# EFFECT OF SIZE, COATING AND CONCENTRATION OF ZINC OXIDE NANOPARTICLES ON ANAEROBIC DIGESTION OF MUNICIPAL WASTEWATER SLUDGE

by

Wesley Badith Olaya

B.Sc., in Mechanical Engineering, Escuela Superior Politécnica del Litoral, 2012

# A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

### MASTER OF APPLIED SCIENCE

in

# THE COLLEGE OF GRADUATE STUDIES (Civil Engineering)

# THE UNIVERSITY OF BRITISH COLUMBIA

(Okanagan)

July 2019

© Wesley Badith Olaya, 2019

The following individuals certify that they have read, and recommend to the College of Graduate Studies for acceptance, a thesis/dissertation entitled:

# EFFECT OF SIZE, COATING AND CONCENTRATION OF ZINC OXIDE NANOPARTICLES ON ANAEROBIC DIGESTION OF MUNICIPAL WASTEWATER SLUDGE

submitted by Wesley Badith Olaya

in partial fulfillment of the requirements of

the degree of Master of Applied Science

Dr. Cigdem Eskicioglu, School of Engineering, UBCO

#### Supervisor

Dr. Lukas Bichler, School of Engineering, UBCO

#### **Supervisory Committee Member**

Dr. Sumi Siddiqua, School of Engineering, UBCO

#### Supervisory Committee Member

Dr. Thomas Johnson, School of Engineering, UBCO

#### **University Examiner**

#### Abstract

The effect of average particle size (30 nm and 100 nm) and type (coated and uncoated) of ZnO nanoparticles (NPs) was studied on anaerobic digestion treatment process utilizing municipal wastewater sludge under mesophilic (35±2oC) and thermophilic (55±2oC) conditions. The effect was investigated in two stages with different digester feeding regime: (1) batch biochemical methane potential (BMP) assays, and (2) semi-continuously fed reactors.

In the first stage with BMP assays, the inhibition of biogas (methane) producing cultures due to presence of ZnO NPs was investigated. A total number of 72, 160 mL serum bottles containing anaerobic inocula acclimatized to mixed sludge from Kelowna's municipal wastewater treatment plant (WWTP) were operated for a period of about 90 days. Three different ZnO dosages were used leading to concentrations of 6 (low), 75 (medium) and 150 (high) mg NP/g mixed sludge total solids (TS).

In the second stage, four mesophilic and four thermophilic semi-continuously fed digesters were set-up with an effective volume of 500 mL. The effect of NPs on the performance of biogas/methane production, organic solids removal, and biogas composition in terms of presence of odorous volatile sulfur compounds was assessed at the low dosing concentration. The eight digesters were operated during 60 days with a solid retention time of 20 days.

The results showed that ZnO NPs do not inhibit methane forming bacteria at the lowest concentration regardless of their average particle size, but they are inhibitory at higher levels. Thermophilic bacteria were more sensitive to ZnO compared to mesophilic bacteria, as at elevated digester temperatures. Coated NPs created less inhibition than non-coated NPs, possibly due to

decreased purity (Zn) of the inhibitor per mass in coated NPs. Batch reactors dosed with medium and high concentrations of coated NPs partially recovered after 25 days of digestion. For the noncoated ZnO NPs, only the mesophilic batch assays were able to recover at the medium concentration and the thermophilic reactors presented chronic inhibition and could not recover. As a beneficial outcome, coated ZnO NPs significantly reduced odour causing volatile sulfur compounds in digester headspace in comparison to the non-coated NPs by chemical precipitation.

#### Lay summary

Nanoparticles (NPs) used in consumer products could be released to the environment and reach municipal wastewater treatment plants (WWTPs). Most of the NPs accumulate in the wastewater sludge that may create toxic effects to microorganisms that are biological engines in treatment processes such as anaerobic digestion. This study focused on potential inhibition effects caused by a common NP, ZnO, when dosed in anaerobic digesters. The inhibition was assessed in terms of biogas (methane) production and other factors included organic solids removal and odor causing compounds, which are a by-product of the process. The results showed that ZnO NPs can cause inhibition but at concentrations higher than what is observed/expected at WWTPs today. However, as a beneficial effect, even small dosages of ZnO NPs showed reduction in the trace compounds of biogas, such as hydrogen sulfide, which is responsible for the foul odor of rotten eggs.

#### Preface

The research presented in this thesis is the original work performed by the author. This thesis was supervised by Dr. Cigdem Eskicioglu at the Bioreactor Technology Group in the School of Engineering, University of British Columbia.

An abstract summarizing the results of this research was accepted and the research was disseminated as a podium presentation at the 6<sup>th</sup> International Conference on Engineering for Waste and Biomass Valorisation (Olaya, W., Eskicioglu. C. 2016. The effect of ZnO nanoparticles on anaerobic sludge digestion under mesophilic and thermophilic conditions. The 6<sup>th</sup> International Conference on Engineering for Waste and Biomass Valorisation, Albi, France) organized in Albi, France in May 23-26, 2016.

An abstract summarizing the results of this research was accepted and the research was disseminated as a podium presentation at the 10<sup>th</sup> International Society for Environmental Biotechnology (ISEB) Conference (Olaya, W., Akgul, D., Eskicioglu. C. 2016. Concentration, size and coating effects of ZnO nanoparticles on mesophilic and thermophilic sludge digestion. The 10<sup>th</sup> International Society for Environmental Biotechnology Conference, Barcelona, Spain) held in Barcelona, Spain in June 1-3, 2016.

# **Table of Contents**

Abstract	III
Lay summary	V
Preface	VI
Table of Contents	VII
List of tables	X
List of figures	XI
List of abbreviations	XIII
Acknowledgement	XV
Dedication	XVI
Chapter 1: Introduction	1
1.1. Background	1
1.2. Objectives	3
1.3. Thesis organization	4
Chapter 2: Literature Review	5
2.1. Properties and detection methods of NPs relevant in wastewater	5
2.2. Use of nanoparticles	6
2.3. Nanoparticle release into the environment	9
2.4. Wastewater and sludge treatment	9
2.4.1. Primary treatment	11
2.4.2. Secondary treatment	12
2.4.3. Sludge treatment	12
2.4.3.1. Anaerobic digestion	
2.4.3.1.1. Principles and operating factors of anaerobic digesters	14
2.5. Removal, fate and impact of nanoparticles on wastewater and sludge treatment	17

2.5.1.	Removal pathways for nanoparticles	17
2.5.2.	Fate of nanoparticles in wastewater and sludge treatment	19
2.5.2.1	. Primary treatment	
2.5.2.2	. Biological treatment	
2.5.	2.2.1. Activated sludge treatment	
2.5.	2.2.2. Anaerobic treatment for wastewater treatment	21
2.5.2.3	. Anaerobic digestion for sludge treatment	
2.5.3.	Impact of nanoparticles on wastewater and sludge treatment	22
2.5.3.1	. Impact of nanoparticles on secondary treatment	
2.5.3.2	. Impact of nanoparticles on anaerobic sludge digestion	
2.6. Sum	mary	
Chapter 3:	Materials and methods	29
3.1. Subs	trate and inocula	29
3.2. Nano	particles and dispersant	30
3.3. Expe	rimental plan for anaerobic digestion inhibition studies	30
3.3.1.	Biochemical methane potential (BMP) assays	30
3.3.2.	Semi-continuous flow anaerobic sludge digesters	33
3.4. Anal	ytical methods for sample characterization and digester monitoring	35
3.4.1.	Biogas measurement	35
3.4.2.	Biogas composition	36
3.4.3.	Volatile sulfur compound	36
3.4.4.	Total solids (TS) and volatile solids (VS)	
3.4.5.	Chemical oxygen demand (COD)	
3.4.6.	Alkalinity	
3.4.7.	Ammonia	
3.4.8.	Volatile fatty acids (VFAs)	
3.4.9.	Dewaterability	
Chapter 4:	Results and discussion	41

4.1. Bate	ch digesters41
4.1.1.	Characterization of sludge, inocula and NPs for BMP41
4.1.2.	Effect of ZnO NPs on BMP of municipal sludge41
4.2. Sem	ai-continuous flow anaerobic sludge digesters
4.2.1.	Characterization of waste sludge stream
4.2.2.	Effect of ZnO NPs on biogas production
4.2.3.	Effect of ZnO NPs on other digester performance parameters
Chapter 5:	Conclusions and recommendations for future work
References	
Appendices	74
Appendix	A: Sample calibration curves74
Figure A	A.1 Chemical oxygen demand calibration curve74
Figure A	A.2 Ammonia calibration curve74
Appendix	B: Statistical analysis75
Table B	.1 Anova for the effect of the dispersant in the BMP assays75
Table B	.2 T-test for the effect of the dispersant in the BMP assays under mesophilic conditions
Table B	.3 T-test for the effect of the dispersant in the BMP assays under thermophilic conditions76
Table B	.4 Split-plot analysis for biogas production in the BMP assays considering the effect of NP
type, ter	nperature and concentration77
Table B	.5 Anova for biogas production in the semi-continuous reactors
Table B	.6 Anova for VSC removal of two uncoated ZnO NPs with different average diameters in the
semi-co	ntinuous reactors
Table B	.7 Anova for VSC removal of coated and uncoated ZnO NPs with different average diameters
in the se	emi-continuous reactors79
Table B	.8 Anova for biogas production in the semi-continuous reactors

## List of tables

Table 2.1 Use of nanoparticles based on Adawi et al. (2018), Contado (2015), Project on	
Emerging Nanotechnologies (2013) and journal articles	8
Table 2.2 Volatile fatty acids to alkalinity ratio (Adapted from WEF 2007) 1	б
Table 4.1 Sludge and inocula characterization for the set-up of the BMP reactors	1
Table 4.2 Sludge and inocula characterization for the set-up of the semi-continuous reactors 5	0
Table 4.3 Results for semi-continuous flow anaerobic digesters operating at 20 d SRT during	
steady state	3

# List of figures

Figure 1.1 Schematic diagram of NPs pathway in the environment when released from
products containing them 1
Figure 2.1 Flowchart of typical unit operations and processes for municipal wastewater and
sludge treatment 10
Figure 2.2 Anaerobic process according to the Anaerobic Digestion Model (ADM) No. 1
(Adapted from IWA 2002)14
Figure 2.3 TEM images of activated sludge exposed to NPs in the presence of EPS (a–d) and
absence of EPS (e-h). Control (a and e), 10 mg L-1 Ag NPs (b and f), 10 mg L-1
TiO2 NPs (c and g), and 10 mg L-1 SiO2 NPs (d and h). Arrows indicate NPs
(synthetic wastewater, 3000 mg L-1 MLSS, 8 h, 100 rpm, 25C) (From Park et al.
2013, used with permission of the publisher Elsevier)
Figure 3.1 BMP assay configuration
Figure 3.2 BMP bottles before and after the set up (72 bottles in total)
Figure 3.3 Semi-continuous flow anaerobic digester configurations
Figure 3.4 Lab-scale semi-continuous flow anaerobic digester (8 digesters in total) 35
Figure 4.1 Specific cumulative biogas yield from BMP assays under (a) mesophilic and (b)
thermophilic conditions at STP (1 atm, 0°C)
Figure 4.2 pH change in BMP bottles under (a) mesophilic (b) thermophilic conditions
Figure 4.3 Total VFA concentration change in BMP bottles under (a) mesophilic (b)
thermophilic conditions

Figure 4.4 Specific daily biogas production for (a) mesophilic and (b) thermophilic semi-
continuous flow anaerobic digesters
Figure 4.5 Methane percentage in biogas under mesophilic and thermophilic conditions at
steady-state
Figure 4.6 Volatile sulfur compounds (VSC) concentration and their removal in the digester
headspace under mesophilic and thermophilic conditions
Figure 4.7 H <sub>2</sub> S concentration and their removal in the digester headspace under mesophilic
and thermophilic conditions
Figure 4.8 TS removal efficiency of anaerobic digesters under mesophilic and thermophilic
conditions
Figure 4.9 VS removal efficiency of anaerobic digesters under mesophilic and thermophilic
conditions
Figure 4.10 TCOD removal efficiency of anaerobic digesters under mesophilic and
thermophilic conditions
Figure 4.11 Total VFAs in anaerobic digesters under mesophilic and thermophilic conditions 60
Figure 4.12 Specific capillary suction time (CST) for the digestates under mesophilic and
thermophilic conditions61

## List of abbreviations

AD	Anaerobic Digestion
ANOVA	Analysis of Variance
BMP	Biochemical Methane Potential
С	Coated
COD	Chemical oxygen demand
CST	Capillary suction time
DO	Dissolved Oxygen
EPA	Environmental Protection Agency
EPS	Extra-cellular Polymeric Substances
FPS	Fermented primary sludge
HRT	Hydraulic Retention Time
IC	Inhibitory Concentration
М	Mesophilic
MBR	Membrane Bioreactor
NMA	Normalized Methanogenic Activity
NC	Non-coated
NP	Nanoparticle

OLR	Organic Loading Rate
SBR	Sequencing Batch Reactors
SCOD	Soluble Chemical oxygen demand
SRT	Solid Retention Time
SSA	Specific Surface Area
STP	Standard Temperature and Pressure
Т	Thermophilic
TCOD	Total Chemical oxygen demand
TS	Total Solids
TSS	Total Suspended Solids
TWAS	Thickened Waste Activated Sludge
UASB	Upflow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acid
VS	Volatile Solids
VSC	Volatile Sulfur Compound
WEF	Water Environmental Federation
WWTP	Wastewater Treatment Plant

#### Acknowledgement

I would like to express my deepest gratitude to my supervisor Dr. Cigdem Eskicioglu for her guidance, support, patience and encouragement throughout the course of my studies. She has dedicated so much time to help and motivate me to pursue my research. I would also like to thank my committee members, Dr. Sumi Siddiqua and Dr. Lukas Bichler, for their feedback and suggestions. In addition, I would like to thank to the staff of UBCO who helped when I most needed it.

Additionally, I would like to thank to my colleagues from the UBC Bioreactor technology Group. Special thanks to the ones who trained me and helped me during my experiments including Fahmida Islam, Deniz Akgul, Tim Abbott and Muneer Ahmed.

I would also like to acknowledge funding from the Ecuadorian government and Natural Sciences and Engineering Research Council (NSERC) of Canada (Strategic Project Grant #396519-10) for this research.

Finally yet importantly, to my family who supported emotionally in spite of being in a different country far from them.

To my family

#### **Chapter 1: Introduction**

#### 1.1. Background

In the last decade, the scientific world has enhanced their knowledge in material sciences by working in smaller scales and opening up opportunities in the field of nanotechnology. Nanotechnology implies the use of nanoparticles (NPs) that refer in most definitions to be particles having one dimension less than 100 nm (PAS71 2005) or at least two of their dimensions between 1 and 100 nm (ASTM 2006). However, NPs are not new in our society; for example, silver (Ag) and copper (Cu) NPs have been detected in Italian renaissance luster pottery dated from the 16<sup>th</sup> century (Borgia et al. 2002).

Nevertheless, consumer products and industrial processes are using modified or engineered NPs to improve some characteristics. These NPs may possess health effects to biota when they are in the environment. A common pathway of NPs to reach natural sources is presented as a schematic diagram in Figure 1.1.



Figure 1.1 Schematic diagram of NPs pathway in the environment when released from products containing them

First, it may start with the use of some products containing NPs that can make contact with water, such as cosmetics or textiles. The usual contact with water can be just through rainfall or washing that can release the NPs into the environment (Benn and Westerhoff 2008; Kaegi et al. 2008). Thus, the released NPs may be discharged into the sewage collection system and can eventually reach to conventional municipal wastewater treatment plants (WWTPs). WWTPs have been designed without a complete awareness of the presence and removal efficiency target for these pollutants.

Studying wastewater and sludge treatment processes with NPs removal focus requires awareness of the release of these pollutants into the environment. In this context, the impact of NPs on the performance and/or by-products of the different treatment processes is important to understand the capability of the treatment processes to resist or adapt to these changes from the incoming NPs concentrations.

Within the different treatment processes used in WWTPs (i.e. physical, chemical and biological), biological treatment processes have shown the greatest interaction between NPs and biomass. Different studies have demonstrated a decrease in the WWTP effluent concentration of different NPs due to the accumulation in the biomass during secondary treatment (Kiser et al. 2009; Wang et al. 2012; Westerhoff et al. 2011). This biomass proceeds to sludge treatment where most of the NPs may end up in the dewatered sludge or "biosolids" commonly disposed in landfills or land applied as soil amendment depending on the concentration of pathogens and trace metals.

Sludge stabilization is one of the required treatments that sludge must undergo before final disposal. The main goal of sludge stabilization is to reduce pathogens, odor and organics. Anaerobic digestion is one common process for sludge stabilization where NPs could end up and accumulate at higher concentrations. Different studies (Garcia et al. 2012, Luna-delRisco et al.

2011, Gonzalez-Estrella et al. 2015, Mu and Chen 2011, Mu et al. 2011, Yang et al. 2012) have examined the effects of different NPs in anaerobic digestion with a focus on mesophilic temperatures (30-38°C). However, only a few studies reporting on the effect of NPs in anaerobic digestion under thermophilic conditions (50-60°C) can be found (Abdelsalam et al. 2016).

The effects of dosing NPs to the most common processes found in WWTPs has been studied in batch, semi-continuous flow and continuous flow reactors. The common procedure is to analyze a variety of NPs or focus on a particular NPs species with more in-depth analysis. However, the influence of different NPs properties, such as average particle diameter or coating, has not been explored in depth and warrants more studies.

#### **1.2. Objectives**

The main objective of this research is to evaluate the impact of ZnO NPs with different properties (i.e. average particle size and presence or absence of coating) on anaerobic digestion utilizing municipal sludge under mesophilic and thermophilic conditions. The specific objectives are as follows:

- Evaluate the inhibition of ZnO NPs with three different concentrations, two different average particle sizes, and the presence of surface coating on the performance of mesophilic and thermophilic anaerobic digestion under batch feeding conditions,
- Verify the ZnO NPs effects observed from the batch digestion under semi-continuous flow conditions, that simulate full-scale digestion more closely, at mesophilic and thermophilic temperatures,

#### **1.3.** Thesis organization

This thesis is divided into five chapters. In this first chapter, Chapter 1, the occurrence of NPs in the environment and wastewater treatment processes is summarized along with the proposed objectives for the study. In the next chapter, Chapter 2, the source, pathway and effect of NPs on wastewater and sludge treatment processes are reviewed. Moreover, a general summary of the typical wastewater and sludge treatment processes is presented with a special emphasis on anaerobic sludge digestion. In Chapter 3, the experimental configuration, materials, equipment and analytical assays used for this research are described. The results from the different research stages are presented and discussed in Chapter 4. Finally, the conclusions and recommendations for future research are summarized in Chapter 5.

#### **Chapter 2: Literature Review**

The following sections in this chapter aim to review the stages that NPs will go through wastewater and sludge treatment processes in conventional WWTPs and its effects on those processes. A brief introduction to the significant properties and provenience of NPs is presented first regarding the comprehension of the effects in those processes. Furthermore, the components and principles involved in the different wastewater and sludge treatment processes is also revised with special focus on anaerobic digestion.

#### 2.1. Properties and detection methods of NPs relevant in wastewater

An adequate characterization of NPs is essential to understand their behavior in any area of research. Properties such as mass concentration, size, surface charge, specific surface area (SSA), coatings and agglomeration provide useful information and comparative results for studies (Neale et al. 2013; Reidy et al. 2013). Information regarding the characterization is usually reported, but sometimes not all of these properties are fully presented.

Sizing NPs requires certain beam wavelength and focus which can be obtained by different sources (i.e. electron, laser, X-ray and neutron) that apply three different techniques (Lu 2012). The first technique is microscopy that includes Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). SEM and TEM are used for visual characterization of biomass (Garcia et al. 2012; Kiser et al. 2009; Wang et al. 2012; Westerhoff et al. 2011). Dynamic light scattering (DLS) is the second and most suitable technique used to determine the size and size distribution of NPs in wastewater and sludge samples (Garcia et al. 2012; Kiser et al. 2009; Lombi et al. 2012; Mu et al. 2011; Yang et al. 2011). Its principle is based on the movement of the NPs through the medium (Lu 2012). X-ray diffraction (XRD) line broadening is the third technique

which is applied in nanocrystallite size measurement (Lu 2012). Each technique may be suitable for a different purpose and can report different sizes according to its measurement method.

The coating present in NPs could be an important characteristic for their fate and removal in environmental systems. Experiments have shown different removal efficiencies when comparing coated and uncoated NPs. For example, Kiser et al. (2010) reported that uncoated Ag and titanium dioxide (TiO<sub>2</sub>) NPs might have higher removal efficiency than their coated counterparts. This may be attributed to the coatings that allow the NPs to be more physical stable in their nanosize preventing agglomeration.

NPs can be held together by strong bonds which can form aggregates or by weak forces of van der Waals which can form agglomerates. Ultrasonication can be used to separate these agglomerates either by using probe or bath ultrasonication. However, depending on the media and type of NPs, using probe ultrasonication for a prolonged time has shown that even though agglomerates are separated, further interaction among particles can increase the hydrodynamic size (Jiang et al. 2009). Furthermore, NPs can be agglomerated back in the presence or absence of different media within time. For example, in a study by Wang et al. (2012), TiO<sub>2</sub> NPs experienced a high agglomeration resulting in 70% removal efficiency in a sequencing batch reactor (SBR) in the absence of biomass while Ag NPs did not interact during the process. This result shows that TiO<sub>2</sub> NPs may tend to aggregate into larger particles and can settle out during the treatment process even without the presence of biomass.

#### 2.2. Use of nanoparticles

NPs are used in many different applications and eventually they are released into the environment. For example, Schmid et al. (2010) surveyed Swiss manufacturing industries and

found out that a few of them were using NPs within their processes, although it was at a low consumption rate. However, not many of them stated that their products contained NPs.

Some of the environmental applications of NPs have also been reported in literature. Iron NPs have shown the capacity to degrade halogenated hydrocarbons and remove metallic pollutants (i.e. arsenic, lead and chromium) and inorganic contaminants (i.e. selenium and nitrates) (Li et al. 2006). Therefore, they can be added directly to the contaminated media and kept dispersed as long as mixing is provided (Zhang 2003). Moreover, they can be used in combination with resins (Cumbal and SenGupta 2005) for removal of phosphates (Blaney et al. 2007).

The employment of NPs in consumer products composition has been listed in Adawi et al. (2018), Contado (2015) and Project on Emerging Nanotechnologies (2013). A summary of their inventory of NPs usage along with findings from other journal articles is provided in Table 2.1. These studies confirm NPs' presence, and/or their discharge to the sewage system. It is also remarkable that only a few studies have been conducted on this topic, which might be due to the cost and the lack of knowledge of the NPs presence in the products.

The mass of NPs present in the products varies according to the type or the application. For example, Ag NPs have been detected in the range of 20 to 31241  $\mu$ g per sock in five different brands of textiles (Benn and Westerhoff 2008). Benn et al. (2010) found that fabrics (especially for medical purposes) have shown the highest presence of Ag NPs as ug Ag per g of product when compared with other products like foam of a soft toy, small humidifier and personal care products.

Nanoparticles	Product/Application	References
Âg	Textile	(Benn and Westerhoff
C	Plastics	2008; Benn et al. 2010)
	Cosmetics (soap, toothpaste)	(Kumar and Münstedt,
	Cleaning products	2005)
	Paint	(Benn et al. 2010)
	Food containers	
	Medicinal products	
Au	Cosmetics (toothpaste)	
С	Textile	
Cu	Cosmetics (facial spray)	
	Paint	
Si	Textile	
	Cosmetics	
Pt	Cosmetics	
SiO2	Textile	
	Cosmetics (toothpaste)	
	Cleaning products	
	Paint	
	Food powders	
TiO2	Cement (size < 100nm)	
	Sunscreen	(Botta et al.2011)
	Paint	(Kaegi et al. 2008)
	Cosmetics (toothpaste, shampoo)	
	Medicine	(Kiser et al. 2009)
	Cleaning products	(Serguei et al. 2011)
	Food (chocolate, pudding, mints,	
	pasta, cheese)	
ZnO	Cement	
	Sunscreen	(Lombi et al. 2012)
	Textile	
	Cosmetics (lip painting)	
	Paint	
	Food packages	

Table 2.1 Use of nanoparticles based on Adawi et al. (2018), Contado (2015), Project on Emerging
Nanotechnologies (2013) and journal articles

#### 2.3. Nanoparticle release into the environment

Products containing NPs may encounter water at some point during their lifetime whether through washing or by rainfall. Even after their lifetime, they may end up in landfills where the possibility of interaction with water is not over due to the leachate found in those facilities (Benn et al. 2010). Industrial wastewater may also contain NPs listed in Table 2.1 at different concentrations; however,  $TiO_2$  NPs are the one with the greatest presence (Westerhoff et al. 2011).

Ag NPs released during washing have been analyzed under lab conditions. For instance, Ag NPs contained in textiles are more likely to be released when washing with ultrapure water rather than tap water (Benn and Westerhoff 2008). Another study showed that plastics containing polyamide / silver composites released greater amounts of  $Ag^+$  when concentration and immersion time were higher (Kumar and Münstedt 2005). Medical clothes, which contained the highest amount of Ag among different products, released the highest value of 46 ug Ag per g of product after washing with water (Benn et al. 2010).

In paint of facades,  $TiO_2$  NPs within a diameter range between 50 nm to more than 200 nm have been observed in buildings.  $TiO_2$  NPs are released into runoff water mostly with a diameter range of 20 to 300 nm for new or aged facades; and considering the urban area, half of the  $TiO_2$  is larger than 300 nm probably due to other sources. Therefore,  $TiO_2$  can easily reach to storm or sanitary sewers ending up in WWTPs or surface water (Kaegi et al. 2008).

#### 2.4. Wastewater and sludge treatment

Wastewater requires treatment before it is discharged to rivers, lakes, oceans or reused for irrigation. Wastewater treatment is a combination of different unit operations or processes depending on its characterization (i.e. level of pollution). Wastewater generated from residential areas is usually treated in conventional municipal WWTPs. Conventional municipal WWTPs consist of preliminary, primary, secondary, and tertiary or advanced treatment as shown in Figure 2.1. Most of these treatments produce sludge (as a by-product) that requires additional treatment before its final disposal.



Figure 2.1 Flowchart of typical unit operations and processes for municipal wastewater and sludge treatment

Preliminary treatment aims to remove any large materials, sand and grease that could negatively affect the operation or maintenance of the different processes and equipment involved in the treatment of wastewater. Preliminary treatment involves unit operations such as screening, grit removal and flow equalization. Screening is the first unit where most WWTPs segregate coarse materials from wastewater. Two types of screens used, as part of preliminary treatment, are coarse and fine screens (openings greater and smaller than 6 mm, respectively).

In most of the cases, grit removal follows the screening treatment. Many types of systems for the removal of grit (e.g. sand, gravel, seeds, and eggshells) are available such as horizontalflow grit chambers, aerated grit chambers, vortex-type grit chambers and cyclone degritter.

The goal of using equalization tank is to maintain a constant wastewater flowrate sent to primary and then to the biological reactors in secondary treatment. This would ensure an optimum treatment efficient and avoid inhibitory conditions to biomass that could be caused by constituents in wastewater characterization. Another advantage of an equalization unit is that it improves the performance of other processes like filtration or chemical treatment.

#### 2.4.1. Primary treatment

In primary treatment, primary clarifiers allow the removal of a portion of suspended solids and organic matter. Primary clarifiers or primary sedimentation tanks have two typical designs, which are rectangular or circular. Primary treatment is mostly a physical treatment process, but chemical dosing is possible to enhance the settling of the suspended solids. From the primary clarifiers, the wastewater goes to the secondary treatment processes and the settled solids (under gravitational force in a turbulent free environment) are removed periodically. This solid byproduct is called primary sludge (PS) and requires further treatment as discussed in the following sections.

#### 2.4.2. Secondary treatment

Secondary treatment aims to remove dissolved biodegradable organic matter and suspended solids. In order to accomplish this goal, secondary treatment consists of physical, chemical and biological mechanisms occurring in different bioreactors or units. These bioreactors can be classified according to the way that the microorganisms are retained in the system and grow. The two categories are suspended growth and attached growth bioreactors. Figure 2.1 shows a typical secondary treatment configuration with an activated sludge process which falls into the suspended growth bioreactor category. For this scenario, the biological treatment consist of a well-mixed aeration tank (aerobic basin) and it is followed by a secondary sedimentation tank where the settled sludge is recycled to the aeration basin and the excess is removed for further treatment. The extracted sludge is called waste activated sludge (WAS) and needs to be treated as discussed in the next section. For industrial wastewater with much higher dissolved organics than municipal wastewater, high-rate bioreactor systems, such as up-flow anaerobic blanket reactors (UASB) or fixed film aerobic or anaerobic reactors can be used.

#### 2.4.3. Sludge treatment

Sludge has to go through different treatment stages before it can be land applied as fertilizer or disposed in a landfill, which is no longer allowed for organic waste for most of the Provinces in Canada. For instance, preliminary unit operations such as screening, grinding, degritting and blending are necessary. Next, thickening is mostly used to reduce the volume of sludge especially from secondary sedimentation tanks. Then, sludge can be treated by one of the four different stabilization methods. These four processes are alkaline stabilization, anaerobic digestion, aerobic digestion and composting. The main purpose of stabilization is to decrease the presence of pathogens and odours in the sludge. The focus of this research is anaerobic digestion, and it is discussed in more detail in the following subsection. Finally, dewatering (reduction of moisture) of sludge is achieved by centrifuge, belt-filter press, drying beds or lagoons.

#### 2.4.3.1. Anaerobic digestion

Anaerobic digestion (AD) is a method used for waste stabilization where biodegradable organic matter and pathogen content are decreased in the absence of oxygen (US EPA 1979). The anaerobic processes consist of different biochemical reactions (Droste 1997). Three steps to classify these different biochemical reactions in AD is hydrolysis, acidogenesis and methanogenesis (Droste 1997; Metcalf and Eddy 2003). A more detailed model is shown in Figure 2.2. The process starts with some portion of large molecules and suspended solids transformed into smaller, easily biodegradable substances and ends with the production of methane. These processes achieve the mass/volume reduction of organic solids in the sludge and it is one of the main advantages of AD (US EPA 1979). Biogas production is another outcome of the anaerobic process. The term biogas is used to describe methane (60-70%), carbon dioxide (30-40%), hydrogen, hydrogen sulfide and other gases in low amounts (Droste 1997; US EPA 1979). Biogas is a renewable energy source which can be used for generation of heat and/or electricity. The main energy source in biogas is methane.



Figure 2.2 Anaerobic process according to the Anaerobic Digestion Model (ADM) No. 1 (Adapted from IWA 2002)

#### 2.4.3.1.1. Principles and operating factors of anaerobic digesters

Anaerobic digestion can be designed to operate under different conditions (i.e. low-rate digestion, high-rate digestion, anaerobic contact and separate digestion) (Metcalf and Eddy 2003; US EPA 1979). Low-rate digesters are larger and less efficient than the high-rate anaerobic digesters (US EPA 1979). Separate digesters treat primary and secondary sludges in different basins (Metcalf and Eddy 2003).

Furthermore, anaerobic digesters can have different configurations which include singlestage, two-stage and two-phase digesters (Metcalf and Eddy 2003). The first term implies the number of reactors present in the system to achieve different purposes (Droste 1997). The "stage" is used to describe whether a second reactor is added for biodegradation and the "phase" describes the application of different biochemical processes (i.e. acid or methane production) or temperature ranges (i.e. mesophilic around or thermophilic conditions) in the reactors (Taricska et al. 2007). Environmental factors are important to understand the theory behind the design of anaerobic digesters (Droste 1997). Temperature, pH, alkalinity and inhibitory substances are discussed below in terms of their impact to AD. Usually these parameters are not specific to a specific type of digester and they might apply for the different conditions and configurations mentioned before.

Mesophilic conditions (temperature between 30 and 40°C) are usually applied for the design of high-rate digesters which can be found in full-scale plants. However, thermophilic conditions (temperature between 50 and 60°C) can also be applied even though they are less common (Metcalf and Eddy 2003; Taricska et al. 2007; US EPA 1979; WEF 2007). During operation, the temperature should not vary more than 0.6°C to maintain the growth of methanogens (US EPA 1979; WEF 2007). Thus, a good heating and insulated system for the reactor vessel is necessary.

Sludge coming from the primary and secondary wastewater treatment processes to the anaerobic sludge digesters might not have neutral pH. A pH range between 6.5 and 7.5 could provide optimum conditions for AD, but it is not restricted to that limit (Droste 1997). The pH can be adjusted by the addition of different chemicals externally and calculation for the chemical dosages can be found elsewhere (US EPA 1979; WEF 2007).

Inhibition of acid and methane forming bacteria can be an issue when high concentrations of toxic substances are present in the sludge. Ammonia, sulfate, light metal ions, heavy metals and organics can cause a collapse of the system when they reach certain levels (Droste 1997; US EPA 1979). Chen et al. (2008) provided an extensive review of the different mechanisms and substances that could upset the AD process. However, it is noteworthy that under certain concentrations, some compounds can also provide positive impact on anaerobic digestion (US EPA 1979).

Different digestion operational factors are well described in a manual published by Water Environmental Federation (WEF 2007). These factors are related with the environmental aspects mentioned before. As mentioned earlier, during operation, pH can be controlled by the addition of chemicals that would increase the alkalinity (US EPA 1979). The pH control is linked to volatile fatty acids (VFAs) and alkalinity present in the digester (WEF 2007). The ratio between VFAs and alkalinity ratio is considered a good indicator for the working conditions in the anaerobic digester and is presented in Table 2.2.

Volatile fatty acids : Alkalinity	Condition
mg/L : mg/L	
0.1 - 0.2	Working well
0.3 - 0.4	Upset condition
> 0.8	Inhibition

Sufficient solid retention time (SRT), as one of the important design criteria, is necessary to keep a balance between biomass growth and excess biomass withdrawal from the system (US EPA 1979). SRT for a high-rate anaerobic digester can be as low as 12 days, but it is common to find STRs in the range between 15 and 20 days (WEF 2007). The Code of Federal Regulations (1999), that is the current U. S. regulation, establishes the necessary SRT for Class B biosolids for land application as fertilizer. For instance, a minimum SRT of 15 days should be considered when working with temperatures higher than 35°C (US EPA 2003).

Loading factor or organic loading rate (OLR) is another important design/operation criterion and is based on the volatile solids coming to the digester. A typical range of OLR is 1.6 - 4.8 kg VSS/m<sup>3</sup>.d for a high-rate anaerobic digester under mesophilic conditions (Metcalf and Eddy 2003). Literature also provides tabulated information that relates the sludge concentration, and SRT with the volatile solids OLR for design purposes (Metcalf and Eddy 2003).

#### 2.5. Removal, fate and impact of nanoparticles on wastewater and sludge treatment

#### **2.5.1. Removal pathways for nanoparticles**

The removal pathway for NPs, under several circumstances, has shown to be adsorption (Benn and Westerhoff 2008; Kiser et al. 2009, 2010; Limbach et al. 2008; Mu et al. 2011). Therefore, higher NP removal may be obtained in higher concentration of biomass (Kiser et al. 2010; Wang et al. 2012).

Adsorption of TiO<sub>2</sub> NPs has been tested in a batch adsorption system, showing the capacity of wastewater bacteria to accumulate TiO<sub>2</sub> NPs with a removal up to 85% and a having a Freundlich coefficient (1/n) equal to 0.53 (Kiser et al. 2009). In another study, silicon dioxide (SiO<sub>2</sub>) NPs adsorption to the biomass also followed the Freundlich isotherm with 1/n = 1.1 (Kiser et al. 2010). The Freundlich isotherm models used to describe the adsorption may be suitable as the model considers heterogeneous surface (i.e. biomass surface).

Park et al. (2013) analyzed the influence of contact time in activated sludge using de-ionized (DI) water for the removal of NPs. Batch adsorption tests indicated that the longer the exposure, the lower the NPs concentration in the effluent from the system. TiO<sub>2</sub> NPs and SiO<sub>2</sub> NPs concentration of 10 mg/L in DI water showed a removal efficiency up to 95% for a contact time of 1 h. Removal efficiencies of Ag NPs in DI water varied from 90% to 99% for concentrations of 1 mg/L and 10 mg/l, respectively.

TiO<sub>2</sub>, Ag and coated fullerene NPs removal efficiency was not affected by the increase of natural organic matter (NOM) or reduction in extracellular polymeric substances (EPS) for a low

biomass concentration up to 400 mg/L of total suspended solids (TSS) in batch reactors which simulated WWTP processes (Kiser et al. 2010). However, Park et al. (2013) found that EPS increased the removal efficiency of NPs at a higher biomass concentration of 3000 mg/L mixed liquid suspended solids (MLSS). As shown in Figure 2.3, EPS is important when NPs have higher physical stability (e.g. Ag NPs coated with citric acid) as it will affect the removal pathway and higher biomass is present in the treatment process. EPS matrix might help to entrap chemically stable NPs (Figure 2.3b) rather than only being adsorbed to the microorganism surface in the absence of EPS (Figure 2.3f). In addition, higher accumulation of NPs to the biomass has been evidenced when biomass dosage concentration increased in adsorption batch experiments (Kiser et al. 2010).



Figure 2.3 TEM images of activated sludge exposed to NPs in the presence of EPS (a–d) and absence of EPS (e–h). Control (a and e), 10 mg L–1 Ag NPs (b and f), 10 mg L–1 TiO2 NPs (c and g), and 10 mg L–1 SiO2 NPs (d and h). Arrows indicate NPs (synthetic wastewater, 3000 mg L–1 MLSS, 8 h, 100 rpm, 25C) (From Park et al. 2013, used with permission of the publisher Elsevier).

#### 2.5.2. Fate of nanoparticles in wastewater and sludge treatment

Once NPs are released into water, they travel through the sewage system and most likely arrive to a municipal WWTP. As described earlier in Section 2.4, conventional municipal WWTPs have primary and secondary wastewater treatment units from where waste sludge is taken for further processing for volume reduction, pathogen destruction in other biological (i.e. anaerobic digestion) or chemical treatment units before final disposal via landfilling, incineration or used as fertilizer. The following sections provide the pathway of NPs in the aforementioned treatment units in order to predict their effect on the receiving environmental system (e.g. lake, river, soil) upon their disposal.

#### 2.5.2.1. Primary treatment

Primary sedimentation tanks has shown to be able to remove  $TiO_2$  with a low efficiency (i.e. 47-60%) compared to secondary treatment (i.e. 84-92%); however, most of the removed portion is in a size range above 700 nm (Kiser et al. 2009). Common overflow rates used in these tanks do not allow the NPs to settle within the hydraulic retention times unless they are attached to larger substances (Westerhoff et al. 2011).

As mentioned before, coatings in NPs are important as they may change its behavior in treatment systems. Jarvie et al. (2009) found that coated and uncoated  $SiO_2$  NPs were physically stable in ultrapure water, but a different respond was found in the presence of wastewater. Uncoated  $SiO_2$  NPs passed through the treatment processes as agglomeration was not induced by any wastewater component. On the other hand, coated  $SiO_2$  NPs were removed as a result of agglomeration with other components that occurred fast enough and enabled settling within a short time.

In the case of using real wastewater from a WWTP, simulated primary clarifier, running for 15 d, achieved a removal efficiency of 6% and 10% at 1 mg/L of uncoated Ag NPs after 30 min of settling with 269 and 730 mg/L of SS in the influent, respectively (Hou et al. 2012).

All the previous results indicate that NPs are mostly removed in posterior wastewater treatment processes. Prolonged retention times and higher biomass concentrations in WWTPs promote higher removals due to adsorption and those conditions are found in activated sludge processes or sludge treatment units as described in the following sections.

#### 2.5.2.2. Biological treatment

Wastewater, most of the time, would go through a biological treatment due to the presence of biodegradable materials before it is discharged to surface waters As mentioned earlier, activated sludge treatment process is one of the most commonly used biological treatment processes applied in the wastewater stream.

#### 2.5.2.2.1. Activated sludge treatment

NPs removal by activated sludge processes has been reported from lab-scale to full-scale WWTPs. In lab-scale systems operating as sequencing batch reactors (SBRs), Ag NPs removal efficiencies varied from 49% to 88% for biomass concentrations of 1.1 and 1.8 g TSS/L, respectively. Meanwhile, TiO<sub>2</sub> NPs removal efficiency was 97% with biomass concentration of 1.3 g TSS/L (Wang et al. 2012). An aeration tank in a model WWTP dosed with 100 ppm of cerium oxide NPs showed similar results. The effluent concentration of cerium oxide NPs after the secondary sedimentation tank was reported to be between 2 and 5 ppm with removal efficiencies of 95-98% (Limbach et al. 2008).
Wastewater samples taken from different stages of a WWTP show that the highest  $TiO_2$  NPs removal occurs in the activated sludge system. One of the reasons seems to be the prolonged contact time between the biomass and the  $TiO_2$  NPs helping the biomass to adsorb more quantities of NPs (Kiser et al. 2009).  $TiO_2$  NPs also tend to aggregate into larger particles even without the presence of biomass (Wang et al. 2012). Therefore, high concentrations of  $TiO_2$  NPs in the secondary sludge were found (Kiser et al. 2009).

Hence, in a WWTP, the removal of NPs comes from the removal of the biomass by secondary sedimentation or filtration processes; and, in different full-scale WWTPs, filtration has been found to accomplish a better performance (Westerhoff et al. 2011). Kiser et al. (2009) reported removal efficiencies for a full-scale and an experimental bench-scale SBR, around 69% and 88%, respectively. Bench-scale testing can be a good approach as it provides a controlled environment to assess impact of different variables (i.e. NPs type, size, concentration) on biological treatment performance. Nevertheless, bench-scale reactors should be operated for a prolonged period in order to simulate the real WWTP operation as close as possible (Wang et al. 2012).

#### 2.5.2.2.2. Anaerobic treatment for wastewater treatment

As one of the most popular anaerobic reactors for industrial wastewater treatment, UASB reactors with an hydraulic retention time (HRT) of 12 d have shown to provide a 62% and 82% removal efficiency of ZnO NPs when they were dosed with synthetic wastewater with concentrations of 0.32 and 34.5 mg Zn/L, respectively (Otero-Gonzalez et al. 2014). In the same study, the removal of VFAs, a representative of organics removal performance leading to biogas conversation, varied depending on the VFA species. Butyric acid has showed to be more likely removed in UASB when compared to acetic and propionic acid. Even when a total inhibition was

observed at the highest ZnO NPs concentration (34.5 mg Zn/L) in the influent, butyric acid was removed up to 50%.

## 2.5.2.3. Anaerobic digestion for sludge treatment

In a batch anaerobic digester, Ag NPs were highly removed by sorption into the biomass showing a remaining concentration less than the 10% of the influent within a few hours (Yang et al. 2012). Under a realistic scenario of the presence of Ag with a concentration of 50 mg/kg in the sludge entering the anaerobic digester, the removal efficiency was 85% regardless of the conditions of NPs coatings or size (Lombi et al. 2013). In an anaerobic digester fed with sewage sludge with ZnO NPs concentration of 1 mg/g, most of the ZnO NPs were retained by 85-90% in the sludge (Lombi et al. 2012). Furthermore, the literature indicates that the sludge leaving the anaerobic digester may no longer contain Ag and ZnO in the form of NPs. Instead, speciation tests have revealed that Ag can be found as Ag<sub>2</sub>S (Lombi et al. 2013, Ma et al. 2014) and Zn can be found as ZnS, Zn-Fe oxy/hydroxides and Zn<sub>3</sub>(PO<sub>4</sub>) (Ma et al. 2014).

# 2.5.3. Impact of nanoparticles on wastewater and sludge treatment

NPs are most likely to interact with biomass present in secondary treatment systems (i.e. activated sludge, SBRs) used for biological wastewater treatment or anaerobic digestion reactors used for biological sludge treatment. Furthermore, most of the NPs are accumulated in the sludge and high concentrations are expected when dealing with sludge treatment processes. Therefore, microbial activity in biological treatment processes may be affected if NPs have toxic effects and the performance can vary according to the presence of different kinds of NPs. In the following sections, the effects of the presence of NPs on secondary treatment and anaerobic digestion processes are discussed in terms of their influence on the performance/stability of those systems.

## 2.5.3.1. Impact of nanoparticles on secondary treatment

Chemical oxygen demand (COD) and TSS removals in SBRs have not been affected significantly by the addition of Ag,  $TiO_2$  and fullerene NPs at concentrations between 0.5 and 2.5 mg/L; despite the fact that for the first operational cycles, Ag and fullerol NPs presented different results for the effluent TSS and COD concentrations, respectively. These behaviors may suggest that the microbial community has the potential to acclimatize to incoming NPs at these concentrations (Wang et al. 2012). In another study, COD removal efficiency has not been affected by the addition of 5 mg/L of ZnO NPs to SBRs (Hou et al. 2013).

The nitrogen removal efficiency can vary from positive to negative according to the NP type, concentrations, and retention times in SBRs. Ni et al. (2013) found that at a concentration of 10 mg/L, magnetic NPs did not influence the total nitrogen removal efficiency significantly in a short term (7 days). However, higher concentrations showed a decrease of the total nitrogen removal efficiency (e.g. 4% reduction at 50 mg/L NPs). Interestingly, at the end of the long term test (60 days), the total nitrogen removal efficiency differed from 80.3% to 94.4% between the control and the reactor dosed with 50 mg/L magnetic NPs (Ni et al. 2013).

In another study, for Ag NPs, ammonia removal was negatively affected in the first cycle of a SBR; meanwhile, no significant changes were found in a longer period of 15 days (Hou et al. 2012). On the other hand, Zheng et al. (2011b) found that concentration of TiO2 NPs at 50 mg/L reduced the total nitrogen efficiency from 80.3% (without NPs) to 24.4% in SBRs with anaerobic and low dissolved oxygen (DO) stages in a long term period (70 days). Variation in the nitrogen removal was observed until day 16 and only during the low DO stages of SBRs. Hou et al. (2013) and Zheng et al. (2011a) found that ammonia removal efficiency of SBR may be able to recover from inhibition for ZnO NPs concentration of 1 mg/L. Nevertheless, higher concentrations can

cause inhibition on the biomass and the ammonia removal can be less efficient (Hou et al. 2013; Zheng et al. 2011a). When conventional nitrogen removal is present at high DO levels (7.2 mg/L), the inhibition seems to be predominant in the nitrification stage rather than in the denitrification (Hou et al. 2013). However, under anaerobic and low DO conditions (DO concentrations between 0.15 and 0.50 mg/L), denitrifiers can not achieve the same nitrate removal (Zheng et al. 2011a) resulting in a lower nitrogen removal efficiency.

In addition to nitrogen removal, SBRs with anaerobic and low DO stages showed that phosphorus removal efficiencies may not be affected by  $TiO_2$  NPs (Zheng et al. 2011b), but it may be reduced in the presence of ZnO NPs with a concentration higher than 10 mg/L (Zheng et al. 2011a).

In order to understand the aforementioned effects on biological nutrient (i.e. nitrogen and phosphorus) removal, studies have been also performed on biomass responsible for treatment. Ammonia-oxidizing, nitrite-oxidizing and heterotrophic bacteria activities did not present negative effects at concentrations of 50 mg/L magnetic NPs in long term tests (Ni et al. 2013). However, in a SBR fed with 50 mg/L of TiO<sub>2</sub> NPs, ammonia oxidizing and nitrite oxidizing bacteria presence declined from 8% and 6% to 1% and 3% of the total biomass, respectively. This decline in *Nitrosomonas sp.* (ammonia oxidizing bacteria) due to the presence of 50 mg/L TiO2 NPs may explain the reduction in the nitrogen removal efficiency observed in other studies as well (Zheng et al. 2011b).

There were other observations in SBR systems exposed to NPs. Secretion of EPS was increased and sludge volume index was decreased due to the adsorption of magnetic NPs to biomass at concentration of 50 mg/L (Ni et al. 2013), indicating faster settling profile.

24

Besides SBR systems, membrane bioreactors (MBRs) were also assessed in terms of their performance variation when exposed to NPs. Pollutant removal performances (carbonization, nitrification, denitrification and phosphorus) in MBRs were reported to not being affected by dosages up to 10 mg ZnO NPs/g SS during the 6 month period of the experiment (Wang et al. 2014). Wang et al. (2014) suggested that microorganisms in batch experiments tend to be shocked and inhibited by high dosages, instead a gradual increment in the ZnO NPs could be tolerated as it is the case for the MBRs operating with an SRT of 100 days.

Zhou et al. (2014) studied the effect of the presence of 50 mg/L of  $TiO_2$  NPs in a submerged MBR and found that fouling was delayed by hindering the initial cake layer formation.  $TiO_2$  NPs may be readily deposited into the initial cake layer causing a postponement of the deposition of other inorganic foulants compounds such as  $SiO_2$ . Wang et al. (2014) also found that the total resistance in an MBR is decreased as the external fouling resistance is reduced even though the pore blocking resistance is increased.

For low concentrations of 0.32 mg Zn/L in UASB, the acetoclastic methanogens were partially inhibited meanwhile hydrogenophilic methanogens were not affected when compared with the control. Otero-Gonzalez et al. (2014) suggested that the long term inhibition found in the study was due to high accumulation in the biosolids with an SRT higher than 160 days. This caused a decrease in the methane production for the low concentration close to the end of the experiments. At high concentration of 34.5 mg Zn/L, the reactor stopped producing biogas after a period of 7 days. The half-maximal inhibitory concentration (IC50) for acetoclastic and hydrogenotrophic methanogenesis was reported as 12.2 and 229 mg Zn/L respectively based on the normalized methanogenic activity (NMA). The NMA is based on the maximum specific methanogenetic activity ratio between the NP dosed reactor and the control. This is contradictory to the results

from MBRs where accumulation was possible, but studies with low concentrations did not produce any negative impact (Wang et al. 2014; Zhou et al. 2014)

## 2.5.3.2. Impact of nanoparticles on anaerobic sludge digestion

Ag NPs have been studied at concentrations lower than 40 mg/L and showed that the methane production volume from anaerobic mesophilic or thermophilic digesters was not significantly affected. This was attributed to the low release of Ag ions in the digesters (Yang et al. 2012).

In another study, CeO<sub>2</sub>, TiO<sub>2</sub>, Au and Ag NPs with a concentration of 640, 1120, 100 and 170 mg/L respectively were tested under mesophilic and thermophilic sludge digester conditions. CeO<sub>2</sub>, TiO<sub>2</sub>, Au and Ag NPs were prepared from different compounds at average diameter size of 12, 7.5, 20 and 30 nm, respectively. The biogas production did not change significantly in the presence of Au and Ag NPs; however, a 10% increase was detected in the presence of TiO<sub>2</sub> NPs and a negative impact in the presence of CeO<sub>2</sub> (Garcia et al. 2012).

The effects of anatase TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub> and SiO<sub>2</sub> NPs (average particle size of 185, 130 and 110 nm, respectively) on the production of methane gas and the different stages of mesophilic anaerobic digestion have shown that concentrations below 150 mg/g-TSS had an insignificant impact. However, ZnO NPs (average particle size of 140 nm) with concentrations above 3 mg/g-TSS could produce a substantial decrease in the production of methane gas when the fermentation time increases (Mu et al. 2011). Furthermore, at concentration of 150 mg ZnO NPs/g-TSS, there is a lower performance in the hydrolysis, acidogenesis and methanogenesis stage (Mu et al. 2011). Mu and Chen (2011) measured the methane production in a semi-continuous flow mesophilic anaerobic digester with an SRT of 20 d, operated for a period of 105 days. They found that the methane production was reduced by 18.3% and 75.1% from the control in the presence of 30 and

150 mg ZnO NPs/g-TSS (feed), respectively. A high part of the inhibition is usually attributed to the release of  $Zn^{2+}$  from the ZnO NPs. The effect from  $Zn^{2+}$  released from the ZnO NPs was also studied and the reduction due to  $Zn^{2+}$  was exhibited to be 9.4% and 63.8% for the equivalent of 30 and 150 mg ZnO NPs/g-TSS (feed) dosage when compared to the control, respectively. This would indicate that at higher ZnO NPs dosages, the inhibition is mainly caused by the release of  $Zn^{2}$  rather than the NPs.

From the different stages of anaerobic digestion which includes hydrolysis, acidification and methanogenesis; hydrolysis at high ZnO NPs concentration was slightly affected, but methanogenesis had the main inhibition effect (Mu and Chen 2011). Therefore more studies have been performed so far to understand the impact of NPs on the methanogenes.

A comprehensive study on NPs including Ag, Al<sub>2</sub>O<sub>3</sub>, CeO<sub>2</sub>, Cu, CuO, Fe, Fe<sub>2</sub>O<sub>3</sub>, Mn<sub>2</sub>O<sub>3</sub>, SiO<sub>2</sub>, TiO<sub>2</sub>, and ZnO has tested the toxicity on acetoclastic and hydrogenotrophic methanogens at concentrations of 1500 mg/L. After a first dose of 1500 mg/L of Mn<sub>2</sub>O<sub>3</sub> NPs, a reduction up to 52.4% in the NMA was obtained for the acetoclastic activity; however, after the application of a second dose at the similar concentration, the NMA went back to 94% showing adaptability of the biomass. The results confirmed the previous studies about inhibition resistance for all the mentioned NPs except for Cu, ZnO and CuO which affect the bacterial activity chronically under anaerobic conditions (Gonzalez-Estrella et al. 2013). A batch anaerobic reactor fed with cattle manure showed that half-maximal inhibitory concentration (IC50) of methane production was 57.3 mg Zn/L and 30.2 mg Cu/L for ZnO and CuO NPs, respectively (Luna-delRisco et al. 2011).

The presence of sulfate in the digester feed has shown to help reducing the inhibitory effect of NPs on acetoclastic methanogens when compared to a sulfate-free feed for concentrations no greater than 0.6 mM ZnO NPs (Gonzalez-Estrella et al. 2015). Also, ZnO NPs and ZnCl<sub>2</sub> have similar effect on the acetoclastic methanogenic activity regardless the presence of sulfates. The presence of sulfates has shown to decrease the amount of soluble Zn ( $Zn^{2+}$ ) due to the generation of biogenic sulfide in anaerobic digesters. Free soluble Zn has been shown to be higher in the presence of ZnO NPs than ZnCl<sub>2</sub> when added in concentrations greater than 0.1 mM.

## 2.6. Summary

This chapter presented a review of the research involving the fate and effects of NPs in the environment. From a chronological point of view, the first studies detecting the release of NPs from daily use products started about a decade ago. Then, studies has been focusing in the removal efficiency of different wastewater and sludge treatment processes from lab scale to real WWTPs. Moreover, some of these common NPs (e.g TiO<sub>2</sub>, ZnO, Ag) have shown impact in the performance parameters for some biological treatment processes. Studies that involved spiking different NPs to these systems usually exhibited some negative effects; however, most of the processes demonstrated to recover after a long period at lower concentrations

One of the last processes of WWTPs is sludge treatment; consequently, higher concentrations of NPs is expected to arrive to this system. Therefore, this study aims to examine the effects three different factors (concentration, average size and coatings) of of ZnO NPs during mesophilic and thermophilic anaerobic digestion of municipal waste sludge.

# **Chapter 3: Materials and methods**

#### 3.1. Substrate and inocula

Sludge was collected from a municipal WWTP located in Kelowna (British Columbia, Canada). Fermented primary sludge (FPS) was taken from a primary sedimentation tank based on gravity sedimentation. Thickened waste activated sludge (TWAS) came from dissolved air flotation clarifiers that follow after a biological nutrient removal process consisting of anaerobic, anoxic and aerobic zones. At the Kelowna WWTP, FPS and TWAS are usually mixed in a ratio of 33:67% (v/v) with a typical 4.5% of total solid (TS) concentration before being centrifuged for final disposal.

At the Kelowna WWTP, anaerobic digestion is not being implemented. Therefore, anaerobic inocula to start the bench-scale anaerobic digester testing for this study had to be obtained from other locations. Thermophilic anaerobic inoculum (temperature of  $55 \pm 2^{\circ}$ C) was obtained from Annacis Island WWTP located in Vancouver (British Columbia, Canada). The digesters at Annacis Island WWTP have been operating around SRT of 20 days. Mesophilic anaerobic inoculum (temperature of  $35 \pm 2^{\circ}$ C) was obtained from an automated 7-L fermenter being operated at UBC's Bioreactor Technology Group for more than two years.

Prior to setting up bench-scale digesters for NP impact testing, anaerobic inocula needed to be acclimated to sludge from Kelowna's WWTP. Inocula acclimation was set up in two (one mesophilic, one thermophilic) semi-continuously fed anaerobic digesters with 1.2 L of effective volume. The semi-continuous flow digesters were fed once day for 7 days a week. Both acclimation digesters were fed with the mixed sludge, at PS:WAS volumetric ratios used at the Kelowna's WWTP, with a 20 days SRT and OLR based on volatile solids (VS) of  $1.66 \pm 0.5$  g VS/L/d for a period of at least 80 days.

## 3.2. Nanoparticles and dispersant

Three different ZnO NPs were obtained as dry powder. Two different types of ZnO NPs were purchased from SkySpring Nanomaterials (Houston, TX, USA) with median diameter (D50) of 10-30 nm uncoated (surface specific area of 30-50 m<sup>2</sup>/g) and treated (coated) with silane coupling agents (surface specific area >  $60 \text{ m}^2/\text{g}$ ). The third ZnO NP was purchased from Sigma Aldrich (St. Louis, MO, USA) with an average particle size less than 100 nm (SSA 15-25 m<sup>2</sup>/g). ZnO NPs solution with concentration of 10-20 g/L was prepared by using Type 1 water containing 0.1 mM sodium dodecylbenzene sulfonate (SDBS) (Sigma Aldrich) as a dispersant. Type 1 water is research grade water treated by softening, reverse osmosis and ultraviolet disinfection processes at UBC's Bioreactor Technology Group. The solution was sonicated using Fisher ultrasonic dismembrator model 500 for a period of 1 h and it was dosed to the anaerobic digesters once a day within an hour of preparation.

The zeta potential of the NPs solution containing the dispersant was measured using a Zetasizer Nano ZS (Malvern Instruments Ltd.). The zeta potential is determined by measuring the velocity at which particles moves when an electric field is applied. The technique used by the instrument is phase analysis light scattering. A sample from each NP solution was added to a disposable capillary cell (Malvern model DTS1070). Then, the cell was placed in the Zetasizer Nano ZS for 1 min in order to let the temperature of the sample to reach 25°C. The NPs zeta potential for each sample was determined for at least 10 times.

# 3.3. Experimental plan for anaerobic digestion inhibition studies

## 3.3.1. Biochemical methane potential (BMP) assays

BMP assays were stablished by Owen et al. (1979) as a low cost and quick way to determine biodegradability potential and levels of toxicity in terms of methane production from a sample.

The potential inhibition due to ZnO NPs added to the Kelowna waste sludge was tested in 72 batch 160 mL (total volume) serum bottles (BMP assays) containing inocula acclimatized to mixed sludge from Kelowna's WWTP (33% FPS + 67% TWAS) with a food (waste sludge) to microorganism (anaerobic inoculum) ratio of 2 g VS/g VS. The effective (liquid) volume in each serum bottle was 85 mL.

A total of 12 BMP combinations (displayed in Figure 3.1) with each combination studied in triplicates yielded a total number of 36 assays operated under mesophilic ( $35 \pm 2 \,^{\circ}$ C) and another 36 assays operated under thermophilic ( $55 \pm 2 \,^{\circ}$ C) digestion conditions.



Figure 3.1 BMP assay configuration

In terms of NPs added to sludge, two different ZnO NPs with 30 and 100 nm average diameter sizes (i.e. S30 and S100) and coated (C) or non-coated (NC) options available for S30, yielded a total of three combinations; NC(S30), NC(S100) and C(S30). Each combination of ZnO NPs were dosed to BMP assays at three different dosages to achieve concentrations of 6 (low), 75 (medium) and 150 (high) mg NP/g feed TS (i.e. C6, C75 and C150). Among 12 combinations, 9 of them included mixed sludge, anaerobic inoculum and NPs at different type/concentration levels. The "Control" bottles (Figure 3.1) set-up with mixed Kelowna sludge were not dosed with any NPs and Type 1 water was used instead to achieve the same effective volume as the rest of the bottles. Another combination, named "Dispersant" (Figure 3.1), was used same as control but instead of using Type 1 water, 0.1 mM SDBS was added to mixed sludge to assess if any inhibition would come from the dispersant used to prepare NPs. Also, "Blank" bottles were used containing anaerobic inocula and Type 1 water (no mixed sludge) to quantify biogas produced from inocula only. At the end of the assays, the biogas production from Blank bottles were subtracted from other bottles containing biogas production from the mixture of mixed sludge and inoculum to obtain biogas production from mixed sludge only.

Additionally, external alkalinity was added to each BMP bottle in the form of sodium bicarbonate (99.7% purity) and potassium bicarbonate (99.7% purity) up to a total concentration of 4000 mg/L in order to avoid accumulation of VFAs (inhibitory to methane forming bacteria) as a result of a potential pH drop (Droste 1996). Once all the materials were added to the BMP bottles, N<sub>2</sub> was used to purge any oxygen left in the mixture and headspace for about 5 min to provide anaerobic environment. Immediately after N<sub>2</sub> purging, the BMP bottles were sealed with rubber stoppers and aluminum crimp caps using a vial crimper. Additionally, the stoppers were punctured with a needle to release extra pressure from N<sub>2</sub> purging. Finally, the BMP bottles were placed in

thermophilic and mesophilic shakers operated at 90 rpm to supply ideal temperature and mixing. BMP bottles were monitored until they stopped producing biogas. Figure 3.2 displays the serum bottles used for BMP assays before and after the set-up.



Figure 3.2 BMP bottles before and after the set up (72 bottles in total)

# 3.3.2. Semi-continuous flow anaerobic sludge digesters

After BMP assays were completed and analyzed, four mesophilic and four thermophilic semi-continuous flow lab-scale anaerobic digesters were set up to study the effect of NPs at low concentrations following the combinations shown in Figure 3.3. These combinations were selected based on the results from the BMP assays, as discussed in detail in the Results and Discussion section. Semi-continuous flow digestion simulates full-scale anaerobic sludge digestion more accurately compared to the BMP assays set-up in batch mode. Therefore, they are highly valuable to verify the effects seen in BMP assays in environmental engineering field.



Figure 3.3 Semi-continuous flow anaerobic digester configurations

The anaerobic digesters were set up in 1 L glass Erlenmeyer flasks by adding 450 mL of acclimated inocula to NPs and 50 mL of mixed sludge feed to achieve a typical sludge digester SRT of 20 d. The organic loading rate of the semi-continuous flow digesters was 1.5 gVS/L/d. The feed was prepared daily for each digester (i.e. control, NC(S30), C(S30), NC(S100)) by adding FPS and TWAS at a 33 to 67% by weight. The different feeds were spiked with ZnO NPs solution, prepared as described before, to achieve 6 mg NP/g feed TS for the reactors with NPs (Figure 3.3). Similar to BMP assays, for the "Control" digesters, reactors included mixed sludge and Type 1 water, instead of NPs to achieve the same volume as the rest of the vessels. All eight digesters were purged with N<sub>2</sub> gas to remove residual oxygen in the liquid/headspace during the set-up to

provide anaerobic conditions. The flasks were sealed from both openings by using rubber stoppers and clamped tubes. The rubber stoppers had two holes with glass tubes used for two different purposes (i.e. taking effluent sample out and collecting biogas using 2-L Teddlar® bags). The other flask opening was used to feed the digesters. All digesters were kept in the shakers at 90 rpm under mesophilic (35°C) and thermophilic temperatures (55°C) and were monitored for a total duration of 60 days. Figure 3.4 displays one of the eight semi-continuous flow vessels used.



Figure 3.4 Lab-scale semi-continuous flow anaerobic digester (8 digesters in total)

#### 3.4. Analytical methods for sample characterization and digester monitoring

## 3.4.1. Biogas measurement

For BMP bottles, the stoppers were punctured with a syringe connected to a manometer to quantify biogas volume accumulated in the bottle headspace between two measurements. For the semi-continuous flow digesters, the Teddlar® bags were emptied every day into a different manometer as it required the use of a pump to transfer the higher volume of biogas generated daily.

The readings were obtained as a displacement of a column of water that was used in a calibration curve based on known volumes of gas. Then, the biogas volume was converted to standard pressure and temperature (STP) of 101.3 kPa (1 atm) and 0°C, respectively. The conversion to STP was done as a normal practice in the field to compare results at different temperature and pressure conditions.

# **3.4.2.** Biogas composition

Biogas composition was determined by quantifying the percent of methane, carbon dioxide, oxygen and nitrogen in digester headspace samples using a gas chromatograph (GC) that was calibrated according to the method developed by van Huyssteen (1967). The equipment used was an Agilent 7820 GC with a packed column (Agilent G3591-8003/80002) and a thermal conductivity detector (TCD). The biogas was sampled for composition analysis by puncturing (with a syringe) either the stoppers of the serum bottles for the BMP test or the gas line connected to the Teddlar® bags for the semi-continuous digester testing. From the syringe, 0.5 mL of biogas was injected to the GC that used helium as a gas carrier at flowrate of 25 mL/min.

# 3.4.3. Volatile sulfur compound

Eight different odour causing volatile sulfur compounds (VSCs) in biogas were measured using another GC (Agilent 7820) with 40 meter J&W G3903-63002 DB-column and an Agilent 355 sulfur chemiluminescence detector (SCD). The instrument uses helium as a carrier gas at a flow ate of 5 mL/min. The VSCs analyzed included hydrogen sulphide, methyl mercaptan, ethyl mercaptan, carbon disulphide, dimethyl sulphide, ethyl methyl sulfide, 1- propanethiol, and dimethyl disulphide. Using a 100 mL syringe, 60 mL of biogas was extracted from the Teddlar® bags attached to semi-continuous flow digesters and 30 mL was injected to GC-SCD through a 1 mL sulfur loop.

## **3.4.4.** Total solids (TS) and volatile solids (VS)

Total solids (TS) and VS were measured in feed and effluent sludge streams of the semicontinuous flow digesters every week following Standard Methods 2540 B and 2540 E (APHA 2005) respectively. For BMP assays, TS and VS were measured in the beginning of the assay for sludge and inoculum separately and at end of the assay for the remaining sludge containing inoculum in serum bottles. For preparation, crucibles were taken after being acid cleaned and fired at 550°C in a muffle furnace and the weight was obtained by using an analytical balance. Well mixed sludge samples were poured into crucibles that were weighed before being put in a drying oven at 98  $\pm$  2°C to allow for water vaporization. After an hour, the temperature was raised to 105  $\pm$  2°C and samples were left in the oven overnight. Next day, samples were transferred to a desiccator to reach room temperature. Then, the crucibles weight was measured to obtain the mass of the dry sample and transferred to the furnace operating at 550°C again for an hour. Finally, the crucibles were cooled down and the final weight was measured. TS and VS were calculated using the following equations, respectively:

$$TS(\%, g/g) = \frac{Wet \max(g) - dry \max(g) + 105^{\circ}C(g)}{Wet \max(g)} * 100 \qquad \text{eq (1)}$$

$$VS(\%, g/g) = \frac{Dry \text{ mass after } 105^{\circ}C(g) - burned \text{ mass after } 550^{\circ}C(g)}{Wet \text{ mass } (g)} * 100 \qquad \text{eq } (2)$$

#### **3.4.5.** Chemical oxygen demand (COD)

Chemical oxygen demand (COD) was measured by following Standard Method 5220D based on closed reflux colorimetric method (APHA 2005). Total and soluble COD was tested on the semi-continuous digester feed and effluent samples once a week. The method is based on the reduction of chromium ion from hexavalent to trivalent that is directly proportional to the change of absorbance. To measure the absorbance, a Thermo Scientific<sup>™</sup> GENESYS<sup>™</sup> 10S UV-Vis spectrophotometer, set at a wavelength of 600 nm, was used and a calibration curve was developed

using known COD concentrations of a standard potassium hydrogen phthalate solution (Sigma BioXtra >99.95% pure) within a range of 100 to 700 mg/L (Appendix A. Figure A.1).

For soluble COD (SCOD), sludge samples were centrifuged for 20 min at 10,000 rpm and the supernatant was filtered through a 0.45 µm pore size membrane. During analysis, well mixed sludge samples for total COD (TCOD) and filtered samples for SCOD were added to glass tubes at desired dilution ratios using reverse osmosis (RO) water. COD digestion solution and COD catalyst were added to the diluted samples in COD tubes. COD digestion solution was prepared by mixing potassium dichromate, mercuric sulfate, sulfuric acid (> 95%) and Type 1 water and COD catalyst was prepared by mixing silver sulfate with sulfuric acid (> 95%). The COD tubes were then vortexed and placed in a temperature controlled chamber at 150°C for 2 h to allow for digestion. Then, the absorbance of digested sample was measured using the spectrophotometer.

## 3.4.6. Alkalinity

Alkalinity was measured for the semi-continuous digester feed and effluent samples once a week by following Standard Method 2320B (APHA 2005). Sludge samples were first centrifuged for 20 min at 8,000 rpm. The pH of 10 mL of supernatant obtained from centrifugation was monitored during a titration when 0.1 N sulfuric acid was added until the pH dropped to 4.6. Alkalinity was calculated according to the equation (3) below:

$$Alkalinity\left(\frac{mg \ as \ CaCO_3}{L}\right) = \frac{0.1*50000*mL \ of \ acid \ consumed}{mL \ of \ sample} \qquad eq \ (3)$$

#### 3.4.7. Ammonia

Ammonia concentration was measured for the semi-continuous digester feed and effluent samples once a week by following Standard Method 4500-NH<sub>3</sub> D (APHA 2005). Sludge samples were centrifuged for 20 min at 8,000 rpm. The supernatant was diluted with Type 1 water at a

volumetric ratio of 1:4. Additionally, a strong base (i.e. 10 N NaOH) was added to increase the pH above 11 in order to allow for the conversion of dissolved ammonia (NH<sub>3(aq)</sub> and NH<sub>4</sub><sup>+</sup>) into NH<sub>3(aq)</sub>. The method used an ammonia probe that sensed the change of pH of an ammonium chloride solution due to the diffusion of NH<sub>3(aq)</sub>, present in the samples, through the probe membrane. The measurement reading was obtained using a dual channel pH/ion meter (Accumet excell XL25) and a calibration curve was developed by using four different concentrations of standard ammonia solution (i.e. 10, 100, 500 and 1000 mg/L) (Appendix A. Figure A.2).

## **3.4.8.** Volatile fatty acids (VFAs)

Three different VFAs (i.e. acetic, propionic and butyric acid) were quantified in the semicontinuous digester feed and effluent samples once a week. VFAs were also quantified for sludge in BMP assays with time to ensure that they do not reach to levels inhibitory to methane forming bacteria. Sludge samples were first centrifuged for 20 min at 10,000 rpm and the supernatant was filtered through 0.22 µm pore size membrane. The filtered samples were added to glass GC vials and mixed with an internal standard solution containing iso-butyric acid. An Agilent 7890A GC equipped with a flame ionization detector and a 25-meter Agilent 19091F-112 polyethylene capillary column was used for quantification based on method developed by Ackman (1972). The GC used helium as a carrier gas at a flowrate of 40 mL/min.

# **3.4.9.** Dewaterability

Capillary suction time (CST) was measured to assess dewaterability rate of semi-continuous flow digester effluent samples by following Standard Method 2710G (APHA 2005). Chromatography paper was set on a capillary suction timer (Model 440, Fann Instrument Company) and a cylindrical metal tube was placed vertically over the paper. Then, 5 mL of sludge samples at room temperature (22°C) were poured inside the cylinder and the CST was recorded from the time that the liquid drained on the paper takes to travel a defined distance by the position of two electrodes. As recommended by APHA (2005), the CST values (in second) were then normalized by the TS value of the sludge sample tested and reported as second per % TS (by weight).

# **Chapter 4: Results and discussion**

# 4.1. Batch digesters

# 4.1.1. Characterization of sludge, inocula and NPs for BMP

The characterization of the inocula (microbial culture) and mixed sludge (reactor feed) used to set up the BMP bottles is shown in Table 4.1. The pH of the feed was slightly acidic (5.83) with low values of ammonia and alkalinity.

Table 4.1 Sludge and inocula characterization for the set-up of the BMP reactors <sup>1</sup>					
Parameters	Mixed	Mesophilic	Thermophilic		
	sludge	inoculum	inoculum		
pH	$5.83 \pm 0.01^{\texttt{2}}$	$7.53\pm0.01$	$7.99\pm0.01$		
TS (% by weight)	$4.04\pm0.01$	$2.26\pm0.01$	$2.11\pm0.01$		
VS (% by weight)	$3.41\pm0.01$	$1.67\pm0.04$	$1.46\pm0.07$		
Ammonia (mg/L)	$216\pm16$	$942 \pm 3$	$1428\pm4$		
Alkalinity (mg as CaCO <sub>3</sub> /L)	798	3284	4755		
TCOD (mg/L)	$53518 \pm 476$	$22935\pm72$	$22193\pm261$		
SCOD (mg/L)	$4517\pm81$	$465 \pm 11$	$193 \pm 2$		

<sup>1</sup>TS/VS: Total solids/volatile solids, TCOD/SCOD: total/soluble chemical oxygen demand <sup>2</sup>Data indicate arithmetic mean ± standard deviation of three replicates

The zeta potential for the non-coated ZnO NPs with 30 and 10 nm average diameter was -  $24.2 \pm 0.4$  and  $-25.6 \pm 0.4$ , respectively. The zeta potential for the coated ZnO NPs with 30 nm average diameter was  $-26.8 \pm 0.3$ . These results validated that the ZnO NPs were within the range of colloidal stability (Moderna et al. 2019).

# 4.1.2. Effect of ZnO NPs on BMP of municipal sludge

The BMP bottles were operated until biogas production stopped (for a period of 87 days). In Figure 4.1, the specific cumulative biogas yield from mixed sludge in terms of mL of biogas per g of VS added was graphed for the different ZnO NPs and concentrations.



(a)



(b)

Figure 4.1 Specific cumulative biogas yield from BMP assays under (a) mesophilic and (b) thermophilic conditions at STP (1 atm, 0°C)

[T: thermophilic, M: mesophilic, NC: non-coated, C: coated, S30/100: nanoparticle size of 30 and 100 nm average diameter respectively, Concentration: nanoparticle concentration of 6, 75, and 150 mg/g TS of sludge respectively. Data represents arithmetic mean and error bars represent standard deviation of the three replicates].

From the analysis of variance (ANOVA) (Appendix B, Table B.1), the dispersant used in the test showed that there was statistically significant difference between the dispersant and the control. T-test analysis (Appendix B, Table B.2 and Table B.3) was used to determine which factors were statistically significant; showing that the dispersant didn't affect the mesophilic biogas production, but the dispersant inhibited the thermophilic control bottles (p = 0.01 < 0.05) with a 9% reduction of cumulative biogas yield after 87 days.

The multi factor ANOVA and the split plot design analysis (Appendix B, Table B.4) showed that concentrations, type of NPs and temperature were statistically significant different (p = 0.03 < 0.05). In terms of type of NPs, it was found that the difference between the non-coated ZnO NPs (i.e. NC(S30) and NC(S100)) was statistically insignificant (p = 0.82 > 0.05).

At low NP concentration (6 mg NPs/g TS of sludge), there was a small difference between the mesophilic and thermophilic BMP reactors in terms of specific cumulative biogas yield. The mesophilic BMP bottles with low concentration of NPs produced similar amounts of biogas when compared to the control (p = 0.20 > 0.05) by having cumulative biogas only 2% below the control (Figure 4.1a). The mesophilic biogas production rate was high and steady during the first 9 days and then a slow rate was kept for the rest of the experiment. In the thermophilic BMP reactors (Figure 4.1b), biogas production was lower by 3.9% (p = 0.008 < 0.05) in comparison with the control. This slight inhibition could be due to the ZnO NPs addition and the small amount of dispersant present in the solution. It was evident that the thermophilic BMP assays produced less biogas in the long run.

At medium concentration (75 mg NPs/g TS of sludge), a lag-phase was visible during the first 21 days of operation in both mesophilic and thermophilic reactors as shown in Figure 4.1. This suggests that a more severe inhibition caused by NPs occurred at medium concentration

compared to that of small concentration. A partial recovery occurred after 21 days as the cumulative biogas yield kept increasing at a constant rate until the end of the experiment. The statistical analysis showed that there was no significant difference between the two non-coated ZnO NPs with 30 and 100 nm average diameters (p = 0.95 > 0.05), but it was different from the coated ZnO NPs with 30 nm dimeter (p = 0.0005 < 0.05). BMP bottles spiked with coated ZnO at medium concentration experienced less severe reduction in their biogas production compared to that of non-coated NPs. Nevertheless, the three types of NPs caused acute inhibition to the reactors at the medium spiking dosage. Compared to mesophilic (Figure 4.1a), spiked thermophilic BMP assays (Figure 4.1b) were inhibited to a higher extent and they did not present a sudden increase (recovery) in the biogas production rate, but instead were slowly producing biogas after the first 20 days. These results are in agreement with studies that report that thermophilic anaerobic cultures are more sensitive to environmental disturbances (i.e. presence of toxic compounds, temperature/pH variations) due to reduced microbial diversity therefore less resilience at elevated temperatures (Labatut et al. 2014). For example, in the case of the non-coated ZnO NPs, it produced slightly more biogas than the blank bottles set-up with no mixed sludge (117 and 135 vs 83 ml/g VS added) under the thermophilic conditions suggesting that ability of methane forming bacteria to utilize mixed sludge was destroyed at the medium NPs concentration (Figure 4.1b).

At high concentration (150 mg NPs/g TS of sludge), three different patterns were observed regarding the influence of the different variables involved in the experiment. The inhibition of NPs was discernable not only for thermophilic, but also for mesophilic cultures at high concentration regardless of whether particles were coated or non-coated. The two non-coated ZnO NPs presented similar results with cumulative biogas productions similar to the blank under mesophilic conditions (Figure 4.1a) and lower than the blank under thermophilic conditions (Figure 4.1b).

This indicated that the anaerobic microorganisms were not able to process the available biodegradable substrate in mixed sludge for biogas production as they could barely produce what the blank (which only contained the microorganisms) produced, implying that the NP doses were higher than what the microorganisms could tolerate to eventually partially or fully recover. Similar to the behavior observed at the medium concentration, the BMPs with coated ZnO NPs had a higher biogas production than the BMPs with non-coated ZnO NPs, but they were still highly inhibited. In all scenarios with high NP concentration, the biogas production was highly inhibited due to the presence of the ZnO NPs; however, thermophilic reactors were the ones who presented the major inhibition in comparison to the mesophilic reactors. Inhibition from ZnO NPs could be used to stop biogas production when other technologies might not be able to achieve it.

The pH of the mesophilic and thermophilic batch digesters was also monitored with time during digestion days of 3, 6, 9, 25 and 87 of the experiment. Reactors at both mesophilic and thermophilic temperature conditions had a pH slightly basic after the first 3 days (7.2 - 8.2 and 7.3 - 8.7, respectively). Afterwards, the pH stayed within a neutral range in mesophilic and thermophilic BMPs (6.8 - 7.3 and 7.2 - 7.8, respectively) until the end of the experiment as shown in Figure 4.2. Thermophilic digesters had a higher pH than the mesophilic digesters which was an initial characteristic of the inoculum presented in Table 4.1. The higher pH presented in the thermophilic digesters was also due to the low solubility of CO<sub>2</sub> at higher temperatures. Furthermore, thermophilic batch digesters exhibited a greater change in the pH (Figure 4.2b) than the mesophilic batch digesters (Figure 4.2a) showing the tendency to be a more sensitive system to the presence of NPs, which is in agreement with biogas production results. As suggested in the literature (WEF 2007; Appels el al. 2008; Chen et al. 2008), in this study, the operational pH range

was controlled with the addition of potassium and sodium bicarbonate buffers which could be an important factor in the partial recovery of BMP bottles spiked at medium NPs concentration.





# [T: thermophilic, M: mesophilic, NC: non-coated, C: coated, Disp: dispersent, S30;S100: nanoparticle size of 30 and 100 nm average diameter, C6;C75;C150: nanoparticle concentration of 6, 75, and 150 mg/g TS of sludge].

Accumulation of VFAs in BMP bottles is another indication of inhibition to methane forming archaea that utilize VFAs for methane conversion. Therefore, the total VFA concentration in BMP assays was monitored during digestion days of 3, 6, 9, 25 and 87 as shown in Figure 4.3. As pH was kept in the neutral range during the whole experiment, the VFA accumulation would not cause inhibition to the fermentative bacteria in charge of hydrolysis/acidogenesis stages (Veeken et al. 2000). As the hydrolysis and acidogenesis rates were not affected, the process carried on producing more VFAs that would cause the slight pH drop in the first 5-25 days of digestion depending on NPs levels and assay temperature as evidenced in Figure 4.2. The total VFA concentration produced during hydrolysis in the mesophilic BMP reactors was mostly consumed by archaea during methanogenesis stage by the 9<sup>th</sup> day for the control, blank and low concentration of NPs to values lower than 25 mg/L. The complete consumption of VFAs was reflected in the high biogas (methane) production rate during those days. The rate of VFA consumption by archaea for the thermophilic control, blank and BMPs with low concentration of NPs was slower as the total VFA concentration was higher than 750 mg/L by the 9<sup>th</sup> day; nevertheless, total VFA concentration was below 25 mg/L by the 25<sup>th</sup> day (Figure 4.3).

On the other hand, BMP reactors with NPs at medium concentrations (i.e. 75 mg NPs/ g TS of sludge) showed an increase of the VFA concentration up to the 25<sup>th</sup> day. The presence of VFAs during those days could indicate that the hydrolysis and acidogenesis processes might not be strongly affected by the dose of NPs at those concentrations. Moreover, the presence of high content of acetic acid could suggest that the partial inhibition occurred to archaea mainly during the methanogenesis process and resulted in biogas being produced continuously at a slow rate. A similar outcome has been reported in which acidogens have showed to resist higher doses of NPs than methanogens without presenting major inhibition (Mu and Chen 2011). Furthermore, the total VFA was being consumed from day 25 until the end of the experiment which supported the biogas production (methanogenesis) recovery displayed in Figure 4.1.



(a)



(b)

Figure 4.3 Total VFA concentration change in BMP bottles under (a) mesophilic (b) thermophilic conditions

[T: thermophilic, M: mesophilic, NC: non-coated, C: coated, Disp: dispersent, S30;S100: nanoparticle size of 30 and 100 nm average diameter, C6;C75;C150: nanoparticle concentration of 6, 75, and 150 mg/g TS of sludge. Data represents arithmetic mean and error bars represent standard deviation of the two replicates]. For the high concentration of NPs, the total VFA concentration was higher at the end of the experiment except for the coated ZnO NPs. The non-coated NPs showed a continuous slow production of total VFA up to approximately 2000 mg/L. The coated ZnO NPs instead revealed a delayed production of VFA happening between day 9 and 25 and then a slow drop until the end of the experiment. The trend of VFA production/consumption for the BMPs with the coated ZnO NPs at medium concentration was similar to that of BMPs with the high concentration with the exception that the total VFA concentration was higher in BMPs with the high NP concentration (Figure 4.3).

## 4.2. Semi-continuous flow anaerobic sludge digesters

Among three NPs dosages studied with BMP assays, the medium and high dosages are unlikely to occur often and represent special cases based on literature. In order to validate BMP results generated with the low dosage (6 mg NPs/g TS of mixed sludge), semi-continuously fed anaerobic sludge digesters that simulate full-scale operation more closely were operated for a duration of 60 days. The results are presented and discussed in the following sections.

# 4.2.1. Characterization of waste sludge stream

The characterization of the inocula and digester feed (mixed sludge) used to the set-up of the semi-continuous flow digesters is shown in Table 4.2. An attempt was made to keep sludge and inocula characteristics as similar as possible between BMP (Table 4.1) and semi-continuous flow (Table 4.2) digester operations. The pH did not differ more than 5% with the BMP experiment as it is an important factor determining the performance of the anaerobic digestion processes (Appels et al. 2008; Veeken et al. 2000). Additionally, the OLR was set to  $1.53 \pm 0.04$  g VS/L/d during the entire experiment to provide a steady process without an accumulation of VFAs that could potentially decrease the pH because of organic overloading. The OLR was based on recommended

organic loading rates found in literature that were within the range of 0.5 to 7 g VS/L/d (Appels et al. 2008).

Table 4.2 Sludge and inocula characterization for the set-up of the semi-continuous reactors <sup>1</sup>				
Parameters	Mixed	Mesophilic	Thermophilic	
	sludge	inoculum	inoculum	
pH	$5.56\pm0.01^{\textbf{2}}$	$7.35\pm0.01$	$7.86\pm0.01$	
TS (% by weight)	$3.86\pm0.08$	$2.00\pm0.03$	$2.04\pm0.01$	
VS (% by weight)	$3.14\pm0.08$	$1.35\pm0.04$	$1.32\pm0.01$	
Ammonia (mg/L)	$166 \pm 9$	$590 \pm 2$	$958 \pm 4$	
Alkalinity (mg as CaCO <sub>3</sub> /L)	644	2877	4437	
TCOD (mg/L)	$52799 \pm 273$	$21531\pm308$	$22265\pm753$	
SCOD (mg/L)	$4674 \pm 71$	$600 \pm 5$	$182 \pm 2$	

<sup>1</sup>TS/VS: Total solids/volatile solids, TCOD/SCOD: total/soluble chemical oxygen demand <sup>2</sup>Data indicate arithmetic mean ± standard deviation of three replicates

# 4.2.2. Effect of ZnO NPs on biogas production

The biogas measured during the experiments was reported in terms of the specific daily biogas production at STP (0°C and 1 atm, respectively). The statistical analysis was performed with the data collected after having a steady state where values would not fluctuate more than 5% which was reached after one SRT of 60 days. Among the different factors (i.e. type of NPs dosed and temperature), the analysis results (Appendix B, Table B.5) showed that the daily biogas production values obtained at different temperatures were statistically different (p = 0.003 < 0.05). However, the biogas results from controls (unspiked) and digesters with different ZnO NPs were not statistically different (p = 0.06 > 0.05).

In Figure 4.4, the specific daily biogas production for the mesophilic (a) and thermophilic (b) digesters had a similar trend with peaks and troughs happening in the same days as new feed (mixed sludge) from Kelowna's WWTP was obtained and fed to digesters every couple of weeks. At both digester temperatures, both spiked and unspiked (control) digesters produced similar specific daily biogas productions. The average mesophilic and thermophilic specific biogas

productions were  $0.515 \pm 0.017$  and  $0.521 \pm 0.018$  L/g VS<sub>added</sub>-d, respectively. These results correspond to average mesophilic and thermophilic specific biogas yield of  $1.05 \pm 0.01$  and  $0.91 \pm 0.01$  L/g VS<sub>removed</sub>-d, respectively. Similar results have been reported in the literature for digesters utilizing mixed sludge feed (Turovskiy and Mathai 2006). At elevated temperatures, a beneficial increase of the hydrolysis rate and biological reaction rate has been reported (Appels et al 2008) which may suggest that a higher daily biogas/methane production is expected at thermophilic digester temperature. However, in this study, the yields were quite similar at different temperatures possibly due to a safe SRT of 20 days provided to both digesters. The specific daily biogas production and other performance parameters measured for each reactor are included in Table 4.3.

The biogas composition (i.e. % of CH<sub>4</sub>, CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub>) was also monitored in digester headspace. The statistical analysis for the gas composition results showed that there was no significant difference (p > 0.05) among the type of ZnO NPs and control, but the results were statistically different (p < 0.05) at different digester temperatures. The mesophilic and thermophilic digesters had an average of 67 and 70% of CH<sub>4</sub> and 29 and 23% of CO<sub>2</sub> respectively, which were in agreement with literature (Vindis et al. 2009). Digesters had trace amount of O<sub>2</sub> (1-2%) and N<sub>2</sub> (2-3%) which indicated that anaerobic conditions were maintained thought the operation without a major leak.



Figure 4.4 Specific daily biogas production for (a) mesophilic and (b) thermophilic semi-continuous flow anaerobic digesters

[M: mesophilic, T: thermophilic, NC: non-coated, C: coated, S: average diameter size in nm].

Mesophilic Thermophilic							
	G ( G G G )						
Parameters M-Control M-NC(S30) M-NC(S100) M-C(S30) T-Control T-NC(S30) T-NC(S100) T-	C(S30)						
Biogas volume and composition							
<b>Specific biogas</b> 0.517 (0.02) 0.517 (0.029) 0.516 (0.02) 0.509 (0.01) 0.519 (0.02) 0.523 (0.02) 0.521 (0.02)	518 (0.02)						
$(L/g VS_{added}-d)$							
Methane (%) 67.26 (0.74) 67.41 (0.77) 67.77 (0.55) 67.22 (1.16) 70.10 (0.41) 69.90 (0.33) 70.25 (0.54) 70	.13 (0.58)						
<b>TVSC (mg/L)</b> 1292 (183) 201 (66) 185 (65) 63 (25) 775 (52) 166 (33) 150 (35) 54	(27)						
Overall removal efficiency							
<b>TS (%)</b> 41.3 (1.9) 41.0 (1.3) 38.7 (1.4) 38.8 (2.4) 46.5 (0.5) 46.6 (1.5) 46.1 (1.0) 47	.0 (0.7)						
<b>VS (%)</b> 49.0 (2.9) 49.4 (2.6) 49.2 (1.9) 49.2 (1.8) 56.3 (1.5) 57.9 (1.5) 57.2 (1.1) 57	.3 (1.7)						
<b>TCOD</b> (%) 53.0 (2.1) 53.7 (3.4) 53.9 (2.0) 53.6 (2.3) 57.4 (1.3) 58.1 (2.3) 58.7 (1.5) 58	.7 (1.8)						
Effluent characteristics							
<b>pH</b> 7.28 (0.04) 7.28 (0.02) 7.28 (0.03) 7.28 (0.03) 7.93 (0.03) 7.90 (0.03) 7.91 (0.03) 7.8	38 (0.03)						
<b>SCOD</b> (mg/L) 500 (28) 495 (18) 500 (32) 528 (20) 2449 (160) 2362 (132) 1898 (81) 21	92 (41)						
NH <sub>3</sub> -N (mg/L) 934 (51) 921 (30) 922 (57) 906 (66) 1348 (71) 1358 (52) 1333 (39) 13	20 (37)						
Alkalinity 3415 (180) 3474 (129) 3403 (201) 3384 (170) 4706 (181) 4691 (184) 4583 (184) 46	79 (158)						
(mgCaCO <sub>3</sub> /L)							
<b>TVFA (mg/L)</b> 21.5 (3.2) 17.7 (4.6) 13.0 (2.8) 16.3 (3.4) 31.5 (6.2) 30.5 (5.7) 24.2 (4.3) 25	.6 (5.5)						

<sup>1</sup>TVSC: Total volatile sulfur compounds, TS/VS: Total solids/volatile solids, TCOD/SCOD: total/soluble chemical oxygen demand, TVFA: Total volatile fatty acids.

<sup>2</sup>Data indicate arithmetic mean  $\pm$  standard deviation of five to twelve replicates

The average headspace methane percentage results reported above were used to convert specific biogas volumes to specific methane volumes. Even though, a lower methane composition was found for the mesophilic digesters (as illustrated in Figure 4.5), the specific methane yield was still higher for the mesophilic digesters. The average specific daily methane yield was 705 and 638 mL CH<sub>4</sub>/g VS<sub>removed</sub>-d for the mesophilic and thermophilic reactors which indicated that thermophilic methanogens could be more sensitive to the variation of the feed sludge or NP addition (Song et al. 2004).



Figure 4.5 Methane percentage in biogas under mesophilic and thermophilic conditions at steadystate

# [M: mesophilic, T: thermophilic, NC: non-coated, C: coated, S: average diameter size in nm. Data represents arithmetic mean and error bars represent standard deviation of five replicates].

In this study, odour causing VSCs were also monitored in digester biogas. The VSCs measured were summation of eight different compounds including  $H_2S$ , as described in the methodology section. The effect of dosing ZnO NPs was evident in terms of VSCs produced by

the digesters as shown in Figure 4.6. The control mesophilic and thermophilic digesters produced  $1292 \pm 183$  and  $775 \pm 52$  ppm VSCs, respectively. At the top part of Figure 4.6, the VSC removal (%) due to the presence of ZnO NPs with respect to the control digester was also shown. At both digester temperatures, the highest VSCs removal was achieved with the coated ZnO NPs (30 nm average diameter) with 95% and 93% removal for mesophilic and thermophilic digesters respectively; meanwhile, the lowest removal was with the non-coated ZnO NPs (30 nm average diameter), which presented 85% and 79% lower VSCs compared to controls, respectively.



Figure 4.6 Volatile sulfur compounds (VSC) concentration and their removal in the digester headspace under mesophilic and thermophilic conditions

[M: mesophilic, T: thermophilic, NC: non-coated, C: coated, S: average diamater size in nm. Data represents arithmetic mean and error bars represent standard deviation of five replicates].

Among the eight different odor-causing compounds measured in this experiment, H<sub>2</sub>S was

the most predominant compound found in the biogas with higher percentage reduction in the

spiked digesters than the reductions in total VSCs. The  $H_2S$  removal predominance might be due to the precipitation of ZnO into ZnS that could be found in the digested sludge (Lombi et al. 2012).



Figure 4.7 H<sub>2</sub>S concentration and their removal in the digester headspace under mesophilic and thermophilic conditions

# [M: mesophilic, T: thermophilic, NC: non-coated, C: coated, S: average diamater size in nm. Data represents arithmetic mean and error bars represent standard deviation of five replicates)].

From the statistical analysis (Appendix B, Table B.6 and Table B.7), the VSC removal results from two uncoated ZnO NPs with different average diameters were not statistically significant (p = 0.41 > 0.05) but the digesters spiked with coated ZnO NPs yielded statistically significant results compared to the digesters with uncoated ZnO NPs (p < 0.05). The coated ZnO NPs were not pure ZnO, but instead, the presence of the coating silane might have improved their dispersion and availability to react sulphide in the liquid phase and precipitate as ZnS. This would reduce the VSCs formation in the headspace of spiked digesters as observed in Figures 4.6 and 4.7. These results indicate that coated NPs with small diameters can be dosed to anaerobic sludge
digesters at low concentrations to control/mitigate odour-causing VSCs as an alternative to other off-the-shelf chemicals used (Akgul et al. 2017).

#### 4.2.3. Effect of ZnO NPs on other digester performance parameters

The total/organic solids (TS/VS) removals are other important parameters to assess digester performance. The TS removal, shown in Figure 4.8, was similar among the different configurations under mesophilic conditions as well under thermophilic conditions; however, mesophilic anaerobic digesters presented a slightly lower TS removal of 38.8-41.3% compared to thermophilic anaerobic digesters TS removal of 46.5-47.0%. The VS removal, shown in Figure 4.9, presented the same tendency as the TS removal with a VS removal of 49.0-49.4% for mesophilic digester compared to 56.3-57.9% for thermophilic digester. The organic removal efficiency results obtained from the digesters utilizing mixed sludge under the SRT of 20 d (OLR =  $1.53 \pm 0.04$  g VS/L/d) were in agreement with literature (Appels et al. 2008; Wahidunnabi and Eskicioglu 2014). Thus, these results indicate that daily addition of various (coated or non-coated at 30 or 100 nm diameter) ZnO NPs at the dose of 6 mg NPs/g TS of sludge does not cause a discernable inhibition on anaerobic cultures at mesophilic or thermophilic temperatures for an operation time of 60 days.



Figure 4.8 TS removal efficiency of anaerobic digesters under mesophilic and thermophilic conditions



Figure 4.9 VS removal efficiency of anaerobic digesters under mesophilic and thermophilic conditions

[M: mesophilic, T: thermophilic, NC: non-coated, C: coated, S: average diamater size in nm. Data represents arithmetic mean and error bars represent standard deviation of twelve replicates].

The total COD (TCOD) removal was presented in Figure 4.10 as an additional way to evaluate if the organic solids removal would be affected by the presence of NPs. The TCOD removal was 53-54% for the mesophilic digesters while 57-58% for the thermophilic digester. The presence of ZnO NPs at 6 mg NPs/g TS of sludge did not show any discernable difference on the TCOD removal when compared to the control digester. These results supported TS/VS removal results presented in Figures 4.8 and 4.9. In general, TCOD removals were also within the range reported in literature for sludge digesters operated under similar organic loading (Appels et al. 2008).





[M: mesophilic, T: thermophilic, NC: non-coated, C: coated, S: average diamater size in nm. Data represents arithmetic mean and error bars represent standard deviation of ten replicates].

As studies for BMP assays, total VFA accumulation in digesters is another factor that would

help understand whether inhibition can occur under the presence of NPs. In Figure 4.11, the total

VFA concentrations in digesters were different between mesophilic and thermophilic conditions. Total VFAs ranged in mesophilic digesters from 14.1 to 18.1 mg/L while thermophilic digesters showed a wider range (25.3 to 33.9 mg/L). These results are consistent with previous studies that suggested a higher presence of VFAs in thermophilic anaerobic digesters due to higher sensitivity of methane forming bacteria (utilizing VFAs) to different intermediate compounds (Zinder, 1989).





Sludge treatment and disposal have been reported to cost up to 60% of the total operation cost of a WWTP (Foladori et al. 2010). Thus, improvement in dewaterability of digester effluent has been considered an important factor in reducing such high sludge disposal cost. In this study, the capillary suction time (CST) was determined to assess the dewaterability rate of the digester effluent (digestate) with and without the presence of NPs as presented in Figure 4.12. As recommended in the literature, CST results were then normalized by TS concentration (% by weight) of digestate sample. The multi-factor ANOVA (Appendix B, Table B.8) showed statistically significant differences between mesophilic and thermophilic digesters (p < 0) with a CST of 665 and 978 second/% of TS (by weight), respectively. However, there was no statistically significant difference among the normalized CST results of the digestate from the control and the different ZnO NPs dosed digesters (p = 0.85 > 0.05). Digester temperature was the only factor showing statistically significant difference with thermophilic effluent having a higher normalized CST value compared to mesophilic digester. Studies have shown that higher temperature promoting higher rate of protein and polysaccharides release and the release of other high soluble microbial products to liquid phase could cause a slower dewaterability rate (Novak et al. 2003).



Figure 4.12 Specific capillary suction time (CST) for the digestates under mesophilic and thermophilic conditions.

[M: mesophilic, T: thermophilic, NC: non-coated, C: coated, S: average diamater size in nm. Data represents arithmetic mean and error bars represent standard deviation of five replicates].

#### **Chapter 5: Conclusions and recommendations for future work**

This research investigated the effect of ZnO NPs on anaerobic digestion of mixed sludge in two stages. The first stage of experiments included twenty-four different BMP combinations to identify important factors that could cause inhibition on anaerobic microorganisms when municipal sludge (anaerobic digester feed) contains ZnO NPs from consumer products/industrial applications. The factors for the first stage were average NPs particle size (30 nm and 100 nm), NPs type (silane coated and non-coated), NPs concentration (6, 75 and 150 mg NPs/g TS of feed), use of dispersant (sodium dodecylbenzene sulfonate) and digester operating temperature (mesophilic at  $35 \pm 2^{\circ}$ C and thermophilic at  $55 \pm 2^{\circ}$ C).

The second stage testing included only eight different NPs/digester operation combinations since some of the parameters, identified as not statistically significant based on the results from the first-stage, were excluded. The goal of the second stage was to verify the impact of NPs at low ZnO NPs concentration (6 mg NPs/g TS of feed) from the batch tests on the performance of semi-continuous flow anaerobic reactors that resemble full-scale operation. Furthermore, the second stage allowed for the testing and evaluation of more performance parameters that are essential to anaerobic digestion operation.

Based on the experiments results and analysis presented in the previous chapters, the following conclusions were drawn:

• NPs at the low concentration (6 mg NPs/g TS of digester feed) with different average particle sizes (30 nm and 100 nm) did not have statistically significant effect on the biogas production from BMPs assays under mesophilic and thermophilic

temperatures. The low concentration represents expected NPs concentration at wastewater treatment plants at today's society,

- Both silane coated and non-coated NPs at the medium and high concentrations (75 and 150 mg NPs/g TS of digester feed) had statistically significant negative impact on the production of biogas under both mesophilic and thermophilic conditions. However, thermophilic bacteria operated at digester temperature of 55°C were more sensitive to ZnO compared to mesophilic bacteria at 35°C, as at elevated digester temperatures, bacterial diversity and therefore tolerance to environmental disturbances decline,
- Coated NPs created less inhibition than non-coated NPs, possibly due to decreased purity (Zn) of the inhibitor per mass in the coated NPs. Batch reactors dosed with the medium (75 mg ZnO/ g TS) and the high concentrations (150 mg ZnO/ g TS) of coated NPs partially recovered after 25 days of digestion. For the non-coated ZnO NPs, only the mesophilic batch assays were able to recover at the medium concentration and the thermophilic reactors presented chronic inhibition and could not recover. However, the production of biogas from batch reactors dosed with coated NPs slightly improved from its inhibitory condition at the medium and high concentration compared to the non-coated NPs,
- For the semi-continuous flow lab-scale anaerobic reactors, steady-state daily biogas production, TS and VS removal, biogas methane and carbon dioxide composition and dewaterability were not significantly affected by the low concentration of ZnO NPs under mesophilic and thermophilic temperatures,

• In addition to the aforementioned detrimental effects of NPs, as a beneficial outcome, coated ZnO NPs significantly reduced odour causing volatile sulfur compounds in digester headspace in comparison to the non-coated NPs by chemical precipitation.

Based on the conclusions presented and limitations aroused during this study, the following further research are recommended:

- Analysis of other NPs species with the presence and absence of coating to assess whether this property can have reproducible effects on the performance of the different wastewater and sludge treatment processes,
- The ability of NPs to remove odor causing compounds in the reactor headspace should be further investigated and optimized with different type of NPs with coating and various dosing concentrations,
- The interactions of various NPs in municipal sludge feed with emerging pretreatments via thermal (i.e. conventional heating, microwave), mechanical (i.e. sonication, high-pressure homogenization) and biological (i.e. state-digestion, enzyme addition) methods for enhanced methane production should be assessed.

### References

- Abdelsalam, E., Samer, M., Attia, Y.A., Abdel-Hadi, M.A., Hassan, H.E., and Badr, Y.2016.
  Comparison of nanoparticles effects on biogas and methane production from anaerobic digestion of cattle dung slurry. Renewable Energy, 87(1): 592-598. doi: 10.1016/j.renene.2015.10.053
- Ackman, R.G. 1972. The analysis of fatty acids and related materials by gas-liquid chromatography. Progress in the Chemistry of Fats and other Lipids, **12**: 165–284
- Adawi, H. I., Newbold, M. A., Reed, J. M., Vance, M. E., Feitshansd, I. L., Bickford, L. R., and Lewinski, N. A. 2018. Nano-enabled personal care products: Current developments in consumer safety. Nanoimpact, 11: 170–179. doi: 10.1016/j.impact.2018.08.002
- Akgul, D., Abbott, T., and Eskicioglu, C. 2017. Assessing iron and aluminum-based coagulants for odour and pathogen reductions in sludge digesters and enhanced digestate dewaterability. Science of The Total Environment, **598**: 881-888. doi: 10.1016/j.scitotenv.2017.04.141
- American Society for Testing and Materials. 2006. Standard terminology relating to nanotechnology. E 2456-06. West Conshohocken, PA.
- American Public Health Association, APHA. 2005. Standard methods for the examination of water and wastewater. Washington DC, USA
- Appels, L., Baeyens, J., Degrève, J., and Dewil, R. 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Progress in Energy and Combustion Science, 34(6): 755–781. doi: 10.1016/j.pecs.2008.06.002
- Benn, T.M., and Westerhoff, P. 2008. Nanoparticle silver released into water from commercially available sock fabrics. Environmental Science and Technology, 42(11): 4133-4139. doi:10.1021/es7032718
- Benn, T., Cavanagh, B., Hristovski, K., Posner, J.D., and Westerhoff, P. 2010. The Release of Nanosilver from Consumer Products Used in the Home. Journal of Environmental Quality, 39(6): 1875-1882. doi:10.2134/jeq2009.0363

- Blaney, L.M., Cinar, S., and SenGupta, A.K. 2007. Hybrid anion exchanger for trace phosphate removal from water and wastewater. Water Research, 41(7): 1603-1613. doi:10.1016/j.watres.2007.01.008
- Borgia, I., Brunetti, B., Mariani, I., Sgamellotti, A., Cariati, F., Fermo, P., et al. 2002. Heterogeneous distribution of metal nanocrystals in glazes of historical pottery. Applied surface science, 185(3): 206-216. doi:10.1016/S0169-4332(01)00659-6
- Chen, Y., Cheng, J. J., and Creamer, K. S. 2008. Inhibition of anaerobic digestion process: a review. Bioresource technology, **99**(10): 4044-4064
- Code of Federal Regulations. 1999. Title 40: Protection of Environment. Part 503 standards for the use or disposal of sewage sludge. Appendix B to Part 503—Pathogen Treatment Processes.
- Contado, C. 2015. Nanomaterials in consumer products: a challenging analytical problem. Frontiers in Chemistry, **3**. doi:10.3389/fchem.2015.00048
- Cumbal, L., and SenGupta, A.K. 2005. Arsenic removal using polymer-supported hydrated iron (III) oxide nanoparticles: role of Donnan membrane effect. Environmental science & technology, **39**(17): 6508-6515. doi:10.1021/es050175e
- Droste, R. L. 1997. Theory and practice of water and wastewater treatment. Wiley, New York
- Foladori, P., Andreottola, G., and Ziglio, G. 2010. Sludge Reduction Technologies in Wastewater Treatment Plants. IWA publishing, London, UK
- Ganidi, N., Tyrrel, S., and Cartmell, E. 2011. The effect of organic loading rate on foam initiation during mesophilic anaerobic digestion of municipal wastewater sludge. Bioresource Technology, **102**(12): 6637-6643. doi:10.1016/j.biortech.2011.03.057
- García, A., Delgado, L., Torà, J.A., Casals, E., González, E., Puntes, V., et al. 2012. Effect of cerium dioxide, titanium dioxide, silver, and gold nanoparticles on the activity of microbial communities intended in wastewater treatment. Journal of Hazardous Materials, 199–200: 64-72. doi:10.1016/j.jhazmat.2011.10.057

- Gonzalez-Estrella, J., Sierra-Alvarez, R., and Field, J.A. 2013. Toxicity assessment of inorganic nanoparticles to acetoclastic and hydrogenotrophic methanogenic activity in anaerobic granular sludge. Journal of Hazardous Materials, 260: 278-285. doi:10.1016/j.jhazmat.2013.05.029
- Gonzalez-Estrella, J., Puyol, D., Sierra-Alvarez R. and Field, J. 2015. Role of biogenic sulfide in attenuating zinc oxide and coppernanoparticle toxicity to acetoclastic methanogenesis. Journal of Hazardous Materials, 283: 755-763. doi:10.1016/j.jhazmat.2014.10.030
- Hou, L., Li, K., Ding, Y., Li, Y., Chen, J., Wu, X., and Li, X. 2012. Removal of silver nanoparticles in simulated wastewater treatment processes and its impact on COD and NH4 reduction. Chemosphere, 87(3): 248-252. doi:10.1016/j.chemosphere.2011.12.042
- Hou, L., Xia, J., Li, K., Chen, J., Wu, X., and Li, X. 2013. Removal of ZnO nanoparticles in simulated wastewater treatment processes and its effects on COD and NH4+-N reduction. Water Science & Technology, 67(2): 254-260. doi:10.2166/wst.2012.530
- IWA Task Group on Modelling of Anaerobic Digestion Processes. 2002. Anaerobic Digestion Model No. 1 (ADM1). IWA Publishing, London
- Jiang, J., Oberdörster, G., and Biswas, P. 2009. Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies. Journal of Nanoparticle Research, 11(1): 77-89. doi: 10.1007/s11051-008-9446-4
- Jarvie, H.P., Al-Obaidi, H., King, S.M., Bowes, M.J., Lawrence, M.J., Drake, A.F., Green M., and Dobson, P.J. 2009. Fate of silica nanoparticles in simulated primary wastewater treatment. Environmental Science and Technology, 43(22): 8622-8628. doi:10.1021/es901399q
- Kaegi, R., Ulrich, A., Sinnet, B., Vonbank, R., Wichser, A., Zuleeg, S. et al. 2008. Synthetic TiO2 nanoparticle emission from exterior facades into the aquatic environment. Environmental Pollution, **156**(2): 233–239. doi:10.1016/j.envpol.2008.08.004
- Kim, M., Ahn., Y. H., and Speece, R. E. 2002. Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. Water Research, 36(17): 4369-4385. doi.org/10.1016/S0043-1354(02)00147-1

- Kiser, M.A., Westerhoff, P., Benn, T., Wang, Y., Perez-Rivera, J., and Hristovski, K. 2009. Titanium nanomaterial removal and release from wastewater treatment plants. Environmental Science & Technology, 43(17): 6757-6763. doi:10.1021/es901102n
- Kiser, M.A., Ryu, H., Jang, H., Hristovski, K., and Westerhoff, P. 2010. Biosorption of nanoparticles to heterotrophic wastewater biomass. Water Research, 44(14): 4105-4114. doi:10.1016/j.watres.2010.05.036
- Kumar, R., and Münstedt, H. 2005. Silver ion release from antimicrobial polyamide/silver composites. Biomaterials, **26**(14): 2081-2088. doi:10.1016/j.biomaterials.2004.05.030
- Labatut, R. A., Angenent, L. T., and Scott, N. R. 2014. Conventional mesophilic vs. thermophilic anaerobic digestion: A trade-off between performance and stability? Water Research, 53: 249-258. doi:10.1016/j.watres.2014.01.035
- Li, L., Fan, M., Brown, R.C., Van Leeuwen, J., Wang, J., Wang, W., et al. 2006. Synthesis, properties, and environmental applications of nanoscale iron-based materials: a review. Critical Reviews in Environmental Science and Technology, **36**(5): 405-431. doi:10.1080/10643380600620387
- Limbach, L.K., Bereiter, R., Muller, E., Krebs, R., Galli, R., and Stark, W.J. 2008. Removal of oxide nanoparticles in a model wastewater treatment plant: Influence of agglomeration and surfactants on clearing efficiency. Environmental Science & Technology, 42(15): 5828-5833. doi:10.1021/es800091f
- Lombi, E., Donner, E., Tavakkoli, E., Turney, T. W., Naidu, R., Miller, B. W., and Scheckel, K.G.
   2012. Fate of zinc oxide nanoparticles during anaerobic digestion of wastewater and post-treatment processing of sewage sludge. Environmental Science & Technology, 46(16): 9089-9096. doi:10.1021/es301487s
- Lombi, E., Donner, E., Taheri, S., Tavakkoli, E., Jämting, Å.K., McClure, S., et al. 2013. Transformation of four silver/silver chloride nanoparticles during anaerobic treatment of wastewater and post-processing of sewage sludge. Environmental Pollution, **176**: 193-197. doi:10.1016/j.envpol.2013.01.029

- Lu, K. 2012. Nanoparticulate materials: Synthesis, characterization, and processing. Wiley, Somerset, N.J.
- Luna-delRisco, M., Orupõld, K., and Dubourguier, H.C. 2011. Particle-size effect of CuO and ZnO on biogas and methane production during anaerobic digestion. Journal of hazardous materials, **189**(1): 603-608. doi:10.1016/j.jhazmat.2011.02.085
- Ma, R., Levard, C., Judy, J. D., Unrine, J. M., Durenkamp, M., Martin, B., et al. 2014. Fate of Zinc Oxide and Silver Nanoparticles in a Pilot Wastewater Treatment Plant and in Processed Biosolids. Environmental science & technology, 48(1): 104-112. doi:10.1021/es403646x
- Metcalf and Eddy, Tchobanoglous, G., Burton, F.L., Stensel, H.D. 2003. Wastewater Engineering, Treatment and Reuse. McGraw-Hill, New York
- Modena, M. M., Rühle, B., Burg, T. P., and Wuttke, S. 2019. Nanoparticle Characterization: What to Measure? Advanced Materials. doi:10.1002/adma.201901556
- Mu, H., and Chen, Y. 2011. Long-term effect of ZnO nanoparticles on waste activated sludge anaerobic digestion. Water research, **45**(17): 5612-5620. doi:10.1016/j.watres.2011.08.022
- Mu, H., Chen, Y., and Xiao, N. 2011. Effects of metal oxide nanoparticles (TiO2, Al2O3, SiO2 and ZnO) on waste activated sludge anaerobic digestion. Bioresource Technology, 102(22): 10305-10311. doi:10.1016/j.biortech.2011.08.100
- Neale, P. A., Jämting, Å. K., Escher, B. I., & Herrmann, J. 2013. A review of the detection, fate and effects of engineered nanomaterials in wastewater treatment plants. Water Science & Technology, 68(7): 1440-1453. doi:10.2166/wst.2013.388
- Ni, S.Q., Ni, J., Yang, N., and Wang, J. 2013. Effect of magnetic nanoparticles on the performance of activated sludge treatment system. Bioresource technology, 143: 555-561. doi:10.1016/j.biortech.2013.06.041
- Novak, J.T., Sadler, M.E., and Sudhir, N.M. 2003. Mechanisms of floc destruction during anaerobic and aerobic digestion and the effect on conditioning and dewatering of biosolids. Water Research, 37(13): 3136-3144. doi.org/10.1016/S0043-1354(03)00171-4

- Nyberg, L., Turco, R. F., and Nies, L. 2008. Assessing the impact of nanomaterials on anaerobic microbial communities. Environmental science & technology, 42(6): 1938-1943. doi:10.1021/es072018g
- Otero-Gonzalez, L., Field, J. and Sierra-Alvarez, R. 2014. Fate and long-term inhibitory impact of ZnO nanoparticles during high-rate anaerobic wastewater treatment. Journal of Environmental Management, **135**: 110-117. doi:10.1016/j.jenvman.2014.01.025
- Owen, W., Stuckey, D., Healy Jr., J., Young, L., and McCarty, P. 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Research, **13**:485-492.
- Park, H., Kim, H.Y., Cha, S., Ahn, C. H., Roh, J., Park, S. et al. 2013. Removal characteristics of engineered nanoparticles by activated sludge. Chemosphere, 92(5): 524-528. doi:10.1016/j.chemosphere.2013.03.020
- PAS71. 2005. Publicly Available Specification. Vocabulary- Nanoparticles. British Standards Institution (BSI).
- Project on Emerging Nanotechnologies. 2013. Consumer Products Inventory [online]. Available from http://www.nanotechproject.org/cpi [cited 15 June 2019]
- Reidy, B., Haase, A., Luch, A., Dawson, K.A., and Lynch, I. 2013. Mechanisms of Silver Nanoparticle Release, Transformation and Toxicity: A Critical Review of Current Knowledge and Recommendations for Future Studies and Applications. Materials, 6(6): 2295-2350. doi:10.3390/ma6062295
- Schmid, K., Danuser, B., and Riediker, M. 2010. Nanoparticle usage and protection measures in the manufacturing Industry - A representative survey. Journal of Occupational and Environmental Hygiene, 7(4): 224-232. doi:10.1080/15459621003609127
- Serguei, M.K., Nikolai, S.D., and Kirill, M.S. 2012. Safety in large-scale manufacturing of nanoparticles for bio-medical use. Emerging Materials Research, 1(1): 49-60. doi:10.1680/emr.11.00005

- Song, Y. C., Kwon, S. J. and Woo, J. H. 2004. Mesophilic and thermophilic temperature co-phase anaerobic digestion compared with single-stage mesophilic- and thermophilic digestion of sewage sludge. Water Research, 38(7): 1653-1662. doi.org/10.1016/j.watres.2003.12.019
- Taricska, J. R., Long, D. A., Chen, J. P., Hung, Y. T., and Zou, S. W. 2007. Anaerobic digestion.*In* Biosolids Treatment Processes. Handbook of Environmental Engineering. *Edited by*Wang L.K., Shammas N.K., Hung YT. Humana Press. pp. 135-176
- Turovskiy, I.S., and Mathai, P.K. 2006. Wastewater sludge processing. John Wiley & Sons, Inc, Hoboken, New Jersey, USA
- US Environmental Protection Agency. 1979. Process design manual for sludge treatment and disposal. U. S. Environmental Protection Agency.
- US Environmental Protection Agency. 2003. Control of Pathogens and Vector Attraction in Sewage Sludge, EPA 625/R-92/013. US Environmental Protection Agency: Washington, D.C. (revised 2003).
- Van Huyssteen, J. J. 1967. Gas chromatographic separation of anaerobic digester gases using porous polymer. Water Research, **1** (3): 237-242.
- Veeken, A., Kalyuzhnyi, S., Scharff, H., and Hamelers, B. 2000. Effect of pH and VFA on Hydrolysis of Organic Solid Waste. Journal of Environmental Engineering, 126(12): 1076– 1081. doi:10.1061/(asce)0733-9372(2000)126:12(1076)
- Vindis, P., Mursec, B., Janzekovic, M., and Cus, F. 2009. The impact of mesophilic and thermophilic anaerobic digestion on biogas production Manufacturing and processing Manufacturing and processing. Journal of Achievements in Materials and Manufacturing Engineering, 36(2): 192–198.
- Wahidunnabi, A.K, and Eskicioglu, C. 2014. High pressure homogenization and two-phased anaerobic digestion for enhanced biogas conversion from municipal waste sludge. Water Research, 66: 430-446.
- Wang, Y., Westerhoff, P., and Hristovski, K. D. 2012. Fate and biological effects of silver, titanium dioxide, and C60 (fullerene) nanomaterials during simulated wastewater treatment

processes. Journal of Hazardous Materials, **201–202**(16-22): 16-22. doi:10.1016/j.jhazmat.2011.10.086

- Wang, Z., Huang, F., Mei, X., Wang, Q., Song, H., Zhu, C., and Wu, Z. 2014. Long-term operation of an MBR in the presence of zinc oxide nanoparticles reveals no significant adverse effects on its performance. Journal of Membrane Science, 471: 258-264. doi:10.1016/j.memsci.2014.08.024
- Water Environment Federation. 2007. Operation of Wastewater Treatment Plants. 6th ed. Manual of Practice no. 11, vol. 3, Chaps. 27-33. Water Environment Federation, Alexandria, VA.
- Westerhoff, P., Song, G.,Hristovski, K., and Kiser, M.A. 2011. Occurrence and removal of titanium at full scale wastewater treatment plants: Implications for TiO2 nanomaterials. Journal of Environmental Monitoring, 13(5): 1195-1203. doi:10.1039/c1em10017c
- Yang, Y., Chen, Q., Wall, J.D., and Hu, Z. 2012. Potential nanosilver impact on anaerobic digestion at moderate silver concentrations. Water Research, 46(4): 1176-1184. doi:10.1016/j.watres.2011.12.024
- Zhang, W. X. 2003. Nanoscale iron particles for environmental remediation: an overview. Journal of nanoparticle Research, **5**(3-4): 323-332.
- Zheng, X., Wu, R., and Chen, Y. 2011a. Effects of ZnO nanoparticles on wastewater biological nitrogen and phosphorus removal. Environmental science & technology, 45(7): 2826-2832. doi:10.1021/es2000744
- Zheng, X., Chen, Y., & Wu, R. 2011b. Long-term effects of titanium dioxide nanoparticles on nitrogen and phosphorus removal from wastewater and bacterial community shift in activated sludge. Environmental science & technology, 45(17): 7284-7290. doi:10.1021/es2008598
- Zhou, L., Zhang, Z., Xia, S., Jiang, W., Ye, B., Xu, X., et al. 2014. Effects of suspended titanium dioxide nanoparticles on cake layer formation in submerged membrane bioreactor. Bioresource technology, 152: 101-106. doi:10.1016/j.biortech.2013.11.006

Zinder, SH. 1984. Thermophilic waste treatment systems. *In* Thermophiles: general molecular and applied biology. *Edited by* Brock TD. Brock TD. Wiley-Interscience, New York. pp. 257–77

## Appendices

### **Appendix A: Sample calibration curves**





Figure A.2 Ammonia calibration curve



### **Appendix B: Statistical analysis**

### Table B.1 Anova for the effect of the dispersant in the BMP assays

SUMMARY	Control	Dispersant	Total			
Mesophilic						
Count	3	3	6	-		
Sum	1814.69	1767.629	3582.319			
Average	604.8967	589.2097	597.0532			
Variance	135.783	85.90718	162.501			
Thermophilic				-		
Count	3	3	6			
Sum	1917.75	1746.562	3664.311			
Average	639.2499	582.1872	610.7186			
Variance	107.9382	394.0729	1177.651			
Total						
Count	б	6		-		
Sum	3732.44	3514.191				
Average	622.0733	585.6984				
Variance	451.5313	206.7866				
ANOVA						
Source of Variation	55	đf	MS	Б	D voluo	E orit
Tomporatura	560 226	1	560 226	3.006	0.117	5 318
Dispersant	300.220	1	3060.220	21 030	0.002	5 318
presence	3969 398	1	3909.390	21.939	0.002	5.516
Interaction	1283 961	1	1283 961	7 097	0.029	5 318
Within	1/17 /02	8	180 925	1.071	0.027	5.510
	7260.000	0	100.723			
Total	/200.988	11				

Anova: Two-Factor With Replication for the dispersant

# Table B.2 T-test for the effect of the dispersant in the BMP assays under mesophilic conditions

t-Test: Two-Sample Assuming Equal Variances (Mesophilic)

	Control	Disp
Mean	604.8967	589.2097
Variance	135.783	85.90718
Observations	3	3
Pooled Variance	110.8451	
Hypothesized Mean		
Difference	0	
df	4	
t Stat	1.824855	
P(T<=t) one-tail	0.071037	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.142074	
t Critical two-tail	2.776445	

## Table B.3 T-test for the effect of the dispersant in the BMP assays under thermophilic conditions

t-Test: Two-Sample Assuming Equal Variances (Thermophilic)

	Control	Disp
Mean	639.2499	582.1872
Variance	107.9382	394.0729
Observations	3	3
Pooled Variance	251.0055	
Hypothesized Mean		
Difference	0	
df	4	
t Stat	4.411199	
P(T<=t) one-tail	0.005795	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.01159	
t Critical two-tail	2.776445	

# Table B.4 Split-plot analysis for biogas production in the BMP assays considering the effect of NP type, temperature and concentration

Split-plot a	nalysis					
		df	SS	MS	F	р
	Replicate	2	1463	731		
А	Type NP	2	151726	75863	37.68	< 0.01
Error(a)	Error (type)	4	8054	2014		
В	Temperature	1	63938	63938	39.75	< 0.01
AxB	Type x Temp	2	8842	4421	2.75	
Error(b)	Error (Temp)	6	9651	1608		
С	Concentration	2	2205065	1102532	4.24	< 0.05
AxC	Type x Concen	2	77700	38850	0.15	
BxC	Temp x Concen	2	89449	44724	0.17	
	Type x Temp x		28089	7022	0.03	
AxBxC	Concen	4				
Error(c)	Error (Concen)	24	6245173	260216		
	Total	53	8889149			

#### Table B.5 Anova for biogas production in the semi-continuous reactors

Anova for the semi-continuous reactors in terms of gas production

SUMMARY	Control	NC(S30)	C(S30)	NC(S100)	Total
Mesophilic					
Count	41	41	41	41	164
Sum	21199.12	21193.12	21189.16	20860.5	84441.89
Average	517.0517	516.9053	516.8088	508.7927	514.8896
Variance	322.1559	359.9372	328.9947	168.9765	302.0607
Thermophilic					
Count	41	41	41	41	164
Sum	21316.78	21463.16	21376.1	21231.14	85387.17
Average	519.9214	523.4916	521.3682	517.8327	520.6535
Variance	253.3036	340.8291	359.2596	314.5288	315.4372
Total				_	
Count	82	82	82	82	
Sum	42515.9	42656.27	42565.26	42091.64	
Average	518.4865	520.1985	519.0885	513.3127	
Variance	286.2619	357.0364	345.1399	259.4506	

ANOVA							
Source of							
Variation	SS	df	MS	F	P-value	F crit	
Temperature	2724	1	2724	8.903	0.003	3.871	Significant
Type	2297	3	766	2.503	0.059	2.633	Insignificant
Interaction	435	3	145	0.474	0.700	2.633	Insignificant
Within	97919	320	306				C
Total	103376	327					

 Table B.6 Anova for VSC removal of two uncoated ZnO NPs with different average

 diameters in the semi-continuous reactors

Anova: Two-Factor With Replication

SUMMARY	NC(S30)	NC(S100)	Total			
Mesophilic						
Count	5	5	10	-		
Sum	422.2599	428.817	851.0769			
Average	84.45199	85.7634	85.10769			
Variance	20.8905	21.82991	19.46457			
Thermophilic						
Count	5	5	10			
Sum	392.6204	402.9933	795.6137			
Average	78.52407	80.59867	79.56137			
Variance	19.18823	19.49697	18.38896			
Total						
Count	10	10				
Sum	814.8803	831.8103				
Average	81.48803	83.18103				
Variance	27.57394	25.77708				
ANOVA						
Source of	aa	10	МС	Г		E
Variation	22	df	MS	F	P-value	F crit
Temperature	153.8086	1	153.8086	7.557641	0.01426	4.493998

5236 4 49399	0
J <u>2</u> JU <del>1</del> .7/J/J	8

### Table B.7 Anova for VSC removal of coated and uncoated ZnO NPs with different average diameters in the semi-continuous reactors

17

24

29

### **Anova: Two-Factor With Replication**

Within

Total

SUMMARY	NC(S30)	NC(S100)	C(S30)	Total	
Mesophilic					_
Count	5	5	5	15	-
Sum	422.2599	428.817	474.2242	1325.301	
Average	84.45199	85.7634	94.84484	88.35341	
Variance	20.8905	21.82991	6.154644	36.8457	
Thermophilic					_
Count	5	5	5	15	
Sum	392.6204	402.9933	464.7527	1260.366	
Average	78.52407	80.59867	92.95053	84.02442	
Variance	19.18823	19.49697	13.68558	58.41491	
Total				_	
Count	10	10	10		
Sum	814.8803	831.8103	938.9769		
Average	81.48803	83.18103	93.89769		
Variance	27.57394	25.77708	9.814654		
ANOVA					
Source of					
Variation	SS	df	MS	F	P-value
Temperature	141	1	141	8.33	0.01
Туре	906	2	453	26.84	0.00
Interaction	23	2	11	0.68	0.52

405

1474

F cr<u>it</u>

4.26

3.40

3.40

### Table B.8 Anova for biogas production in the semi-continuous reactors

Anova: Two-Factor With Replication

SUMMARY	Control	NC(S30)	NC(S100)	C(S30)	Total
Mesophilic					
Count	5	5	5	5	20
Sum	3326	3400	3341	3228	13295
Average	665	680	668	646	665
Variance	2821	3949	524	2632	2251
Thermophilic					
Count	5	5	5	5	20
Sum	4794	4942	4892	4942	19570
Average	959	988	978	988	979
Variance	3244	4592	1079	8356	3791
Total					
Count	10	10	10	10	
Sum	8120	8342	8233	8170	
Average	812	834	823	817	
Variance	26628	30221	27429	37545	

ANO	VA
-----	----

Source of						
Variation	SS	$d\!f$	MS	F	P-value	F crit
Temperature	984383	1	984383	289.56	1.36E-17	4.15
Type/control	2763	3	921	0.27	0.85	2.90
Interaction	3250	3	1083	0.32	0.81	2.90
Within	108786	32	3400			
Total	1099181	39				