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Low temperature Anaerobic Digestion of Wastewater Sludge

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Summary

The management of waste activated sludge (WAS) may represent up to 50% of the operating costs in a wastewater treatment plant (WWTP). Moreover, the annual sludge production was predicted to grow in European Union from 11.5 M tons dry solids (DS) in 2010 to 13.0 M tons DS in 2020, mainly due to the implementation of Directive 91/271/EC and to restrictive limits on nutrients removal. The implementation of sludge management strategies respecting the waste hierarchy (Directive 2008/98/EC) and enabling its energy recovery (European 20-20-20 Targets) is crucial. Among different management strategies, anaerobic digestion (AD) is a well-established technology for the stabilisation of WAS, converting biodegradable matter into biogas, with the aim of reducing sludge volumes, pathogens and putrescible matter, while producing a renewable energy source. Therefore, medium-scale WWTPs are generally served by mesophilic (35-40°C) digesters. However, in cold-weather countries as Canada, these processes are limited by high requirements of thermal energy. In Italy 70% WWTPs have a size below 20,000 equivalent inhabitants (p.e.), and in this perspective a mesophilic AD process is not energy self-sufficient. In this context, developing low temperature AD of WAS could be strategic, having as main challenge the low rate of AD, mainly due to rate-limiting hydrolysis. AD in psychrophilic conditions has been studied for the stabilization of wastewater, animal wastes and municipal biowaste, but there is still a lack of knowledge about low temperature AD of WAS due to its lower degradability. Moreover, many methods to overcome the limitations of hydrolysis have been introduced as pre-treatments for mesophilic and thermophilic processes, but there is a knowledge gap in literature about the integration of pre-treatments and a low temperature process.

The main focus of this thesis is investigating the technical feasibility of AD at low temperature (22°C) of raw and pre-treated WAS from a Canadian wastewater treatment plant (LaPrairie, QC, 240,000 p.e.). The experimental procedures were conducted at Environmental Engineering Laboratories of the Department of Civil Engineering at McGill University (Montreal, QC, Canada) during a 5-months period of mobility. Data analysis and discussion of results were performed at DIATI.

The experimental assessment consisted of the two steps: increasing of WAS solubilisation by means of different pre-treatments and subsequent enhancement of WAS biodegradability during anaerobic digestion. Firstly, three different pre-treatments were selected, optimised and compared by means of COD solubilisation (S_{COD}) and Disintegration Rate (DR, i.e. the increment in COD soluble fraction due to the pre-treatment) evaluation. Thereby, a thermal (TH) treatment (115-118°C, 30 min), an ozone (OZ) treatment (190 mg O_3/L), and a thermo-alkaline (TA) treatment (0.09 g NaOH/g TS, 70°C, 60 min) gave back respectively a mean DR of 20.7 ± 4.1%, 3.6 ± 0.8%, and 33.4 ± 3.5%, and a mean S_{COD} of 26.2 ± 3.6%, 10.2 ± 2.9%, and 37.8 ± 4.0%. Overall, in terms of increased COD solubilisation the performances of the pre-treatments were found in the order: TA > TH > OZ. Thus, TH and TA significantly enhanced COD solubilisation of WAS, in agreement with literature. Secondly, the biodegradability of raw and pre-treated WAS samples was evaluated during AD tests, carried out in triplicates at 22°C in semi-continuous feeding mode of 3% TS and 15-days SRT, by means of total solids (TS) and volatile solids (VS) removal and biogas production. After three SRTs, the anaerobic digestion of raw, TH, OZ, and TA WAS resulted respectively in mean TS and VS removals of 1.0 ± 0.1% and 5.5 ± 1.2%, 26.5 ± 7.0% and 9.8 ± 1.8%, 9.4 ± 0.8% and 8.0 ± 1.0%, 14.1 ± 1.1% and 22.3 ± 1.8%. Furthermore, after three SRTs, the specific biogas production (SBP) was 0.21 ± 0.03 Nm^3/kg VS_{IN} for raw WAS, 0.30 ± 0.03 Nm^3/kg VS_{IN} for TH, 0.25 ± 0.02 Nm^3/kg VS_{IN} for OZ, and 0.36 ± 0.001 Nm^3/kg VS_{IN} for TA. The higher SBPs for TH and TA WAS were comparable with literature data about mesophilic AD and seemed consistent with larger COD solubilisation for TH and TA.

In conclusion, even though promising enhancements of COD solubilisation were found for both TH and TA pre-treatments, a significant improvement of AD at 22°C was not clearly observed. Thereby, further investigations are needed for evaluating the technical feasibility of AD of pre-treated WAS at low temperature in comparison with conventional mesophilic AD. In addition, new methodologies for technical, energetic, economic and environmental analyses should be developed and applied.

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List of abbreviations

AD	Anaerobic digestion
BP	Biogas production
BOD5	Biochemical Oxygen Demand
COD	Chemical oxygen demand
DR	Disintegration rate
DS	Dry Solids
EU	European Union
FS	Fixed solids
HPH	High-pressure homogenization
HRT	Hydraulic retention time
LCFAs	Long chain fatty acids
LHV	Lower heating value
MEC	Microbial electrolysis cell
MW	Microwave
OLR	Organic loading rate
OZ	Ozone treatment
PE	Population equivalent
RVS	Volatile solids removal
RTS	Total solids removal
SBP	Specific biogas production
sCOD	Soluble COD
SRB	Sulfate reducing bacteria
SRT	Solid retention time
SS	Sewage sludge
TOC	Total organic carbon
TPAD	Temperature phased anaerobic digestion
US	Ultrasonication
VFAs	Volatile fatty acids
VS	Volatile solids
VSS	Volatile suspended solids
tCOD	Total COD
TA	Thermo-alkaline treatment
TH	Thermal treatment
TS	Total solids
WAS	Waste activated sludge
WV	Working volume
WWTP	Wastewater treatment plant

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1. Introduction

Waste activated sludge (WAS) is a problem of growing importance, produced in large quantities as a by-product of municipal and industrial wastewater treatment. In a wastewater treatment plant (WWTP) up to 50% of the operating costs may be linked to the management of wastewater sludge. Moreover, the annual sludge production was predicted to grow in European Union from 11.5 M tons dry solids (DS) in 2010 to 13.0 M tons DS in 2020, mainly due to the implementation of Directive 91/271/EEC and to the restrictive limits on the removal of nutrients and emerging contaminants. The implementation of sludge management strategies respecting the waste hierarchy (Directive 2008/98/EC) and enabling its energy recovery is crucial, because energy efficiency is a legal requirement for a WWTP (European 20-20-20 Targets). Among different management strategies, the stabilisation of sludge can be performed by means of anaerobic digestion (AD), which is the biological conversion of biodegradable matter into biogas, with the aim of reducing sludge volumes, pathogens and putrescible matter, while producing a renewable energy source which can partly cover the energy demand of the WWTP. Medium-scale WWTPs are usually served by mesophilic (35-40°C) digesters. However, in cold-weather countries as Canada, mesophilic processes are limited by high requirement of thermal energy. In Italy 70% WWTPs have a size below 20,000 p.e., and in this perspective a mesophilic AD process is not energy self-sufficient. In this context, developing low temperature AD of WAS with lower energy costs could be strategic, having as main challenge the low rate of AD, mainly due to rate-limiting step of hydrolysis particularly sensitive to low temperatures. AD in psychrophilic conditions has been studied and developed for stabilization of wastewater, animal wastes and municipal biowaste, but there is still a lack of knowledge about low temperature AD of WAS due to its lower degradability. Moreover, many methods to overcome the limitations of hydrolysis have been introduced as pre-treatments for mesophilic and thermophilic processes, but there is a knowledge gap in literature about the integration of pre-treatments and a low temperature process.

This master thesis aims to investigate the technical feasibility of anaerobic digestion (AD) at low temperature (22°C) of raw and pre-treated WAS from a wastewater treatment plant without primary sedimentation (LaPrairie, QC, Canada, 240000 p.e.). The experimental assessment consisted of two steps: increasing of WAS solubilisation by means of different pre-treatments and subsequent enhancement of WAS biodegradability during anaerobic digestion. Firstly, three different pre-treatment methods were selected, optimised and compared by means of COD solubilisation: a thermal (TH) treatment (115-118°C, 30 min), an ozone (OZ) treatment (190 mg O_3/L), and a thermo-alkaline (TA) treatment (0.09 g NaOH/ g TS, 70°C, 60 min). Secondly, the biodegradability of raw and pre-treated WAS samples was evaluated during AD tests, carried out in triplicates at 22°C in semi-continuous feeding mode of 3% TS and 15-days SRT, by means of total solids (TS) and volatile solids (VS) removal and biogas production. This phase consisted of two parts, the start-up phase for the adaptation of mesophilic biomass at 22°C, lasted two SRTs, and the anaerobic digestion test, conducted for more than three SRTs. During the AD test, pH, TS and VS and COD were monitored on both the fed and digested sludge, as well as biogas production.

All the experimental procedures were conducted at the Environmental Engineering Laboratories of Department of Civil Engineering and Applied Mechanics of McGill University (Montreal, QC, Canada) under the co-supervision of Professor Dominic Frigon during a 5-months period of mobility funded by Politecnico di Torino. Data analysis and discussion of results were performed at DIATI, Politecnico di Torino.

2. State of the Art

2.1. Wastewater sludge

2.1.1. Definition, characteristics and origin

Wastewater sludge is generally defined as the solid or semi-solid by-product of municipal and industrial wastewater treatments (Fijalkowski et al., 2017). It is a complex mixture of microorganisms, inorganic materials, moisture and undigested organics, containing proteins, lipids and polysaccharides, plant macromolecules and organic micro-pollutants, as well as simpler sub-molecules (Tyagi and Lo, 2013).

As shown in Figure 2.1, wastewater can be treated through physical, biological and chemical processes, thus, sludge, depending on the step of the treatment, is classified as primary, secondary and tertiary.

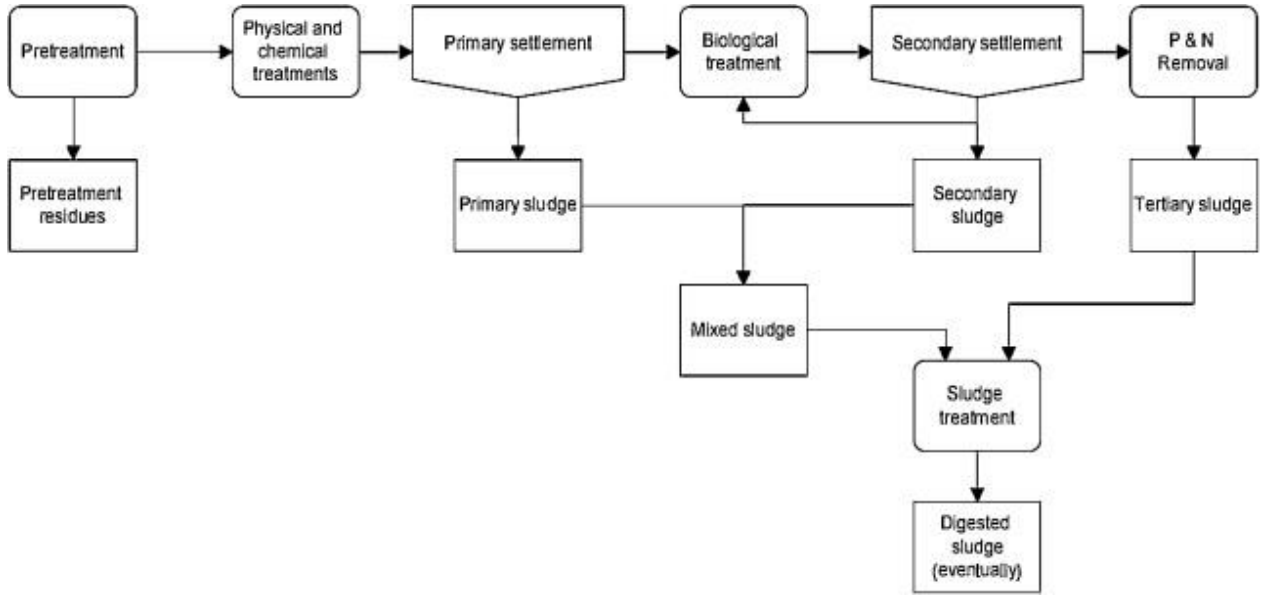


Figure 2.1: Traditional wastewater treatment scheme and sludge origin (Manara and Zabaniotou, 2012).

Primary sludge, removed in primary settler, consists mainly of settleable solids, while secondary sludge, also known as waste activated sludge (WAS), is separated from wastewater after biological processes; finally, tertiary sludge is produced during tertiary or treatments, for reaching a high-level decontamination. Consequently, primary sludge generally exhibits a higher degradability than WAS (Manara and Zabaniotou, 2012), as shown in Table 2.1, that reports typical characteristics of primary and secondary sludge.

Table 2.1: Characteristics of primary and secondary sludge (Burton et al., 2013).

Parameter	Primary sludge		Secondary sludge	
	Range	Typical	Range	Typical
Total solids, TS (%)	1-6	3	0.4-1.2	0.8
Volatile solids, VS (% TS)	60-85	75	60-85	70
Nitrogen, N (% TS)	1.5-4	2.5	2.4-5.0	3.8
Phosphorus, P_2O_5 (% TS)	0.8-2.8	1.6	2.8-11	5.5
Potassium, K_2O (% TS)	0-1	0.4	0.5-0.7	0.6
Grease and fats (% TS)	5-8	6	5-12	8
Proteins (%TS)	20-30	25	32-41	36
Cellulose (%TS)	8-15	10	-	
Organic acids (mg/L as acetate)	200-2000	500	1100-1700	1350
Iron (%TS)	2.0-4.0	2.5	-	
Silica, SiO_2, (%TS)	15-20	-	-	
pH	5.0-8.0	6	6.5-8.0	7.1
Alkalinity (mg/L as $CaCO_3$)	500-1500	600	580-1100	790
Calorific value (MJ/kg TS)	23-29	25	19-23	20

2.1.2. Current production and management strategies

European Union

The implementation of the Urban Waste Water Treatment (UWWT) Directive 91/271/EEC (Council of the European Communities (CEC), 1991) determined the improvement of wastewater treatment systems in all Member States. As a consequence, the annual sludge production in EU-15 (old Member States) increased by 50% between 1992 and 2005 (Kelessidis and Stasinakis, 2012) and it was predicted to grow in EU-27 from 11.5 M tons dry solids (DS) in 2010 to 13.0 M tons DS in 2020 (Milieu Ltd et al., 2008), mainly due to the implementation of the Directive 91/271/EEC by EU-12 (new Member States) (Panepinto et al., 2016). Thus, obviously, the difference between productions of EU-12 and EU-15, respectively 13% and 87% of the total between 2006 and 2010 (European Commission Eurostat), is likely to continue declining.

In Europe sewage sludge is subjected to a large number of management strategies which respect the waste hierarchy: prevention, preparing for re-use, recycling, other recovery and disposal (European Parliament and Council of the European Union, 2008). Starting from the first steps of the hierarchy, among the sludge stabilization methods aerobic and anaerobic digestion appear as the most diffuse in EU-27 countries, secondly, sludge dewatering, mainly mechanical, seems to be a key stage in sludge management; moreover, thermal drying has an important role in EU-15 and long-term storage is also used as an easy and cheap method (Kelessidis and Stasinakis, 2012). The evolution in sewage sludge reuse, recovery and disposal strategies in EU-15 between 1992 and 2015 is shown in Figure 2.2. According to this data, agricultural reuse and compost are the most commonly used methods, reaching 58% between 2011 and 2015, incineration shows a clear increase from 11% to 28% between 1992 and 2011-2015 and landfilling presents a steady decline from 33% to 9% during the same period.

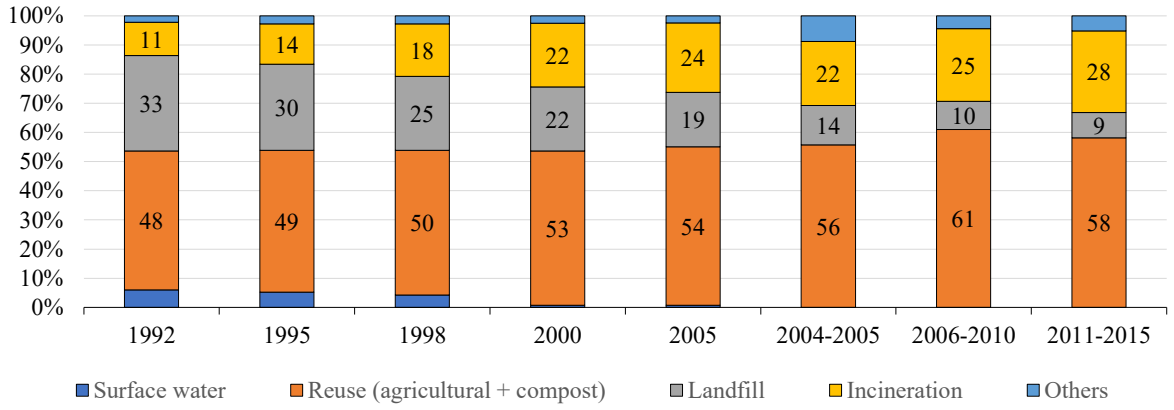


Figure 2.2: Sewage sludge reuse and disposal methods in EU-15 countries between 1992 and 2015. Italy and Sweden aren't included in annual data from 1992 and 1995, while the averages of annual values between 2004 and 2005, 2006 and 2010, 2011 and 2015 comprehend all EU-15 (European Commission, 1999, European Commission Eurostat).

Finally, Figure 2.3 shows the differences in sludge disposal methods between EU-15 and EU-12 countries in 2006-2010. According to these results, the most common method in EU-15 was the use in agriculture (47%). On the other hand, in EU-12 landfilling represented 25% of sludge, even if 32% corresponded to unspecified methods.

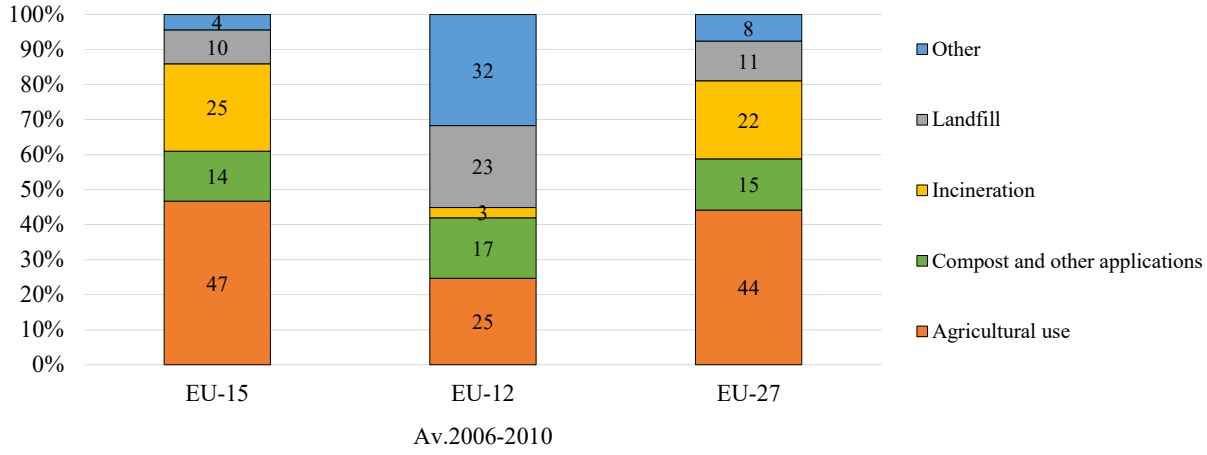


Figure 2.3: Sewage sludge reuse and disposal methods in EU-15, in EU-12 and EU-27 countries between 2006 and 2010 (European Commission Eurostat).

Italy

According to ISPRA, in Italy more than 3 M Tons of sewage sludge were produced in 2015, in particular almost 450 thousands of Tons in Lombardia and 410 thousands of Tons in Emilia-Romagna (ISPRA - Istituto Superiore per la protezione e la ricerca ambientale, 2017). Figure 2.4 shows the different sewage sludge disposal and reuse methods in use in Italy in 2015. Among reuse strategies, recycling of organic

substances was the most important method with 35%, followed by land treatment with benefits for agriculture with 11%; moreover, among different disposal options, biological treatment and landfilling represented respectively 29% and 13%, while incineration was only 2%.

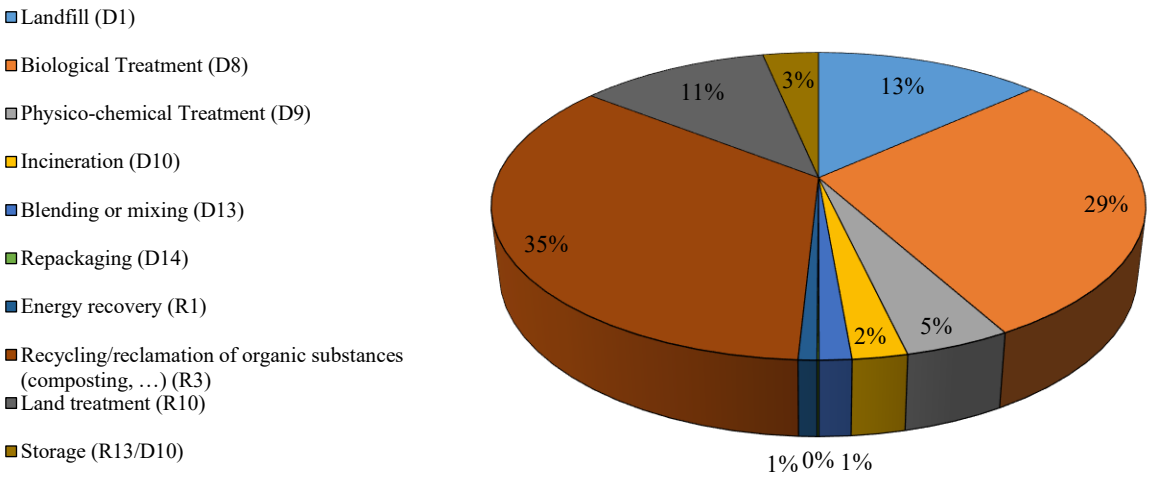


Figure 2.4: Sewage sludge management methods in Italy in 2015. (ISPRA - Istituto Superiore per la protezione e la ricerca ambientale, 2017).

Canada

In Canada there are no detailed national statistics on wastewater management system because its regulatory control is provided by the competent authorities of the 13 Provincial and Territorial Governments (LeBlanc et al., 2009). Moreover, there is a variegated reality, considering that approximately 25% of the population is served by systems such as septic tanks, removing the so called "septage" from the liquid, while the other 75% is connected to the municipal sewage collection system (Canadian Council of Ministers of the Environment (CCME), 2012b). In the approximately 3000 facilities there are more than 4000 sewage treatment facilities (LeBlanc et al., 2009), producing more than 660 thousands of Tons DS of sludge and biosolids per year, where the latter is defined as treated sludge (Canadian Council of Ministers of the Environment (CCME), 2012a). This production is destined to several management methods. According to a survey of 2001 (Canadian Water and Wastewater Association (CWWA), 2001), over 40% of biosolids was land used, 30% was disposed in landfill, and just 15% incinerated (Ryerson University, 2015).

2.1.3. Current legislation on wastewater sludge

European Union

The variegated and evolving scenario regarding the management of sewage sludge, as exposed in the previous section, was the result of the last thirty years of European Policy. The most common form of EU legislation is a Directive which defines the guidelines to be adopted and implemented by all Member States within a specified period (LeBlanc et al., 2009).

The first text regarding the sludge management has been the Sewage Sludge Directive 86/278/EEC (Council of the European Communities (CEC), 1986), which promotes the use of sewage sludge in agriculture and sets all the requirements to avoid harmful effects on humans, animals, vegetation, soil, surface and groundwater (Christodoulou and Stamatelatou, 2016). After more than twenty years, in most of EU countries

these requirements have been exceeded (LeBlanc et al., 2009) result that the European Commission is currently considering a review of the Directive (European Commission, 2016).

Secondly, the Landfill Directive 99/31/EC (Council of the European Union (CEU), 1999) promotes the reuse and recycling of sludge by means of obligation to reduce by 65% the amount of organic wastes disposed in landfills by 2016 and several Member States established stringent limits about the TOC content of acceptable wastes in landfills (Panepinto et al., 2016). Moreover, according to Directive 2000/76/EC on the incineration of wastes (European Parliament and Council of the European Union, 2000), sewage sludge can be considered as waste and, as such, can be incinerated, respecting numerous standards and requirements (Milieu Ltd et al., 2010).

Lastly, under new Waste Framework Directive 2008/98/EC certain types of sewage sludge might cease to be waste by respecting the "End-of-waste" criteria, even if definition of "bio-waste", which should be especially destined to digestion and/or composting, doesn't include SS (Milieu Ltd et al., 2010). However, many Member States currently produce significative amounts of compost from SS and the final report by IPTS (Saveyn and Eder, 2014) considered SS as an input material for composting and anaerobic digestion (Mininni et al., 2015).

Italy

Sewage sludge is considered to be a waste under article 127 of Legislative Decree No 152 of April 3, 2006 (Italian Republic, 2006). Consequently, a certain EWC code (European Waste Catalogue) is used to identify that specific type of SS, e.g. code 190805 corresponds to sludge from treatment of urban waste water, considered as non-hazardous waste (Italian Republic, 2006). Depending on the EWC code distinct management strategies may be applied, in accordance with the already mentioned Waste Hierarchy.

In particular, under Legislative Decree No 99 of January 27, 1992, which transposes Directive 86/278/EEC, sewage sludge can be used in agriculture by respecting several conditions, e.g. after being subjected to previous treatments, not exceeding certain quantities and respecting limits of several parameters (Italian Republic, 1992). In addition, other management methods are ruled by Ministerial Decree of February 5, 1998, which specifies simplified procedures for treating some non-hazardous wastes, such as anaerobic digestion, composting or energy recovery for EWC code 190805 (Italian Republic, 1998). Finally, Legislative Decree No 36 of January 13, 2003 states that wastes are allowed to be landfilled only after treatment and introduces other limitations (Italian Republic, 2003), resulting in the reduction of landfilled SS.

Canada

In Canada different authorities provide the governance of sludge management, from federal to provincial or territorial, until municipal levels. Provinces and Territories generally provide regulations over biosolids management, except in federal lands, e.g. national parks and aboriginal lands, regulated by Environment Canada. Moreover, in most provinces, the wastewater management system is mostly operated by municipalities, even if some facilities are managed by other public or private bodies (Canadian Council of Ministers of the Environment (CCME), 2010).

In addition, other organisations, such as the Canadian Council of Ministers of the Environment (CCME) and the Bureau de Normalisation du Québec (BNQ), provide guidelines and standards as reference for new provincial and federal regulations (Canadian Council of Ministers of the Environment (CCME), 2010).

For instance, CCME published the Canada-wide Approach for the Management of Wastewater Biosolids, known as the Approach, in 2012 with the aim to specify the beneficial uses of wastewater sludge, septage

and biosolids, not intended as wastes but as sources of nutrients, organic matter and energy. Thus, energy production, composting, agricultural and forestry applications as fertilizer and land reclamation are considered beneficial uses of biosolids, while landfilling or combustion without benefits are defined disposal options (Canadian Council of Ministers of the Environment (CCME), 2012a).

Although in Canada some biosolids are still disposed, e.g. in Québec approximately 35% have been landfilled and 45% have been incinerated (LeBlanc et al., 2009), provincial regulations are generally trying to reject disposal options. In Québec, therefore, a green tax against all residuals landfilled has been introduced (LeBlanc et al., 2009) and a strategy for the banning the disposal of municipal biosolids by 2020 have implemented according to provincial regulation (Bakhshi et al., 2018).

2.1.4. Current management strategies

In the previous sections there was evidence that sludge management is a problem of growing importance. Moreover, in a wastewater treatment plant (WWTP) up to 50% of the operating costs may be linked to the management of wastewater sludge, requiring some methods for reducing its volume along with the presence of putrescible matter and pathogens, as well as improving its quality (Appels et al., 2008). Typical sludge treatments are shown in Figure 2.5, dividing processes into primary and secondary treatments, and final management.

Thus, according to this scheme, firstly the gravity or mechanical thickening significantly reduces the water content, secondly wastewater sludge is subject to biological stabilization, aerobic or anaerobic, and then undergoes mechanical dewatering, reducing as much as possible its final volume. Moreover, sludge can be dried, thermal treated or composted before final management routes, such as recycling in agriculture, in land reclamation and in the construction sector, along with energy recovery or disposal (Appels et al., 2008, Kacprzak et al., 2017).

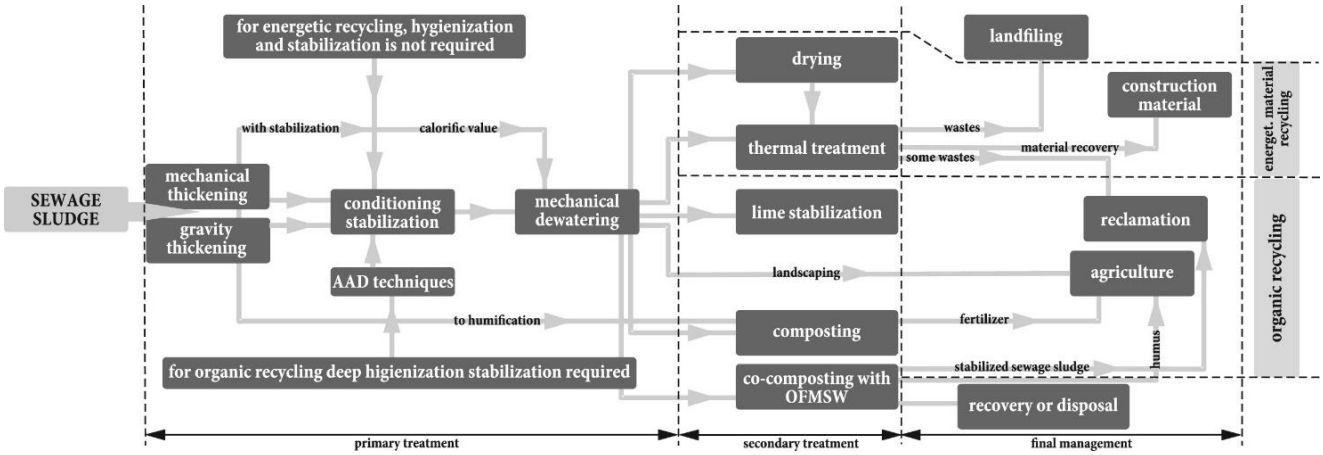


Figure 2.5: Typical wastewater sludge treatments (Kacprzak et al., 2017).

2.2. Anaerobic digestion of wastewater sludge

2.2.1. Definition, benefits and limitations of the process

As mentioned above, biological processes, involving a wide variety of microorganisms, can be exploited as methods for the stabilization of wastewater sludge in a WWTP; with this aim both aerobic and anaerobic digestion can be used. Moreover, some requirements about the management of sludge have been mentioned, e.g. the reduction of volumes and associated costs, along with the removal of pathogens and putrescible matter, avoiding odour problems; these can be met by means of anaerobic digestion (AD).

In general, this process can be defined as the biological conversion of biodegradable matter, composed of organic and sporadically inorganic compounds, mainly to methane, carbon dioxide and new biomass by complex microorganisms communities in absence of a source of oxygen (Batstone and Jensen, 2011). Thus, residual sludge may be suitable for land use (Cieślik et al., 2015). Additionally, the process has also the benefit of transforming organic matter into biogas, a high calorific energy source with the advantage of being renewable. Many WWTPs currently exploit this source to partially cover the energy demand of the plant, contributing to the sustainability of the whole wastewater treatment.

This is one of the main reasons why the anaerobic stabilization is a well-established technology in WWTPs and is usually preferred over aerobic digestion, mediated by other microorganisms in the presence of oxygen, requiring high-energy costs for aeration. Moreover, in this case a large fraction of biodegradable matter is converted to biomass (sludge), instead of being transformed in a useful energy form (biogas) (van Lier et al., 2008). Unfortunately, AD is considered to be cost-effective for relatively large WWTPs (Cieślik et al., 2015), roughly several tens of thousands population equivalent in Italy, due to its slow reaction rates and associated high volumes of the digesters (Appels et al., 2008). It might be appropriate to collect sludge from several WWTPs, but the cost of transport would affect the profits of the anaerobic digestion process (Cieślik et al., 2015).

Other inevitable limitations should be considered, such as the partial removal of organic matter, sufficient to increase the concentration of heavy metals, along with inorganic and recalcitrant compounds in residual sludge (Appels et al., 2008); secondly the process is vulnerable to various inhibitors and is affected by several parameters, requiring a continuous monitoring. Finally, the amount and the quality of biogas can vary considerably depending on many factors.

In conclusion, anaerobic digestion can be considered a crucial step in the management of wastewater sludge, which needs further improvements to current limitations, due to complexity of biological process, as will be explained in following sections.

2.2.2. Microbiology and biochemistry

Anaerobic digestion is a complex multi-step process involving series and parallel biochemical reactions, which can be divided into four phases, namely: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (van Lier et al., 2008). Each phase is mediated by various groups of microorganisms, in some cases standing in syntrophic interrelation (Deublein and Steinhauser, 2011).

Before examining each of them in details, some basic concepts about biochemical activity of microorganisms should be reminded. In general, an organism needs carbon and energy for synthesizing new cellular material, as well as inorganic and organic nutrients. Microorganisms can be classified based on the source of carbon and energy. Thus, *heterotrophs* use organic carbon to produce new cells, while *autotrophs* exploit carbon dioxide, requiring more energy to perform the reduction reaction of carbon dioxide. This results in a lower growth rate for autotrophs. Secondly, microorganisms obtaining energy from light are

named *phototrophs*, in contrast *chemotrophs* can use chemical redox reactions as source of energy (Burton et al., 2013).

There is a distinction between metabolic mechanism, named *respiration*, producing energy through electrons transfer from an electron donor to an electron acceptor which is outside of the cell, and *fermentation*, that uses an internal acceptor and is characterized by lower energy yield than respiration. Finally, reactions involving free and bond oxygen as electron acceptor are called respectively *aerobic* and *anoxic*, while reactions taking place in absence of oxygen are named *anaerobic*. Thus, one can find both aerobic and anaerobic microorganisms, which can be *obliged* or *facultative* (Burton et al., 2013).

These concepts are relevant in the comprehension of the AD complex food web, shown in Figure 2.6. Essentially, hydrolytic and fermentative bacteria convert complex organic matter (proteins, carbohydrates, lipids) into simpler compounds, mainly organic acids, hydrogen and carbon dioxide, which are finally degraded by methanogens to methane (CH4), carbon dioxide (CO2), ammonium (NH_3), hydrogen sulfide (H_2S) and water (van Lier et al., 2008), as explained in following paragraphs.

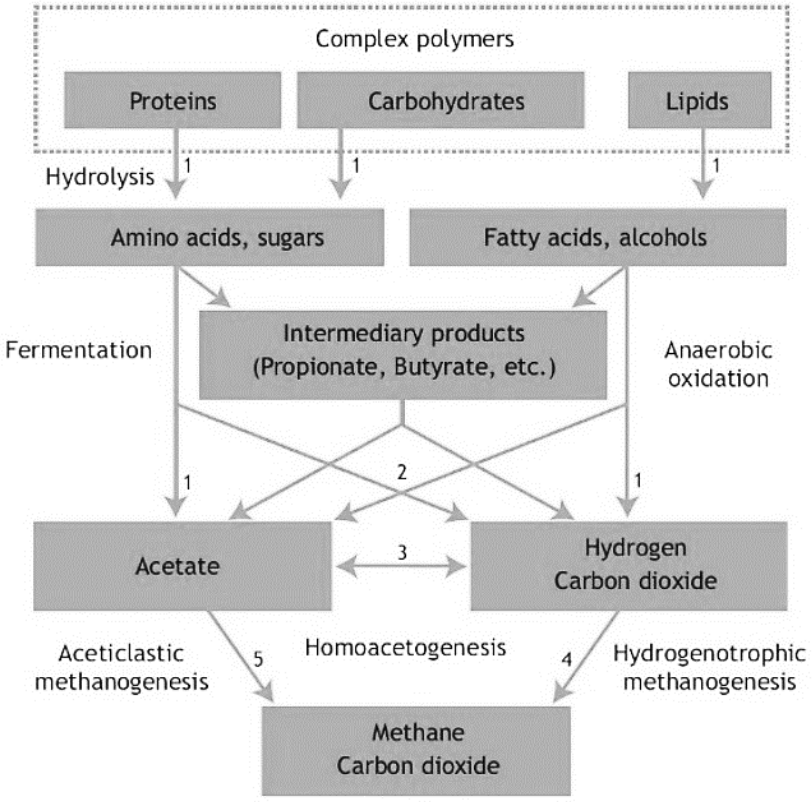


Figure 2.6: Essential phases in anaerobic digestion process. Numbers identify different groups of microorganisms: 1. Hydrolytic and fermentative bacteria, 2. Acetogenic bacteria, 3. Homo-acetogenic bacteria, 4. Hydrogenotrophic methanogens, 5. Aceticlastic methanogens (van Lier et al., 2008).

Hydrolysis

Microorganisms cannot digest directly particulate and insoluble matter, so that the preliminary step of hydrolysis is necessary. This process involves the conversion of complex insoluble polymers, mainly proteins, carbohydrates and fats, as shown in Figure 2.6, consisting of many monomeric molecules linked by unique chemical bonds, into simpler soluble compounds, as amino acids, sugars, long chain fatty acids (LCFAs), and alcohols, which can pass through the cell membranes of acidogenic bacteria. Specifically,

hydrolytic bacteria, along with facultative and obligate anaerobes produce and excrete specific extracellular enzymes, determining the breakage of these unique bonds (Appels et al., 2008, de Lemos Chernicharo, 2017, Gerardi, 2003). A simple scheme of hydrolysis of proteins is shown in Figure 2.7. The time needed for hydrolysis depends on the compound, varying from few hours for carbohydrates to few days for proteins and lipids (Deublein and Steinhauser, 2011).

Moreover, in the case of more complex substrates, such as wastewater sludge, the hydrolysis is preceded by cell lysis, which is particularly critic due to the fact that microbial cells are naturally resistant to lysis and tend to form flocs (Batstone and Jensen, 2011, van Lier et al., 2008). Thus, the hydrolytic phase can be rate limiting for the whole stabilization process whether the quantity of hydrolytic enzymes is not sufficient or the contact with the organics is not effective, in simple terms if the hydrolysis is not functioning properly (Parkin and Owen, 1986). Therefore, the importance of a large and diverse community of microorganisms for ensuring the proper amount and variety of enzymes must be underlined (Gerardi, 2003), as well as an appropriate mixing and temperature, by the moment hydrolysis seems to be particularly sensitive to this parameter and its fluctuations (van Lier et al., 2008).

In addition, it should be noticed that a significative fraction of organic matter cannot be hydrolyzed, due to several factors, such as structure, inaccessibility or nonhydrolyzable linkages. These components are defined nonbiodegradable or refractory and can range from 35-40% to 70-80% of VS of municipal sludge, depending on its origin (Parkin and Owen, 1986).

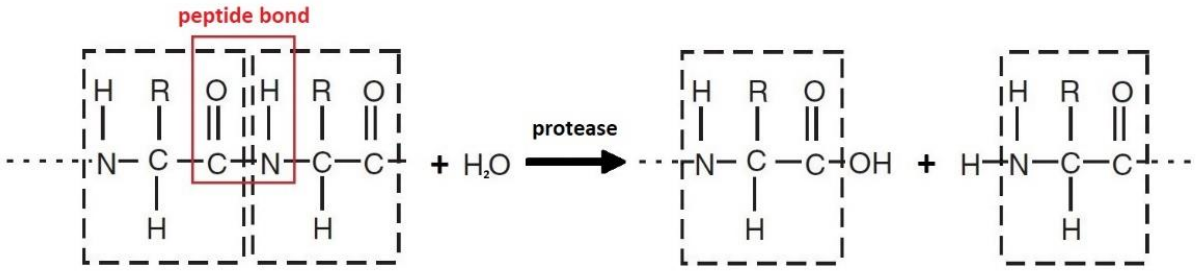


Figure 2.7: The hydrolysis of protein chain to amino acids. Adapted from (Batstone and Jensen, 2011).

Acidogenesis

The products of hydrolysis, mainly amino acids, sugars, LCFAs and alcohols (Figure 2.6), are diffused inside membrane cells of a large family of hydrolytic and other bacteria, then fermented or anaerobically oxidized, and finally excreted by the cells. The products of acidogenesis consist of volatile fatty acids (VFAs), such as propionic and butyric acids, alcohols and lactic acid, as well as carbon dioxide, hydrogen, ammonia and sulfide, along with new bacterial cells (Appels et al., 2008, de Lemos Chernicharo, 2017). Among various end products the most abundant are generally VFAs and carbonic acid, even if the composition of end products depends on the operating and environmental conditions. For instance, if hydrogen is removed by methanogens, acetate will be the main end product, while if methanogenesis is retarded, hydrogen will accumulate and propionate and butyrate, as well as lactate and alcohols may appear (Appels et al., 2008).

The acidogenesis is carried out by a rich population of bacteria, being facultative anaerobes, strict or obligate anaerobes (Parkin and Owen, 1986), and is characterized by the highest conversion rate compared to other steps, resulting in the risk of accumulation of VFAs and of a pH drop in case the following stages would not appropriately occur. Moreover, acidifier bacteria are still active at low pH (Appels et al., 2008). Hydrogen, one of the end products, can be inhibitory to many of the acidogenic bacteria, but luckily is rapidly consumed by methanogenic bacteria as a source of energy (Parkin and Owen, 1986).

Acetogenesis

The compounds produced during acidogenesis phase can be directly used by methanogenic bacteria (carbon dioxide and hydrogen) or can be converted to methanogenic substrates by acetogenic bacteria. Thus, according to scheme of Figure 2.6, VFAs and alcohols are oxidized into acetate, carbon dioxide and hydrogen, while VFAs with chains longer than one unit (e.g. propionic and butyric acids) are converted into acetate and hydrogen, being the most important acetogenic substrates (Adekunle and Okolie, 2015). Carbon dioxide and hydrogen, furthermore, are converted to acetate by homo-acetogenic bacteria. Acetogenic reactions of some of the main substrates are shown in Table 2.2, including homo-acetogenesis process.

Table 2.2: Acetogenic main reactions. Adapted from (Deublein and Steinhauser, 2011).

Substrate	Reaction
Propionic acid	$CH_3(CH_2)COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$
Butyric acid	$CH_3(CH_2)_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$
Glycerine	$C_3H_8O_3 + H_2O \rightarrow CH_3\ COOH + 3H_2 + CO_2$
Lactic acid	$CH_3CHOHCOO^- + 2H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 2H_2$
Ethanol	$CH_3(CH_2)OH + H_2O \rightarrow CH_3COOH + 2H_2$
Homo-acetogenesis reaction	$2CO_2 + 4H_2 \leftrightarrow CH_3COOH + 2H_2O$

As can be noticed from Table 2.2, acetogenic bacteria obligatory produce hydrogen, but their metabolism is inhibited by hydrogen. These reactions, e.g. acetate formation by oxidation of ethanol, butyric and propionic acids, are thermodynamically disadvantageous and can occur only if the hydrogen partial pressure is very low. However, this condition is generally achieved thanks to methanogenic bacteria, which constantly use hydrogen as substrate for methane formation, keeping its pressure at a level suitable for acetogenic bacteria, usually between 120^{-4} – 10^{-6} atm. This microbial interdependence between hydrogen-producing and consuming organisms is called *syntrophic association*, determining the influence of H_2 partial pressure on relative abundances of acetogenic products, and the mechanism is named *interspecies hydrogen transfer* (Deublein and Steinhauser, 2011, van Lier et al., 2008).

Methanogenesis

The final phase of anaerobic conversion of organic matter into methane and carbon dioxide is performed by methanogenic bacteria. These obligate anaerobes can use a limited spectrum of substrates, mainly, acetic acid, formic acid and carbon monoxide, as well as carbon dioxide and hydrogen, besides methanol and methylamines (de Lemos Chernicharo, 2017). Therefore, as explained in Table 2.3, according to affinity for substrates can be divided into two main groups: acetate-using methanogens (*aceticlastic*) and hydrogen-using methanogens (*hydrogenotrophic*) (van Lier et al., 2008). Approximately 70% of the methane formed comes from acetate, while the remaining 30% mainly results from the reaction between hydrogen and carbon dioxide.

The multi-step conversion of complex organic matter to simpler compounds ends in the main final products, methane and carbon dioxide, which essentially separate from sludge as gas. Thus, the removal of degradable matter from sludge occurs just during methanogenic phase (Parkin and Owen, 1986).

Table 2.3: Main methanogenic reactions and the corresponding bacterial groups. Adapted from (Deublein and Steinhauser, 2011).

Group	Reaction
Hydrogenotrophic methanogens	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$
Aceticlastic methanogens	$CH_3COOH + H_2O \rightarrow CH_4 + CO_2$

Finally, it is important to observe that methanogenic bacteria are characterized by slow growth rates, requiring several days to obtain a large population, depending on the temperature, and producing a relatively small amount of bacteria cells (new sludge) (Gerardi, 2003). Moreover, methanogenic bacteria seem to be more sensitive to operating conditions than acidogenic bacteria.

2.2.3. Kinetic and stoichiometry of reaction and biogas production

The AD process utilizes various groups of microorganisms for stabilizing wastewater sludge and for producing biogas. In the following section some kinetic and stoichiometric aspects of AD process as well as some methods for estimating the theoretical biogas production will be discussed.

Kinetics aspects

Anaerobic microbial communities need to consume appropriate substrates as source of carbon and energy to continue growing and surviving. Thus, basically, when putting a small microbial population in a closed system (batch reactor) in contact with an excess of a certain substrate, this will be consumed while the microbial population will evolve according to a defined dynamic, characterized by four sequential growth phases: (1) the *lag phase*, which is the time required for the acclimation of microorganisms in new environmental conditions with negligible growth rate; (2) the *exponential-growth phase*, when bacteria reproduces at their maximum growth rate without limitations in the availability of substrate; (3) the *stationary phase*, with constant biomass concentration due to the equilibrium between growth and death rates of cells; (4) the *death phase*, characterized by a decline in the biomass concentration linked to the depletion of substrate (Burton et al., 2013). In contrast, in an open system one can regulate the flux of substrate and nutrients with the objective of maintaining bacteria in their exponential growth phase for a long time (Echiegu, 2015).

Among different proposed models, the basic equations (2.1) and (2.2) can be used to describe respectively the consumption of substrate in the closed system and the growth of bacteria population, where $-\frac{dS}{dt}$ is the rate of substrate removal (mg $L^{-1}day^{-1}$); k is the maximum specific substrate utilization rate (mg $L^{-1}day^{-1}$); S is the substrate concentration (mg/L); K_S is Monod half-velocity constant (mg/L), i.e. the substrate concentration at one half of the maximum specific growth rate; X is the bacterial concentration (mg/L); $\frac{dX}{dt}$ is the bacterial growth rate (mg $L^{-1}s^{-1}$); Y is the growth yield, i.e. the ratio of bacterial growth rate to substrate consumption rate; b is the bacterial death rate (day^{-1}) (Echiegu, 2015, Parkin and Owen, 1986).

$$-\frac{dS}{dt} = \frac{kSX}{K_S + S} \tag{2.1}$$

$$\frac{dX}{dt} = Y\left(-\frac{dS}{dt}\right) - bX \tag{2.2}$$

The substrate concentration S is generally expressed as Chemical oxygen demand (COD), while the bacterial concentration using Volatile suspended solids (VSS) (Parkin and Owen, 1986).

Finally, substituting equation (2.1) into (2.2) and dividing by X, the specific bacterial growth rate μ (day^{-1}) can be obtained, according to expression (2.3). Thus, the bacterial growth rate depends on the substrate (organic matter) availability, through the kinetic coefficient Y, k, K_S, b (Parkin and Owen, 1986).

$$\mu = \frac{\frac{dX}{dt}}{X} = \frac{YkS}{K_S + S} - b \tag{2.3}$$

Using the previous equations and realizing mass balances around a certain reactor, it is possible to determine relations linking process efficiency, i.e. substrate removal efficiency, bacterial growth rate and biogas production, to reactor hydraulic parameters, such as Hydraulic and Solid retention time (respectively HRT and SRT), revealing a method for controlling process efficiency (Echiegu, 2015, Parkin and Owen, 1986).

Chemical oxygen demand (COD)

The organic matter in wastewater sludge is usually quantified and monitored by exploiting the fact that these compounds can be oxidized by strongly oxidizing agents. In the case of anaerobic digestion processes, the Chemical oxygen demand (COD) test is the most commonly used, consisting in the conversion into carbon dioxide and water of almost all organic compounds by a strong oxidizing agent (bichromate) at high temperature (150°C) (van Lier et al., 2008) The COD is expressed as concentration (g O_2/L) of oxygen equivalent.

Thus, assuming a complete oxidation, the theoretical COD of a generic organic compound can be calculated by the following oxidation reaction (van Lier et al., 2008):

$$C_nH_aO_bN_d + (n + a/4 - b/2 - 3d/4)O_2 \rightarrow nCO_2 + (a/2 - 3d/2)H_2O + dNH_3 \quad (2.4)$$

According to equation (2.4), a mole of organic matter requires $(n + a/4 - b/2 - 3d/4)$ of O_2 to be oxidized. Hence, the theoretical COD can be expressed as follows (van Lier et al., 2008):

$$COD\left(\frac{g\,COD}{gC_nH_aO_bN_d}\right) = \frac{8(4n + a - 2b - 3d)}{(12n + a + 16b + 14d)} \quad (2.5)$$

Finally, it is important to notice that some fraction of the COD of sludge is not biodegradable, hence the COD is generally classified in *biodegradable* and *nonbiodegradable*. In addition, a distinction based on the solubility is also possible, so that COD can be divided into how much is *dissolved* (*soluble*) and how much is *particulate* (Burton et al., 2013, de Lemos Chernicharo, 2017).

COD balance

Section 2.2.2 has shown that during anaerobic digestion there is no substrate (COD) destruction, but just complex substrate rearrangement into simpler intermediates, eventually resulting in methane and carbon dioxide as main final products. Therefore, a COD balance can be utilized for monitoring the changes in the nature of organic matter during AD process (van Lier et al., 2008):

$$COD_{in} = COD_{out} = COD_{eff} + COD_{sludge} + COD_{biogas} \quad (2.6)$$

According to equation (2.6), at steady-state, the COD enters the system is partly degraded, converted to biogas (COD_{biogas}) and new bacterial cell tissue (COD_{sludge}), and partly goes in the effluent flow (COD_{eff}) (Burton et al., 2013). Thus, the fate of COD can be identified performing appropriate analysis of the various outlets of an anaerobic reactor (van Lier et al., 2008).

Biogas production

Biogas from digesters generally contains 65-70% methane, 30-35% carbon dioxide, and impurities such as water vapour, ammonia, hydrogen sulfide, and others (Appels et al., 2008). The quality of biogas depends on the methane content, by the moment methane is characterized by a lower heating value (LHV) of 35.846 MJ/m^3CH_4 (Burton et al., 2013), while the presence of impurities tend to affect its quality. For instance,

carbon dioxide lowers the calorific value, while hydrogen sulfide can cause corrosive effects in equipment and piping systems (Deublein and Steinhauser, 2011). Thus, the composition of biogas depends on both substrate characteristics and AD process efficiency, being also affected by environmental conditions and operational parameters.

Moreover, biogas is considered a promising renewable energy source and can be used in several ways: (1) it can be converted into electric power (fuel cells); (2) it can be burnt for heat generation; (3) it can be used in a CHP generator for the production of both heat and power; (4) it can be fed into the natural gas network; (5) it can be exploited as fuel for vehicles (Deublein and Steinhauser, 2011).

Two theoretical methods are available for estimating the methane production of a given substrate, based on the elemental composition of substrate and on the degraded COD (de Lemos Chernicharo, 2017, Jingura and Kamusoko, 2017).

Firstly, the elemental composition of a substrate can be used to predict the potential quantity of methane. Buswell and Mueller (1952) introduced a stoichiometric relationship relating the carbon, hydrogen and oxygen in an organic compound to methane and carbon dioxide produced in AD (Burton et al., 2013, Buswell and Mueller, 1952), which was further modified including nitrogen and sulfur content to obtain the volumes of ammonia and hydrogen sulfide in the biogas (Achinas and Euverink, 2016, Burton et al., 2013):

$$C_a H_b O_c N_d S_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right) H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right) CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right) CO_2 + dNH_3 + eH_2S \quad (2.7)$$

The theoretical biochemical methane potential (TBMP) of the compound can be estimated from the elemental composition of the substrate by using equation (2.8) derived from equation (2.7) (Achinas and Euverink, 2016):

$$TBMP\left(\frac{std\ L\ CH_4}{gVS}\right) = \frac{22.414\left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)}{12.017a + 1.0079b + 15.999c + 14.0067d + 32.065e} \quad (2.8)$$

For instance, according to (Parkin and Owen, 1986) the chemical composition for primary sludge can be $C_{10}H_{19}O_3N$. Using equation (2.8), this composition results in a TBMP of 0.696 Std L CH_4/g VS, which is the maximum methane production per unit of organic compound destroyed. The measured methane production is usually less than the theoretical value due to several reasons, i.e. a fraction of the substrate is converted to new bacterial material, along with characteristics of the substrate and other process conditions (Achinas and Euverink, 2016, Parkin and Owen, 1986).

Secondly, a simple method based on the degraded COD can be proposed. In fact, from the COD balance of equation (2.6) the term COD_{biogas} can be calculated by measuring COD of both influent and effluent and estimating COD_{sludge} associated to bacterial growth (Burton et al., 2013):

$$COD_{biogas} = COD_{in} - COD_{eff} - COD_{sludge} \quad (2.9)$$

Where COD_{sludge} can be estimated by equation (2.10), by the moment the theoretical COD equivalent for 1 g bacterial VSS can be calculated as 1.42 g COD/g VSS, considering an estimated bacterial composition of $C_5H_7O_2N$ (van Lier et al., 2008), and assuming a growth yield Y= 0.04 g VSS/g COD for primary sludge over the temperature range 20-35°C (Parkin and Owen, 1986):

$$COD_{sludge} = \left(COD_{in} - COD_{eff}\right)\left(1.42\,gCOD/gVSS\right)\left(0.04\,gVSS/gCOD\right) \quad (2.10)$$

Then, assuming all the COD_{biogas} is converted into methane, the COD of methane is the quantity of oxygen necessary to oxidize methane to water and carbon dioxide (Burton et al., 2013). The terms of equation (2.4) can be specified for CH_4:

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \tag{2.11}$$

Hence, from equation (2.11) the COD per mole of methane is 2(32 g O_2/mole) = 64 g O_2/mole CH_4. Moreover, the volume of methane per mole at standard conditions (0°C and 1 atm) is 22.414 L, so that the CH_4 equivalent of COD converted during AD process is 22.414/64 = 0.35 L CH_4/ g COD (Burton et al., 2013). Thus, by multiplying this value by the COD converted into biogas, the theoretical methane production can be estimated.

2.2.4. Control parameters

Biological processes are affected by nutritional requirements, operating parameters and environmental conditions. Thus, for an optimum AD process, several parameters may be taken into account and properly designed (Deublein and Steinhauser, 2011). In this section main control parameters will be described.

Solid retention time (SRT), hydraulic retention time (HRT) and organic loading rate (OLR)

The retention time is a fundamental parameter for anaerobic digestion. There is a distinction between the (1) the Solids retention time (SRT), the average time solids are held in the AD process, and (2) the Hydraulic retention time (HRT), the average time liquid is held in the AD process. Hence, for soluble substrates, it is possible to calculate the SRT as the mass of solids in the reactor divided by the mass of solids wasted daily; in contrast the HRT is obtained by dividing the volume of liquid in the digester by the volume of biosolids removed daily. For anaerobic digesters without recycle, HRT is equal to SRT (Burton et al., 2013).

The SRT is considered as the most important parameter for the design and operation of AD by the moment it defines the relationship between digester operating conditions and the bacterial system. Thus, a higher retention time should increase removal efficiencies and biogas production, as well as it should provide extra resilience to the system (Parkin and Owen, 1986).

From equation (2.3) the relation (valid for completely mixed reactor) between the SRT, microbial kinetic parameters and substrate concentration (S) can be easily shown:

$$\frac{1}{SRT} = \mu = \frac{YkS}{K_s + S} - b \tag{2.12}$$

Equation (2.12) reveals that the specific bacterial growth rate, μ, is equal to the inverse of the SRT, which, therefore, can control the bacterial growth rate affecting removal efficiency of substrate (S) and biogas production (Parkin and Owen, 1986). As shown in section 2.2.2, between various types of bacteria in anaerobic digesters, methanogens and hydrogen-producing acetogens are characterized by the lowest growth rates (μ). Therefore, the selected retention time should be major than a minimum SRT sufficient to allow these microorganisms adequate time to grow and to convert organic matter into methane (Parkin and Owen, 1986) and to allow disintegration/hydrolysis of complex compounds to occur. A much longer SRT is required at lower operating temperature (Burton et al., 2013). For a temperature of 35°C a minimum SRT of 10 days to prevent wash out of methanogens is suggested (Appels et al., 2008), in addition a SRT higher than 10.7 days is recommended for AD of WAS at room temperature and 35°C (Li et al., 2017). Moreover, high SRT values provide more resilience against temperature fluctuations, inefficiency in mixing and effects of

toxic loadings, as well as helping biological acclimation to toxic compounds (Gerardi, 2003, Parkin and Owen, 1986).

However, a longer SRT can be achieved through a higher concentration of solids or a higher volume of the digester, increasing the operating costs (Gerardi, 2003, Li et al., 2017).

The Organic loading rate (OLR) is a parameter related to HRT and it is defined as follows for a continuous process:

$$OLR\left(\frac{gCOD\,or\,VS}{L\ \ day}\right)=\frac{QC_{in}}{V}=\frac{C_{in}}{HRT} \tag{2.13}$$

Where Q is the flow rate (L/day); C_{in} is the influent concentration, expressed as COD (g O_2/L) or VS (g/L); V is the volume of reactor (L). Thus, the OLR can be considered as the mass of solids fed daily per unit of reactor volume, while for a batch process it is defined as the ratio of VS or COD content over the volume (Xie et al., 2017). This parameter should respect a given range varying according to the substrate and operational conditions, since too low concentrations of solids reduce the solids destruction and methane generation, along with increasing volume of produced digestate and heating requirements, while an over load may cause mixing problems, as well as major risk of ammonia and VFAs inhibition (Burton et al., 2013, Deublein and Steinhauser, 2011). Thus, anaerobic digesters in sewage treatment facilities generally operate at an OLR of below 1 g VS $L^{-1}day^{-1}$ (Nghiem et al., 2017).

Temperature

Temperature has important impacts on physicochemical properties of substrate and is also affects the growth rate and metabolism of bacteria (Appels et al., 2008).

Firstly, among physicochemical impacts increased temperature determines lower gas solubility, increased gas transfer rates and higher volumetric gas production, affecting for instance the partial pressure of H_2 in digesters, as well as an increase in liquid viscosity, determining changes in mixing energy requirements. Moreover, the solubility of solids and the acid-base equilibrium are generally affected by temperature changes, such as for NH_4^+/NH_3 system (Batstone and Jensen, 2011).

Secondly, temperature affects microbial activity, generally the higher the temperature the faster the reactions rates, increasing both solids destruction and methane production (Gerardi, 2003). Particularly the rates of enzymatic activity during hydrolysis and methane formation are strongly influenced, so that the minimum SRT should increase with lower temperatures, as shown in Table 2.4 (Burton et al., 2013). Moreover, bacteria are affected by sharp or frequent temperature fluctuations and process failure can occur whether temperature changes more than 1°C/day, thus it should be important to avoid changes in excess of 0.6°C/day (Appels et al., 2008).

Table 2.4: Suggested SRT for complete-mix anaerobic digesters. From (Burton et al., 2013).

Operating temperature (°C)	SRT minimum	SRT desirable
18	11	28
24	8	20
30	6	14
35	4	10
40	4	10

Methane formation can occur in nature over a wide range of temperature, from 0 to 97°C (Dhaked et al., 2010), and anaerobic digestion is generally carried out at three temperature ranges: (1) psychrophilic (10-30°C); (2) mesophilic (30-40°C); (3) thermophilic (40-70°C). This classification is based on optimum temperature and temperature range in which the species can grow and metabolize, as shown in Figure 2.8 (Lettinga et al., 2001). Psychrophilic conditions are mainly environmental, whereas the majority of literature and full scale applications consider mesophilic and thermophilic ranges (Batstone and Jensen, 2011). In particular, thermophilic digestion is reported to have the advantages of increasing organic matter destruction rates, as well as improving destruction of pathogenic bacteria; while it seems to be less stable than mesophilic digestion, due to increased levels of volatile acids, higher sensitivity temperature fluctuations and possible ammonia toxicity (Parkin and Owen, 1986). Moreover, the higher the operational temperature the higher the energy requirements.

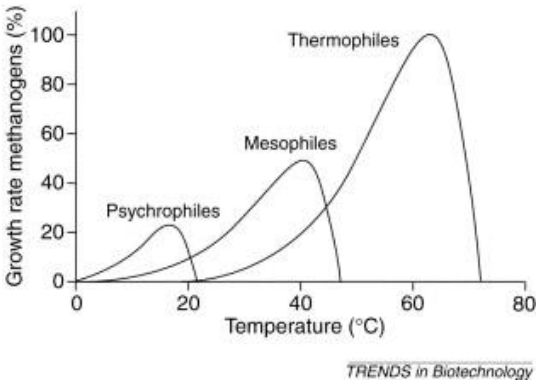


Figure 2.8: Influence of temperature on relative growth rates of different classes of methanogens. From (Lettinga et al., 2001).

A comparison between performances and operating parameters of mesophilic and thermophilic anaerobic digestion of sewage sludge from many experiments has been reported by Kardos et al., as shown in Table 2.5 (Kardos et al., 2011).

Nevertheless, especially in cold weather countries as Canada, for mesophilic and thermophilic processes the usual limitation is the requirement of thermal energy needed to maintain required temperatures, because the influent wastewater sludge is at much lower temperature (5-15°C during the winter season) (Bakhshi et al., 2018, Rajagopal et al., 2017). Moreover, mesophilic AD process is reported to be self-sufficient just after several tens of thousands population equivalent (Cieślik et al., 2015), however in Italy 70% of WWTPs are below 20 thousands of inhabitants (ISTAT - Istituto Nazionale di Statistica, 2018). Thus, in both cases developing psychrophilic AD of wastewater sludge could be strategic requiring lower energy expenditures, having as main challenge the low rate of anaerobic digestion, mainly due to rate-limiting step of hydrolysis which is particularly sensitive to low-temperatures.

AD in psychrophilic conditions has been studied and developed for stabilization of wastewater (Gomec, 2010, Lettinga et al., 2001), as well as treatment of animal manure (Massé et al., 2015, Saady and Massé, 2016, Witarsa and Lansing, 2015) and municipal food wastes (Rajagopal et al., 2017), but there is still a lack of knowledge about low temperature AD of WAS, probably due to lower degradability compared to other cited substrates.

Table 2.5: Comparison between performances and operating parameters of mesophilic and thermophilic AD of wastewater sludge. From (Kardos et al., 2011).

Parameter	Mesophilic conditions	Thermophilic conditions
Optimal temperature (°C)	35-40	55-60
pH	7.2-8.0	7.2-8.5
Temperature fluctuations tolerated (°C)	3-5	1-2
HRT (days)	15-25	3-10
Max COD reduction (%)	65-85	85-95
Biogas production (Nm^3/kg DS)	0.920-0.980	0.950-1.0
Methane content (%)	60-70	70-85
Volatile acids (mg CH_3COOH/L)	1500-2500	3000-4000
Alkalinity (mg $CaCO_3$/L)	4000-6000	3000-5000

pH, alkalinity, FOS/TAC

The pH influences the AD process *directly*, affecting for example different bacterial groups as a result of pH changes, and *indirectly* determining the speciation of a number of compounds, thus affecting their toxicity (Deublein and Steinhauser, 2011).

The pH in a digester initially decreases because of the production of VFAs by acidogenic bacteria, then, as methanogenic bacteria consume the VFAs and alkalinity is produced, the pH increases and later stabilizes (Gerardi, 2003).

Each group of bacteria has a characteristic optimum pH range. Thereby, methanogens are particularly sensitive to pH having an optimum between 6.5 and 7.2, while acidogenic bacteria are less sensitive, living in a pH range between 4.0 and 8.5 (Appels et al., 2008). This fact is particularly important, because during an eventual system imbalance, VFAs produced by fermentative bacteria tend to increase at a faster rate than are decomposed by methanogens, eventually resulting in pH drop and consequent inhibition of methanogens (Parkin and Owen, 1986).

In contrast the AD process has a buffer capacity, intended as the ability of a solution to avoid pH changes (de Lemos Chernicharo, 2017), which depends on the alkalinity of the system, expressed as mg $CaCO_3$/L. In fact, during AD alkalinity is produced in the form of carbon dioxide, ammonia and bicarbonate (Appels et al., 2008), whose concentrations depend on digester pH according to equations (2.14) and (2.15):

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \tag{2.14}$$

$$NH_3 + H^+ \leftrightarrow NH_4^+ \tag{2.15}$$

At pH range 6.0 and 7.5, the buffer capacity is mainly related to carbon dioxide alkalinity and its ability to neutralize volatile acids formed during AD. Hence, process stability can be enhanced by high alkalinity concentrations, whose monitoring becomes more important than pH observation (de Lemos Chernicharo, 2017, Gerardi, 2003). Moreover, it seems especially the stability of the ratio between alkalinity and volatile acids is of much importance rather than its concentration (Appels et al., 2008), suggesting the utility of monitoring parameters such as FOS/TAC, defined as the ratio between Organic Acid Concentration (FOS, expressed as mg acetic acid/L) and Total Alkalinity (TAC) (Fiore et al., 2016). In case of an increase in FOS/TAC ratio, the prevention of excessive acidification can be reached by means of stoppage of substrate supply or addition of diluting water, as well as the addition of neutralizing substances, such as CaO, $Ca(OH)_2$, Na_2CO_3, and NaOH (Deublein and Steinhauser, 2011).

Mixing

The content of an anaerobic digester should be appropriately for ensuring (1) appropriate contact between microorganisms, enzymes, nutrients and fresh substrate; (2) removal of metabolism products and toxic compounds; (3) prevention of temperature gradients; (4) avoidance of foam and stratification. However, bacteria could not survive to a too intensive mixing (Deublein and Steinhauser, 2011, Parkin and Owen, 1986).

Alternative mixing modes can be used in AD processes, i.e. continuous mixing and intermittent mixing, which can reduce the energy demand of the process maintaining similar biogas production as continuous mixing (Lindmark et al., 2014). Moreover, the SRT and the HRT can be controlled by the mixing with a large effect on biogas yield, while in an unmixed digester stratification of substrate can occur determining the decoupling of SRT from HRT (Lindmark et al., 2014).

Nutrients

Compounds and elements needed for bacterial growth can be grouped as *macronutrients*, required in relative large quantities, as nitrogen and phosphorus, and *micronutrients*, such as cobalt, iron, nickel, calcium, and sulfur (Gerardi, 2003).

Since almost all cells present similar chemical composition, requiring the same elements in the same proportions, the nutrient requirements are calculated on the basis the of empirical composition of bacterial cells (de Lemos Chernicharo, 2017). For instance, Table 2.6 reports the chemical composition of methanogens. Two common formula for cellular material can be $C_2H_7O_2N$ or $C_5H_7O_2N$ (Gerardi, 2003, Parkin and Owen, 1986). From literature data it is estimated 4-10% of biodegradable influent substrate (expressed as COD) is converted to bacterial material during AD. Knowing the COD equivalent of bacteria (1.42 g COD/g VS) and the influent COD, one can estimate nutrient requirements (Parkin and Owen, 1986). Moreover, the nutrient requirement can be expressed as C:N:P matter ratio or COD:N:P (Deublein and Steinhauser, 2011).

Commonly, domestic sludge contains enough nitrogen, as protein, urea, and ammonia, as well as phosphorus for sustaining an efficient anaerobic digestion. Nevertheless, industrial sludge or municipal sludge with significative percentages of industrial wastes, presenting more specific compositions, may require the addition of nutrients (Parkin and Owen, 1986).

Finally, it should be noticed that too high concentrations of nutrients can be toxic for microorganisms, as will be explained in the following section.

Table 2.6: Chemical composition of methanogenic microorganisms. From (de Lemos Chernicharo, 2017).

Macronutrients		**Micronutrients**	
Element	**Concentration (g/kg TSS)**	**Element**	**Concentration (mg/kg TSS)**
Nitrogen	65	Iron	1800
Phosphorus	15	Nickel	100
Potassium	10	Cobalt	75
Sulfur	10	Molybdenum	60
Calcium	4	Zinc	60
Magnesium	3	Manganese	20
		Copper	10

2.2.5. Inhibition and toxicity

One of the leading causes of failure of anaerobic reactors is the presence of inhibitory substances, which can determine an adverse shift in the microbial population or inhibition of the bacterial growth (Chen et al., 2008). A wide variety of inhibitory substances are commonly present in AD digesters, including ammonia, sulfide, hydrogen, volatile acids, light and heavy metals, organics, and light (Appels et al., 2008, Chen et al., 2008). Some of them are present in the substrate, others are generated during the process.

Thus, inhibition depends on the composition of the substrate, the adaptation of bacteria to the inhibitor, and its concentration (Deublein and Steinhauser, 2011). In fact, as mentioned in section 2.2.4, many substances can stimulate the process in low concentrations; however, when increasing too much, the effect can become inhibitory (Parkin and Owen, 1986). In this section, main inhibitory substances will be considered more in details.

Ammonia-Nitrogen

Ammonia is produced in the biological degradation of nitrogenous material, mainly in the form of proteins and urea. The two principal forms of inorganic nitrogen present are ammonium (NH_4^+) and free ammonia (NH_3).(Chen et al., 2008). The second one seems to be more toxic since it can diffuse passively through cell membranes, and, into the cell it can determine proton imbalance and potassium deficiency (Appels et al., 2008). Thereby, it has been shown that concentrations of free ammonia higher than 150 mg/L are toxic for methanogens, while for ammonium the limit seems to be 3000 mg/L (de Lemos Chernicharo, 2017). However, methanogenic bacteria can be acclimated to high ammonia concentrations.

The inhibition by free ammonia depends on total ammonia concentration, pH and temperature (Appels et al., 2008). In fact, an increase in pH may determine the shift to higher free ammonia to ammonium ratio (equation (2.15)) resulting in increased toxicity (Chen et al., 2008). Generally, at pH lower than 7.2 the predominant form is ammonium (de Lemos Chernicharo, 2017). Moreover, an increased temperature on the one hand can stimulate bacterial growth, on the other can raise free ammonia concentration (Chen et al., 2008).

Sulfides

Sulfate is widely found in many wastewaters and thereby also in sludges. Under anaerobic conditions, sulfate reducing bacteria (SRB) use sulfate as electron donor transforming it to sulfide. Thus, two levels of inhibition can be distinguished: the *primary inhibition* determined by the competition for substrates from SRB, and *secondary inhibition* by sulfides for different groups of microorganisms (Appels et al., 2008).

Primary inhibition occurs because the main intermediate products can be used by both SRB, obligate hydrogen producing bacteria and methanogens, living under same environmental conditions. The result of the competition depends on the conversion kinetics (van Lier et al., 2008).

Regarding secondary inhibition, non-dissociated hydrogen sulfide is toxic for methanogens, acetogens and SRB since it can freely pass through cell membranes, causing different adverse effects (Appels et al., 2008). Moreover, the quality of biogas is lower in case of presence of significative amounts of H_2S, causing bad smell and corrosion problems, and a lower methane yield may result as a consequence of the larger solubility of H_2S than methane (van Lier et al., 2008).

Furthermore, inhibition by sulfide depends also on temperature and pH. In particular, the chemical equilibrium between non-dissociated and dissociated forms (equation (2.16)) in water solution is strongly affected by pH changes (Deublein and Steinhauser, 2011). At pH lower than 7 the predominant form is H_2S, while between pH 7 and 14 HS^- prevails. Since the non-dissociated form is more toxic, a decrease in pH will result in a higher toxicity (de Lemos Chernicharo, 2017). A concentration of 200 mg/L of H_2S may be toxic at neutral pH (Gerardi, 2003).

$$H_2S \leftrightarrow HS^- + H^+ \leftrightarrow S^{2-} + 2H^+ \tag{2.16}$$

The COD/SO_4^{2-} ratio in influent flow can be used as a control parameter, since for high ratios the amount of organic matter is not enough for a complete reduction of sulfate and methanogenesis can occur (van Lier et al., 2008). Generally, it is assumed that for COD/SO_4^{2-} ratios above 10 toxicity by sulfide should not be a problem (de Lemos Chernicharo, 2017).

Volatile fatty acids (VFAs)

Volatile fatty acids are the main intermediates in AD process, where are consumed by acetogens in symbiosis with methanogens. However, in case of accumulation of VFAs due to a system imbalance, i.e. a temperature change, an overload of substrate, the introduction of toxic compounds, the methanogens are not fast enough to consume hydrogen and VFAs. This can result in VFAs accumulation and consequent pH drop, inhibiting hydrolysis and acetogenesis (Appels et al., 2008). The toxicity depends mainly on non-dissociated acids, which can penetrate into the cells, where they can denaturate the cell proteins. Moreover, the effect of toxicity is increased by pH drop (Deublein and Steinhauser, 2011). Thus, pH control through the addition of alkaline agents is regarded as a valid method while investigating the cause of the imbalance (Parkin and Owen, 1986).

Light metals

Various light metals cations (as Na^+, K^+, Mg^{2+}, and Ca^{2+}) are present in influent substrate of anaerobic reactors, being released during the degradation of organic materials or added as chemicals for pH adjustment. Even if their presence is necessary for bacterial growth, high salt concentrations may cause dehydration of cells because of osmotic pressure, mainly depending on the cation rather than the anion (Chen et al., 2008). Table 2.7 shows stimulating and inhibitory concentrations for various cations.

Thus, whether a cations is present at an inhibitory level, its effect can be reduced by the presence of an antagonist ion, which can be eventually added to the system by means of chloride salts (de Lemos Chernicharo, 2017). Moreover, eventually the acclimation of bacteria to high concentrations of cations could increase the tolerance (Chen et al., 2008), as well as a long SRT.

Table 2.7: Stimulating and inhibiting concentrations for various cations. From (de Lemos Chernicharo, 2017).

	Concentration (mg/L)		
Cation	**Stimulating**	**Moderately inhibiting**	**Strongly inhibiting**
Ca^{2+}	100-200	3500-4500	8000
Mg^{2+}	75-150	1000-1500	3000
K^+	200-400	2500-4500	12000
Na^+	100-200	3500-5500	8000

Heavy metals

Heavy metals, such as chromium, cadmium, nickel, zinc, copper, arsenic, and cyanides among the others, can be found in significative concentrations in wastewater sludge, mainly as the result of industrial contributions (Appels et al., 2008, Chen et al., 2008, de Lemos Chernicharo, 2017). Although trace concentrations are used by bacteria, larger levels can cause toxic effects, generally attributed to the chemical binding of metals to the enzymes and subsequent disruption (Appels et al., 2008). Moreover, a particular feature of heavy metals is that they are not biodegradable, and thereby can accumulate until reaching toxic concentrations (Chen et al., 2008), and this can also cause problems for land applications of digestate.

One of the procedures to control toxicity consists in precipitation of metals by means of the addition of sulfide or other anions. In fact, if sulfides themselves are toxic, when combined with heavy metals, they can form insoluble salts (de Lemos Chernicharo, 2017).

Organics

There are several organic compounds that can inhibit AD processes. In fact, organics that are poorly soluble or adsorbed to sludge particles may accumulate in bacterial membranes, eventually determining cell lysis (Chen et al., 2008). Organics, such as chlorophenols, halogenated aliphatics, N-substituted aromatics, and some LCFAs among the others, can be toxic in a wide range of concentrations (Chen et al., 2008).

Light

Finally, light is not lethal for methanogens, but it can inhibit the formation of methane. Thus, the anaerobic digestion process should occur in absolute darkness (Deublein and Steinhauser, 2011).

2.2.6. Pre-treatments

In section 2.2.2 hydrolysis has been described as the rate-limiting step in anaerobic digestion of wastewater sludge, due to its complex structure. As a result, AD was generally limited by long retention times (20-30 days), low removal efficiencies of organic DS (30-50%) (Appels et al., 2008) and low biogas production. Thereby, many methods to disrupt cell walls and determine the lysis or disintegration of cells have been introduced as pre-treatments (Appels et al., 2008). These include biological, mechanical, thermal, chemical processes and their integration (Zhen et al., 2017), and are reported to allows different advantages, such as the enhancement in hydrolysis rate, the reduction of HRT, the increase in biogas production and solids removal, as well as the raising in dewatering properties (Tyagi and Lo, 2011). Moreover, by the moment hydrolysis is particularly sensitive to low temperatures, pre-treatments seem to be promising to enhance low temperature anaerobic digestion. It has been shown that the integration of ozone pre-treatment and AD of WAS at 20°C could be energy sustainable and more efficient than a conventional mesophilic digester (Bakhshi et al., 2018).

The present section will briefly present the problem of sludge disintegration and will compare different pre-treatments for WAS anaerobic digestion.

Waste activated sludge complex structure

In section 2.1.1 it has been shown that WAS presents a lower degradability than primary sludge, thus pre-treatments mainly concerns this kind of sludge (Carrere et al., 2016). This is due to the fact that during aeration processes in WWTPs the most degradable compounds are consumed and the intense microbial activity (secretion and cell lysis) determines a matrix (flocs) formed by multiphase components, including microbial aggregates, organic and inorganic particles, filamentous bacterial strains, a large fraction of water and extracellular polymeric substances (EPS) (Carrere et al., 2010, Zhen et al., 2017). EPS are generated by microbial activity and are a complex mixture of gel-like and negatively charged biopolymers, constituting the main component of sludge organic fraction. Moreover, this complex matrix constitutes a protective shield for cells preventing cell rupture and lysis (Zhen et al., 2017). In consequence, EPS and cells themselves make sludge difficult to hydrolyze and degrade. Thus, the main objective of pre-treatment consists in disrupting EPS matrix and cell walls, making nutrients rapidly accessible to microorganisms (Zhen et al., 2017).

Biological pre-treatments

Enzymatic hydrolysis, pre-digestion and the use of fungi or bio-surfactants are reported as some of possible biological pre-treatments (Zhen et al., 2017).

The most common is *temperature phased anaerobic digestion* (TPAD), which uses a thermophilic digester prior the main mesophilic AD. Thus, thermophilic conditions accelerate hydrolysis of substrate in the first digester, while symbiosis of acetogenesis and methanogenesis can be optimized in mesophilic AD phase. Among the advantages of TPAD systems over single-stage mesophilic AD there are enhanced solids destruction and methane production, along with increased pathogen removal (Lv et al., 2010) and low energy inputs (Zhen et al., 2017). Although the mentioned advantages, TPAD is reported to be in its infancy, needing further research before considering large scale applications (Zhen et al., 2017). Table 2.8 summarises performances of TPAD among other pre-treatments.

Microbial electrolysis cell (MEC) has been reported as an emerging technique for methane generation through electromethanogenesis. The integration of such a process with anaerobic digesters can enhance methane production and system stability, but there are many critical aspects associated with MEC that need to be solved (Zhen et al., 2017).

Table 2.8: Biological pre-treatment of sludge - TPAD. Adapted from (Carrere et al., 2010, Zhen et al., 2017).

Substrate	Treatment conditions	AD conditions	Results	Reference
WAS	Batch, 1-2 days, 65°C	Batch, 14 d, 35°C	CH_4 prod. to 300 ml/g VS added	(Ge et al., 2011)
WAS	CSTR, 2-3 days, 70°C	Bacth, 37°C	CH_4 prod. to 370 ml/g VS added (+30-50% mesophilic-thermophilic single)	(Bolzonella et al., 2007)
WAS	2 days, 70°C	CSTR, 13 days, 55°C	CH_4 prod. from 40[a] to 55 ml/L.d (+28%)	(Skiadas et al., 2005)
Primary sludge	2 days, 70°C	CSTR, 13 days, 55°C	CH_4 prod. from 146[a] to 162 ml/L.d (+11%)	

a: Performance of AD without pre-treatment

Mechanical pre-treatments

Various mechanical pre-treatments involve the application of external physical stress or pressure on the cells for their disintegration. Presenting various successful industrial scale applications, these methods are considered effective in cell walls disruption, but are associated with high energy consumption (Tyagi and Lo, 2011).

Ultrasonication (US) is a well-established method which consists in the propagation of ultrasound waves in the medium, inducing the occurrence of cavitation, with high pressure and temperature local gradients, and the generation of highly reactive radicals (H· and ·OH). Both the processes contribute to sludge flocs disintegration and liberation of intracellular compounds (Zhen et al., 2017). Generally low frequencies (20-40 kHz) are applied and specific energy ranges from 1 to 16 MJ/kg TS to avoid inactivation of microorganisms (Carrere et al., 2010). Several studies reported US promising results in terms of solubilisation of COD and VS, enhancement in biogas production and solids reduction in anaerobic digesters, as shown in Table 2.9, although high capital and operating costs due to large energy consumption seem to limit the number of actual full scale applications (Tyagi and Lo, 2011).

Microwave irradiation (MW) is a popular technique consisting in the generation of electromagnetic waves (often 900 MHz or 2450 MHz) which can damage sludge cells due to rotation of dipoles inside the medium under oscillating electromagnetic field, inducing thermal and athermal effects. Various researches have proved that MW irradiation can be applied for sludge disintegration before AD with high efficiency and effectively pathogen destruction, as shown in Table 2.9 (Zhen et al., 2017).

High-pressure homogenization (HPH) is realized through a multistage high-pressure pump and a homogenizer pump. Basically, the pump compresses the sludge up to 900 bar into the homogenizer, then, as the sludge approaches to an impact ring, a rapid decrease in pressure occurs (Tyagi and Lo, 2011). This leads to high turbulence, strong shearing forces and cavitation, determining sludge flocs and cell membranes break (Zhen et al., 2017). Thus, this technique is reported as efficient and easy to implement, presenting the main disadvantages of high energy consumption as well as clogging and erosion problems (Tyagi and Lo, 2011).

Moreover, several other methods based on rapid pressure variation are commercially available (Carrere et al., 2010).

Many other techniques are present, such as high voltage electric field methods (electrokinetic disintegration) and other non-electric methods, such as lysis-centrifuges, grinding, collision plates and stirring ball mills among the others (Carrere et al., 2010, Tyagi and Lo, 2011, Zhen et al., 2017).

Table 2.9: Mechanical pre-treatments of sludge. Adapted from (Zhen et al., 2017).

Substrate	Pre-treatment		Anaerobic digestion		References
	Conditions	Effects	Conditions	Performances	
Ultrasonication (US)					
Activated sludge (35.5±0.7 g TS/L)	3380 kJ/kg TS	DR_{COD}: 21%	TPAD-BMP assay (55 °C→ 35 °C)	>+42% CH_4 prod. +13% VS removal	(Riau et al., 2015)
Secondary sludge (31.4 g TS/L)	20 kHz, 750 W, 5742 kJ/kg TS	Increase of SCOD/ TCOD from 0.02 to 0.10	Batch, 35 °C, 30 d	+16.9% VS removal, $+7.89\times10^{-6}$ kWh/g energy output	(Pilli et al., 2016)
Activated sludge (23 g TS/L)	24 kHz, 300 W, ~5000 kJ/kg TS	DR_{COD}: 9%	Semi-continuous, 37◦C, HRT 20 d, 80 d	+35% methane yield	(Braguglia et al., 2011)
Microwave irradiation (MW)					
Thickened sludge (43.6 g TS/kg)	2.45 GHz, 800 W, 1 min, 96 kJ/kg TS	Increase of SCOD: 117%	Semi-continuous, 37 °C, HRT 20 d, 67 d	+ 20% biogas production	(Houtmeyers et al., 2014)
Activated sludge (40.8 g TS/kg)	800 W, 3.5 min, 336 kJ/kg TS	Increase of SCOD: 214%	Semi-continuous, 37 °C, SRT 20 d, 42 d	+50% biogas production, +66.6% DS removal	(Appels et al., 2013)
High-pressure homogenization (HPH)					
Sewage sludge (23 g TS/L)	50 MPa, 2 cycles	sCOD: 2167 mg/L, DR_{COD}: 7.7%	Batch, 35 °C, 7 d	+115% biogas production, +41.17% VS removal, +61.89% tCOD removal	(Zhang et al., 2012)
Concentrated sludge (40 g/L)	150 bar, flow rate 2.7 m^3/h		Full-scale, 36–38 °C	+30% biogas production, +23% sludge reduction	(Onyeche, 2007)

where DR_{COD}: disintegration rate, $DR_{COD} = \frac{sCOD_t - sCOD_0}{tCOD_0 - sCOD_0}$

Thermal pre-treatments

Thermal treatment (TH) is reported to be a well-established and commercially implemented pre-treatment method (Zhen et al., 2017). The application of heat in a wide temperature range (60-180°C) can destroy the chemical bonds of cell walls and membranes, leading to partial solubilisation of intra cellular components and enhancing subsequent anaerobic digestion (Tyagi and Lo, 2011), as shown in many studies in Table 2.10 and full scale applications. In fact, most of studies reports treatment times between 30 and 60 min and an optimal temperature in the range of 160-180°C (Carrere et al., 2010), while many kinds of full-scale processes (CambiTHP™ and Biothelys®) have been diffused worldwide (Zhen et al., 2017). Thus, thermal processes increase hydrolysis rate, inducing HRT reduction, increased solids removal and enhanced biogas production, along with sludge sanitation and odour abatement. However, among the disadvantages, a largely increased soluble inert fraction and a possible increased ammonia inhibition at high temperatures should be remarked (Carrere et al., 2010).

TH pre-treatment has been widely studied in several research projects. For instance, Carrere and co-workers studied the application of thermal process in the range of 60-210°C to six different sludges, revealing an increase of sludge biodegradability up to 190°C. In this temperature range the increased sludge degradability or enhanced methane production was shown to be dependent on COD solubilisation of sludge (Carrere et al., 2008). However, further rise over 170-190°C is reported to lead to lower sludge biodegradability, although achieving high solubilisation levels (Carrere et al., 2010). Moreover, at high temperature ranges, sludge solubilisation has been shown strongly dependent on temperature rather than thermal treatment time (Zhen et al., 2017). However, the most significant disadvantage of high temperature treatment (>100°C) is its high energy request, which can compensate the increased biogas production (Appels et al., 2010). Thus, low temperature TH (<100°C, TPAD) could be considered a valuable alternative to overcome this limitation with significant results in enhancing methane production (Appels et al., 2010, Climent et al., 2007). Moreover, at low temperatures, treatment time has reported to play a more important role than temperature (Appels et al., 2010)

Table 2.10: Thermal pre-treatments of sludge. Adapted from (Carrere et al., 2010, Zhen et al., 2017).

Substrate	Pre-treatment		Anaerobic digestion		References
	Conditions	Effects	Conditions	Performances	
Activated sludge (65.08 ± 2.70 g TS/kg)	70°C 60 min	DR_{COD}: 1.4%	Batch, 35°C, 20 d	Biogas prod. from 34.8 to 35.3 mL/g VS	(Appels et al., 2010)
Activated sludge (65.08 ± 2.70 g TS/kg)	90°C 60 min	DR_{COD}: 17.9%	Batch, 35°C, 20 d	Biogas prod. from 34.8 to 377.6 mL/g VS	
Activated sludge (29.3-32.4 g TS/L)	70°C 9 h	DR_{COD}: 27.9%	Batch, 35°C, 45 d	Biogas prod. from 396 to 523 L/kg VS (+32%) VS removal from 38.9% to 43.7%	(Xu et al., 2014)
Mixed sludge (40-50 g TS/L)	121 ◦C 60 min		CSTR, 36 ◦C, HRT: 20 d	Biogas prod. from 350 to 420 mL/g VSSin (+20%)	(Barjenbruch and Kopplow, 2003)
Activated sludge (38.0 g TS/L)	121 ◦C 30 min	DR_{COD}: 10.5%	Batch, 37 ◦C 7 days	Biogas prod. from 3657 to 4843 L/m^3 sludge (+32%)	(Kim et al., 2003)
Activated sludge (14.26 ± 2.18 g TS/L)	121 ◦C 15 min	sCOD: 4900 mg/L, DR_{COD}: 15.7%	Batch, 37°C 50 d	TSS removal efficiency: 69%	(Salsabil et al., 2010)
Activated sludge (16.9 ± 0.8 g TS/L)	170 ◦C 30 min	DR_{COD}: 35.9%	CSTR, 35 ◦C HRT: 20 d	CH_4 production from 145 to 256 mL/g VSin (+51%) TS removal from 25% to 52% VS removal from 31 to 64%	(Bougrier et al., 2006)

where DR_{COD}: disintegration rate, $DR_{COD} = \frac{sCOD_t - sCOD_o}{tCOD_0 - sCOD_0}$

Chemical pre-treatments

Chemical pre-treatments consist of the addition of strong reagents to destroy the cell wall and membrane, thus enhancing the solubility of intracellular organic matter for enzymatic hydrolysis. The major methods involve the addition of acids, alkali, and oxidants (ozone and peroxides) (Appels et al., 2008, Zhen et al., 2017).

Acid and *alkaline pre-treatments* have been reported to present several advantages, including simplicity of devices and operations, low cost, high methane production and energy efficiency, as well as the possibility of operating at moderate temperatures (Zhen et al., 2017). Acid treatment is generally carried out adding acids, i.e. HCl, H_2SO_4, H_3PO_4, and HNO_3, while alkaline treatment usually involves the use of agents such as NaOH, KOH, $Ca(OH)_2$, CaO, $Mg(OH)_2$, and ammonia (Zhen et al., 2017). At high pH values, the cells cannot maintain a sufficient turgor pressure and disrupt; moreover, the alkali can react with the cell walls in many ways, leading to solubilisation of membrane and subsequent leakage of intracellular organic matter out of the cells (Tyagi and Lo, 2011).

With regard to (COD) solubilisation performances, the alkali agents have been shown in the order NaOH>KOH>$Mg(OH)_2$>$Ca(OH)_2$ (Kim et al., 2003), even if KOH has been reported to be more efficient than NaOH in terms of sludge disintegration (Penaud et al., 1999, Tyagi and Lo, 2011). Thus, alkali methods generally result in increased methane productions, as shown in Table 2.11, and have been reported to significantly reduce the level of pathogens in sludge (Neyens et al., 2003, Tyagi and Lo, 2011). Moreover, solubilisation and biodegradability of sludge increase with alkali dose, however too high concentrations of Na^+ and K^+ may result in inhibition of AD (Carrere et al., 2010). Other drawbacks involve the reduction in the activity of methanogens at alkaline pH (section 2.2.4), requiring subsequent neutralisation by means of an acid (Tyagi and Lo, 2011), as well as increased mineral content of digested sludge (Carrere et al., 2010).

Finally, the alkaline method has been often combined with the cited thermal treatment, with the aim of reducing alkali dose (Ruffino et al., 2016) or process temperature (Rani et al., 2012), and because combined treatments have been shown to increase sludge disintegration (Table 2.11).

Table 2.11: Chemical pre-treatments of sludge. Adapted from (Carrere et al., 2010, Zhen et al., 2017).

Substrate	Pre-treatment		Anaerobic digestion		References
	Conditions	Effects	Conditions	Performances	
Alkali					
Activated sludge (64.2 ± 0.2 g TS/L)	0.157 g NaOH/gTS	DR_{COD}=14.0 ± 0.6%	Batch, 37°C, 35 d	From 141.3 to 183.8 mL CH_4/gCOD (+30%)	(Ruiz-Hernando et al., 2014)
Activated sludge (38.0 gTS/L)	7 g/L NaOH (0.18 gNaOH/gTS)	From 17.6 to 43.5% sCOD/tCOD	Batch, 37 ◦C 7 days	Gas prod. from 3657 to 4147 L/m^3 sludge (+13%)	(Kim et al., 2003)
Activated sludge (10.6 ± 0.1 gTS/L)	NaOH, pH 9-11, 24h		Batch, 37°C, 25 d	+10.7-13.1% TSS removal, +7.2-15.4% biogas yield	(Shao et al., 2012)
Pulp and paper sludge	0.08 gNaOH/gTS	+83% sCOD	Batch, 42 d	+83% CH_4 yield (0.32 m^3CH_4/kg $S_{removed}$	(Lin et al., 2009)
Activated sludge (29.3-32.4 g TS/L)	NaOH, pH 10 8 days	DR_{COD} = 50.4%	Batch, 35°C, 45 d	Biogas prod. from 396 to 560 L/kg VS (+41%) VS removal from 38.9% to 43.5%	(Xu et al., 2014)
Thermal – alkali					
Activated sludge (29.3-32.4 g TS/L)	NaOH (pH 11), 10 h, 90°C	DR_{COD} = 43.7%	Batch, 35°C, 45 d	Biogas prod. from 396 to 605 L/kg VS (+53%) VS removal from 38.9% to 46.2%	
Dairy activated sludge (8.5 g TS/L).	NaOH (pH 12), 1 h, 60°C	COD solub. 23% higher than control	CSTR, 35°C HRT: 15 d	+80.5% TS, +90.4% VS Biogas prod. from 332 to 674 mL/ g VS_{IN} (+103%)	(Rani et al., 2012)
Activated sludge (38.0 gTS/L)	7 g/L NaOH, 30 min, 121°C	From 17.6 to 85.4% sCOD/tCOD	Batch, 37 ◦C 7 days	Biogas prod. from 3657 to 5037 L/m^3 sludge (+38%)	(Kim et al., 2003)
Activated sludge (12.35 ± 0.48 g TS/L)	NaOH (0.1 M), 6 h, 75°C	DR_{COD} = 64.8 ± 2.2%	Batch, 35°C, 21 d	From 112.2 to 191.4 ± 5.3 ml CH_4 prod. (+70.5%)	(Kim et al., 2013)
Activated sludge (5-6% TS)	0.04 gNaOH/gTS, 1.5h, 70°C	DR_{COD} > 25%	Batch, 35°C, 21 d	From 0.236 to 0.299 Nm^3/kgVS gas (+26.8%)	(Ruffino et al., 2016)
Ozonation					
Activated sludge (12.4 g TSS/L)	0.2 g O_3/L (0.02 $gO_3/gVSS_{IN}$)		ASBR, 20°C, HRT: 20 d	+60% VSS removal[a] CH_4 prod.[a] from 62 to 71 mL $CH_4/gVSS_{in}$ (+15%)	(Bakhshi et al., 2018)
Activated sludge (20 g TS/L)	0.16 g O_3/g TS	DR_{COD}>22%[b]	Batch, 35°C, 24 d	CH_4 prod. from 221 to 272 ml $CH_4/g\ COD_{IN}$ (+23%) Mineralisation occured	(Bougrier et al., 2006)
Activated sludge	0.15 g O_3/g TS		Batch, 35°C, 18 d	Gas prod. from 150 to 367 mL/$gCOD_{IN}$ (+145%)	(Bougrier et al., 2007)

where DR_{COD}: disintegration rate, $DR_{COD} = \frac{sCOD_t - sCOD_o}{tCOD_0 - sCOD_0}$; a: Performance of AD at 35°C without pre-treatment; b: estimated value

Ozonation (OZ) is the most used chemical treatment (Carrere et al., 2010) and many ozone systems are commercially available (Zhen et al., 2017). Ozone is a strong oxidant able to kill the microorganisms in WAS and to partially oxidize and hydrolyse the organic matter through sequential decomposition reactions (floc disintegration, solubilisation, oxidation/mineralization of released organics) (Tyagi and Lo, 2011). Many ozone systems are commercially available. Sludge solubilisation effectiveness is related to ozone dose, with an optimum varying between 0.05 and 0.5 g O_3/g TS, depending on properties of sludge and pre-treatment (Zhen et al., 2017), as shown in Table 2.11. In contrast, a too high dose can result in partial oxidation (mineralization) of the liberated organic matter, lowering biogas production (Carrere et al., 2010, Zhen et al., 2017). Moreover, OZ was reported to reduce solids concentration and SRT with increased biogas production, and was studied for pathogens removal (Tyagi and Lo, 2011). However, ozone treatment was shown as energy intensive, needing high energy for production of O_3 and for its transfer to sludge, respectively 12.5 and 2.5 kWh/kg O_3 (Zhen et al., 2017). Conversely, an energy analysis comparing a traditional AD process at 35°C and the integration between OZ treatment and AD at 20°C revealed the latter to produce more energy, suggesting a potentially sustainable option (Bakhshi et al., 2018).

Moreover, other oxidants, such as hydrogen peroxide (H_2O_2) or peracetic acid (PAA), can be used for enhancing solubilisation and biodegradability of sludge (Tyagi and Lo, 2011).

Selection of pre-treatments methods

In this section many pre-treatments to enhance AD were described, revealing that each technique presents some advantages and some drawbacks. However, a comparison between different studies cannot be directly made, because their results depend also on characteristics of sludge and operational conditions (Carrere et al., 2010). Moreover, pre-treatments should be compared based on (1) treatment efficiency, in terms of solubilization and biodegradability of sludge; (2) capital costs; (3) energy and chemicals costs; (4) and others, i.e. pathogens removal and dewatering (Carrere et al., 2010, Tyagi and Lo, 2011). In general, it has been observed an increased number of studies about pre-treatments for mesophilic and thermophilic AD has been conducted in the decades (Zhen et al., 2017), but full-scale applications are still limited, mainly due to high capital and operational costs (Tyagi and Lo, 2011). Hence, additional research is needed for enhancing energy produced as biogas compared to energy used in the treatment, particularly with regards to AD of WAS at low temperatures. In this sense, in addition to the cited ozone (OZ) treatment (Bakhshi et al., 2018), a low thermal (TH) and a thermo-alkaline (TA) pre-treatments have been selected, being easy to implement and relatively low cost (operational and energy) with promising efficiencies (Table 2.10 and Table 2.11).

3. Materials and Methods

In this chapter the laboratory experiments of low temperature AD of waste activated sludge are presented. The effects of a thermal (TH), a thermo-alkaline (TA) and an ozone (OZ) pre-treatments for enhancing AD of WAS at low temperature were studied by means of increased solubilisation of WAS and AD test in semi-continuous mode, comparing solids removals and specific biogas productions.

The present chapter describes the WAS collection and characterization, the design and the procedures of pre-treatments, as well as the operation and control of AD test. The analytical methods are also included. All the experimental procedures were conducted at the Environmental Engineering Laboratories of Department of Civil Engineering and Applied Mechanics of McGill University (Montreal, QC, Canada).

3.1. Inoculum and waste activated sludge

3.1.1. Inoculum

For the reactors start-up, the anaerobic sludge from two sources was used as inoculum. Approximately 2 L of sludge derived from three laboratory scale AD reactors operated at 22°C, located at Environmental Laboratories of McGill University, and 7 L of sludge from a mesophilic full-scale digester, from LaPrairie WWTP (Quebec, Canada).

The mentioned three AD reactors were operated at 22°C in semi-continuous mode for one SRT, equal to 15 days, and fed with ozonated WAS three times per week. The volume of each reactor was 1 L with a working volume (WV) of 80%.

Firstly, the anaerobic sludge from LaPrairie WWTP was collected and stored at 4°C until usage. Then, the two sludges were sampled before and after mixing and then inoculated to 12 semi-continuous digesters. These samples were characterized by means of soluble and total COD (sCOD and tCOD), total and volatile solids (TS and VS).

3.1.2. Waste activated sludge

WAS origin

The waste activated sludge was collected from LaPrairie wastewater treatment plant, located at Saint Catherine (Quebec, Canada) and owned by La Régie de l'assainissement des eaux du bassin LaPrairie (RAEBL), a group of five municipalities.

Collection, storage, dilution and characterization

Generally, once per week, activated sludge was manually collected from a pump located after the thickening sludge unit of LaPrairie WWTP. Then, it was stored at room temperature in a plastic tank while being continuously aerated. After collection, usually every Monday, the raw sludge was diluted, sampled ad subjected to pre-treatments. Thus, the appropriate volume of raw sludge was diluted by means of distilled water from an initial TS content of 4-5% to approximately 3% TS. Hence, a sample of diluted sludge was collected before splitting the 6 L of diluted sludge into four parts (1.5 L per each) which were later subjected to pre-treatments: three of them were treated while one, untreated, was simply stored at 4°C until use as feed

for anaerobic digesters. All the samples for characterization were stored at 4°C until utilization, generally less than 72 h from collection. The volume of each sludge sample was approximately 50 mL for the measurements of the following parameters: pH, total COD (tCOD), soluble COD (sCOD), total solids (TS), volatile solids (VS). The analytical methods will be described in detail in section 3.4.

3.2. Pre-treatments

In the present study three different pre-treatments for enhancing hydrolysis and AD of WAS at 22°C were selected, properly designed, and tested. Thus, in this section the ozone (OZ) treatment, the thermal (TH) treatment and the thermo-alkaline (TA) treatment are presented.

3.2.1. Evaluation of treatment performance

The effectiveness of each treatment was assessed on two levels: firstly, by the increase of substrate solubilisation; secondly, by the enhancement of biodegradability of WAS, by means of subsequent AD test performances (COD and solids removal efficiencies, biogas production). The latter aspect will be explained in detail in section 3.3.

The solubilisation of organic matter induced by treatment was calculated by the disintegration rate (DR), which is the ratio between the increase in sCOD due to pre-treatment and the maximum possible increase in sCOD (Kim et al., 2013, Li et al., 2012), according to equation (3.1), as well as by the solubilisation ratio (S_{COD}), defined as the ration between sCOD and tCOD.

$$DR_{COD}(\%) = \frac{sCOD_T - sCOD_0}{tCOD_0 - sCOD_0} \cdot 100 \tag{3.1}$$

Where the terms $sCOD_T$ is the soluble COD after treatment; $sCOD_0$ and $tCOD_0$ are respectively the soluble and total COD before treatment.

3.2.2. Ozone treatment

The ozone treatment was realized according to (Bakhshi et al., 2018) by using same WAS and equipment, shown in Figure 3.1. Thus, fresh WAS was treated in a 3 L conical glass ozone contactor vessel (Figure 3.1.a) (Ace Glass Incorporated, Vineland, NJ, USA) fitted with two inlet ports equipped with atomizer nozzles supplying ozone (Figure 3.1.b).The sludge inside the contactor was continuously mixed by means of magnetic stirrer for preventing foaming problems and promoting gas transfer. Moreover, using Ultra High Purity 4.3 oxygen (Praxair, Mississauga, ON, Canada) ozone was produced by an ozone generator (model OZO 3VTTL, Ozomax, Canton de Shefford, QC, Canada) (Figure 3.1.c). Finally, the ozone dose was determined by measuring the ozone concentrations in both the WAS and vent (blank) gas flows through an online Mini-HiCon O_3 Analyzer (INUSA Inc. Norwood, MA, USA) (Figure 3.1.c) (Bakhshi et al., 2018).

(a) (b) (c)

Figure 3.1: The ozone treatment equipment: (a) the 3 L glass conical vessel, the direction of the ozone flux is shown by the arrows; (b) stirrer one of the 2 atomizer nozzles for transferring ozone to sludge; (c) on the left the ozone generator, on the right the ozone analyser, at the bottom the magnetic.

Firstly, 1.5 L of WAS was transferred to the glass ozone contactor vessel while 50 mL was collected as sample before ozone treatment. Then, after proper preparation of the system, the sludge was subjected to the ozone stream (air pressure at 13 psi, flow rate at 8 SCF/h) for an exposure time of 50 min. During the exposure time, the concentration of ozone in the outlet stream was recorded by the analyser. These parameters, determining the ozone dosage, were set according to other previous COD solubilisation experiments, in which 3 L of same fresh WAS from LaPrairie WWTP at 3% TS was subjected to growing ozone concentrations. This parameter can be controlled by varying the exposure time. Thus, they varied the latter parameter, and the corresponding soluble COD in the resulting ozonated WAS was measured. Plotting the sCOD as a function of ozone dose, they chose the optimal dose, equivalent to 50 min.

At the end of 50 min of exposure, the reactor vessel was shaken to incorporate surface foam, then, 50 mL of WAS was collected as sample (after OZ) and its pH was immediately measured, while the treated sludge was poured into a glass bottle and stored at 4°C until usage as feed. Finally, the treatment was repeated on the empty contactor vessel keeping same operational conditions to measure the ozone concentration in the outlet vent gas flow. Hence, the ozone dose was determined as difference between areas under the two curves (empty and WAS) of O_3 concentration as a function of time, as follows:

$$Dose_{O_3}\left(ppmv\cdot\min\right)=\left(\sum C_{empty}-\sum C_{WAS}\right)ppmv\cdot 4\sec\cdot\frac{1}{60}\frac{\min}{\sec} \tag{3.2}$$

Where C_{empty} and C_{WAS} are the concentrations of ozone (ppmv) respectively in the "empty" and the "WAS" flows. Thus, from the ideal gas flow (assuming $P = 1\ atm$, $T = 293\ K$, $MW_{O3} = 48\ g/mol$, and $R = 0.08206\ atm/(mol\ K)$), the dose can be converted as:

$$\frac{PV}{nRT}=\frac{Dose_{O_3}\left(ppmv\cdot\min\right)\cdot P\cdot MW}{R\cdot T}=Dose_{O_3}\left(g\cdot ppmv\cdot\min/L\right)=Dose_{O_3}\left(mg\cdot\min/L\right) \tag{3.3}$$

Hence, the $Dose_{O3}$ can be expressed in mg, knowing the gas flow ($Flow_{gas}=8\,SCF/h=3.56\,L/\min$):

$$Dose_{O_3}\left(mg\right)=Dose_{O_3}\left(mg\cdot\min/m^3\right)\cdot Flow_{gas}\left(L/\min\right)\cdot\frac{1}{1000}\left(m^3/L\right) \tag{3.4}$$

Finally, from the volume V (L) and the TS (mg/L) of WAS, the dose of ozone can be expressed as:

$$Dose(mg\,O_3 / mg\,TS) = \frac{Dose(mg\,O_3)}{V \cdot TS} \tag{3.5}$$

3.2.3. Thermal treatment

Description of treatment

The thermal treatment of WAS was performed in a programmable electric pressure cooker (Instant Pot® Company, model IP-DUO80, Ottawa, ON, Canada), shown in Figure 3.2, which is characterized by a rated power of 1200 W and an inner volume of 7.5 L. Firstly, a volume of 0.75 L of WAS (3% TS) was poured into a 1 L Pyrex® Vista™ glass beaker (Figure 3.3.a) while 50 mL was collected as sample before thermal treatment, then the beaker was placed on a stainless steel support inside the inner pot of the pressure cooker (Figure 3.3.b). Moreover, 1-1.5 L of distilled water was also poured into the inner pot. Hence, the steel support prevented contacts between the water bottom layer and the beaker containing WAS (Figure 3.3.c).

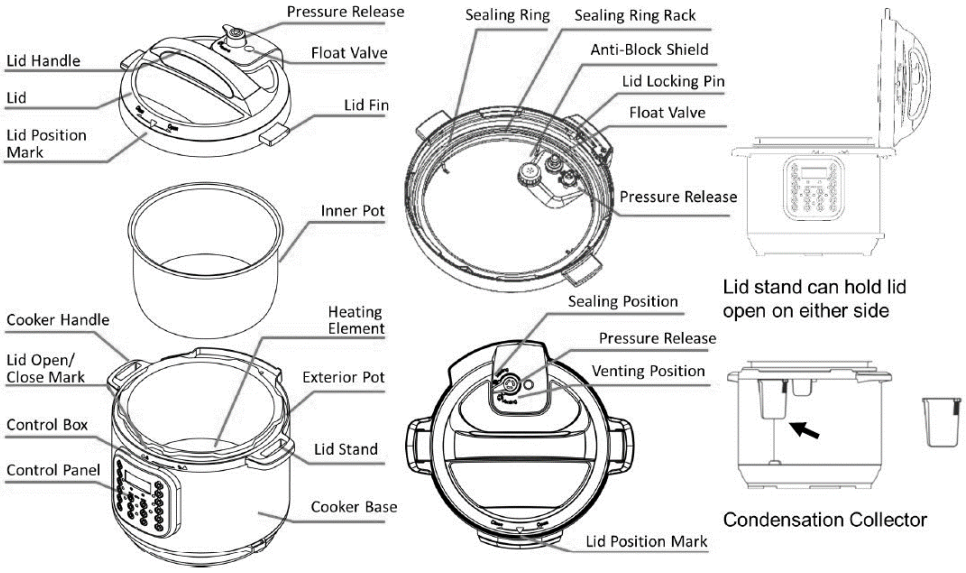


Figure 3.2: Scheme of the programmable electric pressure cooker IP-DUO80. From (Instant Pot® Company, 2017).

(a) (b) (c)

Figure 3.3: Equipment for thermal treatment: (a) 1 L Pyrex® Vista™ glass beaker with WAS; (b) stainless steel support inside the inner pot of pressure cooker; (c) the 1 L beaker inside the inner pot with the water bottom layer.

The pressure cooker was programmed according to "high-pressure settings", corresponding to a working temperature of 115-118°C and a working pressure of 0.70-0.80 bar. Moreover, it was generally necessary 10-15 min for reaching working conditions, when the treatment time started. The optimum treatment time of 30 min was determined by COD solubilisation experiments, as explained in detail in the following section. Thus, at the end of the TH, the beaker containing 0.75 L of WAS was manually mixed for 1 min and covered by an aluminium foil for preventing water evaporation during the cooling phase. Hence, after 60-90 min of cooling time, the WAS was mixed, then 50 mL of sample was collected, pH was measured, and the remaining volume was poured into a glass bottle for storage at 4°C until utilization. Once per week, 1.5 L of 3% TS WAS was treated by means of two thermal treatments (0.75 L each), thus the 50 mL sample collected after the treatment consisted of 25 mL of WAS from each of them.

Definition of optimum treatment time

The optimum time for the thermal treatment of WAS was determined by COD solubilisation experiments, in which 0.3 L of fresh WAS (3% TS) was subjected to TH (115-118°C, 0.7-0.8 bar) with growing times. The corresponding DR_{COD} and pH in the resulting treated WAS were determined and plotted versus treatment times.

Hence, three experiments were conducted, named T1, T2, and T3, in which different treatment times and two cooling methods were tested, as shown in Table 3.1. T1 was carried out prior to starting AD test, so that the optimum treatment time of 30 min was selected referring to this test; furthermore, T2 and T3 were conducted as replicates. The treatment times between 10 and 120 min at working temperatures of 115-120°C were chosen in accordance with literature (Barjenbruch and Kopplow, 2003, Kim et al., 2003, Salsabil et al., 2010), as shown in Table 2.10. Moreover, two cooling modes were investigated, i.e. at room temperature and in ice-bath, aiming to abruptly interrupt the solubilisation of COD at the end of the treatment. Thus, after the thermal treatment, in T1 and T2, the beakers with 0.3 L of WAS were firstly mixed, then covered by an aluminium foil and cooled, respectively at room temperature and in ice bath; while in T3 each sample was mixed and split in two subsamples of approximately 0.15 L, contained in 0.25 mL glass bottles, next these

were cooled following the two mentioned methods. After cooling, for each sample pH was measured and 50 mL of WAS was collected and stored at 4°C until characterization.

Table 3.1: Experimental conditions of COD solubilisation tests for determination of the optimum treatment time.

Test	Operating conditions			
	Heating time (min)	**Treatment time (min)**	**Cooling time (min)**	**Cooling methods**
T1	5-20	30, 60, 90, 120	30-40	Room temperature
T2	5-20	10, 20, 30, 45, 60, 90, 120	15-20	Ice bath
T3	5-10	30, 60, 90, 120	30-40	Room temperature
			15-20	Ice bath

3.2.4. Thermo-alkaline treatment

Once per week, 1.5 L of fresh WAS at 3% TS was subjected to a combined thermo-alkaline treatment, which consisted in the addition of 0.09 g NaOH/g TS by means of 5N sodium hydroxide (NaOH) solution, followed by a thermal treatment in thermostatic water bath at 70°C for 60 min, and a final pH conditioning to pH 7.0-7.5 by means of concentrated hydrochloric acid (HCl).

Preparation of alkali solution and alkaline addition

Sodium hydroxide was selected as alkali agent because it was shown to determine greater (COD) solubilisation than calcium hydroxide and other agents (Kim et al., 2003, Ruffino et al., 2016). The alkali addition was carried out by means of a concentrated solution (5 N) for preventing dilution of WAS. Thus, usually every three weeks, a volume of 100 mL of 5 N solution of sodium hydroxide was prepared from pellets (NaOH, pellets, ≥ 97.0% ACS, Fisher Scientific, NJ, USA). Firstly, 20 g of pellets was weighted in a plastic dish by an analytical balance (model ML104, Mettler Toledo, Mississagua, ON, Canada). Then, operating in fume hood, approximately 50 mL of distilled water was poured into a clean 250 mL Pyrex® beaker, continuously mixed by a magnetic stirrer, and 20 g of NaOH was carefully added to water, remembering to rinse thoroughly the plastic dish with distilled water. Once NaOH was completely dissolved, the solution was hence transferred to a 100 mL glass volumetric flask, rinsing properly the beaker with distilled water. Then, distilled water was added until reaching a final volume of 100 mL, the flask was stoppered and shaken. Finally, the 5 N NaOH solution was transferred into a 100 mL HDPE bottle for storage, remembering to squeeze the bottle before capping to minimize the interior air space.

Once per week, an optimum dose of 0.09 g NaOH/gTS, established by means of COD solubilisation experiments, was added to 1.5 L of 3% TS WAS by means of the proper volume of 5N solution, which was determined from the mentioned data. Firstly, the needed mass of NaOH (M_{NaOH}) was calculated:

$$M_{NaOH}\left(gNaOH\right) = V_{sludge}\left(L\right)\cdot TS\left(g/L\right)\cdot Dose_{NaOH}\left(gNaOH/gTS\right) \tag{3.6}$$

Then, knowing the molecular weight of NaOH (MW_{NaOH} = 40 g/mol), the volume of 5N NaOH ($V_{5N,NaOH}$) was determined:

$$V_{5N,NaOH}\left(mL\right) = \frac{M_{NaOH}\left(g\right)}{MW_{NaOH}\left(g/mol\right)\cdot Conc_{NaOH}\left(mol/L\right)}\cdot 1000\left(ml/L\right) \tag{3.7}$$

Hence, after the collection of 50 mL of WAS as sample before treatment, 1.5 L volume of sludge was poured by means of a 0.5 L glass cylinder into three 0.5 L Pyrex® glass bottles, equipped with screw caps. Then,

operating in fume hood, the appropriate volume of NaOH from the mother solution was added in the bottles by a micro pipette (100-1000 µL, Pipetman, Gilson) fitted with 1 mL tip. Finally, the mixture was manually stirred.

Thermal treatment

Immediately after the alkali addition, the mixture was thermally treated in a thermostatic water bath at 70°C for 60 min. From the literature the temperature of 70°C appeared to be a good compromise between treatment performance and energy cost (Kim et al., 2013, Rani et al., 2012, Ruffino et al., 2016), as shown in Table 2.11, while the optimum treatment time was determined by COD solubilization experiments. The water bath consisted of a stainless-steel container with water, heated by a stirring hot plate (model PC-420D. Corning™, NY, USA) and the temperature was monitored by using a mercury laboratory total-immersion thermometer (±0.5°C). The water bath was prepared and pre-heated to 70°C before adding the samples. Firstly, the mixed samples were partially immersed into the water bath, waiting 10-15 min for stabilizing the temperature closed to 70°C, then the thermal treatment was performed for 60 min, while manually shaking tapped bottles every 15 min. Finally, the glass bottles were manually shaken and cooled at room temperature for 60-75 min. After the cooling, the pH was measured.

pH conditioning

The pH after the TA treatment (pH > 9) was critical for anaerobic digestion process, particularly for methanogenic bacteria, as explained in section 2.2.4, requiring the final pH conditioning to pH 7.0-7.5 by consecutive additions of decreasing small volumes of HCl solution. Thus, operating in fume hood, small volumes of HCl were added by using a micro-pipette (100-1000 µL, Pipetman, Gilson) equipped with 1 mL tip. After every addition, each glass bottle was capped and manually shaken, waiting 5-10 min before the subsequent pH measure. This procedure continued until obtaining a pH value ranged 7.5-8.0, then a period of 30 min was generally waited before final pH adjustment to 7.0-7.5.

After pH conditioning, 50 mL of treated WAS was properly collected for characterization from the three bottles, while the remaining sludge was stored in the capped glass bottles at 4°C until utilization.

Definition of optimum alkali dose and thermal treatment time

The optimum dose of NaOH and the optimum thermal treatment time were defined by COD solubilisation experiments, in which three 0.6 L samples of WAS (3% TS) were subjected to different doses of 5N NaOH solution corresponding approximately to pH 9, 10, and 11, then each sample was split into 5 subsamples of 0.1 L that were thermally treated with different times (0, 30, 60, 90, and 120 min). For each combination of alkali dose and treatment time the corresponding DR_{COD} and pH in the resulting treated WAS were determined and plotted.

Hence, three experiments were carried out, named TA1, TA2, and TA3. However, just TA1 performed prior to starting AD test, so that the optimum dose of 0.09 gNaOH/gTS and optimum time of 60 min were selected, while TA2 and TA3 were replicates.

In each experiment, after dilution of fresh WAS to 3% TS content and collection of 50 mL of sample before treatment, three 0.5 L Pyrex™ bottles equipped with screw caps were filled with 0.6 L of sludge by using a 0.5 L volumetric cylinder. The three 0.6 L samples were subjected to consecutive alkali additions until

reaching round pH 9, 10, and 11. Thus, the starting pH value of each sample was measured and a small volume (corresponding to 0.01 gNaOH/gTS or multiples) of 5N NaOH solution was added by means of a micro-pipette. Each bottle was manually shaken, waiting a period of 5-10 min prior subsequent pH measurement. It was usually needed more than one addition for reaching the target pH value. Moreover, in each test it was not possible to manage more than two 0.6 L samples, resulting that pH 9 and 10 samples were simultaneously treated, while the untreated pH 11 sample was stored at 4°C and tested no more than 24 h later.

Then, each sample, i.e. the one corresponding to pH 9, was split into five subsamples of 0.1 L, contained in five 0.1 L capped glass bottles. One of them was kept at room temperature as control, while the others were thermally treated for 30, 60, 90, and 120 min, according to the procedure above described. During this process the treatment time was properly recorded. Hence, as one subsample was treated, i.e. pH 9 – 30 min, first it was cooled at room temperature for 20-25 min then both pH values of pH 9-30 min and pH 9-control samples were measured. Finally, 50 mL of each sample was collected and stored at 4°C for COD measurements. Table 3.2 reports all the subsamples of each TA test, resulting from the combinations of target pH values (9, 10, 11) and thermal treatment times (0-control, 30, 60, 90, 120 min).

Table 3.2: Thermo-alkaline COD solubilisation experiments: nomenclature of each combination of pH of WAS and thermal treatment time.

	Thermal treatment time (min)				
	0 - control	**30**	**60**	**90**	**120**
pH 9	9-0	9-30	9-60	9-90	9-120
pH 10	10-0	10-30	10-60	10-90	10-120
pH 11	11-0	11-30	11-60	11-90	11-120

3.3. Anaerobic Digestion Tests

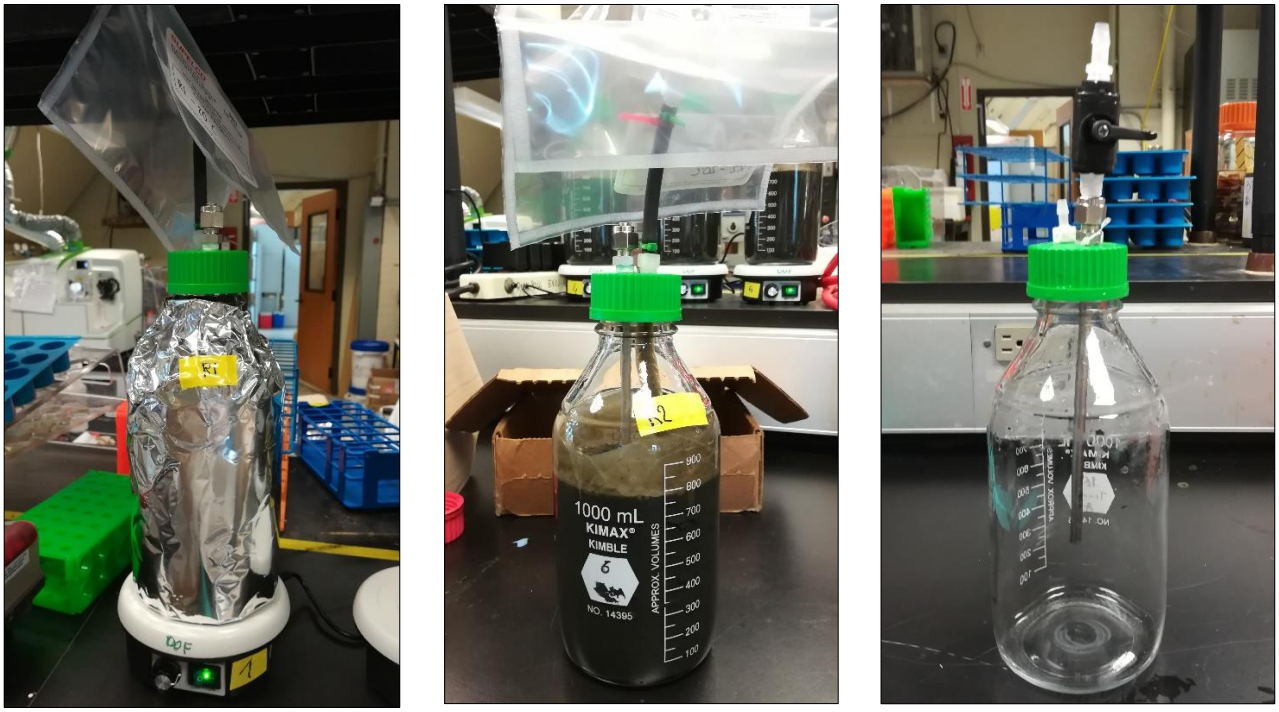
The anaerobic digestion tests were carried out to evaluate the performance of the AD process of WAS at 20°C and to compare the effects of three treatments in enhancing the biodegradability of sludge by means of increased biogas production and solids removals. Thus, twelve 1 L semi-continuous reactors were operated for 77 days with an SRT and a HRT of 15 days. During the start-up phase, lasted around 2 times the SRT (28 days) the reactors were only fed with ozonated WAS, then the AD test was conducted for more than 3 times the SRT (49 days) and consisted in feeding the reactors with WAS subjected to OZ, TH and TA treatments and fresh WAS as control. Thus, three reactors per type of feed were operated as replicates. During the AD test, pH, solids and COD were monitored on both the fed and digested WAS, as well as biogas production was measured. The operational conditions of start-up and AD test phases are summarized in Table 3.3.

Table 3.3: Operational conditions of anaerobic digestion test.

Phase	**Start-up**	**AD test**			
Description	Adaptation of inoculum to 20°C	Feeding reactors with pre-treated and raw WAS			
Duration (days, SRT)	28 (2 x SRT)	49 (3 x SRT)			
Temperature (°C)	22	22			
SRT (days)	15	15			
HRT (days)	15	15			
Number of reactors	12	12			
Reactor volume (L)	1	1			
Working volume (L)	0.8	0.8			
Initial TS (%) of feed	3%	3%			
Feeding mode	Semi-continuous	Semi-continuous			
Feed-waste (times/week)	3 – Monday, Wednesday, Friday	3 – Monday, Wednesday, Friday			
Reactor	R1, …, R12	R1, R2, R3	R4, R5, R6	R7, R8, R9	R10, R11, R12
Feed	Ozone-WAS	Raw-WAS	Therm-WAS	Ozone-WAS	Th.alk.-WAS

3.3.1. Configuration of reactors

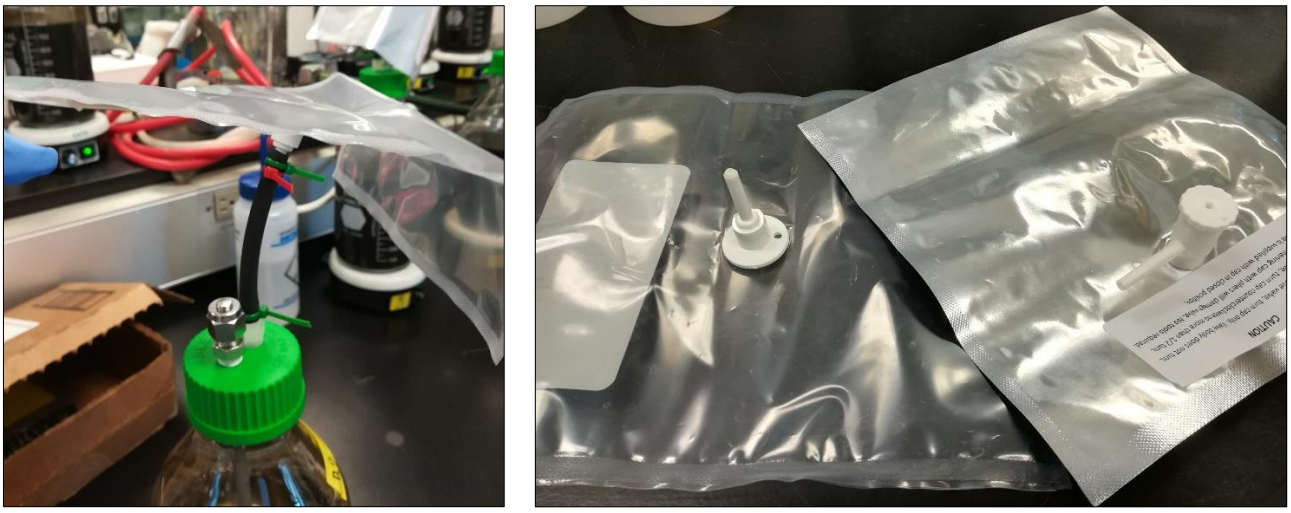
Each reactor consisted of 1 L Pyrex® glass bottle, equipped with a polypropylene screw thread cap (model GL45, DWK Life Sciences), as shown in Figure 3.4. Previously, two drilled holes of about 0.6-0.8 cm were realized in the cap, allowing the insertion of a steel tube for feeding operations and the connection of a gas bag for collection of biogas. Thus, the tube length was designed to collect and inject sludge at about 5 cm from the bottom of the bottle, allowing the mixing by means of a stir bar (Figure 3.4.c).



(a) (b) (c)

Figure 3.4: Configuration of the anaerobic digestion reactor: (a) The reactor placed on the magnetic stirrer; (b) The 1 L reactor filled with a WV of 0.8 L; (c) The 1 L empty reactor.

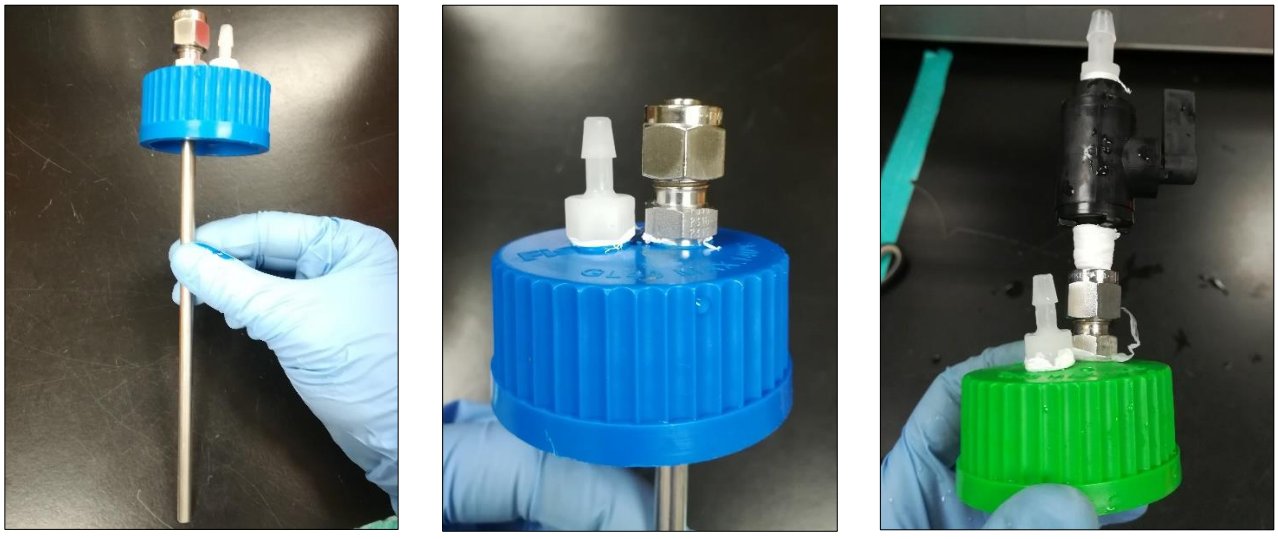
The headspace of the reactor was connected to a gas bag by means of a neoprene tube, closed with plastic ties, as shown in Figure 3.5.a. Two gas bags models were changed during AD test for solving gas leaking problems, first a 1L Tedlar® gas sampling bag (Figure 3.5.b) with a push lock valve (24633, Supelco, Sigma-Aldrich Corporation, S. Louis, MI, USA), then a 1 L Supel™-inert multi-layer foil gas bag equipped with a screw cap valve (30226-U, Supelco, Sigma-Aldrich Corporation, S. Louis, MI, USA). In fact, the screw cap valve of the second gas bag permitted better air-tight locking by means of two plastic ties.



(a) (b)

Figure 3.5: System for the collection of biogas: (a) A neoprene tube, closed by means of plastic ties, connects the headspace of the reactor to the gas bag; (b) on the left the first gas bag equipped with a push lock valve, on the right the second gas bag with a screw cap valve.

Moreover, the valve closing the feeding tube was changed after 3SRTs from the moment of inoculation. Firstly, a stainless-steel swivel nut was adopted, as shown in Figure 3.6.b, which was quickly worn out due to frequent open and close cycles and did not ensure airtight closure. Thereby, after 40 days this system was substituted with a plastic tap valve (Figure 3.6.c), which allowed enhancements in the feeding procedure, as explained in the next section.



(a) (b) (c)

Figure 3.6: The feeding equipment of an AD reactor: (a) The screw cap and the stainless steel tube; (b) The closure by means of a swivel nut; (c) The closure by means of a plastic tap valve.

3.3.2. Start-up phase

On September 29, 2017 twelve 1 L semi-continuous AD reactors were started operating. Firstly, each reactor was filled with 0.7 L of inoculum and 0.1 L of ozone treated WAS, as feed for first two days of operations, with a Working Volume of 0.8 L. Both the inoculum and the ozonated WAS were previously prepared according to procedures explained in previous sections. Then, the reactor content was manually mixed and a stir bar was added prior closure of the screw cap. Hence, for completely removing the air inside the headspace, the reactor was sparged with 100% nitrogen gas for 5-7 min through the feeding valve and isolated from external environment, then the gas bag was rapidly attached to the system. Finally, the reactor was completely wrapped by means of an aluminium foil for ensuring protection from light, marked and placed on a magnetic stirrer, as shown in Figure 3.4.a.

A long start-up phase, lasting approximately two SRTs (28 days), was required for the adaptation of the mesophilic inoculum at room temperature. In fact, the twelve reactors were placed on a bench and kept at room temperature, ranging between 22°C and 23°C, which was daily monitored by using a mercury laboratory thermometer (±0.5°C) partially immersed in a 0.5 L Pyrex® glass bottle filled with water. This was closed by a screw cap and wrapped with an aluminium foil for simulating internal temperature conditions of reactors, as shown in Figure 3.7.

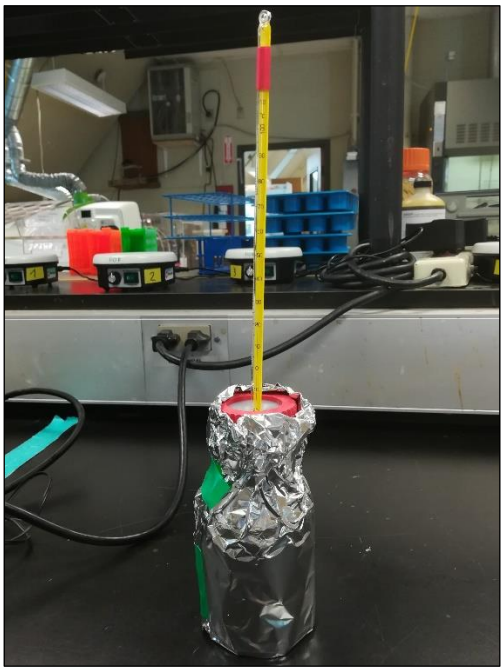


Figure 3.7: The system for monitoring room temperature: a thermometer partially immersed in a glass bottle filled with water and wrapped by means of an aluminium foil.

During the start-up phase, twice per week, 3 L of fresh WAS, previously diluted to a TS content of 3%, was subjected to the ozone treatment. Hence, three times per week, usually on Monday, Wednesday and Friday, the reactors were fed with the appropriate volumes of ozone treated sludge, reported in Table 3.4, ensuring a HRT and a SRT of 15 days, while the same amounts of digested sludge were removed. The volume of sludge needed to be daily added and removed was determined knowing the WV and the HRT:

$$Q_{feed}\left(L/d\right)=\frac{WV}{HRT}=\frac{0.8\,L}{15\,d}=0.053 L/d \tag{3.8}$$

Moreover, the digested sludge was sampled for COD and solids measures once per week, usually on Monday, while the pH was monitored on each sample on Monday, Wednesday and Friday for preventing potential pH drops.

Table 3.4: Schedule of feeding operations.

	Monday	Wednesday	Friday
Feed for these days	Monday, Tuesday	Wednesday, Thursday	Friday, Saturday, Sunday
Fed volume (L)	0.110	0.110	0.160
Wasted volume (L)	0.110	0.110	0.160

3.3.3. Anaerobic digestion test

On October 27, 2017, after the start-up phase lasted 2SRTs, the anaerobic digestion test started, maintaining same operational conditions above described and summarised in Table 3.3. Thus, reactors named R1, R2 and R3 were fed with raw WAS, while the reactors from R4 to R12 with three pre-treated types of WAS, respectively TH, OZ and TA, in groups of three replicates. This phase lasted 49 days, more than 3SRTs, for a total duration of 77 days (5SRTs), because it has been shown that a reactor generally requires more than 3xSRTs for reaching the steady state from the moment of inoculation (Bakhshi et al., 2018, Liao et al., 2006, Masse et al., 2006). Moreover, a SRT of 15 days was considered as appropriate for an AD process (Rani et al., 2012) in accordance with minimum SRTs reported in Table 2.4, assuming a temperature of 22°C.

During this phase, generally on Monday, 1.5 L of fresh WAS at 3% TS was subjected to each pre-treatment, then it was sampled for COD, solids and pH measurements, and stored at 4°C until usage as feed. Moreover, prior every feeding event, the bottles containing WAS were warmed up at room temperature for preventing temperature fluctuations inside the reactors. Thus, every Monday the digested sludge was sampled and characterized according to the same schedule of the start-up phase. In the next section, the feeding operations will be described in detail. The biogas production was measured with a frequency of sampling of 2-4 days, varying with increased or reduced gas production rates, according to procedure presented in section 3.4. Finally, it was occasionally necessary to perform some exceptional measures for solving biogas leaking problems or mixing problems, as explained below. On December 15, 2017 the AD test was terminated, and samples of digested sludge were collected after proper mixing.

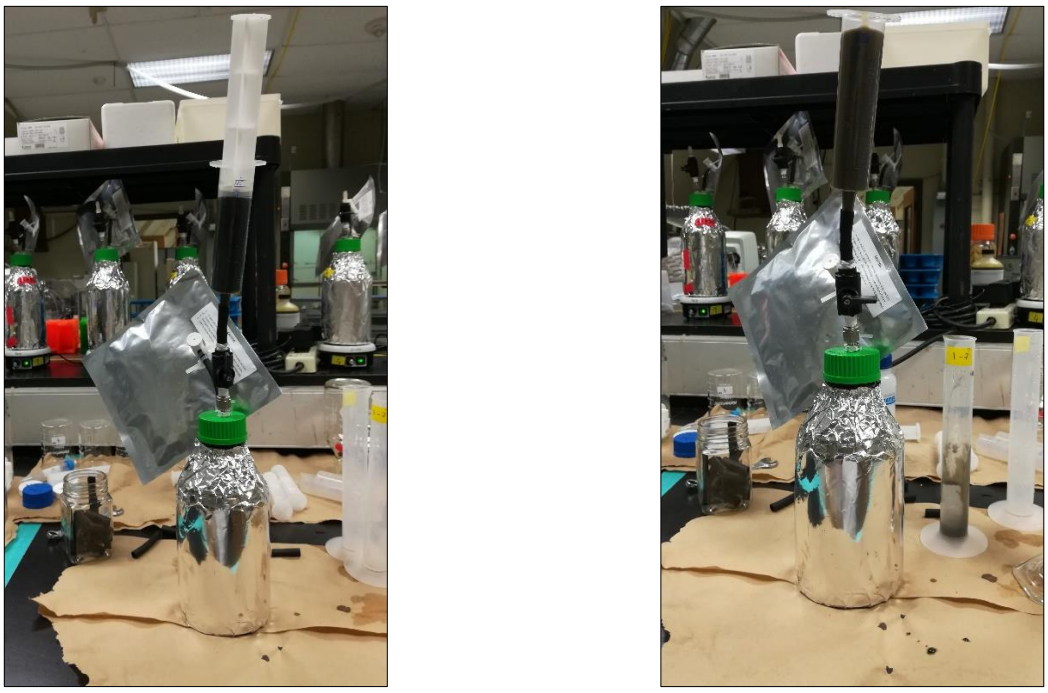
3.3.4. Feeding operations

During the start-up and the AD test phases the twelve 1L reactors were fed in semi-continuous mode according to the schedule reported in Table 3.4. Thus, each feeding event firstly consisted in the removal of defined volume of digested sludge from each reactor after mixing and then in the injection of an equal volume of the appropriate feed by the dedicated tube. As mentioned above, two closing valves were changed during the AD test (Figure 3.6.b and Figure 3.6.c), resulting in different feeding procedures described in this section. Prior starting the procedure, the glass bottles containing WAS were warmed up at room temperature. The feeding and waste operations were carried out using a 60 mL syringe connected by means of a neoprene tube to each reactor, as shown in Figure 3.8. Moreover, the volume of digested sludge and feed was measured by a plastic 100 mL measuring cylinder (± 2.5 mL) per each digester.

First, the reactor was gently shaken for 1 min before opening the closure valve. Then, the appropriate volume of digested sludge was slowly extracted by the 60 mL syringe and measured by the cylinder (Figure 3.8.a), being particularly careful to avoid the induction of sudden pressure gradients in the headspace. Thus, the digested sludge was collected in a 200 mL glass beaker with a stir bar for pH measurements and eventual subsequent sampling. At this point, the swivel nut did not allow the closure while syringe was operating,

while the plastic tap valve did. Thereby, in the first case the appropriate volume of feed had to be slowly injected by the syringe, due to equilibrium between the headspace and the external pressures. In contrast, the closure of the tap valve before disconnecting the syringe preserved the slight internal negative pressure. Thus, in the second case it was sufficient to pour the feed into the syringe lacking the plunger stopper prior opening the closure valve, then wait for the drop of level of WAS and close the tap valve at the appropriate time (Figure 3.8.b). The last procedure ensured no air was injected into the reactor. Finally, the reactor was manually shaken for 1 min and then placed on the magnetic stirrer.

Moreover, the pH of digested sludge samples was measured in half an hour from the moment of collection on Monday, Wednesday and Friday, while the pH of four types of feed was monitored just after the application of treatment (Monday) and generally prior the feeding event on Friday. In addition, on Monday, 50 mL of digested sludge from each reactor was collected for COD and solids measurements and stored at 4°C.



(a) (b)

Figure 3.8: Collection of digested sludge and feeding operations: (a) Collection of digested sludge by a 60 mL syringe with the tap valve open; (b) Feeding operations, the syringed is filled with WAS prior opening the tap valve.

3.3.5. Exceptional operations on reactors

As mentioned above, some exceptional measures on reactors were performed for solving some gas leaks and mixing problems.

Firstly, during the first SRT since the start of AD test, the solids concentration of digested sludge collected during feeding operations significantly varied, as shown in chapter 4, revealing the mixing by means of magnetic stirrers was not sufficient. Subsequently, different stir bars and power levels of magnetic stirrers were unsuccessfully tested. Hence, the reactors were also gently shaken by hand for 1 min once per day, determining an improvement of the mixing.

Secondly, it was frequently observed sudden drops in biogas production, which revealed evident gas leaks, as exposed in chapter 4. Thus, in case of such an event from a certain reactor, firstly the source of the leak was detected, then a different solution was carried out, i.e. the neoprene tube connecting gas bag to cap of reactor were eventually substituted or it had to reseal the valves of the cap with parafilm, as well as the gas bag model was changed if necessary.

3.4. Analytical methods

In order to assess the sludge solubilisation and to determine optimum treatment parameters, samples of WAS before and after treatments were properly collected and stored at 4°C until characterization, which was carried out in any case no longer than 3-4 days from the sampling event. Moreover, during the AD test, generally on Monday, once per week, the four types of feeds were sampled after preparation and treatment (raw WAS, TH, OZ, TA), along with twelve samples of digested sludge from operating reactors. The characterization consisted of the following parameters: total solids (TS), volatile solids (VS), soluble COD (sCOD) and total COD (tCOD). Moreover, the pH was generally measured on the feed samples twice per week (Monday and Friday) and on the digested sludge samples three times at any feeding event. Finally, the biogas production was measured with a frequency of sampling of 2-4 days, varying with increased or reduced gas production rates. In this section, the analytical methods and materials will be described; in addition, the protocols in use at the Environmental Engineering Laboratories of McGill University are reported in the Appendix.

3.4.1. Soluble and Total COD

The chemical oxygen demand (COD) was measured on the soluble and total fraction of samples by means of colorimetric method reported in the lab manual (Appendix), based on method 5220D (APHA, 1998).

Firstly, for determination of COD soluble fraction, approximately 8 mL of sludge sample was mixed, then transferred into 2.0 mL microcentrifuge tubes (model 02-681-344, Fisherbrand™) and centrifuged at 20 x 10^3g for 15 min by using the Sorvall™ Legend Micro 21 centrifuge (Thermo Fisher Scientific, USA). Then, the supernatant was filtered by means of 0.45 µm syringe filters (25 mm, sterile, PVDF 0.45µm, Fisher Scientific). A new filter was used per each sample and the syringe was rinsed for three times by distilled water before each filtration operation.

Subsequently, the samples for total or soluble COD were prepared in triplicates, while the blank and the standard samples were single. This stage consisted in diluting the sample to obtain a 2 mL sub-sample ranged 20-800 mg O_2/L into a borosilicate glass digestion tube, equipped with a Teflon-lined screw cap. Thus, in case of sCOD samples, the filtrated was generally diluted 1:4 or 1:5 with distilled water by using a micro pipette (100-1000 µL, Pipetman, Gilson) fitted with 1 mL tip. In contrast, tCOD samples were firstly diluted 1:50 or 1:100 by two subsequent dilutions and then poured into digestion tubes. Finally, the blank was prepared by pouring 2 mL of distilled water into a digestion tube, while five standards (100, 300, 500, 800, and 1000 mg O_2/L) for the calibration curve were prepared diluting the stock standard solution of potassium-hydrogen-phtalate (KHP) with a COD of 1000 mg O_2/L.

Hence, in the digestion step carried out in fume-hood, firstly 1.2 mL of standard K_2CrO_7 solution and 2.8 mL of sulphuric acid reagent were properly added into each digestion tube, then the tube was digested for 2 h at 150°C in the heating block (COD reactor, model 45600, Hach Company).

Finally, after cooling, the absorbance (-) of samples and standards was measured by using the Spectronic® 20D+ spectrophotometer (Thermo Fisher Scientific, USA) at 600 nm, using the blank for setting the instrument to 100% transmission.

Then, the absorbance of the five standards was used for obtaining the equation of the calibration curve by means of linear regression in data analysis extension of Microsoft Excel 2016, as shown in Figure 3.9. Thus, the absorbance (*Abs*) values of diluted samples were converted to COD, by using the equation of the calibration curve:

$$COD_{diluted}(mg\,O_2 / L) = a \cdot Abs(-) + b \tag{3.9}$$

Where *a* and *b* are respectively the slope and the intercept of the calibration line, i.e. *a* = 2700 and *b* = -13.507 according to equation of Figure 3.9. Then the $COD_{diluted}$ value was corrected with the corresponding dilution ratio for determining the COD of sample before the dilution. Finally, the mean and standard deviation of each triplicate results were performed and attributed as COD value of each sample, expressed as mg O_2/L or g O_2/L.

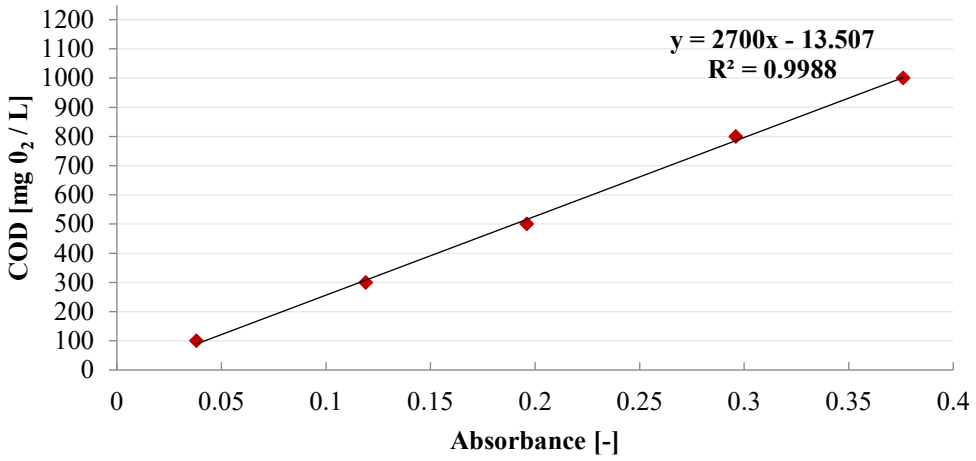


Figure 3.9: Example of COD linear calibration curve, obtained by linear regression of five standards (100, 300, 500, 800, 1000 mg O_2/L).

3.4.2. Total and volatile solids

The total and volatile solids (mg/L) were determined on sludge samples by gravimetric analysis adapted from the method reported in the lab manual (Appendix), based on methods 2540B and 2540E (APHA, 1998).

Essentially, to determine total solids, composed of fixed solids plus volatile solids, a sample in a dried and pre-weighted aluminium dish was weighted as wet, then evaporated and weighted once dried. Subsequently, to calculate the fixed solids, the dried sample was ignited and weighted again. The volatile solids were obtained as difference between total and fixed solids.

Firstly, the aluminium weighing dishes (model 08732101, Fisherbrand™) were marked, then ignited at 500°C for 1 h in the Nabertherm B180 furnace (Nabertherm GmbH, Germany), desiccated and cooled to room temperature in desiccator. Finally, the dishes were weighted (M_{dish}) by an analytical balance (0.1 mg,

model ML104, Mettler Toledo, Mississagua, ON, Canada). Moreover, the dishes were handled by tweezers during operations.

Secondly, three sub-samples (triplicates) of 4 mL were prepared into void dishes from each sludge sample after mixing by using a volumetric serological pipette (10 mL, model 1367811E, Fisherbrand™) and were weighted (M_{wet}). In addition, a blank sample was obtained by pouring 4 mL of distilled water into an aluminium dish. Then, the wet samples were evaporated at 103 °C, overnight, in the Heratherm OGS series oven (Thermo Fisher Scientific, USA). The day after, samples were cooled in desiccator no longer than 30 min and subsequently weighted ($M_{103°C}$). At this point, the total solids (TS) of each sub-sample were determined:

$$TS\left(mg/kg\right) = \frac{M_{103°C}\left(g\right) - M_{dish}\left(g\right)}{M_{wet}\left(g\right) - M_{dish}\left(g\right)} \cdot 10^6 \left(\frac{mg}{kg}\right) \tag{3.10}$$

After weighting, the dishes were ignited at 500°C for 15 min in the furnace, then cooled to room temperature in desiccator and weighted (M_{fixed}). Thus, the fixed solids (FS) and subsequently the volatile solids (VS) were determined:

$$FS\left(mg/kg\right) = \frac{M_{500°C}\left(g\right) - M_{dish}\left(g\right)}{M_{wet}\left(g\right) - M_{dish}\left(g\right)} \cdot 10^6 \left(\frac{mg}{kg}\right) \tag{3.11}$$

$$VS\left(mg/kg\right) = TS - FS \tag{3.12}$$

Then, per each sample, the mean and the standard deviation of TS and VS were calculated from the triplicate measures.

3.4.3. pH measurement

The pH measurements were performed with a benchtop pH/ISE meter (± 0.005 pH, model 710A, Orion™, Thermo Fisher Scientific) equipped with a pH electrode (double junction, model 9102DJWP, Orion™, Thermo Fisher Scientific). Firstly, the autocalibration of the pH meter was carried out before the daily measurements, once per day, by using two buffer solutions corresponding to pH 4.01 and pH 7.00. Then, each sludge sample was poured into a 100 mL or 250 mL glass beaker and continuously mixed by magnetic stirrer during the reading of pH value. Moreover, the electrode was properly rinsed with distilled water and dried by a paper towel between each pH reading. The protocol for pH measurement in use in the laboratory is reported in the Appendix.

3.4.4. Biogas production

During the anaerobic digestion test, the biogas produced by digesters was collected by gas bags and measured for water displacement with a frequency of sampling of 2-4 days, varying with increased or reduced gas production rates. The procedure is explained in detail in this section.

The measuring equipment consisted of a 1L plastic measuring cylinder (± 5 mL), a basin of tap water, a PVC tube and a metallic clamp occluding the tube, as shown in Figure 3.10.a.

For each biogas measure the gas bag valve was closed and disconnected from AD reactor, while the neoprene tube was closed by a metallic clamp. Hence, one end of PVC tube was closed by the metallic clamp while the opposite end was inserted to the bottom of measuring cylinder. Then, the cylinder was filled with tap water and quickly reversed into the basin, inducing vacuum conditions at the top. The water level

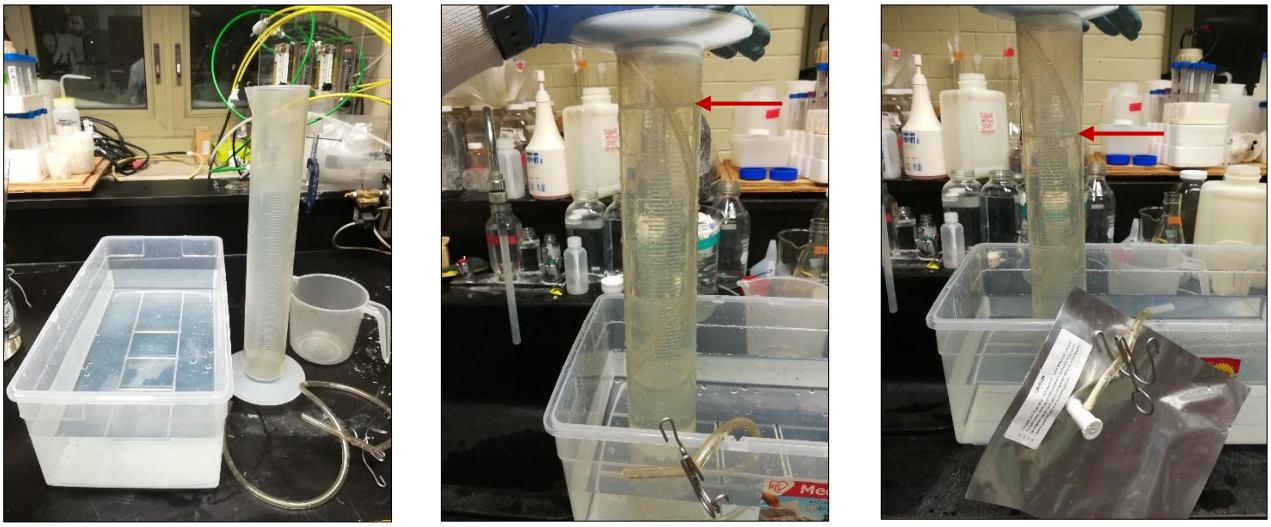
inside the cylinder corresponded to environmental pressure and was subsequently measured on the graduated scale (Figure 3.10.b), expressed as volume (V_{atm}). Hence, the gas bag was connected to the PVC tube, both the valves (gas bag valve and metallic clap) were opened inducing bag voiding and a sudden drop in water level. After the stabilization of water level, the corresponding volume (V_{gas}) was measured (Figure 3.10.c) and the valves were closed. The difference between the two measures provided the volume content of the gas bag. Thus, considering this volume accumulated in N days, the mean daily biogas production (BP) was estimated and expressed in Normal Conditions (1 atm, 0°C):

$$BP\left(Nm^3/d\right)=\frac{V_{gas}\left(L\right)-V_{atm}\left(L\right)}{N}\cdot\frac{273.15}{273.15+T(°C)} \tag{3.13}$$

Where T (°C) is the temperature in the laboratory during the measurements. Finally, the specific biogas production (SPB) was determined:

$$SBP\left(Nm^3/kg\,VS_{in}\right)=\frac{BP\left(StL/d\right)}{VS_{in}\left(g/L\right)\cdot Q_{feed}\left(L/d\right)} \tag{3.14}$$

Where VS_{in} and Q_{feed} are respectively the volatile solids and the volumetric flow of feed.



(a) (b) (c)

Figure 3.10: Biogas production measurement: (a) The equipment; (b) The first water level (V_{atm}) is indicated by the arrow; (c) The second water level (V_{gas}) is indicated.

3.5. Sensitivity Analysis

The data collected during the optimization of pre-treatments and during the AD test were studied by means of different statistical tests, i.e. linear regression, paired and unpaired T-test, and two-way ANOVA. All the tests were carried out by using the data analysis extension of Microsoft Excel 2016.

4. Results and Discussion

4.1. Inoculum and waste activated sludge

4.1.1. Inoculum

For the reactors start-up, the anaerobic sludge from two sources was used as inoculum: 2 L of sludge derived from three laboratory scale AD reactors (R1, R2, R3) and 7 L of sludge from the mesophilic full-scale digester operating for LaPrairie WWTP (Quebec, Canada).

The three 1L AD reactors were operated at 22°C for one SRT, equal to 15 days, and fed with ozonated WAS. Moreover, the anaerobic digester of LaPrairie WWTP consists of two-stages, hydrolysis (24 h) and mesophilic AD (19 days of SRT), and operates at 35°C. The combined system began operating in April 2017 for treating 110 thousand tons DS of secondary sludge per year with a solids removal of more than 55% (NRC - National Research Council Canada, 2017). The operating conditions and the chemical parameters of digested sludges from three reactors and from the full-scale digester, as well as of the inoculum after mixing are reported in Table 4.1.

Table 4.1: Characteristics and chemical parameters of inoculum.

Parameter	**Lab scale reactors**			**Full-scale**	**Mixed inoculum**
(mean ± Std)	**R1**	**R2**	**R3**	**(LaPrairie)**	
Volume (L)	2.0			7.0	9.0
Feed	Ozone WAS			Raw WAS	
SRT (days)	15			1 +19	
Temperature (°C)	22			35	
TS (g/L)	35.9 ± 1.7	35.5 ± 1.1	32.7 ± 1.9	22.4 ± 0.6	25.5 ± 0.5
VS (g/L)	24.1 ± 1.3	23.5 ± 0.6	21.6 ± 1.4	14.6 ± 0.9	17.2 ± 0.4
VS/TS (%)	67.0 ± 0.6	66.1 ± 0.6	66.2 ± 0.5	65.7 ± 1.6	67.3 ± 0.3
sCOD (g O_2/L)	1.1 ± 0.2	1.0 ± 0.2	1.2 ± 0.2	1.4 ± 0.1	1.3 ± 0.1
tCOD (g O_2/L)	29.2 ± 1.4	24.7 ± 0.6	29.1 ± 0.8	15.8 ± 0.5	17.2 ± 0.6

4.1.2. Waste activated sludge

The waste activated sludge was collected from LaPrairie wastewater treatment plant, located at Saint Catherine (Quebec, Canada) and owned by La Régie de l'assainissement des eaux du bassin LaPrairie (RAEBL), a group of five municipalities. The WWTP treats around 60 thousand m^3/d of municipal wastewater, including both domestic and industrial wastes. It presents a simplified scheme, which includes screens and aerated desanders (sand, grease and oil removal) as primary units, longitudinal pre-mixing units, aeration basins and clarifiers as secondary units. Activated sludge from secondary clarifiers is thickened from fewer than 1% to 4-5% TS content before mesophilic AD stabilization (RAEBL - Régie d'Assainissement des Eaux du Bassin de LaPrairie, 2014). The design parameters of the WWTP are reported in Table 4.2. It can be noticed that the population was 90000 inhabitants, while the population equivalent 240000 p.e., including industrial effluents. Moreover, Figure 4.1 reports the trends of influent properties of the plant between July and December of 2017: both Biochemical Oxygen Demand (BOD_5) and Suspended Solids (SS) showed relevant seasonal fluctuations. In addition, in 2017 the WWTP achieved mean removal efficiencies of 96% for BOD_5 and 95% for SS (Aquatech, 2017).

Table 4.2: Design parameters of LaPrarie WWTP. Adapted from (RAEBL - Régie d'Assainissement des Eaux du Bassin de LaPrairie, 2014).

Population (inhabitants)	90000	Influent BOD_5 (mg/L)	190
Population equivalent (pe)	240000	Influent SS (mg/L)	250
Average flow (m^3/d)	65000	Effluent BOD_5 (mg/L)	20
Maximum flow (m^3/d)	88000	Effluent SS (mg/L)	20

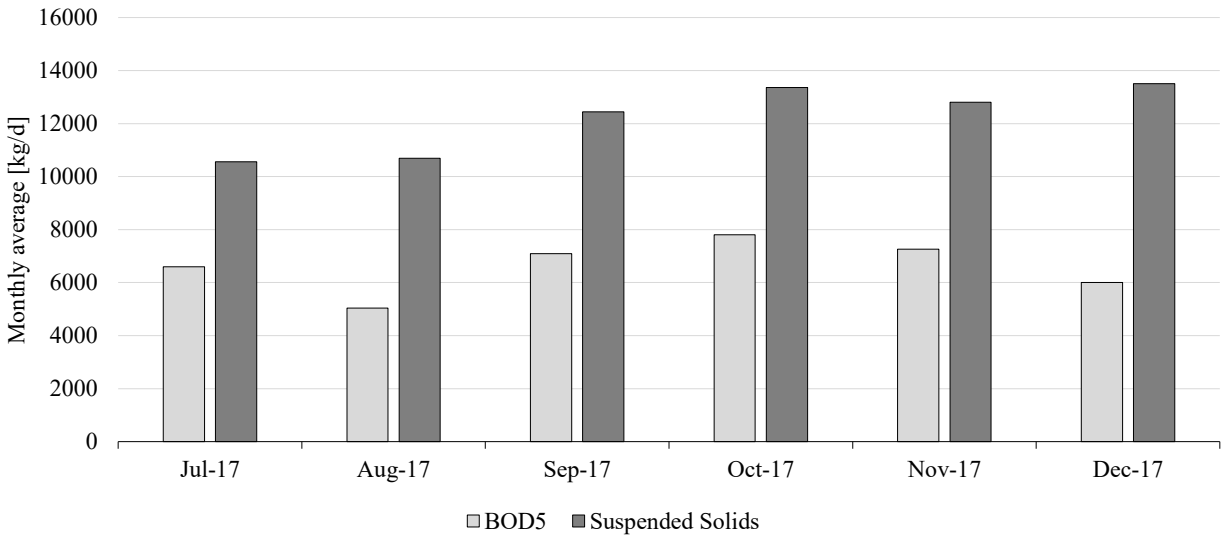


Figure 4.1: Variation of influent properties of LaPrairie WWTP between July, 2017 and December, 2017 (Aquatech, 2017).

During the AD test the WAS presented the mean parameters reported in Table 4.3: some of them are closer to typical parameters of a primary sludge rather than a secondary one, shown in Table 2.1. In fact, in this case the VS/TS is 77% compared with 75% for a primary sludge and 70% for a secondary one, in addition the pH is 6.2 in comparison to respectively 6.0 and 7.1. This fact can be due to the absence of a clarifier at the end of primary units in the WWTP. Moreover, the sCOD/tCOD was around 8%, requiring the integration of AD process with a pre-treatment for increasing the amount of soluble organic matter with the aim of enhancing the rate limiting step of hydrolysis, which is particularly sensitive at 22°C.

Table 4.3: Mean parameters of fresh WAS after dilution to approximately 3% TS, collected from LaPrairie WWTP during the AD test (three SRT).

Parameter	**Mean ± Std**	**Parameter**	**Mean ± Std**
pH	6.2 ± 0.3	tCOD (g O_2/L)	37.7 ± 2.8
TS (g/L)	32.7 ± 2.0	sCOD (g O_2/L)	2.9 ± 1.0
VS (g/L)	25.2 ± 1.3	sCOD/tCOD (%)	7.8 ± 2.9
VS/TS (%)	77.1 ± 1.7		

4.2. Pre-treatments

In the present study three different pre-treatments were selected, properly designed, and tested for enhancing hydrolysis and AD of WAS at 22°C, by evaluation the increased solubilisation of organic matter (DR) and the enhanced AD process (solids removal efficiencies, biogas production). Thus, the first step consisted in the selection of promising treatments, resulting in OZ, low thermal treatment and integration between thermal and alkali treatment. The second step was the design and optimization of operating conditions. Thus, while the OZ treatment was already optimized for WAS from LaPrairie WWTP, three tests were carried out to select the appropriate operating parameters for both the TH and TA treatments. Finally, the performances of optimized treatments were assessed during the AD test in terms of solubilisation of organic matter. In this section the results of mentioned phases are presented and discussed.

4.2.1. Selection of promising pre-treatments

The *ozonation* has been shown as a promising pre-treatment of WAS, due to strong oxidant properties of ozone. The treatment determines sequential floc disintegration, solubilisation and oxidation of released organic matter (mineralisation), generating a dispersion of many micro particles in the supernatant in addition to increased soluble substances (Chu et al., 2009). Many studies reported the effect of ozone doses on the solubilisation of sludge COD: for instance a dose of 0.05 g O_3/g TSS resulted in a sCOD fraction of 19.6%, while 0.1 g O_3/g TSS in 25.7%, starting from a negligible sCOD fraction (Chu et al., 2009, Yeom et al., 2002). Moreover, the results of studies reported in Table 2.11 show significant improvements in biodegradability of OZ sludge. However, in section 2.2.6 the energy cost for production of ozone was mentioned as its main limitation. In contrast, a comparison between a mesophilic AD process of WAS and the integration between OZ treatment and AD at 20°C suggested the latter as a more energetically sustainable option (Bakhshi et al., 2018). In this study the ozonated WAS from LaPrairie WWTP digested at 20°C showed greater biosolids (VSS) destruction and specific biogas production than raw WAS subjected to AD at 35°C (Table 2.11). Thereby, the mentioned ozone treatment was selected and the optimum ozone dose (200 mg O_3/L) was applied on WAS.

In addition, many studies revealed the promising effects of *thermal treatments* on AD of WAS (Table 2.10) in a wide range of temperatures (60-180°C) with an optimum between 160°C and 180°C (Carrere et al., 2010), due to action of temperature in destroying cell walls and membranes (Tyagi and Lo, 2011). Nevertheless, the present study aimed to investigate the viability of AD of WAS at low temperatures to enhance the energy sustainability of the process, thus the introduction of an efficient but energy-intensive treatment was avoided, i.e. mechanical and high-temperature methods (Appels et al., 2010). In terms of energy requirements, a lower temperature seemed to be preferable. Generally, low-temperature treatments (<100°C) need to long durations, i.e. ranging from 10 h to few days at 70°C (Carrere et al., 2010, Ferrer et al., 2009), so that a thermal treatment at an intermediate temperature of 110-120°C was preferred, being easy to implement and relatively low cost with significant performances (Table 2.10).

Finally, in section 2.2.6 the advantages of alkali treatments were exposed, such as simplicity of devices and operations, low cost, and energy efficiency, along with effectiveness in enhancing AD of WAS (Zhen et al., 2017). Moreover, the integration of a *low thermal treatment* (<100°C) and an *alkali* method was reported to enhance AD process (Table 2.11) while limiting the dose of alkali agent and the temperature. Hence, this combined method was adopted and the operating parameters were properly optimized.

4.2.2. Optimization of operating conditions

Thermal treatment

Three tests, named T1, T2 and T3, were performed on WAS (3% TS) to select the optimum treatment time, by determining DR_{COD} and pH per each time. The durations of 30, 60, 90, 120 min were selected in accordance with studies reported in Table 2.10, while the working temperature was 115-118°C. Moreover, the samples were subjected to two cooling methods, at room temperature and ice-bath, with the aim to abruptly interrupt the COD solubilisation at the end of the TH.

Therefore, firstly the effect of the thermal treatment, of growing times and of cooling method on pH of WAS were investigated. Thus, the resultant pH on treated samples of three tests were mediated and plotted as a function of the treatment time and the cooling mode, as shown in Figure 4.2. Overall, the thermal tests seemed to determine a slight decrease of pH between pH 6 for untreated samples and about pH 5.8 for samples treated for 120 min, which perhaps indicated the release of intracellular substances due to temperature. In fact, a significant increased solubilisation of proteins, carbohydrates and lipids was observed with growing temperatures and treatment durations between 15 and 60 min, along with increased VFA concentration (Appels et al., 2010). Moreover, an increased solubilisation of these substances at these temperature (120°C) and time range (0-60 min) was observed in other studies (Noike, 1992, Wang et al., 1997).

A significant negative linear correlation between treatment time and pH was found for both the cooling at room temperature ($r(3) = 0.986$, $p < 0.05$) and in ice bath ($r(3) = 0.913$, $p < 0.05$) as confirmation of the slight pH decrease. In addition, a paired T-test on the two pH trends of Figure 4.2 was performed, showing mean pH in ice bath was significantly greater than mean pH at room temperature (one side, $t(3) = 3.46$, $p < 0.05$). It can be concluded that the slight pH decrease induced by treatment was significantly reduced by the sharp cooling in ice bath.

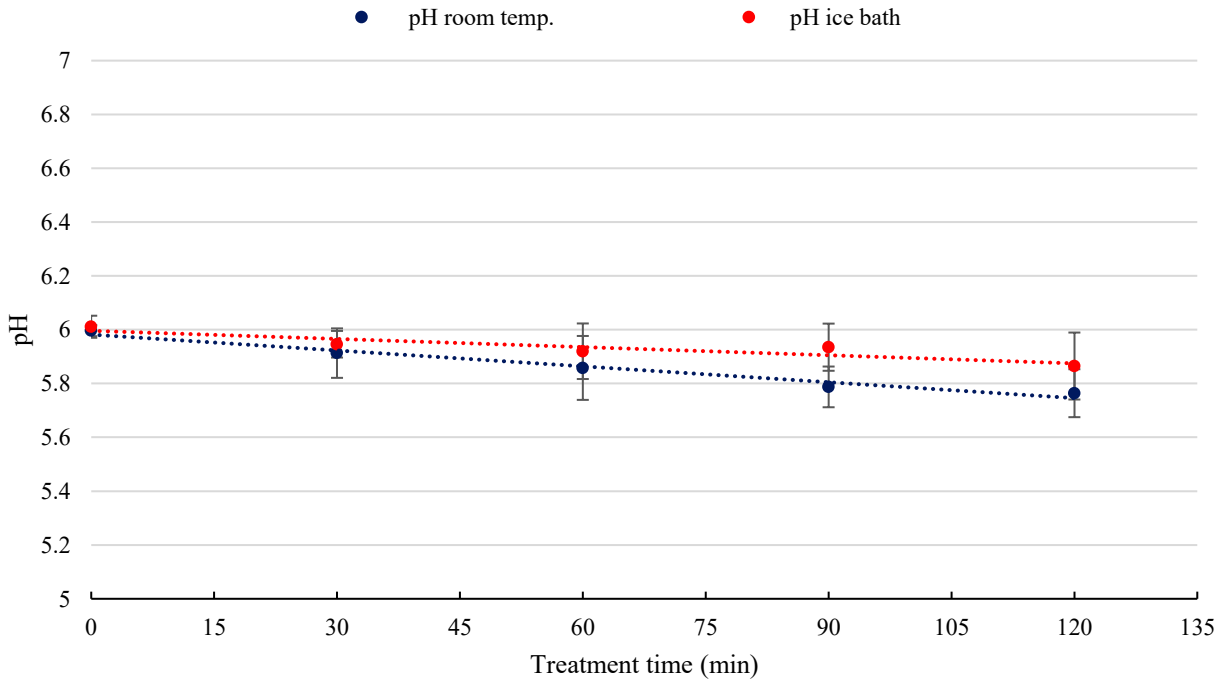


Figure 4.2: Mean pH of WAS (T1, T2, T3) as a function of the treatment time and the cooling mode (room temperature and ice bath).

Moreover, the effect of time and cooling on DR_{COD} was also investigated for determining the optimum treatment duration. The DR_{COD} resulting from T1, T2 and T3 were mediated, but the relative standard deviations (RSD) were higher than 30%, as shown in Table 4.4. This fact is not surprising considering tests were carried out with the same operating conditions on samples of fresh WAS on different dates between September and December of 2017, suffering seasonal fluctuations of WAS, as shown in Figure 4.1. For this reason, the DR of each test were considered and plotted as a function of the treatment times, as shown in Figure 4.3. Hence, the DR ranged between 12% and 13% in case of tests T1 and T2, while in test T3 the DR values surprisingly doubled between 21% and 25% in the considered range of treatment times. According to available data, this variation was not apparently explainable, by the moment the starting sCOD/tCOD ratio of WAS before TH were similar for test T1, T2 and T3, being respectively 8.1 ± 0.2%, 7.9 ± 0.1%, and 7.9 ± 0.2%. In addition, as already mentioned, the WAS was diluted to 3% TS prior TH in all cases. Nevertheless, a DR ranged 12-25% seems to be in accordance with DR values (10-18%) shown in Table 2.10 for activated sludges of different TS concentrations subjected to similar thermal treatments.

Table 4.4: Mean DR during thermal tests T1, T2, T3 and corresponding relative standard deviations (RSD).

Treatment time (min)	Ice bath		Room temperature	
	DR (%)	RSD (-)	DR (%)	RSD (-)
30	17.1	0.35	16.4	0.33
60	17.4	0.39	17.4	0.48
90	18.4	0.46	18.1	0.45
120	18.7	0.46	18.1	0.40

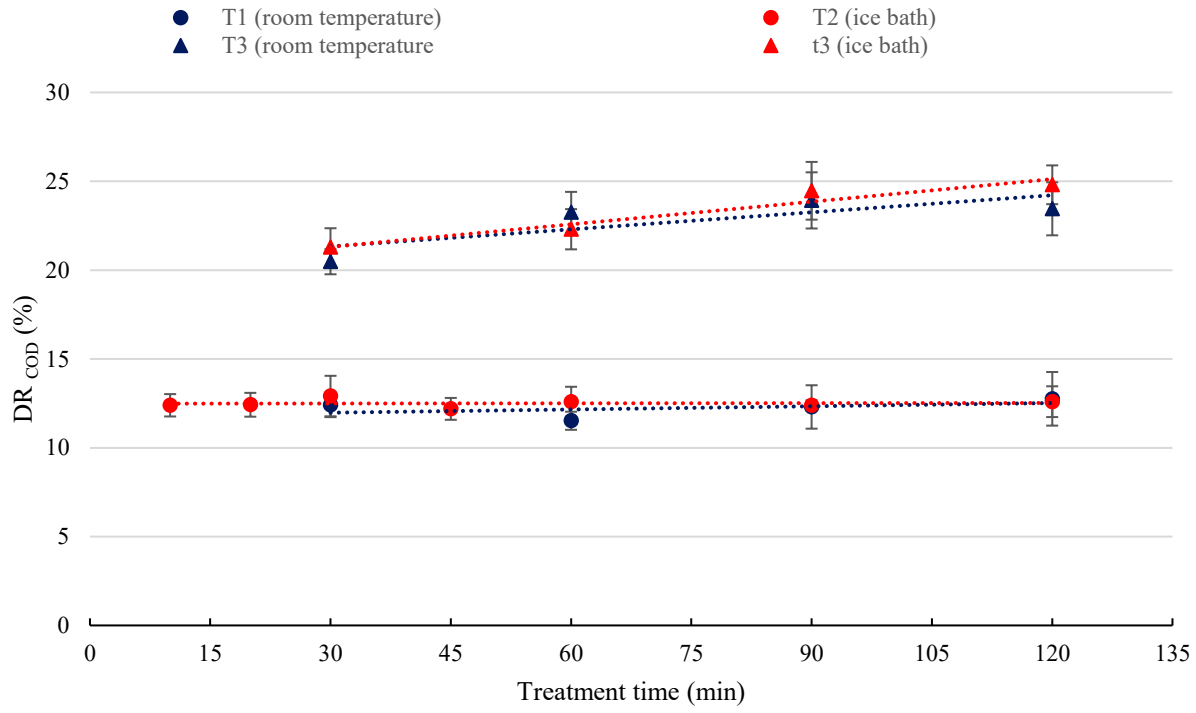


Figure 4.3: Resultant disintegration rate as a function of treatment time in test T1, T2, and T3.

Firstly, it was found a significant positive linear correlation between the duration of treatment and the DR just in test T3 with cooling at room temperature ($r(2) = 0.967$, $p < 0.05$), while in the other cases there was not a significant correlation (for T1 $p = 0.552$, for T2 $p = 0.899$, for T3 in ice bath $p = 0.205$). It can be notice that heating time seemed to have less impact than temperature on sludge solubilisation (Borges and Chernicharo, 2009). Thus, at high temperatures authors generally use short fixed times, while at low temperatures (<100°C) several hours of heating may be required (Borges and Chernicharo, 2009, Zhen et al., 2017). In conclusion, in this case the TH seemed to determine promising COD solubilisation, in accordance with other studies (Table 2.10), but an increase of treatment time did not significantly affect the DR in three cases. Thus, based on test T1, the minimum time of 30 min was selected with the aim of limiting energy consumptions.

In addition, the effect of the cooling mode on DR was investigated in test T3 by means of a paired T-test on the two DR trends shown in Figure 4.3, revealing there was not a significant difference between the mean DR of the two cooling modes (one side, $t(2) = 0.918$, $p = 0.228$). The cooling mode hence did not significantly influence the solubilisation of COD.

Thermo-alkaline treatment

Three thermo- alkaline tests TA1, TA2, and TA3 were carried out on WAS diluted to a TS content of 3% for optimizing the alkali dose and the duration of low temperature treatment. Firstly, the promising sodium hydroxide doses corresponding to pH target values of 9, 10, and 11 were selected starting from results of different authors. Thus, a thermo-alkaline treatment of WAS in a temperature range between 50 and 90°C and pH values of 8, 9, 10 and 11 was investigated (Vlyssides and Karlis, 2004), showing an optimum combination of pH 11 and 90°C. In addition, another thermo-alkaline treatment of dairy WAS was carried out at various temperatures (50, 60, 70, and 80°C) for different times adding various doses of sodium hydroxide to reach pH 10, 11, and 12, obtaining an optimized condition of 60°C and pH 12 (Rani et al., 2012). The selected doses needed for reaching pH 9, 10, and 11 were determined during each test and expressed as g NaOH/g TS. Thus, mean doses of 0.034 ± 0.002, 0.059 ± 0.007, and 0.083 ± 0.006 g NaOH/g TS corresponded respectively to mean pH of 9.2 ± 0.1, 10.1 ± 0.1, and 11.0 ± 0.1. Moreover, pH values as a function of the dose of sodium hydroxide are shown in Figure 4.4. As expected a significant linear positive correlation between alkali dose and pH was found ($r(7) = 0.986$, $p < 0.05$).

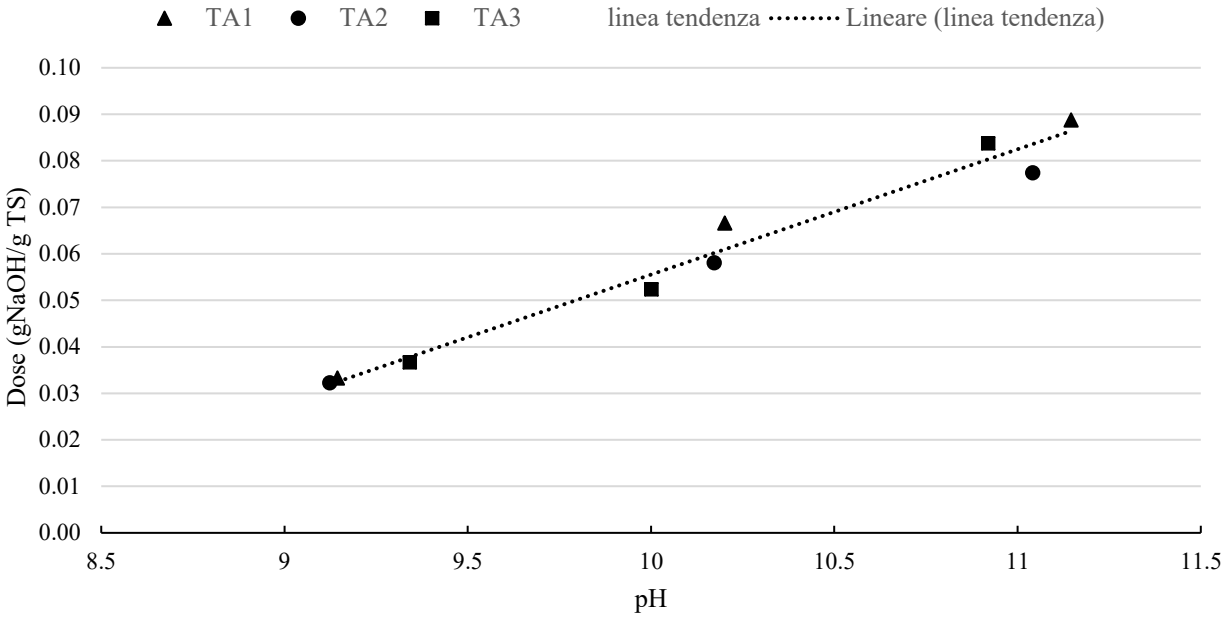


Figure 4.4: Dose of sodium hydroxide as a function of pH during test TA1, TA2, and TA3.

Secondly, as already mentioned, from the literature the temperature of 70°C appeared to be a good compromise between treatment performance and energy cost (Kim et al., 2013, Rani et al., 2012, Ruffino et al., 2016), while the durations of low temperature treatment of 30, 60, 90 and 120 min were chosen in accordance with thermal treatment tests and were consistent with times adopted by other authors (Table 2.11).

Once selected the promising combinations of doses (pH 9, 10, 11) and times (0, 30, 60, 90, 120 min), the three tests were performed determining the pH on the treated WAS and DR per each pair of values.

Thus, firstly the impacts of the thermal treatment and the growing durations on the pH of WAS were examined. The resultant pH on samples of TA1, TA2 and TA3 were mediated and plotted as a function of treatment times and alkali doses, expressed in terms of pH target, as shown in Figure 4.5. On the graph the pH values corresponding to time 0 min were measured after the addition of NaOH and are not to scale with respect to the time axis. Moreover, each combination of dose and time presents two pH values: a sample subjected to thermal treatment and a *control* kept at room temperature. This procedure allowed to investigate the influence of temperature on pH of WAS. In fact, it can be observed a difference of 0.7-0.8 pH units between each pair of pH values on the graph, so that a paired T-test was performed on pH trends of each dose. A difference of 0.6 pH units between mean pH of controls and thermal treated samples was found as significant for pH 9 (one side, $t(3) = 7.821$, $p < 0.05$), pH 10 (one side, $t(3) = 10.246$, $p < 0.05$), and pH 11 (one side, $t(3) = 5.524$, $p < 0.05$). In contrast to thermal tests, the low temperature treatment decreased significantly the pH of WAS samples after the addition of NaOH. Moreover, the effect of treatment time on pH was considered by assessing the significance of linear correlation between time (excluding time: 0 min) and pH, obtaining the results of Table 4.5. In all cases there was a significant slight negative linear relationship between time and pH for both the controls and the thermal treated samples. Hence, it can be concluded that the low temperature treatment seemed to considerably reduce the pH (0.6 pH units) and that the pH of WAS seemed to slowly decrease with time after the alkali addition (slight slopes for both controls and treated samples). Other studies monitored the decrease of pH during the first 24 h from the addition of NaOH. Thus, when starting pH was lower than 10, a decrease of pH to less than 8.5 after 24 h was observed,

while when initial pH was 12-13, pH value was still around 12 after 24 h (Li et al., 2012, YANG et al., 2007). In another study when starting pH was over 10, the subsequent pH drop was almost negligible, suggesting a pH conditioning close to 7.5 for preventing delay or failure of AD process (Li et al., 2012).

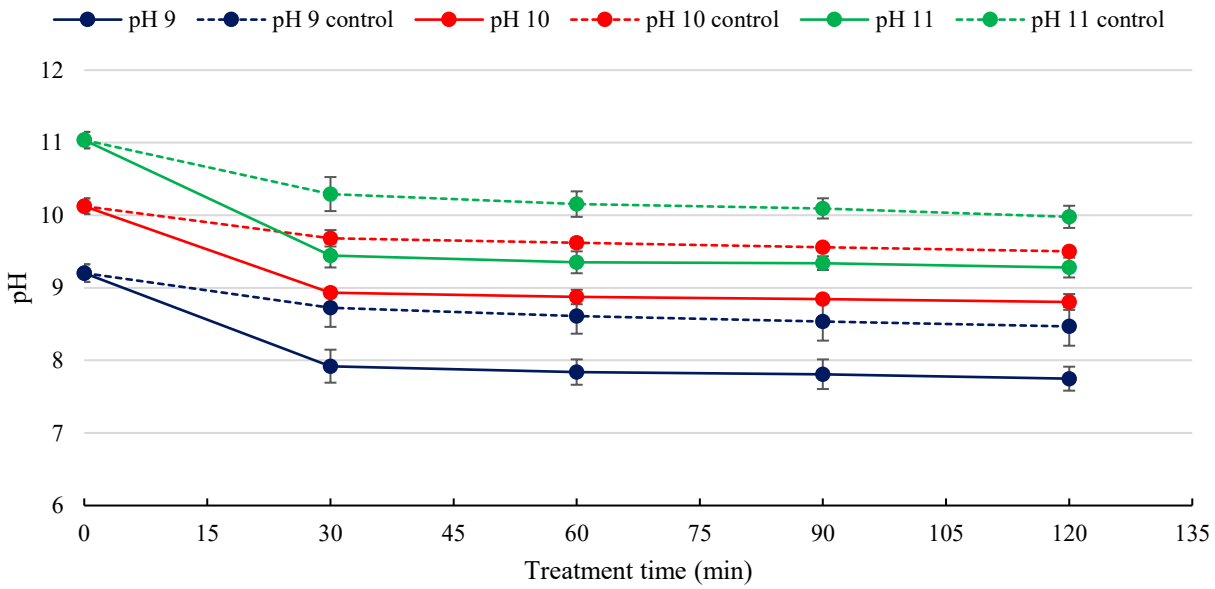


Figure 4.5: pH of treated WAS as a function of treatment time and alkali dose. The control samples were not subjected to thermal treatment; pH values at 0 min were measured after the addition of alkali.

Table 4.5: Results of linear regression between treatment time and pH of treated WAS.

	Control sample			Thermal treated sample		
	***r*(2)**	**Slope (pH/min)**	***p***	**r(2)**	**Slope (pH/min)**	***p***
pH 9	0.992	-0.0028	0.0077	0.985	-0.0018	0.0149
pH 10	1.000	-0.0020	0.0001	0.989	-0.0014	0.0105
pH11	0.990	-0.0033	0.0103	0.959	-0.0017	0.0407

Secondly, the impact of NaOH dose and treatment time on DR_{COD} of sludge was examined by plotting DR of each test per each combination of operating conditions, obtaining the three graphs shown in Figure 4.6, Figure 4.7 and Figure 4.8. In fact, as in the case of thermal tests, the mediated DR presented excessively high RSD, reported in Table 4.6, so that the tests were studied separately. The mean values ranged approximately between 25% and 40%, which seemed to be consistent with COD solubilisations found in other studies (Table 2.11), even if comparisons among different studies are critical because both alkali doses and COD solubilisation are expressed by various measures and units.

The influence of the two parameters on DR of WAS was studied by means of a two-way ANOVA, where the factors were the alkali dose (pH) and the thermal treatment time, and the variable was the DR. As a result, in test TA1 a significant effect of alkali dose was found ($F(2,6) = 6.464$, $p < 0.05$), while the effect of treatment time was not significant. Moreover, in test TA2 it was found a significant effect on DR of both the control parameters (dose: $F(2,6) = 39.896$, $p < 0.05$; time: $F(3,6) = 4.959$, $p < 0.05$). Finally, in TA3 the dose had a significant impact on DR ($F(2,6) = 18.067$, $p < 0.05$), while time had not. Hence, overall, it can be observed that a higher alkali dose determined a significant effect on DR, while an increased time did not in two tests.

In addition, it can be pointed out that thermal treatment determined an increase of sludge solubilisation. In fact, if one looks at graphs of Figure 4.7 and Figure 4.8, in both TA2 and TA3 the difference between each pair of DR at 0 min and 30 min seems to be significant, due to the effect of thermal treatment. However, the duration of the treatment did not appear to be determinant. In fact, it can be recalled that low temperature treatments may require several hours (Borges and Chernicharo, 2009).

Based on test TA1 the combination of pH 11 and 60 min was chosen as optimum, corresponding to the highest DR value, equal to 31.8 ± 2.6%, as shown in Figure 4.6. Therefore, a dose of alkali of 0.09 g NaOH/g TS was used during AD test.

Table 4.6: Mean disintegration rate during thermo-alkaline tests TA1, TA2, TA3 and corresponding relative standard deviations (RSD).

Treatment time (min)	pH 9		pH 10		pH 10	
	Av. DR (%)	RSD (-)	Av. DR (%)	RSD (-)	Av. DR (%)	RSD (-)
0	9.2	0.01	23.6	0.26	28.3	0.06
30	25.4	0.08	32.3	0.14	33.9	0.12
60	27.3	0.10	31.4	0.10	36.4	0.12
90	29.8	0.02	32.3	0.20	38.5	0.23
120	29.4	0.09	34.3	0.13	37.9	0.23

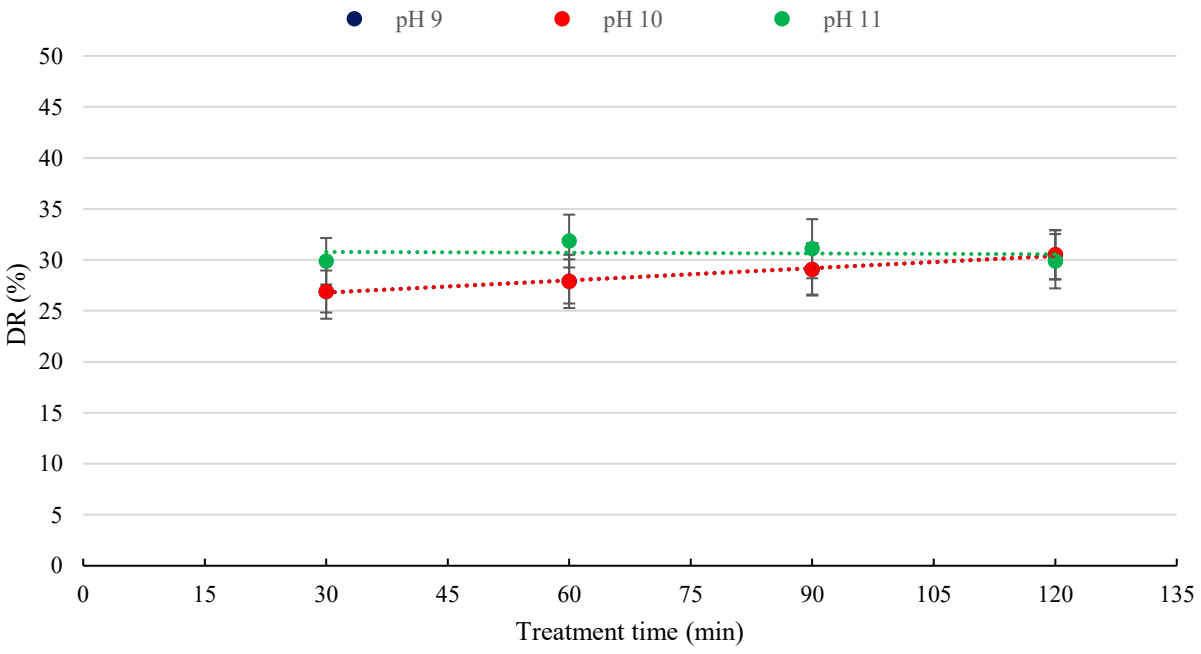


Figure 4.6: Resultant DR_{COD} as a function of treatment time and alkali dose in test TA1.

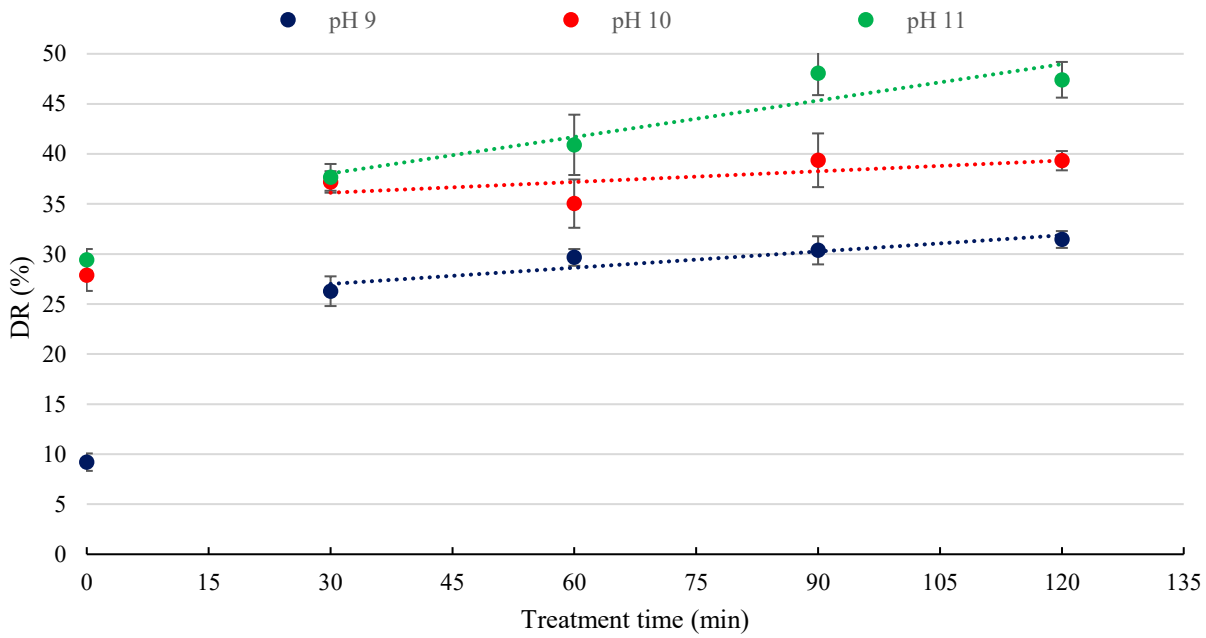


Figure 4.7: Resultant disintegration rate as a function of treatment time and alkali dose in test TA2.

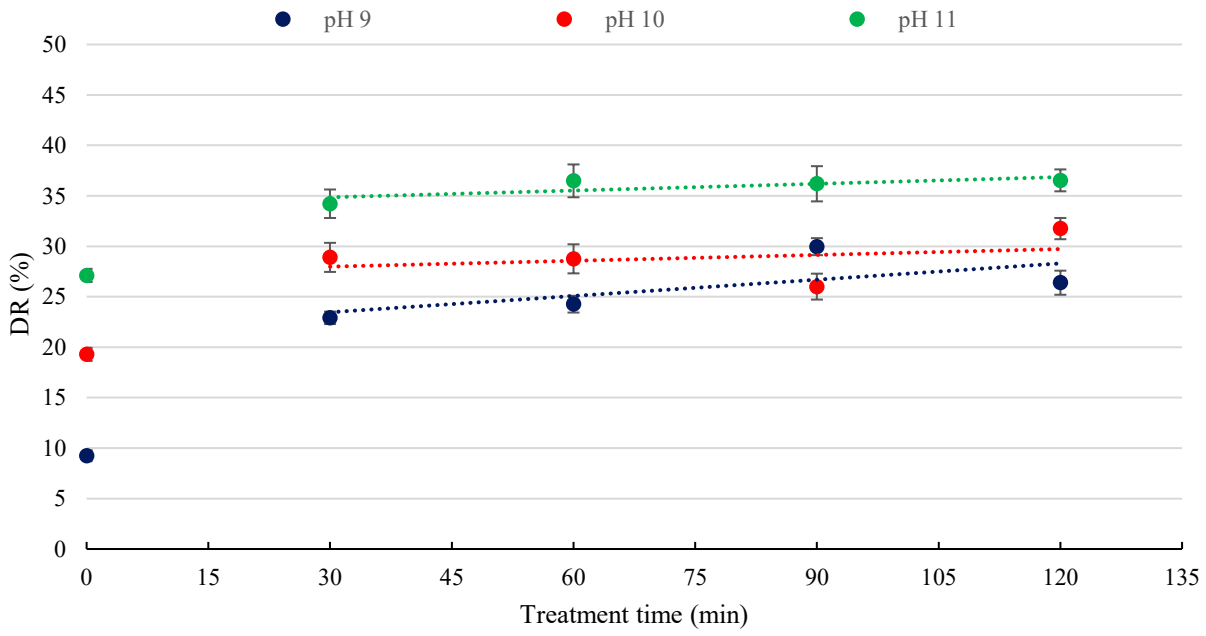


Figure 4.8: Resultant disintegration rate as a function of treatment time and alkali dose in test TA3.

Finally, by the moment the two-way ANOVA revealed a significant effect of an increased dose on DR, this aspect was further examined and the DR of WAS samples which were not subject to the thermal treatment was plotted as a function of the initial pH, as shown in Figure 4.9. It can be observed that an increase of alkali dose from about pH 9 to pH 11 determined a growth of DR from 10% to almost 30%. Moreover, it was found a significant positive linear correlation between the initial pH and the DR ($r(4) = 0.928$, $p < 0.05$).

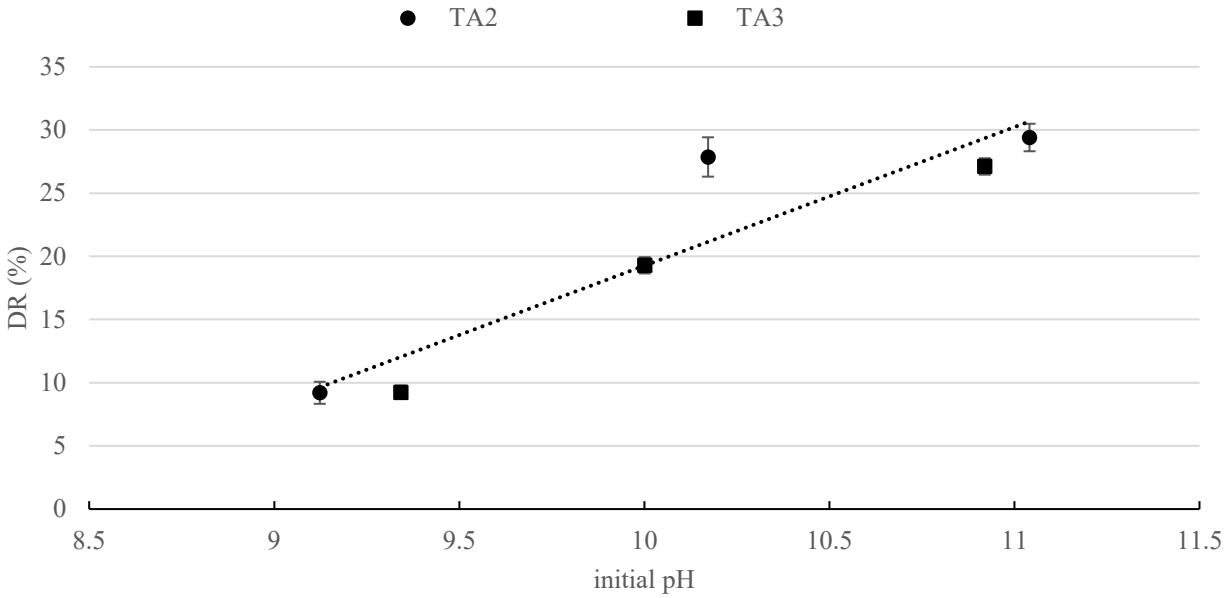


Figure 4.9: Disintegration rate as a function of initial pH values.

4.2.3. Evaluation of solubilisation performances

After the optimization of pre-treatments, the AD test was carried out. Thereby, once per week, fresh WAS (3% TS) was subjected to different pre-treatments and used as feed for the AD rectors during the week. The mean characteristics of WAS after different treatments are reported in Table 4.7. The resulting ozone dose was closed to the target 200 mg O_3/L (Bakhshi et al., 2018), as well as the dose of NaOH, which presented a mean of 0.08 g NaOH/g TS during AD test, compared with a target of 0.09 g NaOH/g TS. Moreover, the mean pH of raw WAS seemed to slightly decrease after thermal treatment. Thus, a paired T-test revealed that the mean pH of raw WAS was significantly greater than mean pH after thermal treatment (one side, $t(5) = 3.527$, $p < 0.05$), while there was not significant difference between pH of raw WAS and pH after OZ.

The pH of 7.3 after TA treatment was closed to the reported optimum range between 6.5 and 7.2 for methanogens (Appels et al., 2008). In addition, the VS concentrations remained fairly constant for all the types of WAS, while the mean sCOD concentrations of pretreated sludge were increased. Thus, the sCOD values of TH, OZ and TA WAS improved respectively by 264%, 47%, and 426% compared to sCOD concentration of raw WAS.

Table 4.7: Operating conditions and mean characteristics of WAS after different pre-treatments.

Pre-treatment	Raw WAS	Thermal	Ozone	Thermo-alkaline
Time		30 min		60 min
Temperature		115-118°C		70°C
Dose			189 ± 53 g O_3/L	0.08 ± 0.01 g NaOH/g TS
pH	6.2 ± 0.3	6.0 ± 0.2	6.1 ± 0.2	7.3 ± 0.3*
TS (g/L)	33.1 ± 1.9	33.2 ± 2.1	33.2 ± 2.0	36.0 ± 2.4
VS (g/L)	25.4 ± 1.4	25.3 ± 1.6	25.4 ± 1.5	25.3 ± 1.5
tCOD (g O_2/L)	37.8 ± 0.3	37.8 ± 2.0	39.2 ± 3.3	38.0 ± 2.7
sCOD (g O_2/L)	2.7 ± 1.0	9.9 ± 1.3	4.0 ± 1.1	14.3 ± 0.8
sCOD/tCOD (%)	7.8 ± 2.9	26.6 ± 3.3	10.1 ± 2.7	36.0 ± 6.0

*After pH conditioning

The effect of pre-treatments on the solubilisation of sludge was examined by means of the disintegration rate (DR), defined by equation (3.1), and by the solubilisation ratio (S_{COD}), defined as the ratio between sCOD and tCOD of sludge.

Disintegration rate

Firstly, the DR of the WAS after pre-treatments were considered. With the aim of investigating the fluctuations of DR over time (three SRTs), the DR values were plotted as a function of time, as shown in Figure 4.10. From the graph, the fluctuations of DR over the 3 SRTs seem to be evident for TH and TA sludges. Thereby, a two-way ANOVA was performed on DR, where the factors were the treatments and the time. As expected overall it was found a significant effect of the treatment on DR ($F(2,12) = 222.74$, $p < 0.05$), but the effect of time was not significant.

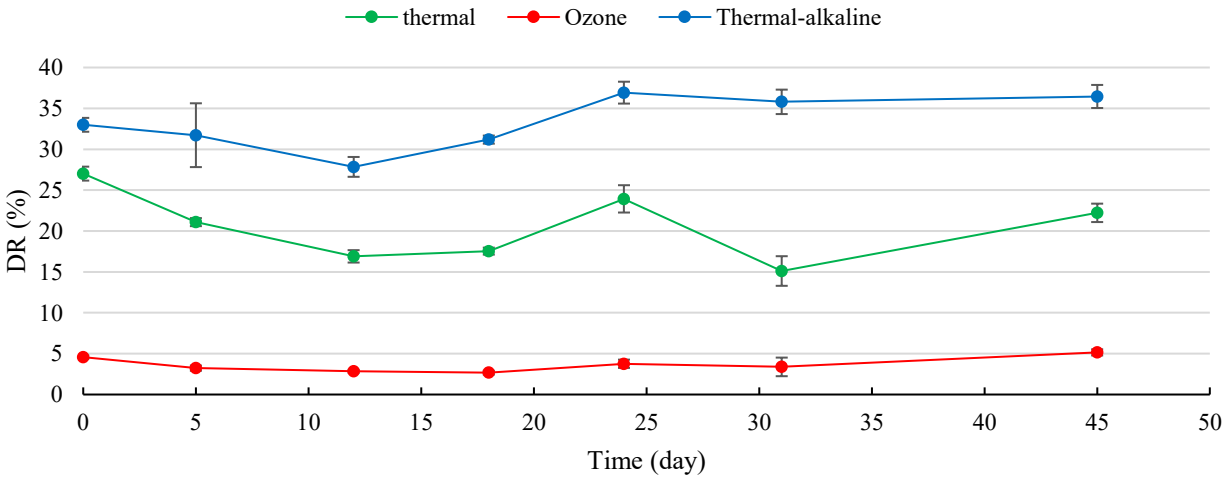


Figure 4.10: Disintegration rate of the WAS after different treatments as a function of time during the AD test.

Hence, Figure 4.11 shows the mean DR of WAS after different treatments, which were in the order $DR_{TA} > DR_{TH} > DR_{OZ}$, resulting the same of sCOD concentrations. Moreover, the DR_{TA} of 33 ± 3% was consistent with 32 ± 3% obtained with the combination pH 11 and 60 min in test TA1. Many studies reported similar DR Table 2.11. Thus, a DR between 25% and 28% was obtained with a dose of 0.04 and 0.08 g NaOH/g TS

(Ruffino et al., 2016), while a DR of 43.7% was reached by keeping a constant pH of 11 for 10 h by adding NaOH (Xu et al., 2014). Moreover, if one looks at Figure 4.3, DR_{TH} of 21 ± 4% was in the expected range, greater than DR_{30min} of both T1 and T2 and closer to DR_{30min} of T3. In accordance with scientific literature (Table 2.10), a TH treatment carried out at 121°C for 30 min determined a DR of 10.5% (Kim et al., 2003), a TH treatment at 121°C for 15 min a DR of 15.7% (Salsabil et al., 2010), and treatment at 90°C for 60 min a DR of 17.9% (Appels et al., 2010). In contrast, the mean DR_{OZ} around 4% was significantly lower than the estimated DR of 22% of WAS treated with an ozone dose of 0.16 g O_3/ g TS (Bougrier et al., 2006). The dose of 190 mg O_3/L used in the present study corresponded to 0.01 g O_3/g TS, which was 16 times lower than the mentioned dose. Moreover, the optimum range was reported between 0.05 and 0.5 g O_3/g TS (Zhen et al., 2017). Thereby, it can be concluded that the dose of O_3 did not seemed to significantly improve the solubilisation of sludge in comparison with TH and TA pre-treatment methods, in terms of DR.

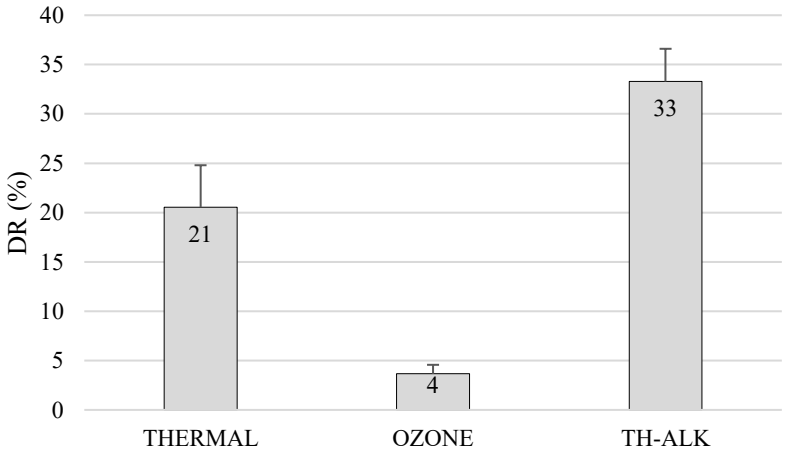


Figure 4.11: Mean disintegration rate of the WAS after different pre-treatments during the AD test.

Solubilisation ratio

In addition, with the aim of comparing the solubilisation of sludge after different treatments with the initial solubilisation of raw WAS, the S_{COD} was determined and plotted over time, as shown in Figure 4.11. It can be noted that the four trends appear to change over time with similar patterns, such that a two-way ANOVA was carried out, where the factors consisted of treatment and time. In contrast to the case of the DR, it was found a significant effect on the S_{COD} for both the treatment ($F(3,18) = 217.282$, $p < 0.05$) and the time ($F(6,18) = 4.338$, $p < 0.05$). Overall, the significant effect of time was likely to be linked with the seasonal fluctuations of WAS, as it can be noticed from the trend of S_{COD} of raw WAS.

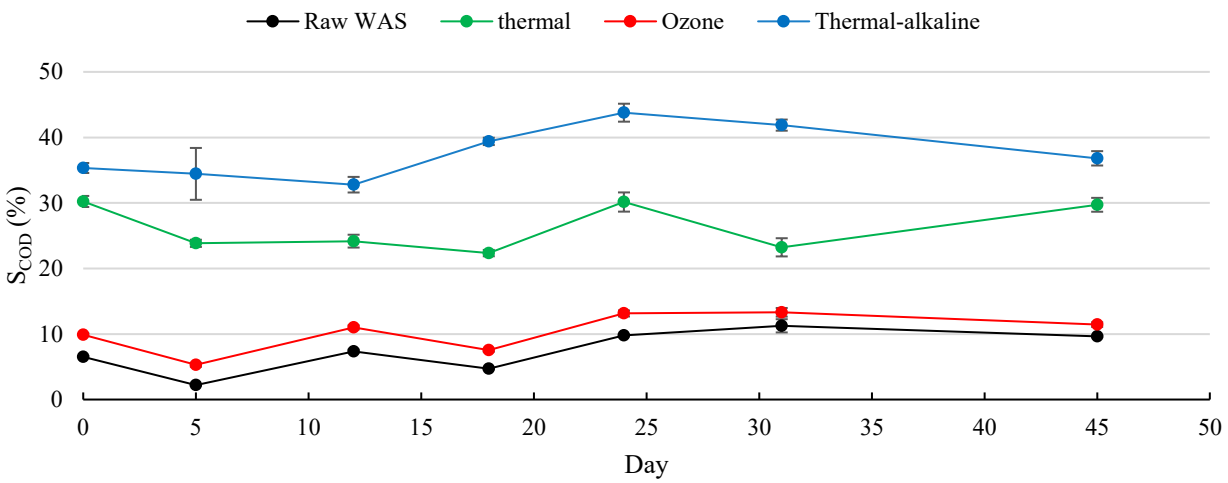


Figure 4.12: Solubilisation ratio of different sludge samples as a function of time during the AD test.

In conclusion, Figure 4.13 shows the mean S_{COD} after different treatments in comparison with initial S_{COD} of raw WAS. As expected the mean S_{COD} were in the order $S_{TH\text{-}ALK} > S_{TH} > S_{OZ} > S_{WAS}$. However, it was performed a paired T-test between the S_{COD} after OZ and the S_{COD} of raw WAS, by the moment the two trends of Figure 4.12 seem to be very closed. It was found that the mean S_{COD} after OZ was significantly higher than mean S_{COD} of raw WAS (one side, $t(6) = 10.90$, $p < 0.05$). Finally, from a second paired T-test resulted that mean $S_{TH\text{-}ALK}$ was significantly greater than mean S_{TH} (one side, $t(6) = 5.988$, $p < 0.05$). Thereby, it can be concluded that the OZ had a significant small effect on the solubilisation of WAS, while the TH and TA treatments improved considerably the solubilisation of sludge. In terms of increased solubilisation of COD of WAS the treatments were in the following order: Thermo-alkaline > thermal > ozone.

However, the increased solubilisation of organic matter is not the only factor affecting the enhancement of AD of WAS. In addition, the increased biodegradability of WAS due to pre-treatments has also to be investigated, by means of analysing subsequent AD test performances (solids removals, biogas production), which will be described and discussed in the next section.

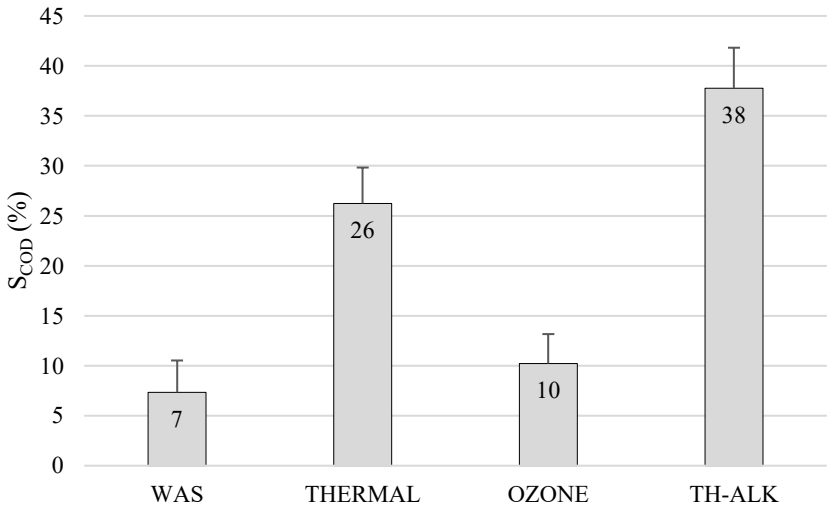


Figure 4.13: Mean solubilisation ratio of different sludge samples during the AD test.

4.3. Anaerobic Digestion Tests

The anaerobic digestion tests were carried out to evaluate the performance of the AD process of WAS at 20°C and to compare the effects of pre-treatments in enhancing the biodegradability of sludge by means of increased biogas production and solids removals. Each test was performed in triplicate, fed with a different sludge, and the subsequent results were mediated. In this section the results of the AD test will be fully described and discussed. Table 4.8 summarizes the mean parameters of digested sludge samples after three SRTs.

Table 4.8: Mean characteristics of digested sludge of each group of triplicate reactors after three SRTs.

Pre-treatment	Raw WAS	Thermal	Ozone	Thermo-alkaline
pH	7.00 ± 0.10	7.09 ± 0.08	7.06 ± 0.05	7.28 ± 0.05
TS (g/L)	32.3 ± 0.04	24.0 ± 2.3	29.6 ± 0.3	28.1 ± 0.3
TS removal (%)	1.0 ± 0.1	26.5 ± 7.0	9.4 ± 0.8	14.1 ± 1.1
VS (g/L)	23.5 ± 0.3	16.6 ± 1.2	20.9 ± 0.4	16.7 ± 0.2
VS/TS (%)	72.5 ± 0.9	69.2 ± 1.4	70.6 ± 0.8	59.6 ± 1.4
VS removal (%)	5.5 ± 1.2	9.8 ± 1.8	8.0 ± 1.0	22.3 ± 1.8
tCOD (g O_2/L)	37.4 ± 0.4	27.9 ± 1.9	31.7 ± 0.4	28.1 ± 1.4
sCOD (g O_2/L)	1.8 ± 0.01	3.6 ± 0.1	2.4 ± 0.1	3.7 ± 0.1
sCOD/tCOD (%)	4.8 ± 0.2	13.1 ± 1.1	7.5 ± 0.6	13.3 ± 1.9

4.3.1. pH

The pH of digested sludge was monitored at each feeding event to detect eventual pH drops, in order to adopt the necessary measures, i.e. interrupting the feeding. However, the pH of digested sludge of all the reactors varied within an acceptable range, as shown in Figure 4.14. In fact, it can be noticed that overall pH values ranged between 6.4 and 7.3 within the recommended range (Appels et al., 2008) as discussed in section 2.2.4. Starting from pH close to neutrality at the end of the start-up phase, the different trends seemed to slight decrease during the first SRT, then they appeared to become distinct and to start growing during the second SRT, finally they seemed to reach the stability during the third SRT. This probably depended on the fact that during the start-up phase all the reactors were fed with OZ sludge and the effects of the change of feeding conditions at day 0 were not significant during the first 15 days of the AD test. Moreover, from the graph it can be observed that starting from the second SRT the pH values of the TA sludge were generally greater than other sludge samples, stabilizing at above pH 7.2 from day 35. In addition, the reactors fed by the TH and the OZ treated WAS reached relatively stable pH higher than 7.0 from days 38. Finally, the pH of reactors fed with the raw WAS shown two drops to pH 6.4 before increasing to values close to neutrality just at the end of the AD test. However, it is generally accepted that AD reactors require more than three SRTs for reaching the steady state from the moment of inoculation or a change of feeding conditions (Bakhshi et al., 2018, Liao et al., 2006, Masse et al., 2006). Hence, with the aim of testing whether the pH values were significantly stable starting from day 38, for each trend the pH values of days 45, 47 and 49, belonging to the fourth SRT, were mediated (M_{4SRT}) and the standard deviation (Std) of the sample was determined. Then, the last pH values belonging to the third SRT were compared with the range $M_{4SRT} \pm Std$. The values within this range were assumed as stable. Based on this criterion, it was possible to estimate the first day within the assumed steady range for each pH trend. The results of the test are reported in Table 4.9. It was found that the pH trends of TH and TA sludges reached a stable level starting from day 38, while raw and ozone sludge samples from day 42. Overall, based on this assumption, it can be concluded the four trends seemed to reach a steady level around the neutrality starting from days 38-42.

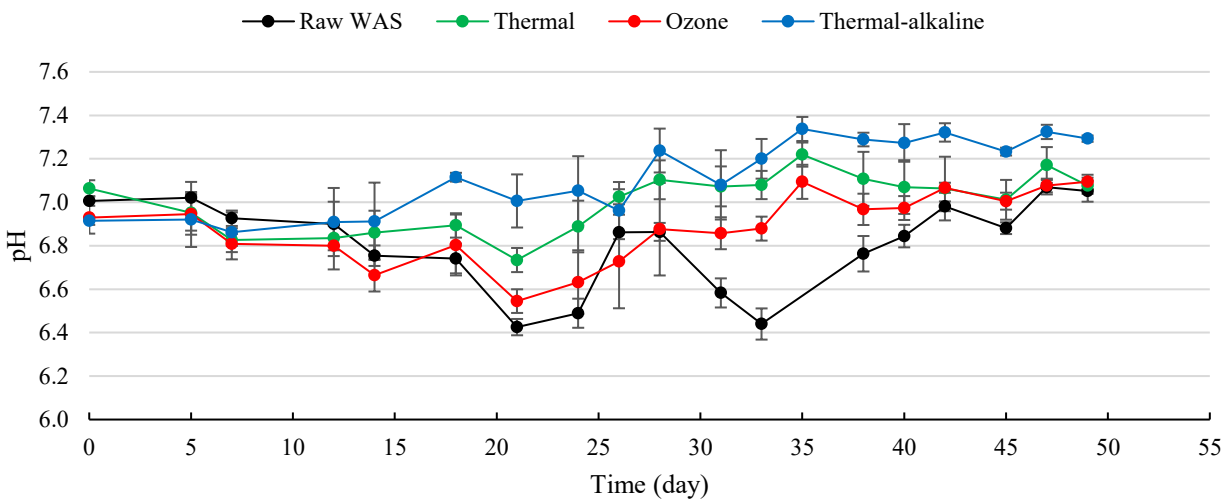


Figure 4.14: Mean pH of digested sludge as a function of time during AD test.

Table 4.9: Results of the test for determining whether pH trends of different sludges reached the steady state.

Pre-treatment	Raw WAS	Thermal	Ozone	Thermo-alkaline
Mean (pH)	7.000	7.086	7.059	7.283
Std (pH)	0.104	0.081	0.047	0.046
Mean – Std	6.896	7.006	7.011	7.237
Mean + Std	7.105	7.167	7.106	7.329
First day within the range (day)	42	38	42	38

4.3.2. Organic loading rate (OLR)

As exposed in section 2.2.4, the organic loading rate was used to prevent inefficiencies of AD process and eventual over load problems. Hence, the daily OLR of each reactor was determined by means of equation (2.13), where C_{IN} was VS of sludge after pre-treatment and Q was calculated by equation (3.8). Then, the daily OLRs were mediated and reported in Table 4.10 for each group of triplicate reactors. A OLR of about 1.7 g VS $L^{-1}d^{-1}$ was found in all cases. Even though the OLR depends on many factors, including the characteristics of substrate and the specific operating conditions, so that a comparison with other studies can be difficult, the performances of mesophilic digesters of WAS from four large Italian WWTPs without primary sedimentation (as LaPrairie WWTP) were reported: the digesters were fed with sludges with a solids content in the range of 2.6-3.9%, operating with a HRT in a range of 20-40 days and an OLR of about 1 g VS $L^{-1}d^{-1}$, determining a removal of VS concentration between 13 and 27% (Bolzonella et al., 2005). Moreover, a pilot scale study of mesophilic, thermophilic and temperature phased AD of WAS at 6% solids content was operated at an OLR of 2.2 g VS $L^{-1}d^{-1}$ and a HRT of 20 days showing promising performances (Bolzonella et al., 2012). In addition, looking at pH trends of Figure 4.14, it can be noticed that the OLR of 1.7 g VS $L^{-1}d^{-1}$ did not seem to determine acidification problems due to over loads of reactors.

Table 4.10: Mean organic loading rate of different reactors during AD test.

Feed	Raw WAS	Thermal	Ozone	Thermo-alkaline
OLR (g VS $L^{-1}d^{-1}$)	1.699 ± 0.009	1.731 ± 0.011	1.725 ± 0.009	1.719 ± 0.008

4.3.3. Removal of solids

Some of the main objectives of the anaerobic digestion of WAS are the reduction of its solids content, assessed by means of the removal of TS (g/L), and the stabilisation of WAS, studied using the degree of reduction of VS content, i.e. the ratio between VS and TS concentrations. Thereby, during the AD test both the removal of TS and VS were determined as follows:

$$R_{TS}(\%) = \frac{TS_{IN}(g/L) - TS_{OUT}(g/L)}{TS_{IN}(g/L)} \cdot 100 \quad (4.1)$$

$$R_{VS}(\%) = \frac{(VS/TS)_{IN} - (VS/TS)_{OUT}}{(VS/TS)_{IN}} \cdot 100 \quad (4.2)$$

Where the *IN* refers to the feed, *OUT* is the value of the digested sludge, and VS/TS is the ratio between VS and TS concentrations (g/L).

In this section, the results of both removal of TS and VS content will be exposed and discussed.

TS removal

Firstly, the removal of TS was calculated once per week for each group of triplicate reactors by means of equation (4.1), using the mean TS concentrations of the three reactors. Then, despite the high volatility of TS removals over time, these values were mediated over the duration of the AD test, obtaining the mean TS removal shown in Figure 4.15.a. It is not possible to compare the mean removals, ranged around 20% for all sludge samples, by the moment the relative standard deviations are excessively high, due to the change of TS removal over time until reaching the steady state. As already exposed, it is generally accepted AD reactors reach steady conditions after three SRTs, corresponding to 45 days for the AD test. Moreover, it was shown that overall pH values of AD reactors seemed to reach relatively stable levels between days 38 and 42.

Hence, the TS removals starting from day 45 were mediated and reported in Figure 4.15.b. It can be noticed that the final mean TS removals of different sludges were in the following order: $R_{TH} > R_{TA} > R_{OZ} > R_{WAS}$. The final TS removals of TH, TA and OZ treated WAS improved respectively by 25, 13 and 8 times compared to the TS removal of raw WAS. However, it can be observed the latter was almost negligible during the last days of AD test. Hence, the different pre-treatments could have a slight effect in enhancing the degradation of solids during AD of WAS. Nevertheless, the final TS removals did not seem to be consistent with the increased COD solubilization of sludge after treatments, which were found in the order: $DR_{TA} > DR_{TH} > DR_{OZ}$.

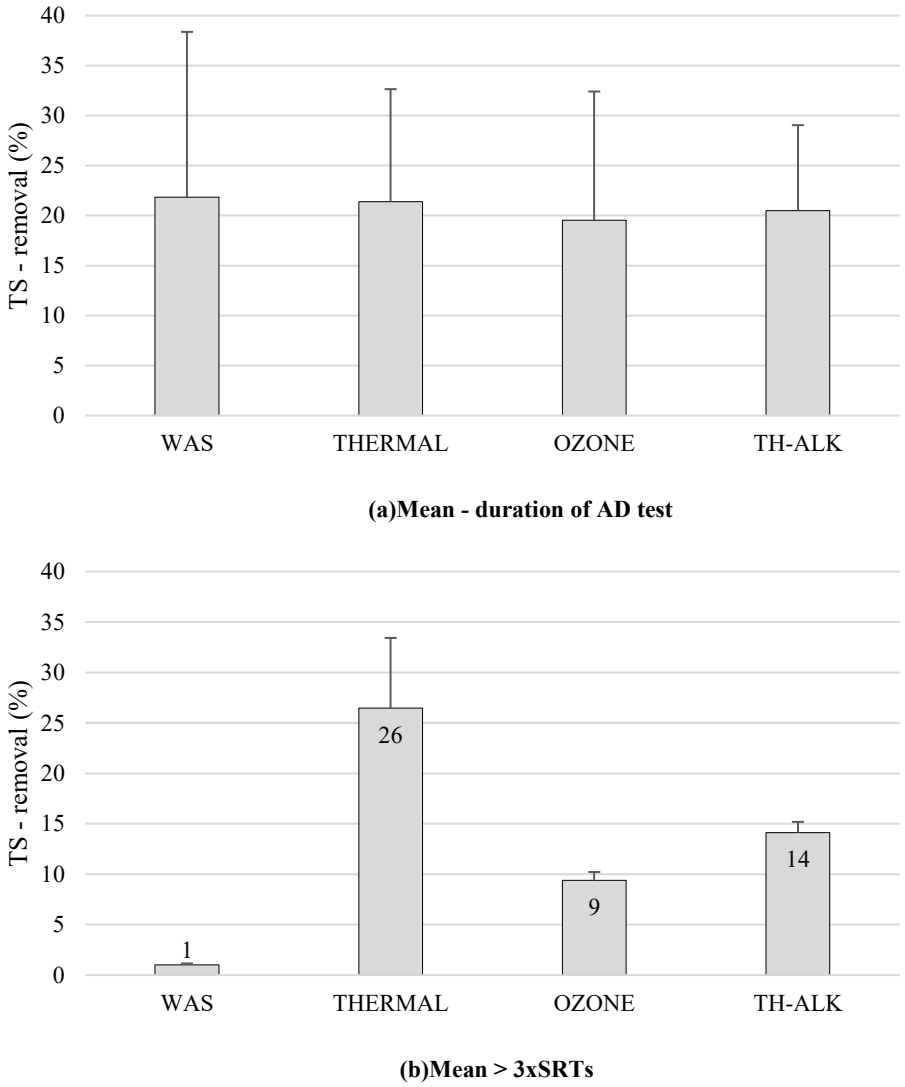


Figure 4.15: Mean TS removals during the AD test: (a) Mean TS removals over the duration of AD test; (b) Mean TS removals starting from day 45 (> 3 SRTs).

VS content removal

With the aim of investigating the degree of stabilization of WAS during the AD test and the effect of different pre-treatments, the VS removal was determined once per week for each group of triplicate reactors by means of equation (4.2). The resultant VS removals (R_{VS}) were hence plotted as a function of time as shown in Figure 4.16. It can be observed that during the first SRT the VS removal of TA sludge seemed to considerably grow to about 20%, while the other trends appeared to maintain in a range between 5% and 10%. During the following SRTs, while the VS removal of TA sludge kept relatively steady round 20%, the VS removals of other sludges varied between 4% and 13%. In addition, for reasons of consistency with the discussion of TS removals, the VS removals were mediated over the duration of the AD test, obtaining the mean TS removal shown in Figure 4.17.a, although it was not possible to make comparisons.

Thereby, as in the previous case, the VS removals starting from day 45 after three SRTs were mediated and reported in Figure 4.17.b. It can be observed that the final mean VS removals appeared to be in the following order: $R_{TA} > R_{TH} > R_{OZ} > R_{WAS}$. Therefore, the TA pre-treatment seemed to enhance the VS removal during

AD of WAS to over 20% in comparison with VS removal of about 6% in case of AD of raw WAS. In addition, it can be noticed that the increased VS removal of TA treated sludge corresponded to the greatest COD solubilization, with a DR_{TA} of more than 30%, but the TS removal of 14% was not the highest value. However, the DR_{TH} of 21% of TH WAS corresponded to the highest TS removal of more than 20% and to a VS removal of about 10% after the anaerobic digestion. Finally, the low DR_{OZ} of 4% of ozone treated WAS corresponded to a TS removal of 9% and a VS removal of 8%. In conclusion, the TH and TA pre-treatments significantly improve the solids removals compared to raw WAS.

Different studies reported the removal of TS and VS concentrations of pre-treated and raw WAS in mesophilic conditions (Table 2.10 and Table 2.11). Thereby, a TA treatment at a constant pH 11 with NaOH for 10 h, at 90°C (DR = 43.7%) and a TH treatment 70°C for 9 h (DR = 27.9%) determined respectively removals of VS concentration of 46.2% and 43.7% in comparison with 38.9% for raw WAS during batch tests (Xu et al., 2014). However, a TA treatment with NaOH at pH 12 and 60°C for 60 min of dairy WAS resulted in a removal of TS and VS concentrations of 25.1% and 33% in comparison with 9.6% and 17% for raw WAS during AD in semi-continuous mode (Rani et al., 2012). Thereby, the performances of AD in terms of TS and VS removals were significantly lower than other studies carried out in mesophilic conditions.

In addition, it was not possible to compare solids removals of VS and TS during AD of WAS at 22°C with other studies performed on WAS in this temperature range. However, other substrates were subject to AD in psychrophilic conditions, such as a psychrophilic dry (21% TS) anaerobic digestion of cow feces and wheat straw mixture in sequential batch reactors at 20°C showed removals of TS and VS concentrations of 3.1% and 1.6% (Saady and Massé, 2016). In contrast, a biomethane potential test of manure (41 g TS/L) at 14°C and 24°C determined a removal of VS concentration respectively of 23% and 32% (Witarsa and Lansing, 2015). Therefore, a comparison with psychrophilic processes to investigate the effect of the operating temperature was not possible because of the gap of literature.

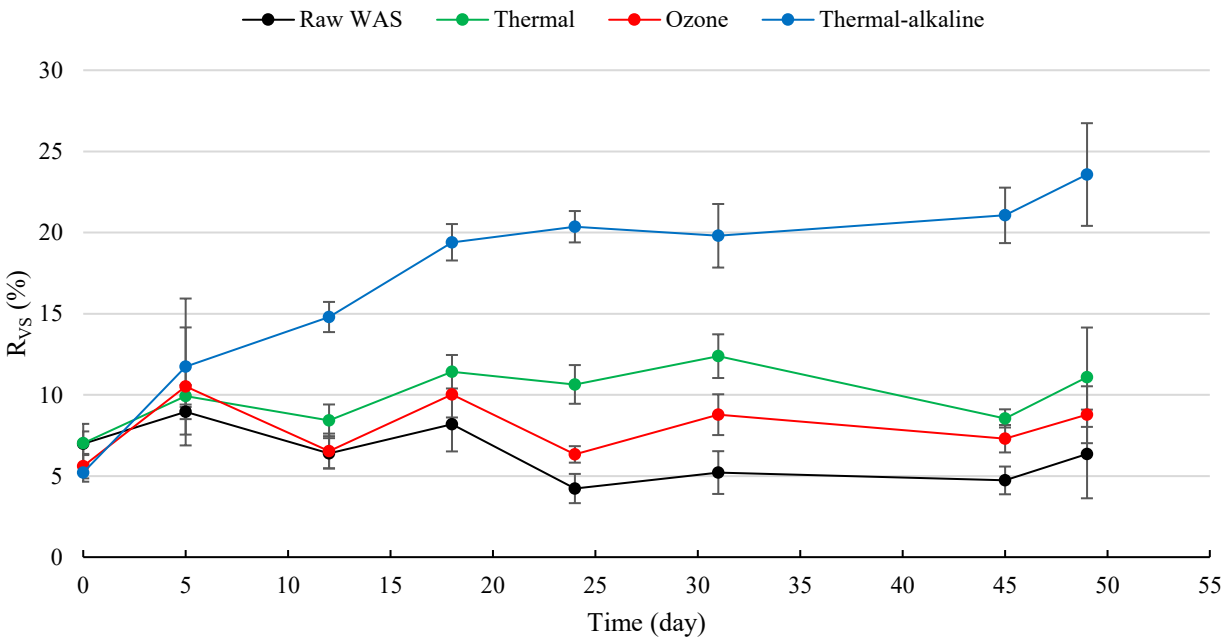


Figure 4.16: VS removals of different sludge samples during the AD test.

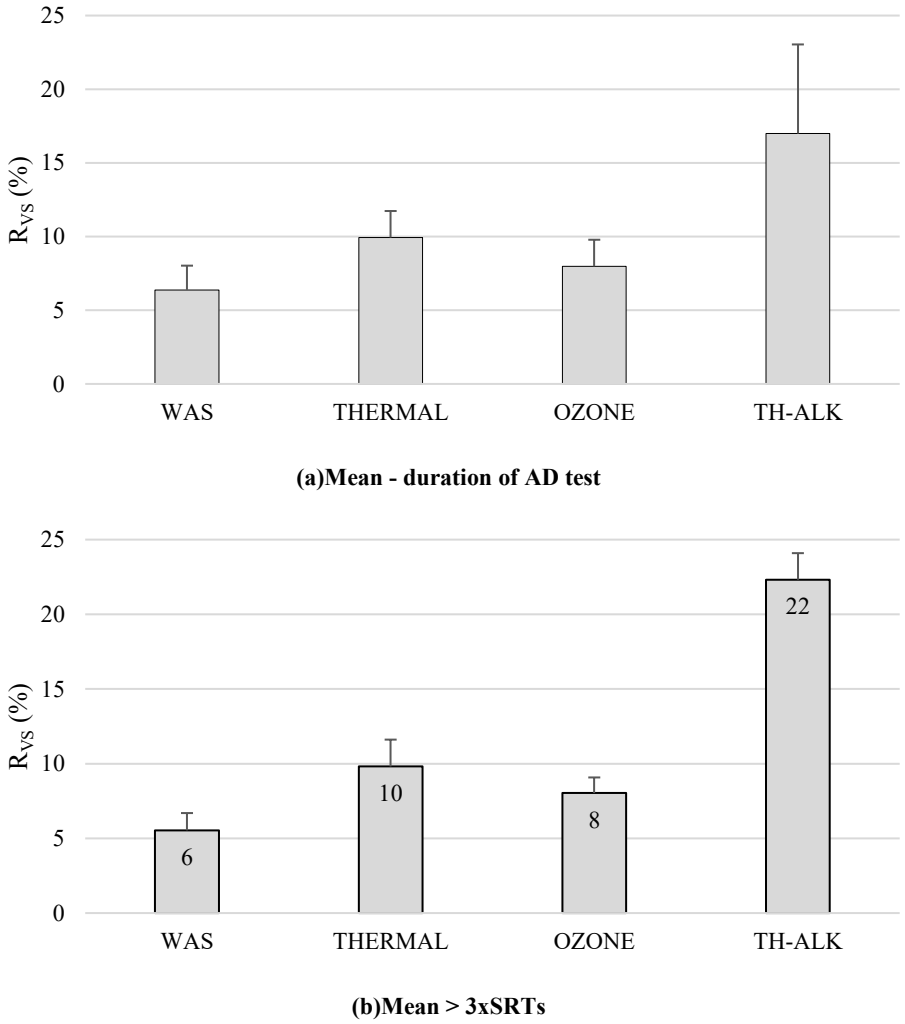


Figure 4.17: Mean VS removal during the AD test: (a) Mean VS removals over the duration of AD test; (b) Mean VS removals starting from day 45 (> 3 SRTs).

4.3.4. Biogas production

The degradability of waste activated sludge after different pre-treatments by means of anaerobic digestion was investigated through the determination of the daily specific biogas productions. Thereby, as exposed in section 3.4.4, the biogas produced by each reactor was collected and measured every 2-4 days. Then, for each reactor the mean daily biogas production (Nm^3/d) was determined by equation (3.13). Subsequently, the values of each group of triplicate reactors were mediated and expressed as specific biogas production ($Nm^3/kg\ VS_{IN}$) using equation (3.14). Finally, the mean SBP were plotted as a function of time as shown in Figure 4.18. It can be observed the lack of some values between day 10 and 20 due to the occurrence of gas leaks for those triplicate reactors. In addition, the presence of variable standard deviations and drops in SBP trends could be affected by some gas leaks. Although the presence of high standard deviations made not possible the determination of significant differences between different trends, it can be noticed that overall at the beginning of AD test the SBP ranged 0.10-0.13 $Nm^3/kg\ VS_{IN}$, while after three SRTs varied between 0.15 and 0.35 $Nm^3/kg\ VS_{IN}$. Moreover, the SBP of raw WAS seemed to be constantly the lowest in comparison with SBP of other sludges varying between 0.15 and 0.21 starting from day 40. In contrast, the TA sludge seemed to reach the highest SMP values between 0.30 and 0.36 $Nm^3/kg\ VS_{IN}$ from day 40, while

the SMP of both the TH and OZ treated sludge appeared to vary between the SMPs of raw WAS and TA WAS.

Finally, after three SRTs, the mean SBP was 0.21 ± 0.03 $Nm^3/kg\ VS_{IN}$ for raw WAS, 0.30 ± 0.03 $Nm^3/kg\ VS_{IN}$ for TH, 0.25 ± 0.02 $Nm^3/kg\ VS_{IN}$ for OZ, and 0.36 ± 0.001 $Nm^3/kg\ VS_{IN}$ for TA. In conclusion, the SBP obtained by the AD of TA WAS could be confident with the highest VS removal (over 20%) and DR (more than 30%), compared with a final SMP of about 0.2 $Nm^3/kg\ VS_{IN}$ and a VS removal of about 6% obtained by the AD of raw WAS at 22°C. However, these results did not seem to be confident with the TS removal, which was higher for TH sludge.

Significant enhancements of the mesophilic AD of WAS by means of a TA pre-treatment found by several authors are reported in Table 2.11. For instance, the pre-treatment of WAS (12 gTS/L) with NaOH (0.1M) at 75°C for 6 h determined a DR of about 65% and corresponded to an increase of 70% in the methane production obtained by a batch test at 35°C (Kim et al., 2013). Moreover, a TA pre-treatment of activated sludge (3% of TS content) by NaOH (pH 11) at 90°C for 10h determined a DR of 44% and an increase of the biogas production from 0.396 to 0.605 L/g VS obtained by means of a batch test (Xu et al., 2014). In addition, a TA treatment by using a dose of 0.04 g NaOH/g TS at 70°C for 90 min on WAS (5-6% solids content), resulting in a DR major than 25%, determined an increase of the biogas production from 0.236 to 0.299 Nm^3/kg VS (+26.8%) by mesophilic AD in batch mode (Ruffino et al., 2016). Moreover, similar biogas productions were found in mesophilic AD of WAS after TH treatments (Table 2.10). For instance, a TH treatment of mixed sludge at 121°C for 60 min enhanced the SBP from 0.35 to 0.42 $L/g\ VSS_{IN}$ (Barjenbruch and Kopplow, 2003), while a TH treatment of WAS at 90°C for 6 min determined an increase of SBP from 0.035 to 0.377 L/g VS (Appels et al., 2010). Overall, the mentioned TA and TH pre-treatments were shown to significantly enhance the mesophilic AD of waste activated sludge. The SBP of 0.36 $Nm^3/kg\ VS_{IN}$ obtained during AD of TH WAS at 22°C and of 0.30 $Nm^3/kg\ VS_{IN}$ of TH WAS were comparable with mentioned mesophilic processes.

In conclusion, the TH (115-118°C, 30 min) and TA (0.09 g NaOH/gTS, 70°C, 30 min) treatments carried out in the present study was likely to determine a significant enhancement in SBPs during the AD of WAS at 22°C, which is comparable with results of other studies carried out in mesophilic conditions (respectively Table 2.10 for TH and Table 2.11 for TA). However, the TS and VS removals were significantly lower than other studies performed at higher temperatures (Table 2.10, Table 2.11). Finally, even though a significant effect in enhancing the (COD) solubilisation of sludge was found for both the TH and the TA treatments, a significant improvement of the AD of WAS at 22°C was not clearly observed.

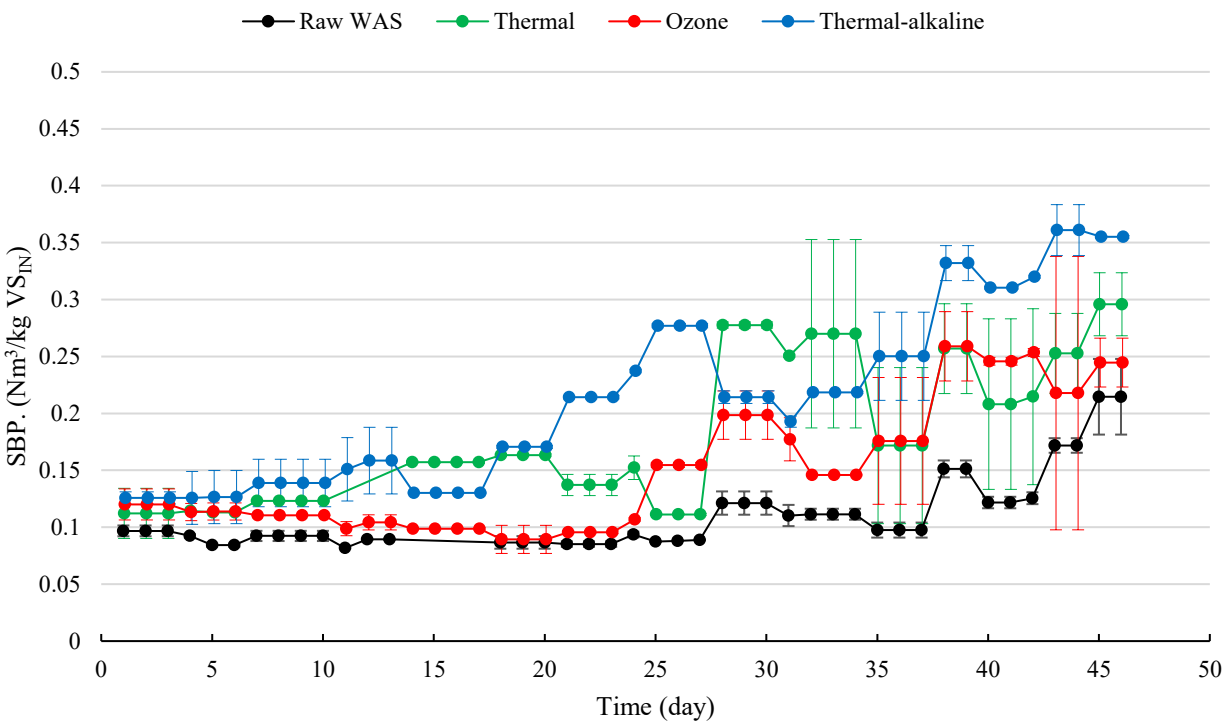


Figure 4.18: Specific biogas production of different sludge samples as a function of time during the AD tests.

5. Conclusions

This thesis aimed to evaluate the technical feasibility of anaerobic digestion at low temperature (22°C) of raw and pre-treated WAS from a Canadian wastewater treatment plant without primary sedimentation (LaPrairie, QC, Canada, 240000 p.e.). The experimental assessment consisted of the two steps, the increasing of WAS solubilisation by means of different pre-treatment methods and the subsequent enhancement WAS biodegradability during anaerobic digestion.

Firstly, three different pre-treatment methods were selected, optimised and compared by means of COD solubilisation (S_{COD}) and Disintegration Rate (DR, i.e. the increment in COD soluble fraction due to the pre-treatment) evaluation. Thereby, a thermal (TH) treatment (115-118°C, 30 min), an ozone (OZ) treatment (190 mg O_3/L), and a thermo-alkaline (TA) treatment (0.09 g NaOH/ g TS, 70°C, 60 min) gave back respectively a mean DR of 20.7 ± 4.1%, 3.6 ± 0.8%, and 33.4 ± 3.5%, and a mean S_{COD} of 26.2 ± 3.6%, 10.2 ± 2.9%, and 37.8 ± 4.0%. Overall, in terms of increased COD solubilisation the performances of the pre-treatments were found in the following order: TA > TH > OZ. Moreover, the OZ treatment determined a negligible COD solubilisation due to the low dose (0.01 g O_3/g TS), while the TH and TA significantly enhanced the COD solubilisation of WAS, in agreement with literature.

Secondly, the biodegradability of raw and pre-treated WAS samples at 3% TS was evaluated AD tests, carried out in triplicates at 22°C in semi-continuous feeding mode of 3% TS and 15-days SRT, by means of total solids (TS) and volatile solids (VS) removal and biogas production. The pH of digested sludges reached a steady level around pH 7.0 – 7.1 for raw, TH and OZ WAS and pH 7.3 for TA WAS starting from days 38-42, about three SRTs. Moreover, the OLR of 1.7 g VS $L^{-1}d^{-1}$ was in agreement with literature. After three SRTs, the anaerobic digestion of raw, TH, OZ, and TA WAS resulted respectively in mean TS and VS removals of 1.0 ± 0.1% and 5.5 ± 1.2%, 26.5 ± 7.0% and 9.8 ± 1.8%, 9.4 ± 0.8% and 8.0 ± 1.0%, 14.1 ± 1.1% and 22.3 ± 1.8%. The solids removals of TH and TA WAS during AD at 22°C were significantly greater than raw WAS but lower than mesophilic processes from literature.

Furthermore, after three SRTs, the specific biogas production (SBP) was 0.21 ± 0.03 Nm^3/kg VS_{IN} for raw WAS, 0.30 ± 0.03 Nm^3/kg VS_{IN} for TH, 0.25 ± 0.02 Nm^3/kg VS_{IN} for OZ, and 0.36 ± 0.001 Nm^3/kg VS_{IN} for TA. The higher SBPs for TH and TA WAS were comparable with literature data about mesophilic AD of WAS and seemed to be consistent with larger COD solubilisation for TH and TA.

In conclusion, even though promising enhancements of (COD) solubilisation of sludge were found for both the TH and the TA treatments, a significant improvement of the AD of WAS at 22°C was not clearly observed.

Thereby, considering the lack of knowledge about AD of WAS at low temperatures and its potential energy and environmental benefits, further investigations are needed for evaluating the technical feasibility of AD of pre-treated WAS at low temperatures in comparison with conventional mesophilic AD. In addition, a comprehensive assessment of the more sustainable option should include economic and environmental aspects. Therefore, new methodologies for technical, energetic, economic and environmental analyses should be developed and applied. In conclusion, further research is needed by the moment low temperature AD could represent a sustainable and energy self-sufficient option for the stabilisation of WAS in small-scale WWTPs (Italy) and in cold weather countries (Canada).

Appendix

In the Appendix the protocols of analytical measurements in use at the Environmental Engineering Laboratories of Department of Civil and Applied Mechanics, at McGill University, are reported, as found on the Laboratory Manual.

COD, Chemical Oxygen Demand: method 5220D, (APHA, 1998)

Principle of measurement

Organic matter is oxidized by a boiling mixture of chromic and sulphuric acids. Oxidation of organic matter is achieved by strong oxidants under acid conditions with Ag^{+} and Hg^{++} as catalysts, at 150 °C.

$K_2Cr_2O_7$ (Cr^{6+}), a strong oxidant, loses 3 O-atoms when reacting with an organic molecule: $Cr^{6+}+3e^{-} = Cr^{3+}$

A sample is digested with a well-defined amount of K-dichromate (Cr^{6+}). As increasing amounts of organic matter are oxidized, the dichromate (Cr^{6+}) decreases and the chromic ion (Cr^{3+}) increases proportionally. Dichromate absorbs at 420 nm, while the chromic ion absorbs at 600 nm.

Low-range COD measures the (CR^{6+}) remaining, but High-range measures the Cr^{3+} produced.

Instrumentation and materials

- Spectrophotometer, visible range at 420 nm and 600 nm.
- Heating block, at 150 $\pm$ 2 °C.
- Digestion tubes or vials, borosilicate glass, with Teflon-lined screw caps.
- 2 mL and 1 mL volumetric pipettes, pipetting bulbs.
- Volumetric flasks, 100 ml; assorted measuring pipettes or micro pipettor.
- Blender; 1 L beaker, magnetic stirrer and bar.
- Wide mouth pipette or other sub-sampling device.
- Filtration equipment (0.45 μm membrane filter).
- Samples.

Reagents:

a. Standard **$K_2Cr_2O_7$ solution**, for **COD 20-800** mg O_2/L, **High-range reagent**

10.216 g $K_2Cr_2O_7$ (0.21 N or 0.035 M)

167 mL conc. H_2SO_4

33.3 g $HgSO_4$ per litre dist. H_2O

b. Standard **$K_2Cr_2O_7$ solution**, for **COD 0-80** mg O_2/L, **Low-range reagent**

10.0216 g $K_2Cr_2O_7$ (00.21 N or 0.0035 M)

167 mL conc. H_2SO_4

33.3 g $HgSO_4$ per litre dist. H_2O

c. **Sulfuric acid reagent**, (for high-range and low-range)

5.5 g Ag_2SO_4/ Kg H_2SO_4 (9.715 g/L H_2SO_4 or 4.852 g/500 mL H_2SO_4)

d. Potassium-hydrogen-phtalate (KHP) stock standard solution,

850 mg/1L with a COD of 1000 µg O_2/mL

Procedure

Prepare standards from KHP stock standard by directly pipetting into digestion tubes.

COD (µg O_2/mL)	Stock Standard Solution (mL)	H_2O (mL)
10	0.02	1.98
25	0.05	1.95
50	0.10	1.90
100	0.20	1.80
300	0.60	1.40
500	1.00	1.00
800	1.60	0.40
1000	2.00	0.00

Alternatively, prepare 100 mL of the standard solutions in volumetric flasks by diluting the 1000 ppm stock standard accordingly.

Prepare samples

- Mix the total available volume of a sample thoroughly.
- For **total COD**, remove 2 mL sub-samples while sample is mixing. If necessary, blend sample in a blender before subsampling.
- For **dissolved COD**, pour a well-mixed subsample into a filter funnel for filtering.
- Remove aliquots from the filter flask.
- Prepare **triplicates** of all samples, but singles of standards and blanks.
- Pipette into at least one digestion tube 1 mL of a sample + 1 mL of a standard that has higher or lower concentration than the sample itself: spiked sample, see QA/QC section.

Digestion - This step is done in a fume-hood, wearing heavy gloves and face shield.

- Using bottle-top dispensers, add 1.2 mL of standard $K_2Cr_2O_7$ solution, using the appropriate concentration (see previous page).
- **Carefully** let sulfuric acid reagent (2.8 mL) run down inside walls of tipped tubes so that an acid layer is formed on the bottom of each of them.
- Cap tubes tightly.
- Using a thick layer of toweling, hold the tube and invert it several times, so that the capped end faces the back of the fume-hood. This will mix the tube contents. If tubes are not mixed before heating, local heating at the bottom of tubes may cause blow-out. Check with paper towel whether tubes are tightly capped.
- Insert tubes in heating block; digest for 2 h at 150 °C.

- Remove tubes from heating block; invert tubes gently several times. Let solids settle; cool to room temperature before colorimetric analysis.

Colorimetric analysis

- Read absorbance and/or transmission with spectrophotometer

  At 600 nm for high concentration standards, samples and spikes, using reagent blank to set instrument to 100% transmission before switching to readings in absorbance mode

  At 420 nm for low concentration standards, samples and spikes, using reagent blank to set instrument to 35% transmission before switching to absorbance mode.

- Prepare a standard curve or use linear regression.
- Evaluate quality control with spikes.

SOLIDS: method 2540, (APHA, 1998)

Principle of measurement

Solids are determined by gravimetric analysis i.e. by weighing the solids (dried) contained in a defined volume of water.

To determine TOTAL SOLIDS, i.e. dissolved salts plus suspended solids, a sample aliquot in a pre-dried and pre-weighed porcelain dish is evaporated and dried to constant weight. To determine SUSPENDED SOLIDS, an aliquot of the sample is filtered through a pre-dried and pre-weighed glass fibre filter, then dried to constant weight. Glass fibre filters, as well as filter papers, provide a deep mat of fibres to retain particles of all sizes ("depth filtration") and should not be confused with filter membranes that have a well-defined pore size, retaining only larger than pore-size particles ("pore filtration").

Instrumentation and materials

- Aluminium and porcelain dishes.
- Glass fibre filter disks (Whatman 934AH, Ahlstrom or equivalents), 47 mm diam.
- Measuring cylinders and ladle; volumetric pipettes.
- Millipore filter apparatus with sidearm flasks and vacuum pump.
- Tweezers, tongs, oven mittens.
- Ovens for 103 °C, 180 °C and 500 °C; desiccator.
- Magnetic stirrer and bars; big beaker.
- Analytical balance (0.0001 g).
- Imhoff cones, glass rod.
- Deionized water.

Procedure – Total Solids and Total Dissolved Solids (TS and TDS)

Preparation of porcelain dishes:

- Mark clean dishes with heat resistant marker.
- Ignite at 500 ± 50 °C for 1 hour.
- Desiccate and cool to room temperature.
- Weigh empty dish immediately: **[A; g]**; store in desiccator until used
-

Treatment of blanks and samples:

- Pour the complete sample into a big beaker and mix well with magnetic stirrer. While mixing, remove sub-sample with a volumetric pipette, or ladle it into a measuring cylinder.
- Pour sub-sample into dish, using volumes based on the expected concentration (dry residue should be 2.5 and 200 mg).
- Use 100 ml deionized water as a blank

- Evaporate:

For TS at 103 °C, overnight

For TDS at 103 °C for 3 h, then dry sample at 180 °C to constant weight.

- Cool in desiccator not longer than 30 minutes; weigh **[B; g]**. Note: Porcelain dishes are highly hygroscopic.
- Ideally, repeat cycle until the loss of weight is less than 4% or 0.5 mg.

TS in mg/l = [B – A] x 10^6 x vol^{-1};

TDS in mg/l = [B – A] x 10^6 x vol^{-1}.

Procedure – Volatile and Fixed Solids (VS and FS)

- After weighing the porcelain dish for TS or TDS, 'ignite' the dish at 500 ± 50 °C for exactly 15 minutes. Use tongs, never fingers, when handling dishes or filters.
- Cool to room temperature in desiccator.
- Weigh **[C; g].**

FS in mg/l = [C – A] x 10^6 x vol^{-1};

VS = TS – FS; also VSS* = TSS – FSS and VDS = TDS – FDS

*Volatile suspended solids. When igniting glass-fibre filters in aluminium dishes for VSS, ensure that the temperature does not exceed 500 °C and that the sample dish and filter are not left for more than 15 minutes in the furnace. **Blanks are critical for VSS.**

pH measurements

Principle of potentiometric pH measurement

When a set of pH electrodes is immersed in an aqueous solution, a voltage is produced that is very precisely related to the hydrogen ion activity (pH) of the solution. This voltage is predictable by the Nerst equation:

$$E_{meas} = E_0 - \frac{2.3}{nF} RTpH$$

E_{meas} = measured voltage

E_0 = total of all constant voltages in the measuring system

N = number of electrons

T = temperature in K

F = Faraday constant

R = gas law constant

A **pH meter** measures small potential differences in a circuit of extremely high resistance as given by the pH electrodes. A pH meter also has to be adjustable to the pH and voltage characteristics of individual electrodes and to other measuring conditions.

As the Nerst equation shows, voltage output is dependent on temperature. Therefore, temperature compensation control has to be possible either by manual compensation or by an automatic control probe (ATC probe). This probe measures the sample temperature and sets the meter to match the Nerstian slope at that temperature.

Electrode chains change their efficiency with use and age. These variations are compensated for by **"slope" or "efficiency" control** settings, which adjust the meter to exactly match the voltage output of the electrodes.

The **glass or indicating electrode** (the pH sensory electrode) is a non-conductive glass body filled with a buffered electrolyte solution (fixed pH and ionic concentration). An internal reference element (Ag/AgCl) is immersed in the electrolyte solution. A bulb of special conductive glass is sealed to the body – this is the actual pH sensory membrane. Thus, a constant potential is formed at the inside of the membrane and on the internal reference element. When the electrode is immersed in a solution of pH 7, the total of the internal fixed voltage equals roughly the voltage that develops on the outer surface of the sensory membrane and the separate reference electrode. That means that the total potential output of the system is 0 mV at pH 7, but at lower or higher pH, the potential on the outer membrane changes proportionally to the sample pH. This voltage change is measured by the pH meter and displayed as pH value.

The **reference electrode** completes the electric measurement circuit and provides a stable reference potential. The constant voltage, no matter what the pH pf the sample may be, is achieved by immersing the reference element (here, too, usually Ag/AgCl, or calomel) in an electrolyte filling solution of fixed ionic concentration. The electrical circuit is completed by a small flow of the electrolyte passing through the porous junction – a small opening – into the sample.

The **combination electrode** combines sensory and reference elements in one single probe body.

pH buffer standards are used to standardize the pH meter/electrode system.

Instrumentation and materials

- pH – meter
- Glass electrodes and reference electrodes or combination electrodes
- Automatic temperature correction (ATC) probes
- Buffers, pH 4, 7, 10 and others
- Thermometer
- Aqueous samples

Procedure

- Make sure that buffers and samples are at the same temperature.
- Connect ATC probe and pH electrodes (reference + indicating, or combination)
- Calibrate instrument according to instructions with two buffers that would bracket the expected sample pH. Calibration instructions vary for each model of pH meter. Check the appropriate operating manual.
- Determine slope, or electrode efficiency, in percent of theoretical slope (should be between 90-110%).
- Measure sample pH.

Correst use of pH electrodes

- Uncover air hole (at the top of electrode) to allow the fill solution to flow through.
- Ensure that the level of electrolyte (fill solution) in reference electrode is above the level of sample. If necessary, fill the electrode with the correct fill solution.
- Ensure to immerse the liquid junction of the combination or reference electrode in the sample.
- Stir sample.
- Rinse electrodes and ATC probe with distilled water before immersing into (next) sample.
- When finished, rinse electrodes well, close filling ole and store in appropriate container.

Presentation of results

Report as pH ….. at ….. °C.

Frequently occurring problems

Problems are usually caused by faulty reference electrodes, less often by indicating electrodes and hardy by defective meters. Long stabilization times or even never-ending drifts of the readouts indicate reference electrode problems. To check, follow these steps:

- A quick movement of a hand towards the electrodes changes pH readings and reverses when the hand is retracted. If drift is not disturbed by movement, the problem is most likely incompatibility of the electrode with the sample, temperature changes, or CO_2 exchange with the atmosphere.
- If stirring the sample seems to cause unstable spreading, turn stirrer off. If reading changes by 0.1 or 0.2 pH units, there is an electrode problem.

A partially or completely clogged liquid junction usually causes failure of a reference or combination electrode. The electrode should be cleaned. Check the operating manual of the specific electrode for cleaning instructions.

- Other sources of problems are:
- Broken wires within the electrode
- Broken lead wires
- Open circuit within the meter

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