses tested. COD solubilisation was also increased for high ozone doses (Figure D.2), but its effect was more pronounced for activated sludge (35%) and digested sludge (15%). Primary sludge reached a low solubilisation (7%). The observed impact of ozonation on anaerobic digested sludge from the activated sludge process is similar to the results reported in Chapter 4 for the CEPT process; ozonation of anaerobic sludge digested, at approximately 210 mg O_3/g COD, achieved a COD removal of 14% with a COD solubilisation of 13%. The primary sludge ozonation; however, resulted in a non-significant solubilisation with a COD removal of 22% at 220 mg O_3/g COD. The results of activated sludge ozonation are in agreement with those from the literature, that have shown the high impact of ozonation on solubilisation; an increase of solubilisation from 15 to 30% for ozone dosages from 60 to 160 mg O_3/g COD (Chu et al., 2008) has been reported. In general, the impact of ozonation on COD removal was strongly influenced by the ozone dose with a similar impact without regard to the origin (CEPT or activated sludge process) and type of sludge used (primary sludge, activated sludge and anaerobic digested sludge). COD solubilisation, however, was influenced by the type of sludge samples and the ozone dose; primary sludges show a low potential for increasing the soluble organic matter, while anaerobic digested sludge and activated sludge resulted in higher solubilisation. The highest impact of ozonation was obtained for the treatment of activated sludge, probably caused by the higher amount of biomass present in the sludge samples.

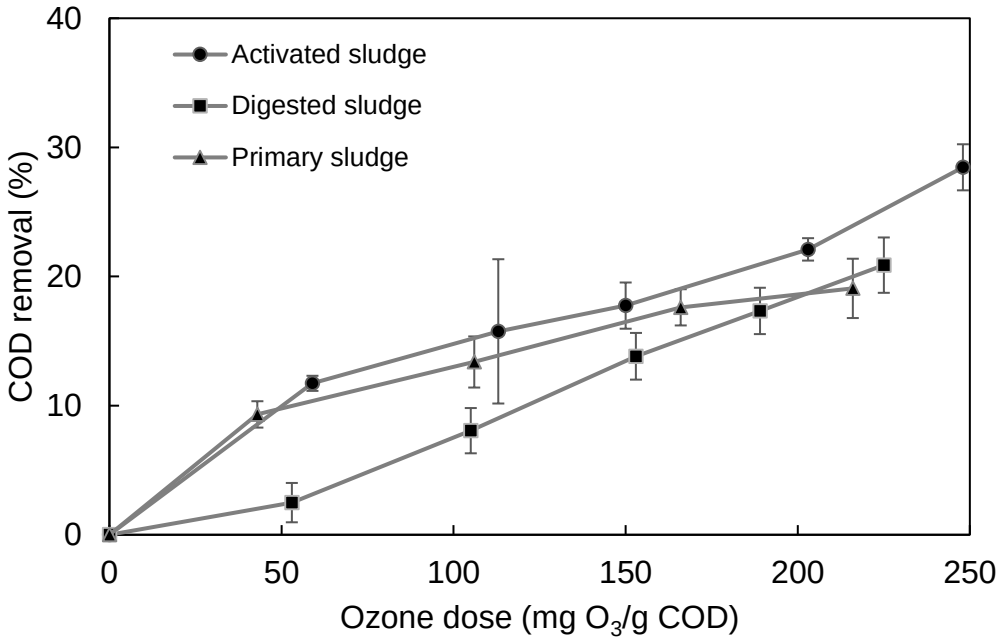

Figure D.1: Influence of ozonation on COD removal.

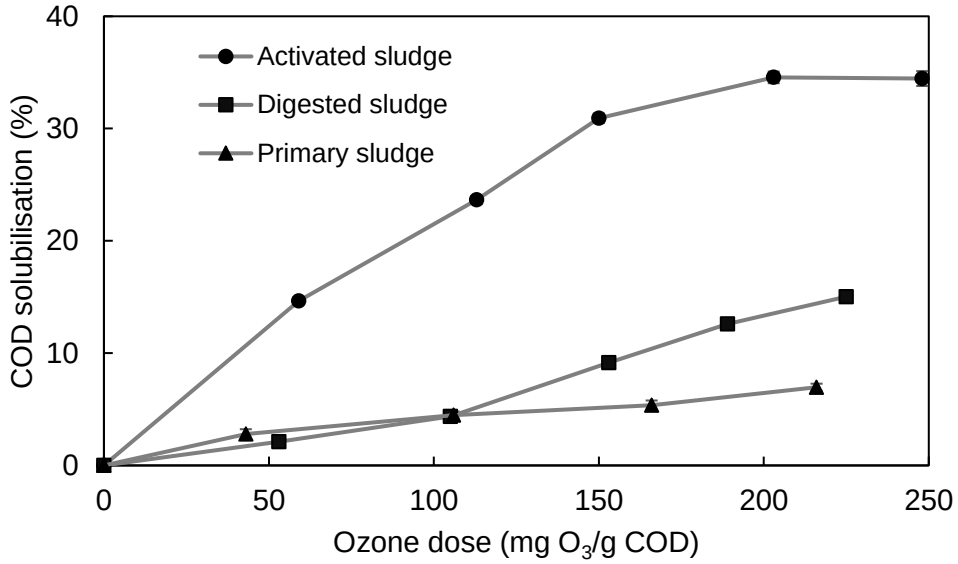

Figure D.2: Influence of ozonation on COD solubilisation.

Methane yield and methane production rates of ozonating samples are presented in Figure D.3. Ozonation increased the methane yield and methane production rate of the activated sludge with a maximum impact at 203 mg O_3/g COD, reaching a methane production of 139 mL N CH_4/g COD (+122 %) and a methane production rate of 10.9 mL N $CH_4 \cdot g\ COD^{-1} \cdot d^{-1}$ (+95 %). A higher ozone

dose resulted in lower increases in methane production. Similarly, the ozonation of anaerobic digested sludge resulted in greater methane yields and methane production rates than the un-ozonated sample; the maximum methane yield reached 52 mL CH_4/g COD (+62%) for an ozone dose of 189 mg O_3/g COD with a methane production rate of 3.3 mL N $CH_4 \cdot g\ COD^{-1} \cdot d^{-1}$ (+14%). Ozonation of primary sludge, however, decreased methane yield and methane production rates of ozonated samples, causing the reduction of methane yield and the methane production rate from 193 to 110 mL N CH_4/g COD and from 15.3 to 6.5 N $CH_4 \cdot g\ COD^{-1} \cdot d^{-1}$, respectively, for an ozone dose of 170 mg O_3/g COD.

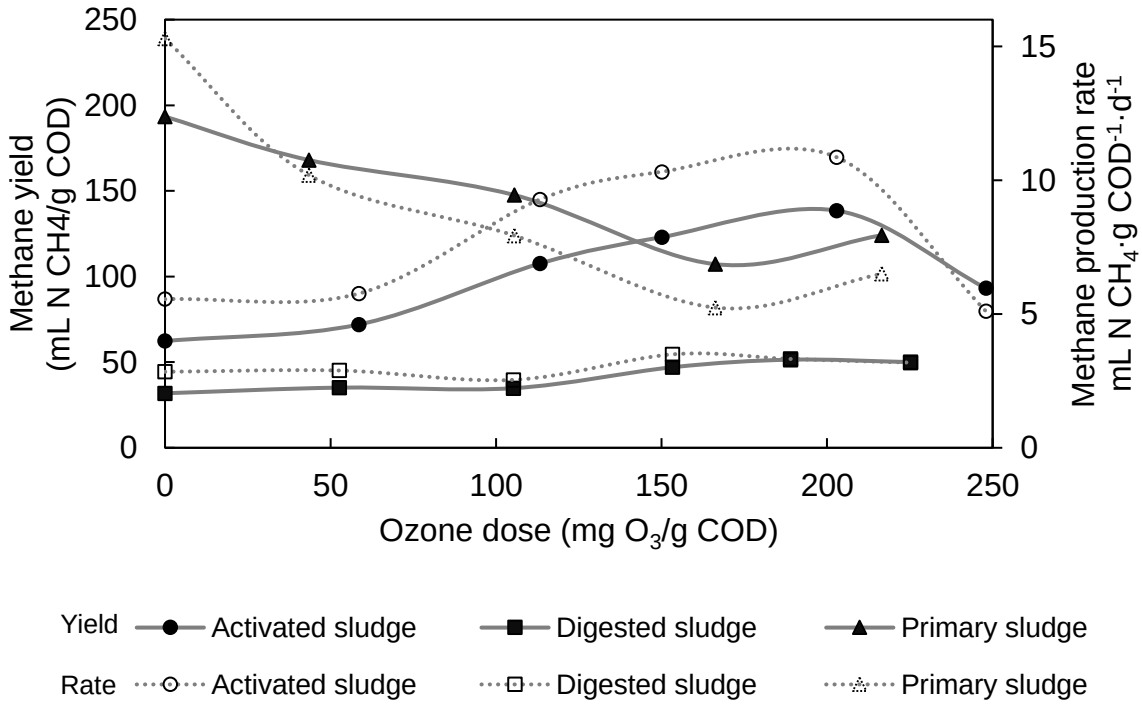

Figure D.3: Effect of ozonation treatment on BMP and specific rate constant of activated sludge, digested sludge and primary sludge.

The impact of ozonation on the methane production of primary sludge and anaerobic digested sludge from samples of the activated sludge system are similar to the results obtained for the CEPT process (Chapter 4); primary sludge and anaerobic digested sludge increased methane yield up to 5% (220 mg O_3/g COD) and 55% (90 mg O_3/g COD), respectively. The impact of ozonation of activated sludge is in agreement with other investigations which have shown a high increase in

methane production. Bougrier et al. (2006) reported that the ozonation of activated sludge resulted in an increase of 125% of biogas (160 mg O_3/g TSS).

This increase in methane production may be associated with increased solubilisation of COD. Solubilisation can increase the accessibility of inert particulate compounds to microorganisms, leading to a subsequent improvement in methane yield and the methane production rate. Ozonation of activated sludge and digested sludge led to high solubilisation with an acceleration of methane production as well as an increase in the quantity produced. On the other hand, primary sludge reached a low solubilisation with a low impact on methane production.