# THE FATE AND TRANSPORT OF ENGINEERED NANOPARTICLES IN WASTEWATER TREATMENT SYSTEMS

by

Soohoon Choi

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Civil Engineering

Fall 2016

© 2016 Soohoon Choi All Rights Reserved ProQuest Number: 10257214

All rights reserved

INFORMATION TO ALL USERS The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 10257214

Published by ProQuest LLC (2017). Copyright of the Dissertation is held by the Author.

All rights reserved. This work is protected against unauthorized copying under Title 17, United States Code Microform Edition © ProQuest LLC.

> ProQuest LLC. 789 East Eisenhower Parkway P.O. Box 1346 Ann Arbor, MI 48106 – 1346

# THE FATE AND TRANSPORT OF ENGINEERED NANOPARTICLES IN WASTEWATER TREATMENT SYSTEMS

by

Soohoon Choi

Approved:

Harry W. Shenton III, Ph.D. Chair of the Department of Civil and Environmental Engineering

Approved:

Babatunde A. Ogunnaike, Ph.D. Dean of the College of Engineering

Approved:

Ann L. Ardis, Ph.D. Senior Vice Provost for Graduate and Professional Education

	I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.
Signed:	Chin-Pao Huang, Ph.D. Professor in charge of dissertation
	I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.
Signed:	Murray V. Johnston, Ph.D. Member of dissertation committee
	I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.
Signed:	Daniel K. Cha, Ph.D. Member of dissertation committee
	I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.
Signed:	Yan Jin, Ph.D. Member of dissertation committee

#### ACKNOWLEDGMENTS

I would like to thank my friends and family for all their help and support. Thanks to you guys it was great to know once again that there is always someone near me that I could lean on.

My highest gratitude and respect to my advisor Chin-Pao Huang. All your help and guidance has made me a better person, inside the lab and in life as well. You will always be a mirror in my conscience, where in tough times I will ask myself "What would C.P. do?" I would also like to thank all the members in my committee. Professor Cha, for his help and advice whenever I needed it, Professor Johnston for all the help in my question in analytical chemistry, and Professor Jin for her general overview and honest opinions on my research. And finally I would like to thank Dr. Hui-Hsien Chang, and Professor Guen Shuh Wang of the National Taiwan University for the work in Taiwan.

Also I would like to thank all the members of the aquatic chemistry group, past and present. We all had a lot of good times together and it wouldn't have been the same without any one of you. Michael Davidson and Rovshan Mahmudov, I could not imagine a day in the lab without you guys. I know I had a ton of help from you and I will always be thankful for it. And finally I would like to thank the EPA for their support on the project R83485901.

And above all my sister Yeon Choi, who would pickup the phone anytime of the day on the other side of the globe for any emotional support she could give. You are a true saint.

## TABLE OF CONTENTS

LIST OF TABLES	ix
LIST OF FIGURES	xiv
ABSTRACT	xx

## Chapter

1	INT	RODU	CTION	1
	1.1	Backg	round	1
	1.2	Engin	eered Nanoparticle Utilization and Daily Exposure	2
	1.3	Runof	f of engineered nanoparticles to the environment	3
	1.4	Curren	nt Research on Engineered Nanoparticles in Wastewater	
		Treatn	nent Plants	4
	1.5	Purpo	se of research	5
	1.6	Overv	iew	6
2	IDE	NTIFIC	CATION AND QUANTIFICATION OF ENGINEERED	
	NAN	NOPAR	TICLES (ENPs) IN WASTEWATER TREATMENT	
	SYS	TMES		
	2.1	Introd	uction	
	2.2	Mater	ials and Methods	15
		2.2.1	Sample collection	15
		2.2.2	Removal of salts and organic decrements	
		2.2.3	ICP Analysis	
		2.2.4	SEM analysis	
	2.3	Result	ts and Discussion	
		2.3.1	Method Development	
		2.3.2	Distribution of engineered nanoparticles in wastewater	01
		233	Engineered nanoparticle detection in the environment. A	
		2.0.0	Taiwan case study	41

		2.3.4	Seasonal variation of engineered nanoparticles in wastewater treatment plants	55
	2.4	Concl	usion	68
3	INT SLU	ERACI IDGE P	TONS BETWEEN ENGINEERED NANOPARTICLES AND ARTICULATES WITH SIZE LESS THAN 1 μM	70
	3.1 3.2 3.3	Introd Theor Mater	uction etical Aspects ial and Methods	70 76 78
		3.3.1 3.3.2 3.3.3	Preparation of engineered nanoparticles Collection and preparation of dissolved organic matter Size measurement	78 78 79
	3.4 3.5	Result Concl	s and Discussion	80 93
4	INT SLU	ERACT IDGE P	TONS BETWEEN ENGINEERED NANOPARTICLES AND ARTICULATES WITH SIZE GREATER THAN 1 $\mu$ M	94
	4.1 4.2	Introd Theor Uptak	uction etical Aspects: Quantification of Engineered Nanoparticle e	94 96
		4.2.1 4.2.2 4.2.3	Sedimentation of engineered nanoparticle adsorbed sludge particulates Analysis of sedimentation curves Determination of free engineered nanoparticle concentration 1	96 97 01
	4.3	Mater 4.3.1 4.3.2	ials and Methods 1 Preparation of engineered nanoparticles and sludge 1 Sedimentation experiment 1	.04 104 106
	4.4	Result	s and Discussion	06
		4.4.1 4.4.2 4.4.3	Effect of sludge particulate size on engineered nanoparticle sorption	.09 128 137
		4.4.4	Effect of Engineered Nanoparticle Type: ZnO, TiO <sub>2</sub> , SiO <sub>2</sub> 1	42

	4.5	Conclusion	. 145
5	MA.	OR FINDINGS AND RESEARCH NEEDS	. 147
	5.1	Major findings	. 147
	5.2	Future research needs	. 148
REFER	ENC	CES	. 151

## Appendix

А	SCHEMATIC OF DIALYSIS SYSTEM	164
В	CONDUCTIVITY OF DESALTED SLUDGE WITH AND WITHOUT	
	WATER CIRCULATION IN SUBMERGED TANK	165
С	OXIDATION OF SLUDGE BY WET PEROXIDE METHOD	166
D	EFFECT OF ACID DIGESTION	167
Е	SPATIAL DISTRIBUTION OF ENGINEERED NANOPARTICLES IN	
	WASTEWATER TREATMENT PLANT B	168
F	SPATIAL DISTRIBUTION OF ENGINEERED NANOPARTICLES IN	
	WASTEWATER TREATMENT PLANT W	169
G	ENGINEERED NANOPARTICLE CONCENTRATION IN SIAO LI	
	RIVER	170
Η	U.S. MONTHLY SALES OF HEALTH AND PERSONAL CARE	
	PRODUCTS	181
Ι	CONCENTRATION OF TITANIUM IN WASTEWATER TREATMEN	Т
	PLANT B	182
J	ATTACHMENT EFFICIENCY STUDIES OF ENGINEERED	
	NANOPARTICLES	188
Κ	ATTACHMENT OF DOM TO ZnO NANOPARTICLES	191
L	SEDIMENT CURVE AND ANALYSIS OF ENGINEERED	
	NANOPARTICLE TO SLUDGE	192
М	SORPTION OF VARIOUS ENGINEERED NANOPARTICLES TO	
	SECONDARY SLUDGE FROM WASTEWATER TREATMENT	
	PLANTS	193
Ν	SEDIMENTATION PROFILE OF NANOPARTICLES TO SLUDGE	194
0	EFFECT OF SLUDGE PARTICULATE SIZE ON NANOPARTICLE	
	ATTACHMENT	210
Р	EFFECT OF IONIC STRENGTH ON THE ATTACHMENT OF TIO2 O	N
	SLUDGE PARTICULATES	212
Q	EFFECT OF MLSS ON NANOPARTICLE ATTACHMENT	213
R	ZETA POTENTIAL VS. pH OF ENGINEERED NANOPARTICLES	217

S	ATTACHMENT OF VARIOUS ENGINEERED NANOPARTICLES TO	
	SLUDGE	218

## LIST OF TABLES

Table 2.1 Identification and quantification of ENP in wastewater    9
Table 3.1 Critical coagulation concentration (CCC) of TiO <sub>2</sub> under various pH and salt conditions. Experimental conditions: [TiO <sub>2</sub> ]= 80 ppm, Temperature = 25°C
Table 3.2 Critical coagulation concentrations of TiO <sub>2</sub> and ZnO under various dissolved organic matter (DOM) concentrations. Experimental conditions: [TiO <sub>2</sub> ] = 50 mg/L; [ZnO] = 50 mg/L; Temperature = 25°C; electrolyte = NaCl; [DOM] = 300 mg/L; pH = 6
Table 4.1 Sorption parameters of TiO <sub>2</sub> and ZnO with sludge particulates of various sizes. The parameters were based on the results shown in Fig. 4.7 and 4.8. Unit of parameters: $K_F = (\mu g/mg)(L/mg)^{1/n}$
Table 4.2 Summary of sorption parameters of TiO <sub>2</sub> to sludge under various ionic strength conditions. Unit of parameters: $\Gamma_{max} = \mu g$ -ENP/mg-Dry sludge, K <sub>L</sub> = L/mg, (L/mol), $\Delta G = Kcal/mol$
Table B1. Conductivity of sludge in dialysis tubes with and without watercirculation in the water tank. (Data for Fig. 2.2)165
Table C1. Removal of organic content in sludge sample with wet peroxide method.         (Data for Fig. 2.3)
Table D1. The effect of acid digestion (AD) on the recovery of engineerednanoparticles. (Data for Fig. 2.4)167
Table E1. Spatial distribution of engineered nanoparticles in wastewater treatmentplant B. (Data for Fig. 2.7 (a), (b), (c))
Table F1. Concentration, Mass flow, and nanoparticle concentration per weight of dry sludge in wastewater treatment plant W. The units of the results are in µg/L, kg/day, and g/kg, respectively (Data for Fig. 2.8 (a), (b), (c), (d), (e))
Table G1. Nanoparticle concentrations of various sample positions in the Sio Li         river. (Data for Fig. 2.11)

Table G2. Nanoparticle analysis from samples collected at the New Fulong tea         factory. (Data for Fig. 2.12(a))
Table G3. Nanoparticle analysis from samples collected at He Sing bridge (Data for Fig. 2.12 (b))
Table G4. Nanoparticle analysis from samples collected at Long Sing bridge (Data for Fig. 2.12 (c))173
Table G5. Nanoparticle analysis from samples collected at Siao Li bridge (Data for      Fig. 2.12 (d))
Table G6. Nanoparticle analysis from samples collected at Bao Shih bridge (Data for Fig. 2.12 (e))175
Table G7. Nanoparticle analysis from samples collected at Fong Hunag bridge (Data for Fig. 2.13 (a))
Table G8. Nanoparticle analysis from samples collected at Jhong Sing bridge (Data for Fig. 2.13 (b))       177
Table G9. Nanoparticle analysis from samples collected at Jhen Sing bridge (Data for Fig. 2.13 (c))         178
Table G10. Nanoparticle analysis from samples collected at Tian Gong monastery         bridge (Data for Fig. 2.13 (d))
Table G11. Nanoparticle analysis from samples collected at Niou Be bridge bridge (Data for Fig. 2.13 (e))       180
Table H1. U.S. monthly sales of health and personal care products. Source:Advanced monthly retail trade report, U.S. census bureau. (Data forFig. 2.15)181
<ul> <li>Table I1. Concentration of titanium in wastewater treatment plant B. The concentration of the titanium is in µg/L. Sampling locations are sited at the far left of the table based on the numbering designation of Fig. 2.1. (Data for Fig. 2.17)</li></ul>
Table I2. Concentration of zinc in wastewater treatment plant B. The concentration of the zinc is in $\mu$ g/L. Sampling locations are sited at the far left of the table based on the numbering designation of Fig 2.1. (Data for Fig. 2.18)

Table I3. Mass flow of titanium in wastewater treatment plant B. The concentration of the titanium is in kg/day. Sampling locations are sited at the far left of the table based on the numbering designation of Fig 2.1. (Data for Fig. 2.19)
Table I4. Mass flow of zinc in wastewater treatment plant B. The concentration of the zinc is in kg/day. Sampling locations are sited at the far left of the table based on the numbering designation of Fig 2.1. (Data for Fig. 2.20)
Table I5. Titanium concentration per weight of dry sludge in wastewater treatment plant B. The units of the results are in g/kg. Sampling locations are sited at the far left of the table based on the numbering designation of Fig 2.1. (Data for Fig. 2.21)
<ul><li>Table I6. Zinc concentration per weight of dry sludge in wastewater treatment plant</li><li>B. The units of the results are in g/kg. Sampling locations are sited at the far left of the table based on the numbering designation of Fig 2.1. (Data for Fig. 2.22)</li></ul>
Table J1. Attachment efficiency (AE) of TiO2 under various pH and electrolyte conditions. (Data for Fig. 3.2, 3.3)         188
Table J2. Attachment efficiency (AE) of TiO2 under various dissolved organicmatter (DOM) concentrations. (Data for Fig. 3.4(a))
Table J3. Attachment efficiency (AE) of TiO2 under various dissolved organicmatter (DOM) concentrations. (Data for Fig. 3.4(b))
Table K1. Attachment of primary and secondary organic matter to ZnO         nanoparticles (Data for Fig. 3.5)
Table L1. Sedimentation cure and analysis of engineered nanoparticle to sludge.         (Data for Fig. 4.1)
<ul> <li>Table L1. Sorption of various engineered nanoparticles to secondary sludge from wastewater treatment plant. Textile #1,2,3 [14], TiO<sub>2</sub> in lab reactor [15], TiO<sub>2</sub>-sludge and ZnO-sludge are each from the current research. (Data for Fig. 4.2)</li></ul>
Table N1. Sedimentation profile of TiO <sub>2</sub> with dissolved organic matter from the primary sediment tank. (Data for Fig. 4.3(a))

Table N2. Sedimentation profile of TiO <sub>2</sub> with dissolved organic matter from the secondary sediment tank. (Data for Fig 4.2 (b))195
Table N3. Sedimentation profile of ZnO with dissolved organic matter from the primary sediment tank. (Data for Fig 4.2 (c))
Table N4. Sedimentation profile of ZnO with dissolved organic matter from the secondary sediment tank. (Data for Fig 4.2 (d))
Table N6. Sedimentation profile of TiO <sub>2</sub> with diameter $100nm < d < 1 \mu m$ from the secondary sediment tank. (Data for Fig. 4.4 (b))
Table N7. Sedimentation profile of ZnO with diameter 100nm < <i>d</i> < 1 μm from the primary sediment tank. (Data for Fig. 4.4 (c))
Table N8. Sedimentation profile of ZnO with diameter 100nm < <i>d</i> < 1 μm from the secondary sediment tank. (Data for Fig. 4.4 (d))
Table N9. Sedimentation profile of TiO <sub>2</sub> with diameter $d > 1 \mu m$ from the primary sediment tank. (Data for Fig. 4.5 (a))
Table N10. Sedimentation profile of TiO <sub>2</sub> with diameter $d > 1 \mu m$ from the secondary sediment tank. (Data for Fig. 4.5 (b))
Table N11. Sedimentation profile of ZnO with diameter $d > 1 \mu m$ from the primary sediment tank. (Data for Fig. 4.5 (c))
Table N12. Sedimentation profile of ZnO with diameter $d > 1 \mu m$ from the secondary sediment tank. (Data for Fig. 4.5 (d))
Table N13. Sedimentation profile of TiO2 in sludge from the primary sedimenttank. (Data for Fig. 4.6 (a))206
Table N14. Sedimentation profile of TiO2 in sludge from the secondary sediment tank. (Data for Fig. 4.6 (b))       207
Table N16. Sedimentation profile of ZnO in sludge from the secondary sediment tank. (Data for Fig. 4.6 (d))       209
Table O1. Effect of sludge particulate size on the attachment on TiO2. The data isshown in a graphical from in Fig. 4.7. (Data for Fig. 4.7)210
Table O2. Effect of sludge particulate size on the attachment on ZnO. The data is shown in a graphical from in Fig. 4.8. (Data for Fig. 4.8)

Table P1. Effect of ionic strength on the attachment of TiO <sub>2</sub> onto sludge particulates. Units: $C_e = mg/L$ , $\Gamma = \mu g$ -ENP/mg-dry sludge. (Data for
Fig. 4.10, 11)
Table Q1. Attachment of TiO2 to primary waste activated sludge of various concentrations. The graph of the following table is shown in Fig. 4.12 (a)
<ul> <li>Table Q2. Attachment of TiO<sub>2</sub> to secondary waste activated sludge of various concentrations. The graph of the following table is shown in Fig. 4.12</li> <li>(b)</li></ul>
Table Q3. Attachment of ZnO to primary waste activated sludge of various concentrations. The graph of the following table is shown in Fig. 4.13 (a)
Table Q4. Attachment of ZnO to secondary waste activated sludge of various concentrations. The graph of the following table is shown in Fig. 4.13 (b)
Table R1. Zeta potential vs. pH of ENP particles and organic material. (Data for      Fig. 4.14)
Table S1. Attachment of various engineered nanoparticles composed of different materials. (Data for Fig. 4.15)         218

## LIST OF FIGURES

Figure 2.1	Wastewater treatment plant (WWTP) studied. Two WWTP were selected in this study, a; WWTP B, and b; WWTP W. The sampling locations are identified by the dual-digit system in the figure. The first digit (before the hyphen) indicates the sedimentation basin, and the second digit (after the hyphen) indicates the (1) inflow, (2) outflow, and (3) waste sludge of each sedimentation basin. For the secondary waste sludge in WWTP B, it is divided into two separate categories where 2.3.1 is the return sludge being re-circulated into the aeration basin and 2.3.2 the waste sludge that undergoes sludge thickening. The flux of the water flow of each sampling location was also	
	displayed in the figures	17
Figure 2.2	Conductivity profile of the dialysis of activated sludge as a function of time. Dialysis was conducted under two different conditions: ( $\blacktriangle$ ) the dialysis tube was submerged in a continuous batch unit (Fig. 2.2) with water circulation; ( $\bigcirc$ ) the dialysis tube was seemed in a continuous bath unit as above (Fig. 2.2) except without water circulation. (Data is illustrated in the Appendix Table B1)	22
Figure 2.3	Removal of organic content in sludge sample by the wet $H_2O_2$ oxidation method. Experimental conditions: [MLSS] = 1000 mg/L, sludge quantity = 100 mL, $H_2O_2$ (30%) = varying, Temperature = $105^{\circ}C$ , time = 1 h 1 <sup>st</sup> cycle ( $\bigcirc$ ), 2 <sup>nd</sup> cycle ( $\bigcirc$ ) (Data is illustrated in the Appendix Table C1).	24
Figure 2.4	The effect of acid digestion (AD) on the recovery of ENP. Each axis shows the observed concentration of ENP as a function of the added concentration of engineered nanoparticles. Open symbols: without acid digestion; Closed symbols: with acid digestion. Experimental conditions: [HNO <sub>3</sub> ] = 15 mg/L, [H <sub>2</sub> SO <sub>4</sub> ] = 5 mg/L, Temperature = 95°C, Time = 2 h. (Data is illustrated in the Appendix Table D1)	26

Figure 2.5	SEM images of oxidized sludge (a) $\sim$ (g). Outset voltage 23kV, (Hitachi 4700 FE-SEM) (a) SEM image of TiO <sub>2</sub> sorbed sludge, (b) magnified image showing aggregated TiO <sub>2</sub> adsorbed to the flocs, (c) SEM image of sludge particulate containing TiO <sub>2</sub> (d) Elemental coloring of TiO <sub>2</sub> imbedded sludge flocs, where organic material is shown in green and Ti shown in orange, (e) SEM image of TiO <sub>2</sub> entrapped in inorganic granulates from wastewater samples
Figure 2.6	SEM image and elemental scanning of $TiO_2$ nano-particles imbedded in sludge particulate. (a) SEM image of sludge particulate, (b) titanium scanning, white dots indicate elemental titanium. (c) elemental scanning of oxygen, (d) overlapping of images (a) and (c). Red dots represent the overlapping elements (titanium and oxygen) 30
Figure 2.7	Spatial distribution of ENP in WWTP B. (November, 2015), (a) liquid phase concentration, (b) concentration per weight of dry sludge, (c) Mass flow rate. (Data is illustrated in the Appendix Table E1)
Figure 2.8	Spatial distribution of ENP in WWTP W. (a) Liquid phase concentration of zinc, (b) Liquid phase concentration of titanium, (c) concentration per weight of dry sludge, (d) Mass flow rate of Zinc, (e) Mass flow rate of titanium. (Data is illustrated in the Appendix Table F1)
Figure 2.9	The detailed map of sampling stations $#1$ ~ $#5$ . The location of the sampling station is; 1 = New Fulong Tea Factory, 2 = De Sing Bridge, 3 = Long Sing bridge, 4 = Siao Li Bridge, 5 = Bao Shih bridge
Figure 2.10	Sampling positions within Taiwan. Sample $6\sim10$ . The location of the sampling station is; $6 =$ Fong Huang bridge, $7 =$ Jong Sing bridge, $8 =$ Hisinchu science park, $9 =$ Tian Gong monastery, and $10 =$ Niou Bu bridge
Figure 2.11	ENP concentration in the river samples filtered with 5- $\mu$ m membrane. The sample numbers represent the ten sampling stations of the Siao Li river (1~5), and the Ke Ya river (6~10). (Data of the figure is illustrated in the Appendix Table G1)
Figure 2.12	Distribution of engineering nanoparticle in the Siao Li river. (a) 1: New Fulong Tea Factory, (b) 2: HeSing bridge, (c) 3: Long Sing bridge, (d) 4: Siao Li bridge, (e) 5: Bao Shih bridge. The sample sub number represents the different size range of particles in the sample. (1: No treatment, 2: > 9 $\mu$ m, 3: > 230 nm, 4: > 65 nm, 5: < 65 nm) (Data is illustrated in the Appendix Table G2~G6)

Figure 2.13	The distribution of engineered nanoparticle concentration in the Ki Ya river. (a) 6: Fong Huang bridge, (b) 7: Jong Sing bridge, (c) 8: Jhen Sing bridge, (d) 9: Tian Gong monastery, (e) 10: Niou Bu bridge. The sample sub number represents the different size range of particles in the sample. (1: No treatment, $2: > 9 \mu m$ , $3: > 230 nm$ , $4: > 65 nm$ , $5: < 65 nm$ ) (Data is illustrated in the Appendix Table G7~G11)	53
Figure 2.14	Monthly inflow of TiO <sub>2</sub> and ZnO nanoparticles into wastewater treatment plant B. Samples were acquired at the inflow of the primary sediment basin after the grit chamber. Samples were collected throughout the year 2012. (Data of the current graph is illustrated in the Appendix Table I1 for titanium and I2 for zinc)	56
Figure 2.15	U.S. Monthly sales of Health and personal care products (01/2012~12/2015) Source: Advanced monthly retail trade report, U.S. census bureau. (The dotted grey lines show the sales in December) (Data is illustrated in the Appendix Table H1)	58
Figure 2.16	Monthly accumulation of $TiO_2$ and ZnO nanoparticles in the aeration basin of wastewater treatment plant B. Samples were acquired at the return sludge flow of the secondary sedimentation tank. Samples were collected throughout the year 2012. (Data of the current graph is illustrated in the Appendix Table II for titanium and I2 for zinc)	59
Figure 2.17	Seasonal variation of total Ti at various sample points in the wastewater treatment plant B. Samples were collected through the year 2012 (Data is illustrated in the Appendix Table I1)	51
Figure 2.18	Seasonal variation of total Zn at various sample points in the wastewater treatment plant B. Samples were collected through the year 2012(Data is illustrated in the Appendix Table I2)	51
Figure 2.19	Total Ti flux at various sampling points in wastewater treatment plant B. Samples were collected through the year 2012 (Data is illustrated in the Appendix Table I3)	54
Figure 2.20	Total flux of Zn at various sampling points in WWTP B. Samples were collected through the year 2012. (Data is illustrated in the Appendix Table I4)	54
Figure 2.21	The total Ti to MLSS mass ratio at different sample points in wastewater treatment plant B. Samples were collected through the year 2012. (Data is illustrated in the Appendix Table I5)	56

Figure 2.22	The total Zn to MLSS mass ratio at different sample points in wastewater treatment plant B. Samples were collected through the year 2012. (Data is illustrated in the Appendix Table I6)
Figure 3.1	Dynamic light scattering (DLS) measurements of $TiO_2$ aggregation. The aggregation profile was measured as a function of salt concentration. Conditions: $[TiO_2] = 10 \text{ mg/L}$ , pH = 8, NaCl used as an electrolyte
Figure 3.2	Stability ratio as a function of various pH conditions. Experimental conditions: $[TiO_2] = 80 \text{ mg/L}$ ; Temperature = 25°C; electrolyte = NaCl. (Data is illustrated in the Appendix Table J1)
Figure 3.3	Stability ratio as a function of electrolyte concentration. Experimental conditions $[TiO_2] = 80 \text{ mg/L}$ ; Temperature = 25°C; pH = 3 (Data is illustrated in the Appendix Table J1)
Figure 3.4	Stability ratio as a function of various DOM conditions. Experimental conditions: $[TiO_2] = 50 \text{ mg/L}$ ; $[ZnO] = 50 \text{ mg/L}$ ; Temperature = $25^{\circ}C$ ; electrolyte = NaCl; $[DOM] = 300 \text{ mg/L}$ ; pH = 6. (Data is illustrated in the Appendix Table J2, and J3)
Figure 3.5	Sorption study of DOM and small organic particulates with ZnO. Dissolved organic matter ( $d < 100$ nm), Small organic particulates (100 nm $< d < 1 \mu$ m), pH = 6.5. (a) Organics obtained from primary waste sludge. (b) Organics obtained from secondary waste sludge. (Data is illustrated in the Appendix Table K1)
Figure 4.1	Sedimentation curve and analysis of ENP to sludge. Conditions: $pH = 6.5$ , $[ZnO] = 80 \text{ mg/L}$ , Sludge = Wilmington Primary sludge. (a) Linear fit for "Type 1" and Type 2" sedimentation. The overlap of the two trend lines indicate $t^*_{12}$ . (b) Linear fit for "Type 2" and Type 3" sedimentation. The overlap of the two trend lines indicate $t^*_{23}$ . (c) Linear fit for "Type 1", "Type 2", and "Type 3"sedimentation. (Data of the figures are illustrated in Appendix 4.1)

Figure 4.2	Sorption of various engineered nanoparticles to secondary sludge from wastewater treatment plants. Silver nanoparticle from textile #1 [14], Silver nanoparticle from textile #2 [14], Silver nanoparticle from textile #3 [14], TiO <sub>2</sub> sorption in wastewater reactor [15], TiO <sub>2</sub> in wastewater of low organic loads, TiO <sub>2</sub> in wastewater of high organic loads, ZnO in wastewater of low organic loads, TiO <sub>2</sub> in wastewater of high organic loads. The results are based on batch reactor tests with continuous inflow of nanoparticles. The mg-biomass is based on the dry weight of the bioreactor sludge. (Data is illustrated in the Appendix Table M1)
Figure 4.3	Sedimentation profile of Zn and TiO <sub>2</sub> with DOM. Primary [DOM] = $400 \text{ mg/L}$ , Secondary [DOM] = $250 \text{ mg/L}$ . Reaction time = 5 hr. pH = $6.8 \text{ (a)}$ Primary DOM + TiO <sub>2</sub> (b) Secondary DOM + TiO <sub>2</sub> . (c) Primary DOM + ZnO (d) Secondary DOM + ZnO. (Data is illustrated in the Appendix Table N1~N4)
Figure 4.4	Sedimentation profile of ZnO and TiO <sub>2</sub> with supernatant. Primary supernatant [MLSS] = 567 mg/L. Reaction time = 5 hr. pH = 6.8 (a) Primary supernatant + TiO <sub>2</sub> (b) Secondary supernatant + TiO <sub>2</sub> . (c) Primary supernatant + ZnO (d) Secondary supernatant + ZnO. (Data is illustrated in the Appendix Table N5-N8)
Figure 4.5	Sedimentation profile of Zn and TiO <sub>2</sub> with large organic particles. Primary [MLSS] = 2242 mg/L, Secondary [MLSS] = 6100 mg/L. Reaction time = 5hr. pH = 6.8 (a) Primary + TiO <sub>2</sub> (b) Secondary + TiO <sub>2</sub> . (c) Primary + ZnO (d) Secondary + ZnO. (Data is illustrated in the Appendix Table N9-N12)
Figure 4.6	Sedimentation profile of Zn and TiO <sub>2</sub> with sludge. Primary [MLSS] = 2942 mg/L, Secondary [MLSS] = 6667 mg/L. Reaction time = 5 hr. pH = 6.8 (a) Primary sludge + TiO <sub>2</sub> (b) Secondary sludge + TiO <sub>2</sub> . (c) Primary sludge + ZnO (d) Secondary sludge + ZnO. (Data is illustrated in the Appendix Table N13-N16)
Figure 4.7	Effect of the sludge particulate size on the sorption on TiO <sub>2.</sub> (Data is illustrated in the Appendix Table O1)
Figure 4.8	Effect of the sludge particulate size on the sorption on ZnO. (Data is illustrated in the Appendix Table O2)
Figure 4.9	Summary of sludge particulate size on the effect of $TiO_2$ and $ZnO$ uptake. (Data is illustrated in the Appendix Table O1, and O2)

Figure 4.10	Effect of ionic strength on the adsorption of $TiO_2$ onto sludge particulates. Experimental conditions: $pH = 6.84$ , $[MLSS] = 2186$ mg/L, electrolyte = NaCl, Secondary sludge (Data is illustrated in the appendix Table P1)
Figure 4.11	Dual sorption model of $TiO_2$ to secondary sludge, under various ionic strengths. The sorption diagram (Total) was divided into two layers (1 <sup>st</sup> , 2 <sup>nd</sup> layer) and separately analyzed for its sorption parameters (model). Experimental conditions: pH = 6.84, [MLSS] = 2186 mg/L (a) [NaCl] = 280mM, (b) 480mM, (c) 680mM, (d) 830mM. (Data is illustrated in the Appendix Table P1)
Figure 4.12	Attachment of TiO <sub>2</sub> to wastewater sludge of various concentrations. (a) Primary sludge (b) Secondary sludge. (Data is illustrated in the Appendix Table Q1, and Q2)
Figure 4.13	Attachment of ZnO to wastewater sludge of various concentrations. (a) Primary sludge (b) Secondary sludge. (Data is illustrated in the Appendix Table Q2, and Q3)
Figure 4.14	Zeta potential vs. pH of ENP particles ( $TiO_2$ , ZnO, $SiO_2$ ). Experimental conditions: [ $TiO_2$ ] = 50 mg/L, [ $ZnO$ ] = 50 mg/L, [ $SiO_2$ ] = 1 mg/L, [Alginate] = 100 mg/L, [Humic Acid] = 100 mg/L. (Data is illustrated in the Appendix Table R1)
Figure 4.15	Sorption of engineered nanoparticles composed of various elements. TiO <sub>2</sub> , ZnO, SiO <sub>2</sub> . (Data is illustrated in the Appendix Table S1)
Figure A1.	Schematic outlay of dialysis test unit. Deionized (DI) water was pumped into a stirred reservoir (2 L) for dialysis. A dialysis tube (DT) containing wastewater samples were submerged in the reservoir for salt removal. The water was pumped into the reservoir at a flow rate of 0.1 mL/h

#### ABSTRACT

To comprehend the engineered nanoparticle release to waster, soil, and air, it is important to understand the discharge from specific points of release. And as a point of release, wastewater treatment plants play a critical role in the collection and redistribution of nanoparticles in municipal waste streams. So to better understand its fate and transport, the current study focuses on three major subjects regarding the quantification, particle-particle interaction, and particle-organic sorption of engineered nanoparticles in wastewater treatment plants.

To quantify engineered nanoparticles, a novel pretreatment method was developed for ICP-OES analysis. Samples were collected from various locations in the treatment plant, mainly to verify the transport pathway, and mass balance of each sedimentation basins. A concentration profile was mapped out, to visualize the distribution of engineered nanoparticles throughout the treatment plant. Additional aspects such as the mass flux, particle to organic ratio, and seasonal variations were also mapped out for further analysis. Results showed an overall 80% removal of titanium and 68% removal of zinc through primary and secondary sludge particulates, respectively, indicating the importance of activated sludge in the fate of engineered nanoparticles. Seasonal effects showed elevated amounts through summer and winter, matching the seasonal consumption patterns of nanoparticle imbedded products.

To understand the mechanism behind the fate of engineered nanoparticles in the wastewater treatment plants, the next sections were focused on the interactions of the particles. The first mechanism observed, was the particle-particle interaction of nanoparticles under high concentrations of dissolved organic matter. The main purpose of the observation was to understand the transport characteristics of nanoparticles through attachment efficiency experiments. The uniqueness of the current study was the fact that experimental conditions were set to simulate field conditions, where dissolved organic matter from field-operated plants were tested under TOC concentrations that match the inflow of wastewater treatment plants. Results showed the influence of organic concentration on the stability of particles, where higher organic loading will increase the stability. The second mechanism observed was the interaction of engineered nanoparticles and wastewater sludge. Based on the distribution results in the wastewater treatment plants, particle-organic sorption experiments were conducted with primary and secondary sludge particulates. The main object was to understand the attachment of engineered nanoparticles to wastewater sludge. Sedimentation experiments were conducted to measure the attachment of both suspended materials under various conditions. Conditions such as ionic strength, organic loading, and nanoparticle compositions were tested to verify the impacts of field conditions. Results showed elevated sorption of ENP under lower organic loads, lower ionic strengths and strongly hydrophobic nanoparticles. The knowledge acquired from the current research, can be a basis for future research and policy regulations.

#### Chapter 1

#### **INTRODUCTION**

#### 1.1 Background

Due to its novel behavior and large specific surface area, nanotechnology has been growing exponentially during the past decades. [1] The utilization of nanomaterials have been expending to commercial products such as filters [2], semiconductors [3], cosmetics [4], microelectronics [5], pharmaceuticals [6], drug carriers [7], energy storage [8] and sensors [9,10]. Development of the technology has started with establishing fundamental knowledge focusing on the documentation of nanomaterials, where currently up to 40 elements have been used, and with 20 or so more elements soon to be synthesize in the market. [11] For the past two decades, the average annual growth of journal publications and patents in nanotechnology has been increased steadily from 23 to 35%. Following this rate, the global market of products that incorporate nanotechnology has been estimated to be worthy of an overall of \$3 trillion by the year 2020 [12]. And with the growth in market size and continuing technological development, the future trend of nanotechnology has shifted from pure material synthesis to integrating knowledge for the production of more complex and dynamic nanosystems. [13] And as a result of the current development, nanotechnology is closer and more incorporated in our daily lives.

#### **1.2 Engineered Nanoparticle Utilization and Daily Exposure**

The utilization of nanoparticles in consumer products spans a wide range, starting from semiconductors and microelectronics to products such as foods, personal care products, textiles, coatings, paints and pigments [14-17]. Due to the excessive amount of ENP in consumer products, the daily exposure  $TiO_2$  alone is estimated to be approximately 0.2~0.7 mgTiO<sub>2</sub>/kgbw/day and 1~2 mgTiO<sub>2</sub>/kgbw/day for adults and toddlers in the U.S. [16]. The main sources for these exposure levels are speculated to be foods and personal care products. For example, titanium dioxide can be found in food products at concentration higher than 3  $\mu$ gTiO<sub>2</sub>/mg food [16]. This is due to the pigmentation effect of TiO<sub>2</sub> where it is used in various foods ranging from deserts such as frosting, chocolates, and chewing gums to daily food products such as instant coffee, noodles, seasonings, and canned products [16, 18]. Aside form foods, personal care products are also known to have high amounts of nanoparticles especially in functional cosmetics and hygiene products. [19, 20] Investigations have shown nanoparticle exposure through sunscreens and moisturization products to be as high as 1.9 g/per-capita/day. [21] The highest amount of exposure is known to be through shampoo products where Loretz et al. detected a maximum exposure of 21.91 g/percapita/day. [20] The results were higher than food products investigated, where as the highest investigated amount of exposure was 3.6 g/per-capita/day through instant noodles [18]. However not all products that contain nanoparticles list the added particle concentration. Nowack et. al, reported that 53% of ENP registered as biocidal silver likely contains nano silver while only 7% mark the presence of silver nanoparticles [17]. And although engineered nanoparticles are becoming a large part of our everyday lives, there is scarce information on the fate and transport of nanoparticles after consumer usage.

#### **1.3** Runoff of engineered nanoparticles to the environment

Ultimately, engineered nanoparticles in commercial products will escape into the environment, namely the municipal wastewater stream, after usage. [22-25] For example there have been numerous reports on the release of titanium and silver from nanoparticle imbedded products [26-28]. Dependent on the type of nanoparticlecontaining product, the characteristics and amount of aquatic discharge will differ, thereby impacting the transport and fate of the nanoparticles.

Textiles imbedded with nanosilver or TiO<sub>2</sub> particles can release significant amounts of particles upon repeated washing, estimated in the range of 20 to 100% of the total amount of functional particles in the product. [14,17] And while the amount of release relies on the individual product itself, the characteristics of the discharged particles rely on the material and functional properties. This is seen in the size and characteristics of the particles where TiO<sub>2</sub> discharge is mainly observed as small aggregates ranging in size of 50 nm to 1  $\mu$ m. [15, 19] Silver on the other hand is mainly discharged as single particulates ranging in a size range of 40 ~ 60 nm. [29]

Personal care products, such as sunscreen and functional cosmetics, containing titanium, will also be discharged directly into municipal waste streams, with particle release of an average of 90% of the total incorporated products. [30] The size of the titanium particles showed less clustering than textiles ranging from 100~600 nm, regardless of the aggregating nature of  $TiO_2$  under neutral pH conditions. This is due to the organic constituents of the products, coating the outer layer of the particles and enhancing the stability.

Aside from products that are discharged directly into municipal streams, a large portion of nanoparticle discharge is from urban runoff. The highest amount is known to be carbon black and  $SiO_2$  from tire wear, which has been reported at

discharge amount matching that of TiO<sub>2</sub> release from sunscreens. [31] Additional runoffs have also been found in coating materials and paints, where TiO<sub>2</sub> concentration of 300~400  $\mu$ g/L in the runoff from exterior paints has also been detected [15].

The nanoparticle discharged into the aquifer were eventually transferred via wastewater treatment plants, which serve as redistribution points of an estimated 26~39% of the total amount of nanoparticle waste [22]. This is due to the attachment of dissolved organic matter to particle surfaces, when particles come in contact with the original product or DOM in the waste stream.

# **1.4 Current Research on Engineered Nanoparticles in Wastewater Treatment Plants**

Aside from building models for predicting the flux of ENP in the environment, various attempts have been made to measure the material mass flow of ENP in WWTP. Westerhoff et. al. quantified, the concentration of  $TiO_2$  in an Arizona wastewater treatment plant [32]. The authors used acid digestion as pretreatment to remove organic materials, followed by using inductively coupled plasma (ICP) technique to analyze the soluble Ti. Results showed an average 843 µg/L of total Ti in the inflow and a maximum 8.46 mg/L of total Ti in the secondary sludge particulates of the wastewater treatment plant studied. Li et al. quantified Silver nanoparticles in nine wastewater treatment plants in Germany [33], using ion exchange resins and cloud point extraction to separate particulates from ionic silver, and graphite furnace atomic adsorption spectrometry to quantify the silver content of particulates. Results showed a maximum daily load of 4.4 g-Ag/d and, more than 95% of the particles were removed in the primary and secondary sedimentation tanks. Various attempts have

been made to study the distribution of other nanoparticles such as cerium, silica, and carbon in laboratory scale wastewater treatment units. Results all showed similar trends that biological sorption dominated the removal of nanoparticles from the liquid phase. Although the key characteristics of the distribution of engineered nanoparticles in wastewater conditions have been revealed by the above methods, there are still a number of limitations in these studies. For example, most of the studies were made using laboratory batch reactors, which might not represent field conditions. Furthermore the studies mainly involved samples having low organic loads. Due to the limitations mentioned above, better quantification of ENP remains a challenge in the field.

#### 1.5 Purpose of research

With the current increase in studying the fate of ENP, there are still numerous limitations in recent work. One of the main limitations in current research is the lack of analytical methods with ENP in wastewater samples. Due to this methodology limitation, research on the fate of ENP in aquatic conditions has been limited to experiments under ideal laboratory conditions. The lack of experimental methods and related field studies had prompted this thesis to investigate the fate of ENP based on field operation of WWTPs.

The specific aim of this research was threefold. The first was to develop an analytical method to qualify and quantify the ENP in wastewater samples. ENP of various material compositions were to be verified and quantified with a rational method. The second was to track the ENP through the WWTP and measure the distribution pathway of the particles. This approach was also used on rivers in Taiwan to verify the fate of ENP in environmental conditions. The final purpose was to understand the mechanism behind the fate of ENP in WWTP. The focus was to verify the mechanisms using field samples under conditions similar to operational WWTPs.

This study will also attempt to provide insights into the interactions of ENP and sludge particulates, as well as the fate of particles under conditions of high organic loads. The insights may aid in future research relating nanoparticle transport and sorption in various environmental conditions.

#### **1.6** Overview

Chapter one is to apprehend the overall transport, distribution and fate of engineered nanoparticles in wastewater treatment plants. The emphasis is on the utilization and disposal of ENP as well as the exposure and impact on the environment.

The second chapter is dedicated to the overall fate and distribution of ENP in WWTPs. The first part of the chapter was focused on the development of a new pretreatment method for ENP qualification and quantification in wastewater samples with high organic loads. A literature survey was conducted on conventional methods, developed for ENP analysis in wastewater samples. Various methods were compared and analyzed for their advantages and limitations. Based on the research, a novel multi step pretreatment method was developed for the quantification of ENP. The developed method was used to measure ENP concentrations at various locations of the WWTP. Based on the measured values, a concentration profile of ENP throughout the WWTP was established and analyzed. Addition to the distribution studies, samples was collected on a monthly basis for a seasonal assessment of ENP distribution. Aside from WWTPs, ENP surveys were also conducted on rivers in Taiwan. Samples

collected throughout the span of two rivers were analyzed to track the pollution pathways as well as the increase and decrease of the ENP during river flow.

The third chapter was focused on the stability and transport of ENP in WWTPs. To verify the stability of ENP in wastewater, a number of aquatic conditions such as the pH, ionic strength and DOM concentrations were tested. Aggregation kinetics experiments were conducted to determine the critical coagulation concentration (CCC) of ENP under given environmental conditions. The main focus of the CCC experiments was to verify the effects of high concentrations of DOM in the WWTP on particle stability.

The final chapter focused on the mechanism behind the high ENP concentrations in the wastewater sludge. The attachment of various ENPs to primary and secondary sludge was investigated under different conditions including ionic strength, MLSS concentration, and sludge origin. The thesis ends with a discussion on the major finding and future research needs.

#### Chapter 2

#### IDENTIFICATION AND QUANTIFICATION OF ENGINEERED NANOPARTICLES (ENPs) IN WASTEWATER TREATMENT SYSTMES

#### 2.1 Introduction

In the present study, attempts were made to determine the concentration of engineered nanoparticles under various aquatic conditions, to assess the advantages and disadvantages of current methods, and to evaluate the applicability of the method at broader scale. Table 2.1 summarizes the results of a wide variety of past studies dealing with the qualification and quantification of engineered nanoparticles in wastewater treatment systems. Emphasis was on the pre-treatment protocols and chemical-analytical methods developed for the specific quantification process.

Nanoparticle	Wastewater	Pre-treatment	Detection	Concentration	Recovery (%)	Ref
nTiO <sub>2</sub>	Field	Acid digestion (HNO <sub>3</sub> +H <sub>2</sub> SO <sub>4</sub> ; Hot plate); 0.7 μm filtration	ICP-OES, SEM, EDX	$\frac{10 \ \mu g/L}{mg/L} \sim 10 \ mg/L \ ^a$	N/A	[32]
nAg	Field	0.45 μm filter; Cloud point extraction (CPE); Ion Exchange Resin (IER)	GFAAS °	$< 30 \ \mu g/L \ ^{a,b}$	Mill Q water: 100%	[33]
nAg nAu nC <sub>60</sub>	Field	HNO <sub>3</sub> digestion (Ag); Aqua regia (Au); liquid-liquid extraction, (nC <sub>60</sub> )	Ag, Au: ICP-OES nC <sub>60</sub> : HPLC	>0.1 mg/L <sup>b</sup>	N/A	[34]
nAg	Laboratory reactor	Microwave digestion (HNO <sub>3</sub> +HCl+H <sub>2</sub> O <sub>2</sub> ); (200°C, 10min)	ICP-MS, Dynamic Light Scattering, TEM	$1.5~\mu g/L{\sim}~3$ mg/L $^{\rm b}$	N/A	[35]

## Table 2.1 Identification and quantification of ENP in wastewater

nAg nTiO <sub>2</sub> nSiO <sub>2</sub>	Laboratory reactor	Centrifugation	ICP-MS, ICP- OES, TEM	0.1 mg/L ~ 10 mg/L <sup>b</sup>	N/A	[36]
nZnO	Laboratory scale reactor	Aqua Regia (1:3 HCl:HNO <sub>3</sub> ) + Microwave digestion	ICP-MS	0.1 mg/L ~ 2 mg/L <sup>a</sup>	N/A	[37]
nCeO <sub>2</sub>	Laboratory reactor	Microwave digestion (HNO <sub>3</sub> +H <sub>2</sub> O <sub>2</sub> )	ICP-OES, Zetasizer ZN (zeta potential)	0.1 mg/L ~ 100 mg/L <sup>b</sup>	N/A	[38]
nAg nTiO <sub>2</sub> nC <sub>60</sub>	Laboratory reactor	$H_2O_2$ + hot plate (Ag); HNO <sub>3</sub> +H <sub>2</sub> SO <sub>4</sub> + hot plate (Ti); Liquid-liquid extraction (glacial acetic acid, toluene) (C <sub>60</sub> )	ICP-OES, UV/VIS, HPLC, LC/MS, SEM, TEM, EDS, ,PALS <sup>d</sup> , X-ray diffraction	$\begin{array}{l} nC_{60}{:}\;10\;\mu g/L\sim 0.5\\ mg/L\\ nAg{:}\;10\;\mu g/L\sim 1\\ mg/L\\ nTiO_2\;:10\;\mu g/L\\ \sim 2\;mg/L^{b} \end{array}$	N/A	[39]
nCeO <sub>2</sub>	Laboratory reactor	Centrifugation; HNO <sub>3</sub> +H <sub>2</sub> O <sub>2</sub> +microwave digestion	ICP-OES	0.5 mg/L ~ 10 mg/L <sup>b</sup>	N/A	[40]
nAg	Laboratory reactor	Filtration (0.4, 0.45µm)	ICP-OES	10 ng/L ~ 4 mg/L <sup>a,b</sup>	N/A	[14]

nTiO <sub>2</sub>	Field, Freeze dried	HCl+HNO <sub>3</sub> +HF+Hotplate; HCl+HNO <sub>3</sub> +HF+Microwave; Ammonium persulfate + HNO <sub>3</sub>	ICP-MS	5 ng/L ~ 200 μg/L <sup>b</sup>	Mill Q water: <u>Hot plate:</u> 88~94% <u>Microwave:</u> 88~94% <u>Ammonium</u> <u>persulfate:</u> 88~94%	[41]
nAg nAu	Laboratory reactor	NCl+HNO <sub>3</sub> + Microwave digestion (Ag); Aqua regia (Au)	ICP-MS	$100~\mu g/L \sim 400~\mu g/L$ $^b$	N/A	[42]
nZnO nAg	Laboratory reactor	Aqua regia + Microwave digestion	ICP-MS	$10~\mu g/L \sim 40$ mg/L $^{\rm b}$	N/A	[43]
nTiO <sub>2</sub> nZnO	Field	Dialysis; centrifugation; oxidation; acid digestion	ICP-OES	Ti : 0.1 ~ 1 mg/L Zn : 0.1 ~ 1 mg/L <sup>a,b</sup>	Ti : 63~100% Zn : 86~100%	e

a : Measured sample

b: Spiked sample
c: GFAAS = Graphite furnace atomic adsorption spectrometry
d: PALS = Phase analysis scattering technique
e: Method developed in current research

Pretreatment methods varied dependent on the chemical composition of nanoparticles and analytical instrumentation used. Most studies on the fate of ENPs in wastewater focused on a group of common nanoparticles such as TiO<sub>2</sub>, Ag, and C<sub>60</sub>. Dependent on the chemical composition of the nanoparticle, acid digestion and particle extraction were the main pretreatment methods. In both cases, the major challenge is dealing with wastewater samples of high organic content, containing soluble metal ions and coarse non-nanoparticles. Results in Table 2.1 reveal that, acid digestion over hot plate or in microwave are the most widely pretreatment methods for metallic nanoparticles. The selection of acid is dependent on the chemical composition of the nanoparticles. For example, water/wastewater samples containing titanium dioxide (TiO<sub>2</sub>) was digested with nitric acid and sulfuric acid according to the Standard Method for Water and Wastewater Analysis, Method 3030 on a hot plate. [16,32,44] Ammonium persulfate along with nitric acid has also been used in a "fusion method" for titanium [41]. For silver nanoparticles, mainly nitric acid was used either in a pure state or in a mixed form such as aqua regia, and digested over the hot plate or in microwave. [14,35,39] Other metal oxides, such as zinc and cerium oxides were also digested with the microwave method using aqua regia and mixture of nitric acid and hydrogen peroxide, respectively. [37,38] Due to its oxidizing property, acid digestion can treat wastewater samples containing high organic loads as well as a wide concentration range of nanoparticles. And with the acid digestion method, samples containing nanoparticles can be quantified with a variety of analytical instruments [32,33,35,36,38-40,43-47]. However without additional particle separation operation metals might be soluble from particles of granular size (a non-engineered one), which may overestimate the level of nanoparticles in the samples [32]. Although some studies have used filtration as a means to separate the coarse particles from the sample, it was mainly conducted after acid digestion. In this case, the purpose of filtration is mainly to prevent clogging of the ICP piping, to prevent overestimate of nanoparticles contributed from coarse particles.

In addition to acid digestion, particle extraction has also been applied as a common pretreatment method [33, 48]. For example, silver NP extraction, cloud point extraction (CPE) and ion exchange resins (IER) have been showed to be promising for the extraction of silver nanoparticles from water. Results showed high silver particle extraction with less than 4% of interference from silver ion. However the studies above used wastewater samples of low organic loads. To the best of our knowledge, there were no results available on the extraction of silver nanoparticles from samples with high organic loads (e.g., sludge).

Liquid-liquid extraction has also been widely used for the study of carbonbased materials such as fullerene ( $C_{60}$ ) or carbon nanotubes. Due to the non-metalic nature of the material, ionic salt separation or coarse particle extraction presents no technical complications for the method, however attempts to extract carbon-based nenomaterials using liquid-liquid extraction have encountered numeral difficulties especially for samples with high organic loads. Membrane filtration and centrifugation have been applied to study the size separation capability [44, 49] of engineered nanoparticles in wastewaters with or without additional treatment. Although the methods are effective in separating particles at specific size, the attachment of organic materials to nanoparticles under high organic loads makes it difficult to successfully retrieve the total mass of engineered nanoparticles in the sample [35, 50]. Many attempts have been conducted to quantify engineered nanoparticles in wastewater samples. High sensitivity, wide detection range, and reliability, inductively coupled plasma (ICP) has been the most widely utilized technique to quantify the metallic engineered nanoparticles [32,34-43]. ICP-OES and ICP-MS have been the most widely used detectors in ICP. The ICP and graphite furnace atomic adsorption (GFAA) spectrometry provide a highly reliable measurement of metals in and chemical composition of ENP materials. As seen in Table 1, the element detection of ICP and AA techniques have shown high recovery over a wide range of concentration. However it must be mentioned that the above methods require various pretreatments, especially, when dealing with wastewater samples.

Additionally, chromatographic methods such as High Performance Liquid Chromatography (HPLC) and Field Flow Fractionation (FFF) mainly have been applied to determine the size and concentration of carbon based ENPs [34,39,51-56]. The results showed high sensitivity at concentration as low as 0.1 mg-C/L; however the methods can only handle a small amount of sample per run.

Electron microscopy such as SEM or TEM has been the most common method for the identification of ENPs [32,35,36,39]. The method is highly reliable in determining the size and the shape of nanoparticles, and in some cases the attachment characteristics between nanoparticles and sludge particulates can be observed as well. Electron microscopy can be reliable for size measurement with the additional advantage of only requiring a small amount of sample for analysis. However small sample size may not be representative of the broad characteristics of the whole ENP particle in a sample.
The present study focused on the quantification of engineered nanoparticles in field wastewaters. The aim was to arrive at a comprehensive methodology to verify and quantify different engineered nanoparticles in wastewater systems using a rational pre-treatment process and enumeration technique. Various procedures were studied and compared to assess the advantages and disadvantages of existing methods. Procedures were designed and refined to separate engineered nanoparticles, coarse particles, and ionic species from the wastewater and sludge particulates. Samples from various process points of two municipal wastewater treatment plants were collected to study the distribution of engineered nanoparticles. The concentration and mass flux of selected nanoparticles at different points in the wastewater treatment systems were determined and analyzed as to understand the fate and distribution of engineered nanoparticles in the wastewater treatment plants.

# 2.2 Materials and Methods

## 2.2.1 Sample collection

Samples were collected from wastewater treatment plant B and W. The two plants were selected based on the location and serving population of the cities that originated the inflow of the plants. Both cities are of the top 30 cites based on the population, with a high population density, and have a continuous population increase indicating a thriving economy. Both WWTP are conventional plants for organic removal with plant W equip with an anaerobic treatment system and tertiary sediment tanks.

To understand the distribution characteristics of ENP in the wastewater treatment plants, samples were collected from different areas of the treatment plant to verify the amounts of ENPs in each section of the plant. Samples were collected from the inflow, outflow, and waste sludge of the WWTP and each of the sediment basins. As seen in Fig. 2.1, each of the samples was labeled with a dual-numbering system. The first digit indicated the sediment basin (primary sediment basin: 1, secondary sediment basin: 2), and the second digit indicated the flow from or to the basin (inflow:1, outflow: 2, return or waste flow: 3). Sampled wastewater was firmly caped and stored in a refrigerated environment of 4°C before experiments. Experiments were conducted within three days after sampling. The MLSS, conductivity and pH of the sludge were measured for each sample in the laboratory after collection.



Figure 2.1 Wastewater treatment plant (WWTP) studied. Two WWTP were selected in this study, a; WWTP B, and b; WWTP W. The sampling locations are identified by the dual-digit system in the figure. The first digit (before the hyphen) indicates the sedimentation basin, and the second digit (after the hyphen) indicates the (1) inflow, (2) outflow, and (3) waste sludge of each sedimentation basin. For the secondary waste sludge in WWTP B, it is divided into two separate categories where 2.3.1 is the return sludge being re-circulated into the aeration basin and 2.3.2 the waste sludge that undergoes sludge thickening. The flux of the water flow of each sampling location was also displayed in the figures.

## 2.2.2 Removal of salts and organic decrements

**Dialysis:** For dialysis, seamless cellulose dialysis tubes with a molecular weight cut-off of 1 kDa (Fisher Scientific) were used. Sludge was inserted into the tubes and sealed with plastic clips on each end to prevent leakage. The dialysis tubes were submerged into a 2L tank with water circulation for 12 hours. Deionized water (18  $\mu$ Ω) was circulated into and out of the tank at a flow rate of 0.1 mL/min. The tank was stirred with a magnetic stirrer throughout the process. A conductivity meter (YSI model 35) was used to monitor the salt content of both samples. Results showed an exponential rate of salt removal, where the conductivity dropped from 3.7 to 0.26 m-Ω within 12 hours.

**Sludge oxidation:** Deionized samples were transferred from the dialysis tubes to glass beakers for the wet hydrogen peroxide method. 10 mL of 30%  $H_2O_2$  were added to the sludge and autoclaved for 1 hour at 105°C. Different amounts of  $H_2O_2$ were introduced into the sludge sample and autoclaved (Yuamato SM52) up to two times to assess the maximum effect of sludge oxidation. To prevent the overflow of sludge due to the rapid oxidation of wastewaters with  $H_2O_2$ , samples containing high concentrations of organic matter were initially digested using 5 mL of  $H_2O_2$  on a hot plate before the wet hydrogen peroxide method. After the samples were taken out of the autoclave, the beakers were sonicated to detach particles that on the beaker wall.

**Centrifugation:** Oxidized sludge samples were transferred to a 20 mL Teflon tube and inserted into a high-speed centrifuge (RC-5 Super-speed Refrigerated centrifuge, DuPont). Prior to the transfer the samples were sonicated with high intensity ultra sound processor (Ultrasonic Homogenizer 4710 Series, Cole-Parmer Instrument Co., Chicago, IL) at an intensity of 24 W for 5 min. The samples were centrifuged at 1,000 g for 3 min for particle separation.

Acid digestion: Liquid and solid samples were digested using HNO<sub>3</sub>/H<sub>2</sub>SO<sub>4</sub> as described by Standard Method 3030 g for water and wastewater analysis. 20mL of oxidized sludge samples were transferred to a flask. 5 mL of HNO<sub>3</sub> (Analytical grade, Fisher Scientific, US) was added to the flask and slowly boiled on a hot plate and evaporated to 15 mL. 5mL of HNO<sub>3</sub> and 10mL of H<sub>2</sub>SO<sub>4</sub> were added to the sample and evaporated until fumes of SO<sub>3</sub> start to show. [31, 33] The samples were heated additionally until the HNO<sub>3</sub> was all removed as well. After the heating process the samples were cooled and diluted with water to its original volume. Samples were also filtered after the acid digestion, before the ICP-OES analysis.

# 2.2.3 ICP Analysis

Inorganic material collected from the sludge was sonicated to prevent the ENP within the samples to attach to the clay particles. Oxidized sludge samples were contained in a 50 mL centrifuge tube with an addition of 5% nitric acid. A quality control standard (Perkin Elmer, 21 Elements, Matrix per Volume 5% HNO<sub>3</sub> per 100 mL) was used for a standard calibration of the selected elements to be detected. Standard curves were constructed with five different concentrations ranging from 0.01 to 1 ppm. All the samples were filtered with a 0.45- $\mu$ m filter (Fisher Scientific, US) to prevent clogging the ICP tubes. Additional filtration with 1.2  $\mu$ m and 0.2  $\mu$ m cellulose acetate filters (Fisher Scientific, US) were used to filter the sample for particle size evaluation. ICP-OES measurements were conducted on a Perkin Elmer Optima 7300. The following ICP-OES parameters were used: nebulizer flow, 0.80 L/min; radio frequency power, 1450; sample introduction, 1.50 mL/min; flush time, 15 sec; delay time, 20 sec; read time, 10 sec; wash time, 60 sec.

# 2.2.4 SEM analysis

Scanning electron microscopy (SEM) images of wastewater sludge samples were taken to verify the existence of engineered nanoparticles and their chemical composition. A Hitachi 4700 FE-SEM equip with a Princeton Gamma Tech EDX system was used for the analysis. To prepare the samples for analysis 100  $\mu$ L of the oxidized sludge was applied onto a SEM stub and air-dried for 24 h before analysis. The samples were then coated with a thin layer of carbon (Denton Bench Top Turbo III, DENTON Vacuum, LLC) to prevent charging under the electron beam. The SEM imaging process was operated at an outset voltage of 23 kV.

Additional to the SEM imaging, the "X-ray mapping" function was used to verify the shape and composition of detected particles. "X-ray mapping" is a combination of X-ray spectroscopy and computer-assisted imaging, where each of the elements is marked on separate maps based on the detected location. The maps are expressed in a "dot on" (element present) or "dot off" (element not present) manners. With the locational distribution of each element organized on separate spreadsheets, each sheet can be superimposed on the SEM image. This will help verify the chemical composition of an engineered nanoparticle as well as its specific shape.

### **2.3 Results and Discussion**

#### **2.3.1** Method Development

As seen in Table 2.1, various attempts have been successful in detecting and measuring ENP in wastewater samples. However all of these methods have limitations in analyzing ENP in wastewater samples, specifically these methods can only analyze a small quantity of sample each time. Furthermore unsuccessful pretreatment to

separate metal ions and unrelated coarse particles may lead to an ill representation of ENPs in the wastewater.

In the present pretreatment study a novel method was developed to overcome some of the limitations of the conventional procedures. A series of pre-treatment processes were studied in a four-step strategy to isolate the nanoparticles for wastewater samples free from organic or inorganic contaminants.

First, the salts and ionic metals ions in the samples were removed by dialysis using a cellulose acetate membrane. Second, organic and biological debris in the waste samples were removed by the wet hydrogen peroxide oxidation. Third, coarse particles (> 200  $\mu$ m) were separated by centrifugation. And finally, nanoparticles free from inorganic salts and organic substances were digested in strong acid to stabilize the metal ions for ICP-analysis.



Figure 2.2 Conductivity profile of the dialysis of activated sludge as a function of time. Dialysis was conducted under two different conditions: (▲) the dialysis tube was submerged in a continuous batch unit (Fig. 2.2) with water circulation; (●) the dialysis tube was seemed in a continuous bath unit as above (Fig. 2.2) except without water circulation. (Data is illustrated in the Appendix Table B1)

Dialysis was conducted in a circulating water tank to facilitate rapid ion separations. (Appendix) For comparison, dialysis with and without water circulation was also conducted. Results showed that after 12 hours of water circulation, the conductivity dropped to 0.26  $\mu$ mho/cm, or 0.52  $\mu$ mho/cm without water circulation. (Fig. 2.3) Compared to DI water, which has a conductivity of 0.1  $\mu$ mho/cm, the dialysis process was successful in removing large amounts of dissolved ions from the sludge.

The optimal amount of hydrogen peroxide to remove organic detritus from waste samples was also established. The effectiveness of  $H_2O_2$  oxidation was determined in terms of TOC removal. Results showed that for a 20 mL waste sample having a MLSS of 2,000 mg/L, it was possible to remove > 99% of organic matters using 2.8 mL of  $H_2O_2$  (30%) in the autoclave for a 1 h operation. One more autoclave run did not result in removing additional TOC removal (Fig. 2.3). Advanced oxidation method helped remove organic detritus without altering the basic chemical or physical properties of the treated samples in terms of generating additional nanoparticles. Controls were run to assess the impact of advanced oxidation on the oxidation of soluble metal ions, specifically Ag, Ti, and Zn, to form solid metal oxides. Results were negative.

After dialysis and oxidation, large amounts of large inorganic particles were observed in the sludge. The separation of course inorganic particles are important due to the mineral composition of the particles, where Ti and other heavy metals have been found in particles larger than 1  $\mu$ m. [32] Centrifugation was selected for the separations, where the operation conditions were selected based on the hydrodynamic diameter and mass density of the particles of interest.



Figure 2.3 Removal of organic content in sludge sample by the wet  $H_2O_2$  oxidation method. Experimental conditions: [MLSS] = 1000 mg/L, sludge quantity = 100 mL,  $H_2O_2$  (30%) = varying, Temperature = 105°C, time = 1 h 1<sup>st</sup> cycle (•), 2<sup>nd</sup> cycle ( $\circ$ ) (Data is illustrated in the Appendix Table C1)

Acid digestion showed more reliable results toward the quantification of ENP. To verify the effect of acid digestion, oxidized wastewater samples were spiked with various concentrations of ENP. Samples were divided into two separate groups with one undergoing acid digestion and the other without. The two different groups where analyzed and plotted on Fig. 2.4.

Acid-digested samples showed a high metal recovery of up to 100%, which a slight over estimation in the lower total metal concentration ranges and a slight under estimation in the higher ranges. Un-acid digested samples showed generally lower recovery due to probably the loss of particles in the nebulizer. At high ENP concentration may result in particle aggregation may take place, making it difficult for large particles to enter the plasma chamber via the nebulizer. However the ionization of metallic nanoparticles prevents material loss in the nebulizer chamber resulting in a high recovery.

The developed method showed good reliability for detecting ENPs at similar concentrations reported in the literature [32,37,44]. As seen in Table 2.1 the developed method shows high degree of reliability in quantifying engineered nanoparticles compared to the other methods reported in the literature. Alongside the reliability, the developed method also has various advantages over many conventional ones. The method studied can analyze aquatic samples with high organic loads, making it possible to quantify ENP in a diverse group of field samples including both, freshwater and wastewater. The current method also enables simultaneous detection of particles with different chemical compositions over a wide range of concentrations.



Figure 2.4 The effect of acid digestion (AD) on the recovery of ENP. Each axis shows the observed concentration of ENP as a function of the added concentration of engineered nanoparticles. Open symbols: without acid digestion; Closed symbols: with acid digestion. Experimental conditions:  $[HNO_3] = 15 \text{ mg/L}$ ,  $[H_2SO_4] = 5 \text{ mg/L}$ , Temperature = 95°C, Time = 2 h. (Data is illustrated in the Appendix Table D1)

Finally, SEM imaging together with X-ray scanning was conducted to identify the size, shape, and composition of existing engineered nanoparticles collected from the two wastewater treatment systems. The X-ray scanning method displays the composition and location of elements in an individual ENP particle, making it easier to identify the chemical composition and shape of the ENP as well. Fig. 2.5~2.6 shows results of the elemental scanning exemplifying by Ti, where spherical particles containing titanium and oxygen were detected. Different elements would be displayed on separate frames where it could be later overlapped to verify the chemical composition of the particle (Fig 2.6 d). The superposition of the images in Fig. 2.6 (d) showed coagulated TiO<sub>2</sub> particles imbedded in small organic clusters reinforcing the sorption of ENP into sewage particulates in wastewaters with high organic loads.



(c)

(d)





Figure 2.5 SEM images of oxidized sludge (a) ~(g). Outset voltage 23kV, (Hitachi 4700 FE-SEM) (a) SEM image of TiO<sub>2</sub> sorbed sludge, (b) magnified image showing aggregated TiO<sub>2</sub> adsorbed to the flocs, (c) SEM image of sludge particulate containing TiO<sub>2</sub> (d) Elemental coloring of TiO<sub>2</sub> imbedded sludge flocs, where organic material is shown in green and Ti shown in orange, (e) SEM image of TiO<sub>2</sub> entrapped in inorganic granulates from wastewater samples.





Figure 2.6 SEM image and elemental scanning of TiO<sub>2</sub> nano-particles imbedded in sludge particulate. (a) SEM image of sludge particulate, (b) titanium scanning, white dots indicate elemental titanium. (c) elemental scanning of oxygen, (d) overlapping of images (a) and (c). Red dots represent the overlapping elements (titanium and oxygen).

### **2.3.2** Distribution of engineered nanoparticles in wastewater treatment plants

Based on the developed method, an overall profile of ENP distribution was mapped out on two wastewater treatment plants. Concentrations of titanium and zinc were measured at the inflow, outflow, and waste sludge of each sediment basin. Samples of the initial inflow to the WWTPs as well as the final discharge was also collected and analyzed. Two treatment plants (B, W) each with secondary and tertiary treatment basins, were selected to verify ENP distribution in wastewater treatment plants.

Fig. 2.7 (a) shows TiO<sub>2</sub> and ZnO concentrations on each of the sampling positions in wastewater treatment plant B. Profiles of the distribution show a general decrease in the concentration toward the outflow of the plant, with high amounts of ENP at the waste and return sludge of each basin. It could be inferred from the results that redistribution of ENP will be directed to mostly the waste sludge. Concentration difference from the primary inflow (1-1) to the final discharge (3) indicate an overall 80% removal of titanium and 68% removal of zinc through primary and secondary sludge. The difference in the inflow and outflow of TiO<sub>2</sub> in the individual sediment basins show that 17% of the total ENP was lost in the primary basin where 51% was lost in the secondary basin. Zn also showed a similar trend where 43% loss during the primary basin and 32% loss in the secondary process. However the overall concentration of Ti was of 2 to 5 times higher than that of Zn throughout the treatment plant. It has been proven in previous studies that the removal of ENP through each of the basins is due to the interaction of ENP through coagulation [40,58], organic/inorganic sorption [40, 59], and biosorption [32, 34, 57, 59, 60].







Figure 2.7 Spatial distribution of ENP in WWTP B. (November, 2015), (a) liquid phase concentration, (b) concentration per weight of dry sludge, (c) Mass flow rate. (Data is illustrated in the Appendix Table E1)

Due to the high organic load, long suspension time, and continuous mixing most of the ENP is removed in the aeration process. Results from WWTP B show that for TiO<sub>2</sub>, 62% of the particles from the primary sediment tank outflow were removed by biosorption where 16% of the initial inflow was removed through coagulation and organic/inorganic sorption in the primary tank. Zinc also showed similar results where the removal was higher with the secondary sediment tanks (57% removal from the primary sediment tank outflow) compared to the primary sediment tanks (43% removal from the initial inflow of the plant). Additionally, after the secondary treatment an additional 12% of the total inflow of ENP was lost in the sand filtration process before final discharge.

To estimate the sorption of ENP to sludge in wastewater, the amount of detected ENP was divided by the organic weight in each of the samples. Fig. 2.7 (b) shows the NP to sludge ratio for each of the sampling positions. Comparing Fig. 2.7 (b) with (a) it can be seen that regardless of the concentration of the NP, maximum adsorption of ENP is of 1 g-NP/kg-sludge in both of the sediment basins. This corresponds to both Ti and Zn particles. In previous research, results of ENP sorption show amounts up to 13.5 mg-NP/g sludge, which are higher than the amount in the current study. [33,57] However in these cases the injected concentration of ENP were higher than the values in the current study. This may be due to the fact that most of the sorption studies were conducted in laboratory reactors. Experiments in laboratory reactors in general inject particles to a system batch reactor tank. This may induce the attachment of particles to sludge, where with WWTPs the particles may be coated in dissolved organic matter in the primary sediment tank. Also in laboratory reactors, the organic and biological constituents are different with the field samples. [34-36, 61]

The outflow of each of the basins (1-2, 2-2) shows higher NP to sludge ratio (2.7 g/kg). However, this can be due to the free NP in the outflow rather than the amount adsorbed to organic matter.

Additional to the concentration profile, the total mass of ENP that flow through the given sampling point (mass flux) of the two WWTP is configured in Fig. 2.7 (c). The mass flux was calculated by multiplying the measured NP concentration to the hydraulic flow. (The hydraulic flow data was provided by the operators at WWTP B.) By factoring in the flux, a more accurate estimation of the ENP in WWTPs was possible. Unlike calculating the particle distribution through concentration profiles, using the mass flow of ENP has various advantages. For example, a more accurate amount of ENP distribution can be accounted for the WWTP. The flow rates of the inflow and outflow in sediment tanks are not equal due to the discharge of waste sludge and the circulation of return sludge. And since the concentration profile does not account for this fact, moderate misrepresentations of ENP distribution can be corrected with the mass flow. From the concentration profile of plant B (Fig. 2.7 a), the removal of Zn was estimated at 43%, however with the mass flux it showed that 37.5% was removed. And with Ti the mass flux showed a 19.14% removal where the concentration difference estimated a 17% removal in the primary clarification tank. The secondary sedimentation also showed different removal results where the mass flux results indicated a 48% of Ti removal compared to the 58% removal with the concentration difference method. Zn however showed a similar result with both methods showing 24% removal with both methods. The final discharge showed similar results where the mass flux showed Ti removal at 80.6% and Zn removal at 83%.

Additionally, the mass flow will display the total amount of particle flow in a given position, making it easier to estimate the particle loss in the treatment system as well as the actual amount of ENP that will be later discharged to landfills or the environment. In the primary sediment basin, Zn showed a 7.5% loss in the mass where Ti showed no sign of particle loss in the primary clarifier. Considering the standard deviation of the measurements the loss of the particles was well in the error range. However in the secondary clarifier, higher amounts of particle loss were indicated where 20% of the Zn inflow and 22% of Ti inflow were unaccounted for in the process. The amount of NP loss in the secondary clarifier is due to the accumulation of NP onto sludge.







Figure 2.8 Spatial distribution of ENP in WWTP W. (a) Liquid phase concentration of zinc, (b) Liquid phase concentration of titanium, (c) concentration per weight of dry sludge, (d) Mass flow rate of Zinc, (e) Mass flow rate of titanium. (Data is illustrated in the Appendix Table F1)

Fig. 2.8 (a) and (b) each show the Zn and Ti concentrations in WWTP W. The concentration profile showed similar trends of that of WWTP B, where higher concentrations are detected in the sludge. The inflow ENP concentrations of the two plants showed similar amounts of Ti and lower amount of Zn. This is reflected in the waste sludge of the primary basin as well. However regardless of the lower amount of initial inflow the accumulation of ENP in the aeration is equal or higher in WWTP W. Compared to Fig. 2.7, the concentration of Zn is similar, where Ti in the secondary return sludge is approximately twice of that of WWTP W. Longer retention times may explain the larger accumulation, as well as the history of inflow where higher concentrations may have been inserted into the plant. This is also reflected in the ENP to organic mater ratio of Fig. 2.8 (c). Higher organic loads of WWTP W shows lower results than Fig. 2.7 (b), but the longer retention time resulted in higher values. Also, concentrations of the inflow of tertiary sediment tanks showed higher values than the outflow of the previous basins due to the mixing of the return sludge. ENP concentrations in the final outflow of the plant showed lower values in WWTP W, where the tertiary treatment reduced the amount of ENP that are introduced into the sand filtration process. And as a result the final outflow of WWTP W showed concentrations lower than the detection limit.

The mass flow of WWTP W was also calculated to evaluate the particles distribution (Fig. 2.8 d, e). Although the ENP concentration of WWTP W was lower, the mass flow of the two plants were similar due to the higher hydraulic flux of the plant W. Results showed similar trends with the Baltimore WWTP where the ENP loss through the primary sediment tanks were over estimated in the concentration profile, and underestimated in the secondary sediment tanks. Also particle loss in

WWTP showed a negligible amount of  $TiO_2$  particle loss, and 10% loss for Zn in the primary sediment tank. The particle loss in the secondary and tertiary showed higher amounts of particles loss compared to plant B as well, due to the retention time.

Additionally, the mass of the secondary and tertiary sediment tank does not show balanced valued between the in and outflow of basin. For the secondary sediment tank, the elevated amounts in the return sludge are due to the accumulation in the sludge from higher internal circulations and higher particle inflow previous to the sampling date. This can be commonly seen with the secondary basin, where higher or lower values of mass can be found in the waste sludge. In Fig. 2.8 (d) and (e) however, shows the mass flux of the re-circulated amount of secondary sludge (2-3) showing exaggerated values. With primary basins on the other hand, having a simple linier inflow to outflow mechanism, results in a simpler match of the mass balance.

The inflow of the tertiary sediment tank is also seen to be abnormally higher, compared to the secondary outflow of the secondary sediment tank. However this is due to the mixing of the secondary outflow and tertiary return sludge, where the access point of the tertiary inflow in the plant was after the initial mixing stage of the tertiary tank.

# 2.3.3 Engineered nanoparticle detection in the environment: A Taiwan case study

16 raw samples from Taiwan was sampled and analyzed with the ICP-MS. The samples were from various areas of Taiwan, adjacent to science parks and highly populated areas. Fig. 2.9 shows an overview of the areas where the samples were collected. The samples come from three major areas where 1~5 were from the Siao Li

brook, samples  $6\sim10$  were from the Ke Ya brook and samples  $11\sim16$  were from Nei Hu wastewater treatment plant. All of the salts from the samples were removed by submerging the samples in deionized water for 24 hours with dialysis tubes (molecular weight cut off of 100 kDa). The organic contents were then removed by adding 0.1 M of hydrogen peroxide and autoclaved for 15 minutes. Each sample was separated into six subgroups and treated in different methods for nanoparticle separation. Centrifuge was used to separate particles based on their size [62,63]. Subgroup #1 was the raw samples taken out of the autoclave undergoing no additional treatment. Subgroup #2 was allowed to settle under quiescent conditions for 2 hr to collect particles larger than 9  $\mu$ m. Subgroup #3 was centrifuged at 3,700 g for 5 hr to separate and collect particles larger than 230 nm. Subgroup #4 was centrifuged at 124,000 g for 1 hr to collect particles larger than 65 nm. Subgroup #5 was the supernatant from subgroup #4 containing particles smaller than 65 nm. The samples were labeled from 1-1 to 16-5 and analyzed with the ICP-MS. The samples with no subgroups were the raw water only filtered by a 5  $\mu$ m glass filter.



Figure 2.9 The detailed map of sampling stations  $#1\sim#5$ . The location of the sampling station is; 1 = New Fulong Tea Factory, 2 = De Sing Bridge, 3 = Long Sing bridge, 4 = Siao Li Bridge, 5 = Bao Shih bridge



Figure 2.10 Sampling positions within Taiwan. Sample  $6\sim10$ . The location of the sampling station is; 6 = Fong Huang bridge, 7 = Jong Sing bridge, 8 = Hisinchu science park, 9 = Tian Gong monastery, and 10 = Niou Bu bridge

To evaluate the amount of nanoparticles in the industry adjacent environments, 3 major areas in Taiwan were selected for NP analysis. The 3 major areas were Siao Li river, Ke Ya river and Nei Hu WWTP. The first sample group was selected from Siao Li river which passes through highly populated areas within Taiwan. Samples 1, 2, 4 and 5 were samples from the three different locations along the riverside. Sample 1 and 2 was from upstream, sample 3 from midstream and sample 4 was in the downstream area of the river. Sample 5 was from and intercepting area of Siao Li river and Fong Shan river. Samples 6 through 10 were from Ke Ya river which flows through the Hsinchu Science Park. Hsinchu Science Park (HSP) is one of the world's most significant areas for semiconductor manufacturing having more than 400 hightech companies. The companies are mainly involved in the semiconductor, computer, telecommunication, and optoelectronics industries, which highly rely on nanotechnology. Fig. 2.10 shows a more detail image of the sampling sites. Sample 6 was collected from Fong Huang bridge which is at the upstream area of the Ke Ya river and sample 7 was collected near the Jong Sing Bridge. Sample 8 was from Jhen Sing bridge which is next to the wastewater discharge point of the Hsinchu Science Park. Sample number 9 and 10 are each from Tian Gong monastery and Nio Bu bridge, respectively which is located at the downstream area of the Ke Ya river. The location of Nei Hu WWTP can be seen in Fig. 2.10.



Figure 2.11 ENP concentration in the river samples filtered with 5-µm membrane. The sample numbers represent the ten sampling stations of the Siao Li river (1~5), and the Ke Ya river (6~10). (Data of the figure is illustrated in the Appendix Table G1)

To measure the amount of NP within the samples a known amount of NP was measured with the ICP-MS. This was used to convert the elemental amount detected to an equivalent concentration of NP within the sample. Fig. 2.11 shows the ICP-MS results of Titanium, Aluminum, Iron, Zinc and Silver NP of 1ppm, 0.5 ppm, 0.1 ppm, 50 ppb, 10 ppb. The results were used to calculate the amount of NP within the samples from Taiwan. Fig. 2.12~2.13 shows the amount of different NP detected within samples 1~10. Results indicate an increasing amount of iron and titanium NP amongst sample 3 and 4 compared with samples 1 and 2. It can be assumed that there is an additional source of pollution along the stream. However it can be observed that the amount of zinc is decreasing throughout the flow of the river. It can be speculated that the zinc in the water is being adsorbed to species in the environment.











Figure 2.12 Distribution of engineering nanoparticle in the Siao Li river. (a) 1: New Fulong Tea Factory, (b) 2: HeSing bridge, (c) 3: Long Sing bridge, (d) 4: Siao Li bridge, (e) 5: Bao Shih bridge. The sample sub number represents the different size range of particles in the sample. (1: No treatment, 2: > 9 µm, 3: > 230 nm, 4: > 65 nm, 5: < 65 nm) (Data is illustrated in the Appendix Table G2~G6)










Figure 2.13 The distribution of engineered nanoparticle concentration in the Ki Ya river. (a) 6: Fong Huang bridge, (b) 7: Jong Sing bridge, (c) 8: Jhen Sing bridge, (d) 9: Tian Gong monastery, (e) 10: Niou Bu bridge. The sample sub number represents the different size range of particles in the sample. (1: No treatment,  $2: > 9 \mu m$ , 3: > 230 nm, 4: > 65 nm, 5: < 65 nm) (Data is illustrated in the Appendix Table G7~G11)

With samples 6~10 there is a define increase in the iron composition as the water body of the river travels downstream. A distinct spike in all the tested elements can be observed with sample 8 (Hsinchu Science Park wastewater discharge point). However samples taken downstream of this point show lower amounts of NP, suggesting the adsorption of NP onto organic or particular silica in the environment [64]. Fig. 2.12a shows the samples treated with different centrifuge speeds. Results from subsample  $1 \sim 4$  shows that the major composition in particle size is in the nano range for aluminum titanium and zinc. However iron is found in both the submicron and nano range. In sample 3 (Fig. 2.12c), it can be observed that zinc and titanium is mostly found as nanoparticles in the samples. However in sample 4 most of the particles are larger than 9  $\mu$ m. By comparing the upstream (sample 2), midstream (sample 3) and downstream (sample 4) we can see a general decrease of concentration in the sub-micro and nano range. However sample 5 shows a higher increase in all the particle size ranges indicating an additional amount of NP in the Fong Shan river. In sample 6 (Fig. 2.13a), which was collected from the upstream area of Ke Ya, river showed concentrations of 80 ppb to 180 ppb of iron. However most of the other elements showed lower concentrations ranging from 10 ppb to 45 ppb in NP concentration. As Fig. 2.13 shows the concentration of aluminum and iron spikes while the water travels to midstream. Iron is mostly found in the submicron size range while aluminum is detected in both the nano and micro size range. As expected sample 8, which was sampled at the wastewater discharge area of the Hsinchu, science park showed high concentrations of particles in all the size ranges tested with an exception of titanium. Further downstream of Ke Ya river sample 9 shows an increase amount of titanium and iron. This may indicate further discharge of NP into the environment do to the additional amounts of industries onside the stream.

# 2.3.4 Seasonal variation of engineered nanoparticles in wastewater treatment plants

Although engineered nanoparticles are found in a wide variety of consumer products, the amount released into municipal wastewater streams differ on a seasonal basis. This is due to the nature of the products, where the use of functional cosmetics and personal care products are tailored for specific seasonal conditions. Sunscreen and cosmetics with SPF functions, body lotions and other moisturizers are an example of products, which are composed on mainly titanium and zinc nanoparticles, with a seasonal variation in its usage. Also particle release from functional textiles through washing may also be higher in extreme weather conditions as well. In this study a monthly analysis of engineered nanoparticle distribution in wastewater treatment plants were conducted to evaluate the seasonal variation of ENP in WWTP.



Figure 2.14 Monthly inflow of  $TiO_2$  and ZnO nanoparticles into wastewater treatment plant B. Samples were acquired at the inflow of the primary sediment basin after the grit chamber. Samples were collected throughout the year 2012. (Data of the current graph is illustrated in the Appendix Table I1 for titanium and I2 for zinc)

Fig. 2.14 shows the monthly inflow of Ti and Zn nanoparticles into WWTP B. Samples were collected on a one-year duration with the sampling time being at the end of the month after rush hours (9~10 am). Results show elevated concentrations of both Ti and Zn in the summer (July ~ September, 2012) and winter (December ~ February, 2012) periods. This may be due to the characteristics of the products used in each of the periods where in summer various UV protection products such as sunscreen lotion, sprays and cosmetics with SPF protection. And for winter, the higher utilization of various moisturizing products may explain the elevations. Fig. 2.15 displays the U.S. Census Bureau's monthly U.S. health and personal care stores sales from 2012 to 2015, showing an annual spike in the sales of personal care products at the end of each year. This may be due to year-end sales and discounts, however the high amounts of sales may also be associated with higher consumption as well.



Figure 2.15 U.S. Monthly sales of Health and personal care products (01/2012~12/2015) Source: Advanced monthly retail trade report, U.S. census bureau. (The dotted grey lines show the sales in December) (Data is illustrated in the Appendix Table H1)



Figure 2.16 Monthly accumulation of  $TiO_2$  and ZnO nanoparticles in the aeration basin of wastewater treatment plant B. Samples were acquired at the return sludge flow of the secondary sedimentation tank. Samples were collected throughout the year 2012. (Data of the current graph is illustrated in the Appendix Table I1 for titanium and I2 for zinc)

Fig1.16 shows the monthly variation of Ti and Zn nanoparticles in the aeration basin of WWTP B. Compared to the results in Fig. 2.14 the seasonal variation of the nanoparticles are lower, showing a more gradual increase and decrease throughout the year. This may be due to the fact that the amount measured in the aeration tank (Fig. 2.16) represents the accumulation of the ENP due to the recirculation of sludge, where the total inflow (Fig. 2.14) only represents the concentration in a certain point of time or day. Also considering the fact that the samples were collected at a time when the inflow amount is high within a day (Westerhoff) the total inflow results may show an overrepresentation of the average inflow to the treatment plant. The difference between the January, July, and August results of the two figures may be a representative example where the values in the tow figures differ the most. Although zinc also shows seasonal variation in the aeration tank the extent was lower than  $TiO_2$ indicating lower accumulation. Comparing the results of the two seasonal results show that concentrations of the aeration basin better represents the inflow of nanoparticles on a long-term matter.



Figure 2.17 Seasonal variation of total Ti at various sample points in the wastewater treatment plant B. Samples were collected through the year 2012 (Data is illustrated in the Appendix Table I1)



Figure 2.18 Seasonal variation of total Zn at various sample points in the wastewater treatment plant B. Samples were collected through the year 2012(Data is illustrated in the Appendix Table I2)

For an overall seasonal assessment of ENP distribution, a monthly analysis of particle concentration was measured throughout wastewater treatment plant B. Fig. 2.17, and 2.18 show ENP distribution of Ti and Zn respectively, in the inflow, outflow, and waste sludge of each the sediment basins. As mentioned in the previous section, summer and winter seasons show higher concentrations throughout the plant due to the inflow, where Zn was higher in summer and Ti higher in winter. This may be due to the difference in the configuration of the products used in the market, where Zn in mainly used in sunscreen products and Ti used in a wider variety of products.

With both particles, summer shows the highest concentrations in the primary sludge. This may be due to the temperature effect, where nanoparticles show higher diffusion, and sorption equilibrium under high temperatures. [65] Along with the temperature effects, attachment of particles to the primary sludge may also be increased through the retention time of ENP in the waste pipeline system, where the hydraulic flow from the waste point to the treatment plant may provide favorable conditions for particle organic interaction. Compared to the ENP concentration in the primary waste sludge, the average concentration in the secondary sludge was similar for Zn and approximately 4 times higher for Ti. The differences between the two sediment basins are not only due to the characters of the sludge but also the accumulation of the particles, where 1-3 reflects the daily inflow to the treatment plant and 2-3 reflects the accumulation of particles throughout a period of time.

Not only the inflow may have influenced the results but the operational conditions and sludge characteristics may also play a role in the particle sorption. For example, in winter season internal circulation in the aeration tank may be longer providing longer time for particles to attach to the sludge. Additional temperature effects may have also influenced the sorption, where in winter, it may be possible that the lower temperatures may impact the floc stability of the sludge making it break down into smaller floc sizes. [65] The larger surface area of the sludge may capacitate larger amounts of ENP onto its surface.

Aside from the concentration, mass flux of ENP was calculated from the monthly ENP concentration and hydraulic flow Data for WWTP B. Due to the consideration of hydraulic flow, the mass flux incorporates the variation of treatment loads, making it a better representation of the total amount of ENP in each location. Fig. 2.19, and 2.20 show the mass flux of Ti and Zn respectively, in WWTP B. Seasonal change of treatment loads showed higher amounts of ENNP in winter, making the difference in summer and winter mass flux lower than that of the concentration difference. The total inflow and aeration basin also shows less seasonal differences in the mass flux, compared to the concentration values. Also the flow to the waste sludge in the secondary sediment basin was higher in winter indicating higher removal in winter seasons as well. However the higher mass flux values in the secondary sediment basin may be due to the higher return sludge flow as well as higher internal circulation of the aeration basin. By comparing the concentration, and mass flux values it can be seen that where the concentration represents the amount of particles in a single point, the mass flux better represents the fate of the particles in the WWTP.



Figure 2.19 Total Ti flux at various sampling points in wastewater treatment plant B. Samples were collected through the year 2012 (Data is illustrated in the Appendix Table I3)



Figure 2.20 Total flux of Zn at various sampling points in WWTP B. Samples were collected through the year 2012. (Data is illustrated in the Appendix Table I4)

To understand the seasonal sorption characters of ENP to sludge, the ENP to mixed liquid suspended solid ratio was calculated and shown in Fig. 2.21, and 2.22. Among the results, the ENP ratio in the primary sediment tank shows higher values than that of the secondary basin. This is due to the lower organic content of the primary sludge than secondary sludge, however this may also be due to ENP attaching to the organic matter through the wastewater stream before reaching the WWTP. The ratio showed the highest values in summer, due to the temperature effect of higher diffusion of the ENP and higher sorption capacities of organic material. Additionally the high winter sorption may be due to the lower sediment speed due to the lower viscosity. For the secondary sediment basin, maximum sorption of ENP was found to be 0.24 g/kg for Zn and 1.28 g/kg for Ti. As mentioned above the higher ratio will be due to the higher inflow of TiO<sub>2</sub>.

Throughout the WWTP it can be seen that the ENP to organic ratio is higher in the outflow than inflow of each basins. It should be mentioned that the higher ratio of ENP is not a cause of particle concentration but of the lower organic concentrations. The ENP to organic ratio may help in future research to predict the amount of ENP to be found in the waste sludge. This will help landfill or incineration plants for operational preparations. Also the sorption of ENP to organics can help predict the seasonal outflow of ENP as well.



Figure 2.21 The total Ti to MLSS mass ratio at different sample points in wastewater treatment plant B. Samples were collected through the year 2012. (Data is illustrated in the Appendix Table I5)



Figure 2.22 The total Zn to MLSS mass ratio at different sample points in wastewater treatment plant B. Samples were collected through the year 2012. (Data is illustrated in the Appendix Table I6)

### 2.4 Conclusion

Analytical methods displayed in the research shows a novel approach to a wider variety of aquatic conditions to quantify ENP. The main focus of the procedure was to analyze ENP in aquatic conditions containing high organic loads. The results of the research also showed the developed method capable of detecting ENP of specific sizes, wide concentration ranges, and various chemical compositions. Each method in the study was applied to serve a specific function. The wet peroxide method was selected to remove high concentrations of organic matter without producing or reducing the oxidized metallic particles. Centrifugation was conducted for size separation, and dialysis to prevent the detection of dissolved metallic ions, as well as preventing particle-particle attachment. Based on the current approach various methods can be developed in different aspects and purposes to widen the capability of ENP detection. Although the current research focused on the quantification of ENP in wastewater samples, the method can be applied or modified for complex aquatic conditions containing high loads of organic material. The organic and biological conditions mentioned in this study may represent conditions such as DOM or algae in the environment. Applications and development of the current method will help aid in understanding the fate of ENP in various environmental conditions.

With the developed method, the fate of ENP in wastewater treatment plants was analyzed in this research. A monthly analysis of the ENP distribution in various locations of the treatment plant was conducted for seasonal inflow, and distribution analysis. Inflow of both Ti and Zn both showed elevated amounts in the summer, and winter due to the use of functional products imbedded with nanoparticles. Distribution results showed that organic and biological material play a major role in the distribution of ENP, where ENP is mainly found in the primary and secondary sludge. Seasonal variations of the inflow were reflected in the waste sludge, with the primary sludge showing higher correlation with the inflow. The secondary sludge showed more general seasonal changes indicating the effect of accumulation within the secondary basin, aside from the primary sludge reflecting the daily inflow of ENP. The accuracy of the conventional methods were compared with the mass flux analysis showing that mass flux analysis was a more accurate method to determine the fate of ENP in WWTP. This is due to the monthly change in treatment loads, and operational conditions better representing the actual quantity of ENP pass through a given position. The ENP to organic matter ratio was also calculated for additional information on the sorption, where results showed the sorption capacities as well as the seasonal and temperature effects of ENP sorption in wastewater treatment plants. The methodology applied in this study may aid in future analysis of environmental systems and the fate of various contaminants.

### Chapter 3

# INTERACTIONS BETWEEN ENGINEERED NANOPARTICLES AND SLUDGE PARTICULATES WITH SIZE LESS THAN 1 $\mu M$

#### 3.1 Introduction

During the last decade, engineered nanoparticles (ENP) have become an essential part of everyday consumer products, exhibiting widespread application in foods, personal care products, textiles, and home appliances. [14-17,28,29,52,66] Studies have shown various pathways of exposure of these particles into the aquifer, ranging from direct outflow through municipal waste to non-point source pathways. And based on the product type and property, some products show as much as 100% direct release where some show a steady release of particles under specific conditions. And eventually the outflow of the particles will result in the exposure to aquatic life forms as well as landfill accumulation.

In aquatic environments the transport and fate of nanoparticles are mainly due to the mobility and interactions of the particles. Aggregation and deposition play a key role in determining the behavior, mobility, and fate of ENP in aquatic environments. According to the DLVO theory the rate of aggregation kinetics depends on the electrostatic double layer of the particles. With the increase of ionic strength, electric double layer compression occurs, lowering the resistance between the particles, resulting in a higher likelihood of particle attachment. The stability ratio (or attachment efficiency) shows the particle attachment as a function of ionic strength concentrations. As the salt concentration increases, the attachment rate increases due to the screening of surface charge. During this stage the electrostatic repulsion decreases while the Van Der Waals attraction stays constant, generally decreasing the net repulsive force. This is known as the reaction-limited regime where the electrostatic interaction is the dominating factor behind the particle attachment, and the success rate of particle attachment per collision increases with the ionic strength. As the ionic strength continuously increases, it reaches the point where the net repulsive force is eliminated. This is called the critical coagulation concentration. Particle collisions at ionic strength from this point and higher directly leads to aggregation. This is also called the diffusion-limited regime, where the physical movement and collision of the particles dominates the aggregation.

Aside from DLVO forces, organic sorption to nanoparticle surfaces brings about steric repulsion, a non-DLVO force. Negatively charged functional groups on the organic matter causes the steric repulsion resulting in the stability of the nanoparticles, decreasing the aggregation kinetics and hence increases the CCC values. [67,68] The influence of organic matter relies on the both the type of organic matter as well as the amount adsorbed onto the particle surface. It has been known that the type of organic matter influences the stabilization of particles, where humic acid (HA) showed the highest repulsion followed by natural organic matter (NOM) and fulvic acids (FA). The amount of organic sorption to the particle surface also follows this order, where the sorption of organic matter is due to the higher ratio of aromatic to aliphatic carbon. [69] In general, an increase in organic sorption will enhance the stability of particles; however it has been proven that at low organic concentrations aggregation processes were increased through organic bridging. [70] This has also been observed with the existence of divalent cations, where  $Ca^{2+}$  induced a bridging effect between the sorbed organic materials resulting in faster aggregation kinetics. [71]

Various aggregation studies have been conducted with different ENPs (ZnO, TiO<sub>2</sub>, Fe<sub>2</sub>O<sub>3</sub>, C<sub>60</sub>, Single walled carbon nanotubes and grapheme oxide) under numerous aquatic conditions to verify the fate and transport of nanoparticles. Both metallic and carbon based nanoparticles show similar characteristics under higher ionic strengths as well as sorption of dissolved organic material onto the particle surface. With NaCl the CCC values of carbon based nanoparticles range from 44~160 mM, and metallic particles ranging from 20~40 mM. Difference in the measured CCC range may be due to the surface characteristics of the particles, where metallic oxides have an even distribution of functional groups on the surface where the carbon based nanoparticles differ on the surface oxidation of the particle surface. Detailed results of the literature research showed CCC values of 25, 15, 20, 160, 160, and 44 mM for TiO<sub>2</sub>, Fe<sub>2</sub>O<sub>3</sub>, C<sub>60</sub>, SWCN, and GO respectively. [67-72, 76]

For carbon-based materials it has been proven that the surface charge relies on the oxidation of the carbon surfaces leading to surface functional groups. [73] Carboxylic and hydroxyl functional groups are the main cause of the surface charge of carbon materials, where the amount of oxidation determines the degree of surface charge and hence the aggregation. [72] However, some studies have shown that the shape of materials also influences the aggregation of the particles. This has been seen in carbon nanotubes having structural irregularities in its scaffold as well as incomplete carbon rings in the end termini, making it more acceptable to oxidation. [74] Graphene oxide and fullerenes also share this trend where the CCC value of 2D graphene oxide (GO) is higher than 1D carbon nanotubes (CNT), and lower than 3D fullerene. [75] Among graphene oxides, the physical crumbling of the particles also showed influence on the surface charge and attachment characteristics. [72] Difference in CCC values based on particle shape has also been seen with ZnO, where non-spherical particles show faster aggregation characteristics compared to spherical particles. [76]

Li et al. tested the attachment efficiency of single walled carbon nanotubes (SWCN) in electrolytes of NaCl,  $CaCl_2$  and  $AlCl_3$ . Aside from the ionic conditions, samples containing humic acid (HA) were also tested to verify the effects of DOM. Results indicated that oxidized single wall carbon nanotubes (O-SWCNT) were relatively stable in water with a CCC of 0.16,  $4.2 \times 10^{-3}$  and  $5.4 \times 10^{-5}$  M for NaCl, CaCl<sub>2</sub> and AlCl<sub>3</sub>, respectively. The CCC ratio for the three different cations was 2962:7.7:1 (Na<sup>+</sup>:Ca<sup>2+</sup>:Al<sup>3+</sup>), which differed from the Schulze–Hardy rule that the CCC is a function of the ionic charge to the sixth power. It must be noted that the Shulze-Hardy rule was applicable only to symmetrical electrolytes, such as NaCl, and may not be applicable precisely to asymmetrical electrolytes such as Na<sub>2</sub>SO<sub>4</sub>. [72,77-80] Furthermore the DLVO theory does not consider contribution from chemical energy. Results highlighted the importance of water chemistry on the stability of ENP and the incapability of the DLVO theory to predict the stability of colloidal particles with complex surface chemistry. The difference in theoretical and experimental values may also be due to the interaction of functional groups and multivalent ions of the SWCN. The zetas potential of the SWCN was -28 mV in DI water and were proven to decrease in absolute values under higher ionic strengths with multivalent ions two to five degrees more sensitive in concentration compared to the monovalent ion, which is in good correlation with the experimental CCC ratio.

The CCC values of grapheme oxide (GO) were 44, 1.3, and 0.9 mM for Na<sup>+</sup>,  $Mg^{2+}$ , and  $Ca^{2+}$  respectively (Jiang et al. [72]). It has been shown that with both divalent cations the CCC value differs due to the smaller size and binding ability of  $Ca^{2+}$  compared to  $Mg^{2+}$ . Although GO tested in the study were not spherical, results showed a good correlation between the sheet like GO and conventional spherical nanoparticles. Compared to oxidized SWCN, GO showed lower CCC values with both Na<sup>+</sup>, and Ca<sup>2+</sup>. Though the pH values were different in both studies, it has been proven in various studies that with carbon material the difference in pH (in a range of pH 3~10 [59]) did not impact the CCC in a meaningful manner. The existence of humic acids also showed a higher impact on the stability compared to oxidized SWCN, where the CCC value raised from 2~5 times when HA was added. However, additional studies from this research with effluent wastewater showed rapid destabilization, indicating additional factors that may contribute to the coagulation of ENP.

Chen et al. showed the CCC value of fullerene with the presence of alginate. [67] The CCC values were of 20 mM NaCl, 180 mM NaCl+HA, 5 mM MgCl<sub>2</sub>+HA, 4.7 mM CaCl<sub>2</sub>+HA. The value of fullerene was smaller than of SWCN and GO. And such as GO, fullerene also showed a higher stability with the existence of organic material. In this study, one fact stands out where the attachment efficiency measured with calcium and alginate showed values higher than 1. Theoretically this would mean that particles attachment frequency would be higher than the collision frequency. However it is assumed in this study, that the existence of calcium and organic material form a  $Ca^{2+}$  bridging effect. Due to the binding capacity of  $Ca^{2+}$  ions with functional groups such as hydroxyl and carbonyl the calcium bridging effect attaches alginate coated on the surface of the ENP. This may explain the attachment efficiencies higher than 1 and how the attachment occurs more rapidly than the known particle collision rate.

Results from various research groups showed that with carbon materials pH does not have much effect on the stability within a certain range. Also, it can be seen that humic acids aid in the stability of particles due to the static repulsion, however the existence of  $Ca^{2+}$  will lead to calcium bridging which enhances the attachment efficiency. These results have been proven with ENP regardless whether it is from carbon based materials or metallic particles. The existence of various salts and dissolved organics may result in the change of surface chemistry of particles in the primary sediment tank. Considerations of these aquatic conditions may aid greatly in the prediction of the fate of ENP in WWTP but in the environment in general as well.

In the current research, particle-particle interactions will be tested for  $TiO_2$  and ZnO under various aquatic conditions. The pH and ionic strength will be a main factor as well as the natural organic matter from the sampled wastewater. The attachment efficiency tests will be conducted to verify the effects of natural wastewater conditions and its effect on the stability and aggregation of the nanoparticles of interest. Additionally, sorption tests of dissolved organic matter to the nanoparticles will also be conducted to assess the amount of organic sorption to nanoparticles and its effect on the stability as well.

#### **3.2** Theoretical Aspects

Aggregation of mono-disperse particles to doublet formation can be described with a kinetic approach with the equation [81,82]

$$\frac{dN_2}{dt} = \frac{1}{2}k_{11}N_1^2 - k_{12}N_1N_2 \tag{3.1}$$

this equation shows the aggregation kinetics of single particle aggregation as well as the dissociation of aggregated particles, where N<sub>1</sub> and N<sub>2</sub> are the number particles of the primary and secondary particles, respectively. The second-order rate constants  $k_{11}$ and  $k_{12}$  are rate constants related to the aggregation and dissociation process of particle interaction, respectively. For early state aggregation where single particle interaction occurs, in absence of dissociation, Eq. 3.1 can be simplified as the following expression

$$\frac{dN_2}{dt} = -\frac{1}{2}\frac{dN_1}{dt} = \frac{1}{2}k_{11}N_1^2 \tag{3.2}$$

Based on the surface chemistry of the particles and aquatic chemistry, particles aggregation may display two different stages. The fast aggregation stage occurs under high salinity conditions or at pH near the ZPC of the particles where the surface charge of the particles is negligible. According to Smoluchowski, [81] in this state, Brownian motion dominates the particle attachment where each collision results in aggregation represented by the following equation.

$$k_{11,fast} = \frac{4k_B T}{3\mu} \tag{3.3}$$

where  $k_{IIfast}$  is the rate constant for fast aggregation,  $k_B$  is the Boltzmann constant, T is temperature, and  $\mu$  is the viscosity of the fluid.

On the contrary, slow aggregation occurs under conditions where the repulsive forces retard the aggregation between the particles lowering the attachment per collision ratio. The relationship between the two stages can be explained by the following equation

$$k_{11}N_1^2 = \alpha k_{11,fast}N_1^2 \tag{3.4}$$

Where  $k_{11}$  is the aggregation rate constant at a given aquatic condition,  $k_{11fast}$  is the rate constant at the fast aggregation stage, and  $\alpha$  is the attachment efficiency. The attachment efficiency is the also known as the reciprocal of the stability ratio (W), where it represents the success of attachment per number of particle collision. The equation can also be stated as

$$\alpha = \frac{1}{W} = \frac{k_{11}}{k_{11,fast}}$$
(3.5)

Through the comparison of aggregation rates, the attachment efficiency ( $0 < \alpha \le 1$ ) can be known without measuring the absolute rate values.

### 3.3 Material and Methods

#### **3.3.1** Preparation of engineered nanoparticles

For the experiments titanium dioxide (P25, Sigma Aldrich), and zinc oxide (Sigma Aldrich, U.S.A.) was used as model ENP. According to the information provided by the supplier, TiO<sub>2</sub> has a diameter of 21 nm with an assay of  $\geq$  99.5% trace metals basis, where ZnO has a diameter of 50 nm and smaller with an assay of  $\geq$  97% trace metals basis. However using the dynamic light scattering (DLS) (Zetasizer nano, Malvern, UK) hydrodynamic radius of the TiO<sub>2</sub> particles was determined to be 200 nm (±20 nm) and ZnO to be 80 nm (±10 nm) in diameter when measured in aqueous conditions. Before each experiment, the powdered form of ENP was suspended in deionized water (18 m $\Omega$ ) and dispersed with a high intensity ultrasound processor (Ultrasonic Homogenizer 4710 Series, Cole-Parmer Instrument Co., Chicago, IL). The electrolyte (NaCl and Na<sub>2</sub>SO<sub>4</sub>) stock solutions were prepared and filtered through 0.2 µm filters and adjusted to the desired pH before use. All samples were, unless otherwise stated, prepared at a pH of 6.0. All chemicals were of analytical grade.

### 3.3.2 Collection and preparation of dissolved organic matter

Wastewater samples were collected from the secondary return sludge of the Wilmington wastewater treatment plant. Sampled wastewater was firmly caped and stored in a refrigerated environment of 4°C before experiments. Experiments were conducted within three days after sampling. To separate the DOM based on the size of the organic matter, the samples were centrifuged at 10,000 g for 10 min (RC-5 Superspeed Refrigerated centrifuge, DuPont) various trials to separate organic particles of diameters smaller than 1 µm. The supernatant was collected from the samples and

analyzed for the organic content with a TOC analyzer. Centrifuged DOM was stored in a refrigerated environment before the experiments.

### **3.3.3** Size measurement

The change is particle diameter was measured by Dynamic light scattering, performed on the Zetasizer nano (Malvern). It was equipped with a He-Ne laser source with a wavelength of 633 nm and 90° fixed angle detector. Before each experiment the ENP was dispersed via sonication for a minimum of 2 minutes for maximum particle dispersion. The stock suspension of  $TiO_2$  and ZnO were added to a disposable methacrylate cuvette (Fisherbrand, Fisher), and diluted with DI water to match the target particle concentration. The desired ionic solution was added moments before the sample was added into the cuvettes and placed in the instrument (Zetasizer) for measurement. For experiments associated to ENP and organic matter, DOM was added to the cuvettes immediately after the ENP.

The measurement was conducted with a 10 second interval with a total measurement time of 30 minutes to an hour. Total detection time was based on the time for the hydrodynamic radius to increase approximately 30%. This provides adequate data to derive the aggregation kinetics of single particle interaction, since the defective doublet hydrodynamic radius has been reported to be about 1.38 times the primary particle hydrodynamic radius. [77,81] All measurements were carried out at 25°C, and the pH of each of the stock solution and DI water were checked before the experiments.

## **3.4** Results and Discussion

The stability of nanoparticles is closely related to the fate, and transport, of nanoparticles. Literature reviews show that aquatic conditions such as ionic strength, organic matter, and divalent cations are the main factors influencing particle stability. However, previous research mainly focuses on a single organic matter under low concentrations, which may not properly represent the field conditions. So in the current study particle stability was tested under conditions simulating filed conditions, such as neutral pH, and high DOM concentrations. As an extension to the previous chapter research was mainly focused on metallic particles such as TiO<sub>2</sub> and ZnO. Conditions such as the pH and type of ions were also considered in the experiment. The results of this study will aid in the comprehension and prediction of ENP in wastewater treatment plants, providing insight to particle interaction under field conditions.



Figure 3.1 Dynamic light scattering (DLS) measurements of  $TiO_2$  aggregation. The aggregation profile was measured as a function of salt concentration. Conditions:  $[TiO_2] = 10 \text{ mg/L}$ , pH = 8, NaCl used as an electrolyte

Fig. 3.1, shows the increase of TiO<sub>2</sub> particle size as a function of time. The experiments were performed in duplicates at each electrolyte concentration showing good reproductively. Results showed an increase in the hydrodynamic radius with time for different electrolyte concentrations. The slope of the hydrodynamic radius versus time curve increased dramatically when the electrolyte concentration was increased from 0.01 M to 0.1 M. Any increase of the electrolyte concentration did not result in additional increase of the curve indicating that NaCl concentration of 0.1 M is in the fast aggregation regime. This is in good agreement with the characteristics of the fast regime where the coagulation rate constant is independent of the electrolyte concentrations. It can also be observed that at higher electrolyte concentrations the particle size distribution increases with time. This is due to multiple scattering, which leads to a diffuse halo around the primary laser beam inside the cell and a reduced intercept of the autocorrelation function resulting in a wider spread in particle size measurement.



Figure 3.2 Stability ratio as a function of various pH conditions. Experimental conditions:  $[TiO_2] = 80 \text{ mg/L}$ ; Temperature = 25°C; electrolyte = NaCl. (Data is illustrated in the Appendix Table J1)

From the slopes obtain in Fig. 3.1 the attachment efficiency was calculated and presented in Fig. 3.2. The stability ratio was shown through the attachment efficiency as a function of electrolyte concentration. Fast coagulation (where the attachment efficiency = 1) was calculated by averaging three of the slopes in the fast regime. The effect of pH on particle aggregation was verified by testing the attachment efficiency of TiO<sub>2</sub> in three different pH conditions (pH 3, 6, 8). The pH was selected based on the zero point charge (ZPC) of the particles, which is known to be approximately pH 6.3. Zeta potential of the particles were measured at +15 mV for pH, close to zero at pH 6, and -20 mV at pH 8. Coagulation of the particles show a strong correlation with the zeta potential where the attachment efficiency was 0.07 mM at pH 6, 8 mM at pH 3, and 20 mM for pH 8. Based on the degree of surface charge, the CCC value rises from one to two degrees of magnitude. The effect of pH can be seen with the results where the surface charge is a two-step protonation of the metal oxides on the particle surface. Since pH 6 is near neutral conditions, a slight increase of ionic strength will result in the coagulation of the particles due to the even distribution of single protonated surface groups. However, with pH 3 the high concentration of dualprotonated surface charges demand a higher degree of anions to suppress the positive surface charge. And with particles in pH 8, the cations will interact with the deprotonated surface for coagulation.



Figure 3.3 Stability ratio as a function of electrolyte concentration. Experimental conditions  $[TiO_2] = 80 \text{ mg/L}$ ; Temperature = 25°C; pH = 3 (Data is illustrated in the Appendix Table J1)

рН	IS	CCC (mM)
3	NaCl	40
6	NaCl	0.007
	Na <sub>2</sub> SO <sub>4</sub>	0.6
8	NaCl	25

Table 3.1 Critical coagulation concentration (CCC) of  $TiO_2$  under various pH and salt conditions. Experimental conditions:  $[TiO_2]$ = 80 ppm, Temperature =  $25^{\circ}C$
Aside from the effect of pH and salinity, multivalent ions are also known to have pronounced effects on the destabilization of colloidal particles [67]. Fig. 3.3 shows the aggregation profiles with different divalent anion concentrations at pH 3 where the particles displayed a positive charge. Results showed a rapid increase in the slope at Na<sub>2</sub>SO<sub>4</sub> concentrations up to 0.5 mM. Compared with the monovalent anions the divalent anions showed a difference in the CCC up to two degrees of magnitude. Shih et. al. [82] have also reported similar results where the CCC of NaCl was 8.2 mM and NaSO4<sup>2-</sup> was 0.05 mM. The results with NaCl showed almost identical results where  $NaSO_4^{2-}$  showed a one degree of magnitude difference. This may be due to the fact that Shih et. al. conducted the experiments in pH conditions ranging for pH 3 to 4, where in pH 4 the zeta potential dropped to 8 mV aiding in the lower CCC value. However in various researches the effect of divalent cations have shown to differ based on the selected ionic species. Results have indicated that will various divalent ionic species the CCC values may differ up to two degrees in magnitude. [83-87] The Schulze-Hardy rule states that the difference between the monovalent and divalent ion CCC values should be 1:64  $(1/1^6 : 1/2^6)$ . However the results from the two different electrolytes (Cl<sup>-</sup> and  $SO_4^{2-}$ ) show a ratio of 1:16.4. The difference between the theoretical and experimental value may be due to the size and symmetry of the sulfate ions, where the Schulze-Hardy rule is based on the assumption of using symmetrical ions, not fitting the tetratomic ions of interest. [88,89]



Figure 3.4 Stability ratio as a function of various DOM conditions. Experimental conditions: [TiO<sub>2</sub>] = 50 mg/L; [ZnO] = 50 mg/L; Temperature = 25°C; electrolyte = NaCl; [DOM] = 300 mg/L; pH = 6. (Data is illustrated in the Appendix Table J2, and J3)

Nanoparticle	[DOM]	CCC (mM)
TiO2	0	0.007
	10% (30 mg/L)	20
	50% (150 mg/L)	9
	100% (300 mg/L)	70
ZnO	0%	5.5
	100% (300 mg/L)	90

Table 3.2 Critical coagulation concentrations of TiO<sub>2</sub> and ZnO under various dissolved organic matter (DOM) concentrations. Experimental conditions: [TiO<sub>2</sub>] = 50 mg/L; [ZnO] = 50 mg/L; Temperature = 25°C; electrolyte = NaCl; [DOM] = 300 mg/L; pH = 6 The effect of dissolved organic matter on particle stability was tested with wastewater organic matter sampled from wastewater treatment plant B. Dissolved organic matter was extracted from secondary waste sludge and tested under neutral pH conditions (pH 6). The DOM showed TOC concentrations of 409 mg/L, where the experimental conditions were set at 300 mg/L due to the addition of ENP and ionic species.

Fig. 3.4 shows attachment efficiency of  $TiO_2$  and ZnO under various DOM concentrations. With the addition of organic matter, results showed that both particles displayed higher CCC values with the existence of organic matter. The overall aggregation kinetics of both particles showed similar results where CCC values where of 100 mM similar to the results found in the literature review above. However as seen in Fig. 3.4 (a), the change in organic matter concentration impacts the aggregation kinetics, where lower organic concentration shows lower CCC values. It has been proven in various researches that the amount of sorption influences the aggregation kinetics of nanoparticles, where the increases of HA from 1 mg/L to 5 mg/L results in a 35% increase in the CCC. This may be due to the compact formation of DOM on particle surfaces where the increase of ionic strength impacts the shape and morphology of organic material. [69] It has been observed that with increasing ionic strength, the steric repulsion of humic acid (HA) decreases, resulting compact sorption layer of HA on the particle surface. [90] The higher ionic strength may also change the shape of the organic material from linear to spherical due to the neutralization of anionic carboxylic and phenolic groups [69, 91] resulting in higher sorption. Hence higher ionic strength and organic concentration may result in the decrease of aggregation kinetics. Additionally the concentration of DOM may also be a factor,

where under higher organic concentrations larger amounts of ionic salts may be needed to reach the CCC values. Due to the increased number of functional sites a higher amount of cations will be needed to reduce the steric effects of the organic material.

Fig. 3.4 shows the influence of dissolved organic matter on the stability of nanoparticles displaying a slightly higher CCC value with ZnO. With the results seen in Fig. 3.4 experiments were conducted under the assumption that the total amount of organic sorption will differ with the ENP surface charge. Results in Fig. 3.5 show that sorption of ZnO display elevated amounts of sorption with higher ENP concentrations. However under the same experimental conditions,  $TiO_2$  showed only trace amounts of sorption. This has also been observed in various studies, where the maximum sorption of humic acid (HA) to ZnO was measured to be 60.48 mg-HA/g-NP [76], and maximum NOM sorption to TiO<sub>2</sub> of 18 mg-NOM/g-NP [69]. Although is has been proven that humic acids have a stronger affinity to metallic particles, the measured difference of other organic matter does not exceed two times the amount. [69] The higher sorption of organic matter to ZnO may indicate a multilayer sorption, where the positive surface charge masks the functional groups of the initial organic layer leading to additional sorption. However, regardless of the additional layers, the results of  $TiO_2$ and ZnO in Fig. 3.4 indicate that the outer organic sorption layers display comparable amounts of functional groups, resulting in the measured CCC values.



Figure 3.5 Sorption study of DOM and small organic particulates with ZnO. Dissolved organic matter (d < 100 nm), Small organic particulates (100 nm  $< d < 1 \mu$ m), pH = 6.5. (a) Organics obtained from primary waste sludge. (b) Organics obtained from secondary waste sludge. (Data is illustrated in the Appendix Table K1)

## 3.5 Conclusion

In the current research the stability of ENP was tested under various aquatic conditions. Particles were tested in various pH, ionic species, and dissolved organic matter concentrations to verify the fate and transport of ENP in WWTP. Results showed that with various pH and ionic species the aggregation characteristics agreed with the DLVO theory, with a slight variation in divalent anion species. Attachment of dissolved organic matter to the particle surface aided in the stabilization process, where higher organic attachment resulted in higher stability. Sorption experiments reveled a multi-layer sorption on ZnO particles proving that relationship between organic sorption and particle charge. However the results have also showed that regardless of the number of sorption layers, the outer sorption layer displayed comparable amounts of functional groups on the outer sorption surface. Additionally it was shown that the aggregation kinetics can be influenced by the concentration of organic species, where lower DOM will result in lower CCC values. With the results obtained from the current research it can be deduced that when ENP travels into wastewater treatment plants, dissolved organic matter will stabilize the particles, regardless of its surface charge, resulting in a plant wide distribution of the ENP.

#### Chapter 4

# INTERACTIONS BETWEEN ENGINEERED NANOPARTICLES AND SLUDGE PARTICULATES WITH SIZE GREATER THAN 1 $\mu M$

#### 4.1 Introduction

With the increased use of engineered nanoparticles (ENP) in consumer products, release of the particles will eventually flow into municipal wastewater systems. Nanoparticles in the municipal stream will then interact with dissolved organic matter resulting in a stable transport to wastewater treatment plant (WWTP), without substantial loss to the sewer biofilm. [92] The WWTP will have a defining impact on the ENP, which will later influence the fate of the particles. As an example, the anaerobic treatment in WWTP may alter the surface chemistry of the particles, reducing the solubility. This is mainly observed with silver and zinc particles where they undergo sulfidation. Sulfidation occurs during lime and heat treatment process, where Zn is mainly transformed into ZnS, Zn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, and Zn associated Fe oxy/hydroxides. Studies have shown that with the total Zn found in the WWTP, up to 70% found in a form of sulfidation. [93,94] Silver on the other hand was mainly transformed into AgS. [94,95] The change in surface chemistry has been known to influence the short-term toxicity due to the chemical alteration of the particles. The sulfidation of the particle surfaces decrease the solubility of the particles rendering further travel in aquatic environments. However, although the toxicity may be reduced due to surface modification, the outflow of particles may impact water born species due to cell deposition. [96] With the lower dissolution nanoparticles may travel longer distances eventually deposing onto aquatic species. The attached particles will eventually dissolve, directly diffusing the particle material into the cells resulting in higher toxicity than ionic metal. [97]

An additional influence of WWTP to ENP is the redistribution of particles through sludge sorption. The ENP that is adsorbed to sludge is later distributed to secondary locations through disposal of waste activated sludge. In the United States, it is estimated that over 50% of WWTP sludge is processed to biosolids for fertilizer. [98] And with the biosolids, it has been reported to contain up to 1,000 mg/kg of TiO<sub>2</sub> with a few hundred mg/kg Zn. [23] The metallic particles may impact the soil where it has been proven that TiO<sub>2</sub> and ZnO both reduce both microbial biomass and diversity. [99] To better understand the fate of ENP and its impact to the environment, the removal of ENP in WWTP through sorption should be further observed.

The main goal of this chapter was to study the interaction of ENP by wastewater and its constituents, such as dissolved organic matter, and sludge particulates. The extent of ENP attachment to sludge and its constituents will be studied by determining the amount of ENP adsorbed as a function of free ENP concentration (sorption isotherms) using differential sedimentation methods. In order to study the sorption of each of the constituents the sludge was separated into four different samples based on the sludge constituents (DOM, DOM + sludge particulates, particulate matter, sludge). Each of the samples underwent a differential sedimentation experiment to observe the absorption isotherm to the particular substance with ENPs. The adsorption isotherms will aid in the explanation of ENP distribution in wastewater treatment plants as well as propose additional insight to future research.

### 4.2 Theoretical Aspects: Quantification of Engineered Nanoparticle Uptake

## 4.2.1 Sedimentation of engineered nanoparticle adsorbed sludge particulates

To measure the attachment of ENP to various materials, there are a limited number of methods developed and currently in use. For example, batch sorption experiments are a widely used method, where nanoparticle and sorbents such as sand and soil [100, 101] are agitated in overhead shakers, and later separated through centrifuge. Column experiments are also a popular method where the nanoparticles are injected continuously or in a pulse method, and sorption parameters are measured from the difference of the inflow and outflow concentration [102-105]. Currently, quartz micro balance have been used as a novel technique to verify the sorption based on the frequency shift of particles to the quartz surface [106-108].

However the experimental mentioned above is based on the attachment of a mobile substance (ENP) onto stationary material. Measuring sorption parameters under conditions where the sorbent and sorbate are both mobile, such as sludge and ENP, is proven to be difficult. And although the actual mechanism of ENP interaction with sludge is attachment, the term sorption will be used interchangeably in the current chapter when describing the quantifiable isotherms. Current research relating ENP to sludge sorption methods mainly rely on laboratory scale batch reactor experiments. ENP is continuously inserted into the batch reactor for a fixed amount of time. The sludge is then extracted and measured for the amount of ENP. Although the method accurately represents the conditions of sediment tanks, it is a time intensive process where the sludge should be sampled and conditioned before the procedure. And while during the conditioning process the sludge characteristics may change, altering the sorption characteristics (parameters). Additionally, particle loss may also

occur due to sweep flocculation during the sedimentation process of the bench reactors. To overcome the limitations of the conventional method, the current research focuses on a differential sedimentation process, where the free particle concentration is extrapolated based on the sediment characters of suspended particles. The process is conducted by mixing sludge and nanoparticles together and left to settle. Nanoparticle attached sludge flocs settle comparatively fast compared to the free nanoparticles in the sample. After the flocs settle the free particles show a linear decrease in their concentration. Based on the time dependent concentration decrease of the free particles a, trend line can be draw where free particle concentration before floc sedimentation can be calculated. The suggested method has an advantage over conventional methods where unconditioned sludge can be used directly, representing the actual sludge conditions of the wastewater treatment plant. Additionally, sorption parameters can be measured without particle loss due to sweep flocculation, and the process is conducted in a more time worthy and simple method. However the experiment is difficult to conduct with trace amount of particles under high sludge concentrations. And although the actual mechanism of ENP interaction with sludge is attachment, the term "sorption" will be used in the current chapter when describing the quantifiable isotherms. In other words, the term "sorption" will be used instead of attachment when explaining sorption parameters.

## 4.2.2 Analysis of sedimentation curves

Sorption is the most crucial aspects in the fate of ENP in WWTP. However due to the difficulty of measuring the attachment of particular mater to suspended organic matter, a non-conventional method was used for sorption measurement. [109] Differential sedimentation of sludge particulates and ENP were measured as a function of time. Sedimentation as a function of time is referred as a sedimentation curve. Sedimentation curves of sludge-ENP mixture were analyzed following the approach developed by Nicolosi et al. (2005) who describes the local concentration as a function of time.

Based on the local conservation equation for mass and linear momentum, the local concentration of the settling particles can be expressed as

$$C_t = C_o e^{-t/\tau} \tag{4.1}$$

where  $C_t$  is the local concentration at the sedimentation time t;  $C_0$  is the concentration at the beginning of the sedimentation;  $\tau$  is the time constant, which is a hydrodynamicchemical characteristic property of particles. Based on the shape of the sediments (where in the current study assumed to be spherical) the time constant can be calculated by the following equation.

$$\tau = \frac{9\eta(\beta - p_f)}{2g^2(\rho_s - \rho_l)^2 R_H^2}$$
(4.2)

where,  $\eta$  is the viscosity,  $\beta$  is related to the solid fluid interaction force and has the dimension of pressure,  $p_f$  is the fluid pressure, g is the acceleration due to gravity,  $\rho_s$  is the solid component density,  $\rho_l$  is the liquid density, and  $R_H$  is the hydrodynamic radius. Due to the size and characteristics of the sludge flocs, the sedimentation process can be separated into three phases. Each phase occurs based on the size of the flocs, where large and small flocs show different sedimentation behavior. After ENP sorption to sludge reaches equilibrium, three different sediment types can be observed,

with large agglomerates of sludge flocs and ENP, followed by small sludge particulates observed with ENP. Additionally, nanoparticles that do not sorb to sludge surfaces also exist in the mixture as well. This can be characterized in the sedimentation curve, where three asymptotic lines can be drawn, each representing the different sediment type. In the current research each sediment type will be characterized as "type 1" and "type 2" aggregates for the large and small sediments, respectively. Free ENP particles are named "type 3" particles, for the sake of differentiation.

Although the turbidity of each sediment type cannot be measured, the total turbidity of the sedimentation process has been measured as a function of time and represented as  $T_{Total(t)}$ . The total turbidity is a summation of each type of sedimentation ("type 1", "type 2" and "type 3") where each type is indicated as  $T_1(t)$ ,  $T_2(t)$  and  $T_3(t)$ .

$$T_{total}(t) = T_1(t) + T_2(t) + T_3(t)$$
(4.3)

Turbidity can be related to concentration by its extinction coefficient  $\alpha$ , which is assumed constant throughout the sedimentation experiment.

$$T(t) = \alpha C(t) \tag{4.4}$$

By combining Eq. (4.3) and (4.4) the sedimentation equation can be rewritten as

$$T_{total}(t) = \alpha_1 C_1(t) + \alpha_2 C_2(t) + \alpha_3 C_3(t)$$
(4.5)

$$T_{total}(t) = \alpha_1 C_1(0) e^{-\frac{t}{\tau_1}} + \alpha_2 C_2(0) e^{-\frac{t}{\tau_2}} + \alpha_3 C_3(0) e^{-\frac{t}{\tau_3}}$$
(4.6)

$$T_{total}(t) = T_1(0)e^{-\frac{t}{\tau_1}} + T_2(0)e^{-\frac{t}{\tau_2}} + T_3(0)e^{-\frac{t}{\tau_3}}$$
(4.7)

where  $\alpha_1$ ,  $\alpha_2$  and  $\alpha_3$  are the extinction coefficient of type 1, 2 and 3 sedimentation, the zero in the quotation mark means the beginning of the sedimentation.

The term  $T_{total}(t)$  can be measured using UV-visible spectrophotometer, whereas  $T_1(t)$ ,  $T_2(t)$  and  $T_3(t)$  are calculated. The difference in the settling properties of "type 1", "type 2" and "type 3" is the priori requirement for their successful separation from each other during data analysis. The difference in sedimentation will allow the determination of the time constants, i.e.,  $\tau_1$ ,  $\tau_2$  and  $\tau_3$  from which  $T_1(0)$ ,  $T_2(0)$ , and  $T_3(0)$  can be calculated. The "separation time" (time at which the slope of the sedimentation changes) from "type 1" to "type 2" aggregates is designated as  $t^*_{12}$ , and  $t^*_{23}$  from "type 2" to "type 3". Since "type 1" sedimentation proceeds ahead of "type 2",  $t^*_{12}$  always arrives before  $t^*_{23}$ . Intuitively, when the sedimentation time reaches  $t^*_{12}$ , it means that "type 1" aggregates have already settled and  $T_1(t)$  becomes zero (Fig. 4.1 (a)). When the sedimentation time reaches  $t^*_{23}$ , "type 2" aggregates completely settle, there both  $T_1(t)$  and  $T_2(t)$  are zero. (Fig. 4.1 (b))

$$T_{total}(t') = T_3(0)e^{-\frac{t}{\tau_3}}$$
(4.8)  
$$lnT_{total}(t') = \ln T_3(0) = t'/\tau_3$$
(4.9)

Now we can go back to calculate  $T_2(0)$  and  $\tau_2$ . When the sedimentation time reaches  $t*_{12}$ , "type 1" aggregates already settle and  $T_1(t)$  is zero. Therefore, when t = t" ( $t > t*_{12}$ ),  $T_2(0)$  and  $\tau_2$  can be obtained according to Eq. 4.12.

$$T_{total}(t') = T_2(t') + T_3(t')$$
(4.10)

$$T_{total}(t') - T_3(t') = T_2(0)e^{-t/\tau}$$
(4.11)

$$\ln(T_{total}(t') - T_3(t')) = \ln T_2(0) - t'/\tau_2$$
(4.12)

Finally,  $T_1(0)$  and  $\tau_1$  can be obtained using Eq. 4.13 Fig. 4.1 (c) shows an example that the turbidity data is well fitted by using this procedure.

$$\ln(T_{total}(t) - T_3(t) - T_2(t)) = \ln T_1(0) - t'/\tau_1$$
(4.13)

#### 4.2.3 Determination of free engineered nanoparticle concentration

By using the calibration curve of turbidity versus ENP concentration,  $T_3(0)$  is used to calculate free ENP concentration, which represents ENP equilibrium concentration, *C* (mg/L). ENP uptake  $\Gamma$  (#-TiO<sub>2</sub>/kg-sludge) was calculated with the mass balance equation

$$\Gamma = \frac{c_0 - c}{x} \tag{4.14}$$

where  $C_0$  is the initial concentration of ENP (mg/L), X is the organic concentration of sludge (g/L). The free ENP concentration was extrapolated by the turbidity value of T<sub>3</sub>(0), using the intercept of the y axis and the trend line of T<sub>3</sub>(0). Once the T<sub>3</sub>(0) value was obtained, the free ENP concentration was extrapolated from a calibration curve representing the relationship between the turbidity and ENP concentration. The calibration curve of ENP concentration was obtained under various DOM concentrations. For accurate conversions, the Toc values of the samples after the sedimentation experiments were also measured for its dissolved organic concentration.





Figure 4.1 Sedimentation curve and analysis of ENP to sludge. Conditions: pH = 6.5, [ZnO] = 80 mg/L, Sludge = Wilmington Primary sludge. (a) Linear fit for "Type 1" and Type 2" sedimentation. The overlap of the two trend lines indicate  $t_{12}$ . (b) Linear fit for "Type 2" and Type 3" sedimentation. The overlap of the two trend lines indicate  $t_{23}$ . (c) Linear fit for "Type 1", "Type 2", and "Type 3"sedimentation. (Data of the figures are illustrated in Appendix 4.1)

#### 4.3 Materials and Methods

#### **4.3.1** Preparation of engineered nanoparticles and sludge

ENP selected for the current experiment were SiO<sub>2</sub>, TiO<sub>2</sub> and ZnO. Commercial grade, nano-sized TiO<sub>2</sub> (> 99.5%) was purchased from Degussa Corp. (Degussa Aeroxide P25, Parsippany, NJ) and was used without any treatment. In addition, ZnO nano-powder (< 50 nm) was purchased from Sigma Aldrich (> 97%) and also used without treatment. Prior to each experiment, nanoparticle solutions were prepared with deionized water, and sonicated with a high powered sonicater (Ultrasonic Homogenizer 4710 Series, Cole-Parmer Instrument Co., Chicago, IL) before inserted into the sludge.

For sorption experiments, sludge was sampled from the primary and secondary sediment sludge tanks of the Wilmington wastewater treatment plant. As listed above sampled sludge was firmly capped and placed in temperature-controlled environment during transportation. The samples were stored in a refrigerated environment of 4°C before experiments. Experiments were conducted within three days after sampling. The preparations for sorption experiments were conducted equally to both primary and secondary sludge samples.

To understand the interactions of ENP and sludge particulate in detail, sludge particulate was separated into four different subsamples based on their size and constituents. The four subsamples were; 1) dissolved organic matter (DOM) with a size of d < 100 nm, 2) small organic particular matter (100 nm  $< d < 1 \mu$ m), 3) large

organic particulates ( $d > 1 \mu m$ ), and 4) sludge that contains all the substances above (sludge that does not undergo any separation process.)

The first subsample was consisted of only DOM, where 20 mL of sludge was centrifuged at 10,000 g for 20 min using a high-speed centrifuge (Marathon 22k, Fisher scientific). Each sample was centrifuged three times for maximum separation. The second sample was of DOM and sludge particles smaller than 1  $\mu$ m in diameter. Sludge particulates were separated by gravity sedimentation in an Imhoff cone for 4hr. The supernatant was then collected which includes the DOM and particles smaller than 1  $\mu$ m in diameter without any DOM or small particulates. Sludge particulates were gravimetrically settled for 4 hr, and after the supernatant was removed the sample was filled with DI water and settled again. The supernatant would be removed and the process would be continued for at least four times to remove all the DOM and small particles from the sludge. The fourth subsample was the original sludge containing all the constituents above (DOM, small and large particulate matter). All sludge samples were poured through a 4.5 mm sieve to remove waste material irrelevant to the experiment. [14]

For sorption experiments conducted under various ionic strengths, the sludge samples were deionized prior to the experimental procedures. Dialysis tubes of 1 kDa were the sludge was bagged and desalinated in a tank of deionized water to lower the ionic strength to that of tab water. NaCl was used to control the ionic strength as well as HCl and NaOH for pH control. Stock solutions of NaCl were mixed in the desalinated wastewater samples, and measured with a conductivity meter for reference.

#### 4.3.2 Sedimentation experiment

For the sedimentation test ENPs of various amounts were introduced into the sludge samples. To obtain a sediment curve a time dependent measurement of turbidity should be conducted. Samples were mixed in 12 mm disposable polystyrene cuvettes, where sludge and ENP were added along with Di water or NaCl, depending on the experiment. The cuvettes where shaken and placed on a shaking plate for 2 hr. The cuvettes where then placed in a UV-visible spectrophotometer (HACH DR5000) to measure the light transmittance at the wavelength of 600 nm. The data was converted to turbidity, T, using the Lambert-Beer law.

$$I/I_o = e^{-Tl} \tag{4.14}$$

where  $I/I_o$  is the transmittance, T is the turbidity and l is the sample length. The light transmittance was recorded every 10 min for the first 1hr, 20 min for the next 1 hr, and measured every 30 min for the next 2 hours. Each experiment was repeated 5 times for accuracy.

#### 4.4 **Results and Discussion**

As it has been revealed in the previous sections, the bulk of the ENP is found in the primary and secondary sludge. From this, it can be deducted that the fate of ENP in WWTP primarily relies on sorption of ENP to DOM and sludge particulates. Various researches have been conducted on the fate of ENP in wastewater treatment plants, where the sorption of particles to sludge has been observed. Fig. 4.2 shows the results of various research groups using a laboratory sequence batch reactor to measure the removal of ENP through sludge and verify the sorption parameters. Silver nanoparticle attachment to secondary sludge of different research groups has shown results within the error range, indicating similar sorption characteristics among the different research groups. Sorption of TiO<sub>2</sub> has also been compared with the data from various research groups, and results from the current research. Although the range of ENP concentration tested was different for each group, the data showed an alignment in the results, indicating that the differential sedimentation method is an interchangeable method with batch reactor methods. So based on the established method, this study will focus on the interaction of ENP and sludge particulates. Factors that influence the attachment such as ionic strength, concentration ratio of sludge particulates to ENP, composition of ENP, and characteristics of the sludge particulates were investigated. The various parameters selected are to represent practical and realistic conditions of a WWTP. Based on this study it will be possible to understand and predict the fate of ENP due to attachment in the primary and secondary sediment tanks.



Figure 4.2 Sorption of various engineered nanoparticles to secondary sludge from wastewater treatment plants. ● Silver nanoparticle from textile #1 [14],
● Silver nanoparticle from textile #2 [14], ● Silver nanoparticle from textile #3 [14], ▲ TiO<sub>2</sub> sorption in wastewater reactor [15], ■ TiO<sub>2</sub> in wastewater of low organic loads, ● TiO<sub>2</sub> in wastewater of high organic loads, ◆ ZnO in wastewater of low organic loads, ● TiO<sub>2</sub> in wastewater of high organic loads. The results are based on batch reactor tests with continuous inflow of nanoparticles. The mg-biomass is based on the dry weight of the bioreactor sludge. (Data is illustrated in the Appendix Table M1)

#### 4.4.1 Effect of sludge particulate size on engineered nanoparticle sorption

Sedimentation experiments were conducted with ZnO and TiO<sub>2</sub> nanoparticles adsorbed to primary and secondary sludge (Fig. 4.3-4.6). As mentioned in the methodology above sludge was partitioned into four fractions based on the particle size of the organic material. Each fraction was separately tested with various concentrations of ZnO and TiO<sub>2</sub> (0, 10, 20, 40, 60 and 80ppm). Note that the pH and ionic strength of all the samples were matched to that of the initial condition of the sampled WWTP.

As seen in the stability research, conducted in the previous section, interaction of DOM with particles resulted in higher particle stability. This is seen with both TiO<sub>2</sub> and ZnO, where mainly "type 3" aggregates (free particles) were the dominate form in the sediment curves (Fig. 4.3a and 4.3b). Considering the fact that the pH of the sludge sample matches the isoelectric point (IEP) of TiO<sub>2</sub>, it can be speculated that the DOM (which is smaller in size compared to TiO<sub>2</sub>, < 10 nm) has formed a coated layer on the particle surface. And through this layer a net charge has been formed aiding in the stability of TiO<sub>2</sub> particles. ZnO particles, however exhibit IEP at pH 10.4 displaying a net positive charge at the given condition of the sample (pH 6.5). Negatively charged DOM attaches to the surface of the ZnO particles neutralizing the net charge of the particles, with the steric repulsion of the organic matter that dominates the stability of the particles. The results showing similar stability characteristics of both particles reinforce the results of the attachment efficiency research, where both particles also showed similar stability with organic attachment.





Figure 4.3 Sedimentation profile of Zn and TiO<sub>2</sub> with DOM. Primary [DOM] = 400 mg/L, Secondary [DOM] = 250 mg/L. Reaction time = 5 hr. pH = 6.8 (a) Primary DOM + TiO<sub>2</sub> (b) Secondary DOM + TiO<sub>2</sub>. (c) Primary DOM + ZnO (d) Secondary DOM + ZnO. (Data is illustrated in the Appendix Table N1~N4)





Figure 4.4 Sedimentation profile of ZnO and TiO<sub>2</sub> with supernatant. Primary supernatant [MLSS] = 567 mg/L. Reaction time = 5 hr. pH = 6.8 (a) Primary supernatant + TiO<sub>2</sub> (b) Secondary supernatant + TiO<sub>2</sub>. (c) Primary supernatant + ZnO (d) Secondary supernatant + ZnO. (Data is illustrated in the Appendix Table N5-N8)





Figure 4.5 Sedimentation profile of Zn and TiO<sub>2</sub> with large organic particles.
Primary [MLSS] = 2242 mg/L, Secondary [MLSS] = 6100 mg/L.
Reaction time = 5hr. pH = 6.8 (a) Primary + TiO<sub>2</sub> (b) Secondary + TiO<sub>2</sub>.
(c) Primary + ZnO (d) Secondary + ZnO. (Data is illustrated in the Appendix Table N9-N12)





Figure 4.6 Sedimentation profile of Zn and  $TiO_2$  with sludge. Primary [MLSS] = 2942 mg/L, Secondary [MLSS] = 6667 mg/L. Reaction time = 5 hr. pH = 6.8 (a) Primary sludge +  $TiO_2$  (b) Secondary sludge +  $TiO_2$ . (c) Primary sludge + ZnO (d) Secondary sludge + ZnO. (Data is illustrated in the Appendix Table N13-N16)

The second series of sediment experiments were conducted with small organic particles (< 1  $\mu$ m) and DOM (Fig. 4.4). Larger organic particles (> 1  $\mu$ m) were settled and removed from the samples, to verify the effects of suspended organic matter on the attachment and fate of ENP. Results mainly showed "type 2" and "type 3" sedimentation, indicating a significant degree of sedimentation with both ZnO and TiO<sub>2</sub>, compared with samples containing only DOM. Results also showed that particles with secondary sludge displayed higher sediment rates, and among the particles ZnO showed a higher sediment rate compared to  $TiO_2$ . The higher amount of sedimentation in secondary sludge is due to the characteristics of the sludge, where primary sludge is mainly consisted of inorganic granular material, and dissolved organic material, and secondary sludge is mainly consisted of sludge flocs. The contribution of sludge flocs to "type 2" sedimentation are assumed to be not only due to the flocs but also to the microbial matter that breaks out of the flocs as well, leading to additional "type 2" sedimentation. Among the particles tested, ZnO showed a higher degree of sedimentation compared to TiO<sub>2</sub>. This may be due to the surface charge of the particles, where the positive charge of the zinc particles results in the charge screening of the sludge flocs or dissolved organic matter. Is can be seen in Fig. 4.4d, where ZnO in secondary sludge shows accelerated aggregation of the flocs resulting in a "type 1" sedimentation in the early stages of the sedimentation process.

Fig. 4.5 shows the attachment of ENP to large organic particulates (> 1  $\mu$ m). Due to the low concentration of DOM in the samples, sedimentation profiles show mostly type 1 and type 3 aggregations. Type 2 aggregations are visible in the process however the amount is small compared to sedimentation experiments with original sludge or small organic particles (Fig. 4.4). With both particle, the "type 2"

sedimentation is mainly seen in the primary sludge. Compared to the experiments with small organic matter, it can be observed that the "type 2" sedimentation occurs in a shorter time frame. This may be due to the composition of the primary sludge where the difference in density of the inorganic and organic particular matter results in a difference in sediment time. It can also be seen that, compared with small organic matter, the residual free particle concentration is lower with the large organic particles, indicating that larger organic particles have a larger impact with ENP sorption. Additionally it can be seen that regardless of the initial concentration, ZnO displays a small difference in the free particle concentrations after the sedimentation process, indicating a stronger affinity of ZnO particles due to the positive surface charge of the particles. With ZnO particles it can also be observed that with the free particles there is a slight decrease in the turbidity throughout the "type 3" stage. This may be due to residual DOM in the sample where it has been proven that low organic concentration aids the coagulation of ENP.

Fig. 4.6 shows the sedimentation profile of the original sludge that did not go under any separation. This profile shows sediment and adsorption characteristics of all the experiments above. The samples from both basins (primary and secondary) show all three type of aggregation (type 1, 2 and 3). Due to the stronger attachment characters ZnO show less deviation in the turbidity with different particle concentrations. By comparing the sedimentation profile of the different sludge partitions it can be observed that the simple summation of the turbidity difference of the sludge different fractions do not equal the turbidity difference of the original sludge. For example with TiO<sub>2</sub> and primary sludge, the turbidity difference with DOM does not show any decrease, where turbidity difference with small organics particulates (< 1  $\mu$ m) + DOM = 0.4, large organics (> 1  $\mu$ m) = 0.8 and un-separated field sludge (DOM + small + large sludge particulates) = 1.4. Hence the sum of the results of the three samples (DOM + small organic +large organic = 1.2) does not equal the total turbidity difference of the original sludge (1.4). This indicates that the adsorption of ENP to sludge is a complex process where the simple summation of sorption to DOM and sludge particulates does not represent the total sorption of ENP.

The Freundlich model was chosen to describe the uptake process as this model is not restricted to a monolayer case and it avoids the assumption of surface homogeneity. The Freundlich model has the form as:

$$\Gamma = K_F C^{1/n_F} \tag{4.15}$$

where  $\Gamma$  is TiO<sub>2</sub> uptake (ppm-TiO<sub>2</sub>/g-Biomass), C is TiO<sub>2</sub> equilibrium concentration (mg/mL) and K<sub>F</sub> is the Freundlich constant (mL/g-Biomass). The parameter 1/n<sub>F</sub> is related to the sorption intensity (Weber et al., 1991) and 1/n<sub>F</sub> is between zero and one. [110] To determine K<sub>F</sub> and 1/n<sub>F</sub>, data are fitted to the logarithmic form

$$ln\Gamma = lnK_F + 1/n_F lnC \tag{4.16}$$

Similar isotherms have been observed for other associations of nanoparticles with cells [40, 44]. The attachment of gold nanoparticles to mammalian cells has the similar pattern as the results shown above [44]: gold nanoparticle attachment to cells increases with the applied nanoparticle concentration, but the saturation of the cellular

attachment can be observed. Studies [40] show that the attachment of CeO2 nanoparticles with E. coli increases with free CeO<sub>2</sub> nanoparticle concentration, and the maximum attachment is  $16 \text{ mg/m}^2$ , which corresponds to a multilayer adsorption: 64  $m^2$  of the surface of CeO<sub>2</sub> nanoparticles for 1  $m^2$  of outer bacteria surface, which equals to 1.6 layer of attached nanoparticles on bacterial surfaces. For the purpose of comparison, the CeO<sub>2</sub> sorption isotherm obtained by Thill et al. (not described by any model) is described in the Freundlich model here: the fitted parameters K<sub>F</sub> and 1/n<sub>F</sub> is around 418 (mL per cell) and 0.15, respectively ( $R^2 = 0.91$  for the fitting). The parameter  $1/n_F$  is related to the sorption intensity. The result suggests that the sorption intensity for CeO<sub>2</sub> and E. coli should be smaller than that for TiO<sub>2</sub> and algae at pH 6.9 but larger than that at pH 4.2. The point of zero charge of CeO<sub>2</sub> nanoparticles is at pH 10.5; under the experimental condition around pH 6, CeO<sub>2</sub> nanoparticles are positively charged [40]. It is inferred that the electrostatic attraction between CeO<sub>2</sub> nanoparticles and negatively charged E. coli promotes the uptake. The above discussion suggests that electrostatic interaction along with other attractions, such as van der Waals, may be influential on pH-dependent TiO<sub>2</sub> uptake by microbes in the sludge. Furthermore, the examination of the interaction in a quantitative manner, i.e., by DLVO theory, may provide the insight of mechanisms of TiO<sub>2</sub> uptake.



Figure 4.7 Effect of the sludge particulate size on the sorption on TiO<sub>2.</sub> (Data is illustrated in the Appendix Table O1)


Figure 4.8 Effect of the sludge particulate size on the sorption on ZnO. (Data is illustrated in the Appendix Table O2)

Nanoparticle	Organic size	n <sub>F</sub>	K <sub>F</sub>
TiO <sub>2</sub>	$100 \text{ nm} < d < 1 \mu \text{m}$	1.45	3.02
	$d > 1 \ \mu m$	0.78	0.29
	Total sludge	0.63	0.042
ZnO	$100 \text{ nm} < d < 1 \mu \text{m}$	0.72	0.093
	$d > 1 \ \mu m$	1.18	0.54
	Total sludge	1.42	0.63

Table 4.1 Sorption parameters of TiO<sub>2</sub> and ZnO with sludge particulates of various sizes. The parameters were based on the results shown in Fig. 4.7 and 4.8. Unit of parameters:  $K_F = (\mu g/mg)(L/mg)^{1/n}$ 

Fig. 4.7 shows attachment results of  $TiO_2$  to small organic particles (100 nm <  $d < 1 \mu m$ ), large organic particles (1  $\mu m < d$ ), and sludge without any separation (total sludge). Results showed the highest overall attachment to the total sludge, with slightly higher affinity to small sludge particulates compared to large sludge particulates. The attachment characters differ with the total sludge as well where the sorption intensity  $(1/n_F)$  is higher with the total sludge. This higher TiO<sub>2</sub> to small sludge particulate attachment may be due to the surface area of the organic particles due to their average size. Also the existence of  $Ca^{2+}$  in the sludge may aid in the attachment through calcium bridging of the organic coated particles as well. Higher sorption intensities can be observed in the total sludge, but also with higher concentration range of TiO<sub>2</sub>. This may be attributed to the aggregation of TiO<sub>2</sub> particles, where the high concentration of particles increases the collision frequency. This may result in attachment of aggregated nano particles to the sludge displaying higher sorption intensities. Fig. 4.8 shows the attachment results of ZnO to various fractions of sludge tested in Fig. 4.7. With ZnO, it can be seen that the size of the sludge particulates has less effect on the sorption properties, compared to TiO<sub>2</sub>. The properties of ZnO attachment may be due to the positive surface charge of the particles, which contributes in two different aspects. First is the lower aggregation and even distribution of the particles. Unlike  $TiO_2$  the charge of ZnO prevents aggregation, resulting in smaller and more mobile particles. This may result in a higher possibility of the particles to attach to sludge surfaces. Second, is the opposing charge of ZnO with the organic matter. Steric attraction of the particles and organic matter due to the charge also aids in the higher affinity of ZnO particles, compared with the TiO<sub>2</sub> attachment.

Fig 4.9 compares the attachment of  $TiO_2$  and ZnO on sludge and its constituents. Results show that with both large and small organic particulates, ZnO display a stronger affinity to sludge than  $TiO_2$ . The difference in affinity is more extreme with large sludge particulates. This may be due to the charge screening of ZnO particles to the sludge particulates, where the compiling of the particles masks the negative charge of the functional groups favoring additional attachment of organic material with ZnO particles. The complexity of ENP sorption to sludge may be due to various reasons such as multilayer sorption, flocculation of organic particles, calcium bridging, and hydrophobic attraction. However, further research is to be conducted on the matter.



Figure 4.9 Summary of sludge particulate size on the effect of TiO<sub>2</sub> and ZnO uptake. (Data is illustrated in the Appendix Table O1, and O2)

### 4.4.2 Effect of ionic strength on engineered nanoparticle uptake

The current chapter focuses on the effect of ionic strength on the attachment characteristics of sludge. Fig. 4.10 shows the attachment of  $TiO_2$  to secondary sludge in various ionic strengths. Sorption experiments were conducted in salt concentrations from 280 mM to 980 mM. Results showed that with higher salt concentrations, an overall lower degree of ENP attachment occurred. However, closer observations show that the attachment results in a two-step manner. This can be seen in all the tested conditions, where the sorption characteristics displays Langmuir type sorption in low particle concentrations (Ce), but increases sharply as the concentration increases. To verify the results in detail, Fig. 4.11 shows the attachment characteristics in each ionic strength condition tested. A dual sorption model was applied for the analysis, separating the initial Langmuir type sorption and the secondary sorption increase. Two main phenomena's were observed in the results as the ionic strength increases. The fist was that as the ionic strength (IS) increases, the secondary sorption starts occurring at higher ENP concentrations. This can be observed with IS conditions from 280 mM to 830 mM, where the initial engagement of the secondary sorption curve occurs from 30 mg/L to 85 mg/L. The second was that as the IS increases, the maximum sorption of the secondary curve decreases. The observed phenomena can be explained by the DLVO theory. With the increase of ionic strength, the electric double layer is compressed with the hydrophobic attraction unchanged. This leads to conditions where hydrophobic attraction becomes the dominating factor with organic interactions [111]. The combined effects result in enhanced organic attachment, and compression leading to higher organic attachment and stabilization of TiO<sub>2</sub> particles. [112]. Increased attachment will influence the particles physically, and chemically due to higher steric repulsion and lower diffusion. [113] The diffusion is a result of the

increase in particle diameter due to organic attachment, where the attached organic matter retards the particle diffusion. The shape and formation of sludge flocs are also influenced by salinity, where higher ionic strength reduces the repulsion of linear organic material resulting in the physical curling of the material. This will aid in the stability of sludge flocs, where at higher ionic strength, the curled up organic materials will form compacted structures preventing floc breakage. Where at lower ionic strengths, linear organic matter will result in the breakage and reattachment of larger flocs. This will encourage  $TiO_2$  sorption through floc entrapment during the rearrangement process. Additionally with linear organic material, bridging of particles mater may also occur increasing particle attachment. The combined effects shown in low ionic strength may result in a multi layer sorption model where it has been reported that  $TiO_2$  may display up to a 5-layer sorption to algal cells [114].



Figure 4.10 Effect of ionic strength on the adsorption of  $TiO_2$  onto sludge particulates. Experimental conditions: pH = 6.84, [MLSS] = 2186 mg/L, electrolyte = NaCl, Secondary sludge (Data is illustrated in the appendix Table P1)









Figure 4.11 Dual sorption model of TiO<sub>2</sub> to secondary sludge, under various ionic strengths. The sorption diagram (Total) was divided into two layers (1<sup>st</sup>,  $2^{nd}$  layer) and separately analyzed for its sorption parameters (model). Experimental conditions: pH = 6.84, [MLSS] = 2186 mg/L (a) [NaCl] = 280mM, (b) 480mM, (c) 680mM, (d) 830mM. (Data is illustrated in the Appendix Table P1)

NaCl (mM)	Sorption Parameter	Primary sorption curve	Secondary sorption curve
280	$\Gamma_{\max}$	15.39	55.25
	K <sub>L</sub>	0.55 (4.4×10 <sup>4</sup> )	5.5×10 <sup>-2</sup> (4392)
	$\Delta G$	6.33	4.97
480	$\Gamma_{max}$	33.14	38.40
	KL	$1.68 \times 10^{-2}$ (1.3×10 <sup>3</sup> )	5.70×10 <sup>-2</sup> (4552)
	$\Delta G$	4.26	4.99
680	$\Gamma_{max}$	50.00	180.76
	K <sub>L</sub>	$1.09 \times 10^{-2}$ (8.7×10 <sup>2</sup> )	9.9×10 <sup>-4</sup> (79)
	$\Delta G$	4.01	2.59
830	$\Gamma_{max}$	97.04	60.04
	KL	3.3×10 <sup>-3</sup> (2.65×10 <sup>2</sup> )	1.01×10 <sup>-3</sup> (81)
	$\Delta G$	3.30	2.60

Table 4.2 Summary of sorption parameters of TiO<sub>2</sub> to sludge under various ionic strength conditions. Unit of parameters:  $\Gamma_{max} = \mu g$ -ENP/mg-Dry sludge,  $K_L = L/mg$ , (L/mol),  $\Delta G = Kcal/mol$ 

Although the attachment characteristics may not show any definite change under lower ENP concentrations, this may be due to a combination of factors including hydrophobic interaction, and larger floc formation canceling each other out. This will result in small changes in attachment compared to the larger IS increase. As the IS increases, the electric double layer compression will occur on both  $TiO_2$  and sludge. Additionally it is known that with the screening of electrostatic repulsion, hydrophobic interaction becomes the dominant mechanism in particle and sludge interaction resulting in higher attachment of particles to sludge [115, 116]. However electric double layer compression and hydrophobic interaction also results in the compression of sludge flocs as well, where the organic materials coils in shape forming a denser floc with increasing IS. On the contrary with lower IS, organic material form linear shapes extending and detaching from the flocs due to higher electrostatic repulsion [117]. The combined effect of EDL compression with added mixing gives the potential for larger floc formation. Additional to floc growth, the shape and hydraulic mobility is also influenced by the ionic strength. It has been proven that with higher ionic strength, flocs also become elongated, adding to the increase of the flocs. [118] As a result, larger floc formation leads to a smaller floc surface area per organic content ratio, decreasing ENP sorption.

With higher salt concentrations the boundary water content of sludge also decreases, lowering the viscosity of the flocs [119, 120]. This may additionally aid in the increase of settling velocities, shortening the time for ENP to attach to sludge surfaces.

### 4.4.3 Effect of MLSS concentration on engineered nanoparticle sorption

Under seasonal conditions or various weathers, sludge may show different compositions and concentrations influencing the attachment characteristics of ENP. To verify the effects of sludge concentration on ENP attachment, the current study focuses on the effects of sludge concentration on ENP attachment. To verify the effects, attachment experiments with  $TiO_2$  and ZnO were tested on both primary and secondary sludge. Fig. 4.12 and 4.13 show the attachment of both nanoparticles under various sludge concentrations. Results showed that with lower organic concentrations, larger amounts of nanoparticle attachment occurred. Although the higher ENP to sludge ratio may contribute to the results, due to the fact that sludge of one degree magnitude showing up to four degrees of magnitude difference in attachment indicates various phenomena contributing to the results. Lower attachment under high sludge concentrations are due to a number of factors including DOM attachment to ENP, larger sludge flocs, and faster sludge sedimentation.

Under high organic loads, attachment of DOM to ENP will result in the stabilization of particles. As it has been proven in the previous chapter, steric hindrance of organic matter stabilizes the ENP, increasing the CCC value up to three degrees of magnitude. DOM such as natural organic matter, organic acids, and extracellular polymeric substances display negative functional groups that aid in steric repulsion [120, 121], resulting in the hindrance of DOM coated ENP and sludge floc interaction. High concentration of organic matter also results in higher viscosity, where the viscosity is known to increase exponentially with organic loading. [119] This may lead to the reduction of particle mobility of ENP resulting in decreased attachment to sludge. With low organic loadings, the increased attachment results are due to the higher particle collision frequency and organic bridging. [122]

Comparatively higher ENP concentrations will lead to a higher frequency in particleparticle interaction and lower particle-DOM interaction. The interaction will result in larger particle aggregates that attach to sludge flocs showing higher attachment results, as shown in Fig. 3.12 and 3.13.



Figure 4.12 Attachment of TiO<sub>2</sub> to wastewater sludge of various concentrations. (a) Primary sludge (b) Secondary sludge. (Data is illustrated in the Appendix Table Q1, and Q2)



Figure 4.13 Attachment of ZnO to wastewater sludge of various concentrations. (a) Primary sludge (b) Secondary sludge. (Data is illustrated in the Appendix Table Q2, and Q3)

The floc size is also a factor in ENP sorption capacity, where under limited organic concentrations, the increase in floc diameter will result in smaller surface areas. Also, with larger sludge flocs the settling velocity of the flocs will increase resulting in less time for ENP attachment. It has been proven that the addition of ENP would enhance the aggregation of sludge flocs, where ENP attachment reducing the internal energy barrier, and leading to an enhancement in the flocculation. [123]

Both ZnO and TiO<sub>2</sub> particles have a stronger affinity to secondary sludge, which are two to three degrees of magnitude higher compared to primary sludge. The higher affinity to secondary sludge is due to the organic composition, and viscosity of the wastewater samples. Primary sludge is composed of various organic acids, sugars, and fatty acids, where secondary sludge is composed of flocculated microbial aggregates [124, 125]. The difference in sludge characters indicates a higher concentration of dissolved organic material in the primary tank, and larger flocculate matter found in the secondary tank. Due to the larger amounts of dissolved organic matter, particles in the primary tank are more likely to interact with the dissolved matter, resulting in the stabilization of the ENP. Additionally the high organic and fatty acid concentration, primary wastewater samples display higher viscosity values, resulting in slower ENP transport toward sludge flocs. On the other hand, ENP in secondary sludge would attach to flocculate matter resulting in attachment.

Aside from sludge concentration and characteristics, the charge of the ENP also contributes to the attachment characteristics. By comparing the results in Fig. 4.2 and 4.13, it can be seen that Freundlich constants of ZnO are more distinctively correlated with the sludge concentration than TiO<sub>2</sub>. As mentioned above, the ZnO

particles display higher dispersion characters under the pH conditions of the wastewaters, resulting in more even distribution onto the sludge.

## 4.4.4 Effect of Engineered Nanoparticle Type: ZnO, TiO<sub>2</sub>, SiO<sub>2</sub>

The effect of the particles chemical composition was also tested with three different nanoparticles commonly found in wastewater treatment plants (ZnO, TiO<sub>2</sub>, and  $SiO_2$  Fig. 4.15). Results showed higher attachment of ZnO, up to one degree of magnitude higher, compared to  $TiO_2$ . This may be explained with the surface charge of the particles, where it can be seen in Fig. 4.14 under pH 6.2 (for which the conditions of the wastewater were samples and experiment was conducted) TiO<sub>2</sub> show a near +10 mV zeta value where TiO<sub>2</sub> shows a near neutral charge. According to the DLVO theory, the range of the surface charge spans up to 50 nm from the particle surface, which is widest among the existing forces. The wide range will be the initial force attracting the ZnO and sludge particles, and would be what results in higher attachment compared to  $TiO_2$ . In the previous chapter it has been shown that regardless of the particle charge, the existence of DOM may result in neutralizing the effect of surface charge in particle-particle interaction. However with the existence of sludge particulates this effect may not be the dominating influence, where the ENP may also interact with sludge particulates instead of DOM. Additionally, the salinity of the tested conditions were in a range higher than 0.1 M, indicating that the steric repulsion will be masked by the ionic strength.



Figure 4.14 Zeta potential vs. pH of ENP particles ( $\circ$  TiO<sub>2</sub>,  $\bullet$  ZnO,  $\checkmark$  SiO<sub>2</sub>). Experimental conditions: [TiO<sub>2</sub>] = 50 mg/L, [ZnO] = 50 mg/L, [SiO<sub>2</sub>] = 1 mg/L, [Alginate] = 100 mg/L, [Humic Acid] = 100 mg/L. (Data is illustrated in the Appendix Table R1)



Figure 4.15 Sorption of engineered nanoparticles composed of various elements.  $\bullet$  TiO<sub>2</sub>,  $\checkmark$  ZnO,  $\blacksquare$  SiO<sub>2</sub>. (Data is illustrated in the Appendix Table S1)

Silicon dioxide showed the highest amount of removal, which was two degrees magnitude higher than ZnO, and three degrees of magnitude higher than TiO<sub>2</sub>. Unlike the other particles, SiO<sub>2</sub> displays a negative charge, making it undesirable for attachment, according to the DLVO theory. However the higher amount of silicon dioxide removal may be due to particle hydrophobicity, regardless of the surface charge. With higher ionic strengths, the absolute value of the surface charge decreases due to charge masking of the ions, but with higher ionic strength, hydrophobic interaction is not influenced resulting as a dominate factor in particle interaction under high ionic strengths. It is well known that pure silica is a strongly hydrophobic material with a contact angle near 90°, where its hydrophobicity decreases with the thickness of its oxide layer thickness [126]. With SiO<sub>2</sub> nanoparticles, due to its low surface oxide thickness, the particles would be strongly hydrophobic, making it susceptible to hydrophobic interactions with sludge particulates. Additionally, due to the high salinity conditions of wastewater, the particles may also experience a salt-out effect increasing sludge-particle interaction as well as particle-particle aggregation.

## 4.5 Conclusion

In the current study, a differential sedimentation method was used to study the attachment of ENP to wastewater, making it possible to test various conditions with a number of different nanoparticles. The effects of ionic strength, sludge concentration, ENP composition, and sludge characteristics on ENP attachment was tested on primary and secondary wastewaters. Results showed that a change in a single variable would impact the attachment characteristics through a series of complex interactions. For example the increase in salinity would result in electric double layer compression as well as an increase in the hydrophobic attraction for both organic matter and ENP.

Repulsion due to surface functional groups will be reduced, resulting in an intrinsic attachment within an organic material forming a coiled shape compound. With the added reduction of ENP surface charge, the sorption of organic matter will be enhanced further stabilizing the ENP. This resulted in the overall decrease of ENP sorption to sludge under higher ionic strengths, where the secondary sorption layer would decrease with higher ionic strengths. The change in organic concentration also showed complex influences, where lower sludge concentrations resulted in smaller flocs, organic bridging, and lower viscosity. At lower organic concentrations sludge particulates would form smaller flocs, displaying larger surface areas and higher mobility. Addition to the floc size, lower organic concentrations also lead to larger number of aggregated ENP, caused by organic bridging, and more rapid diffusion through lower viscosity. This resulted in higher sorption of ENP under lower sludge concentrations.

And finally, the effect of electrostatic double layers and hydrophobic attraction was found to differ based on ENP composition. It was found that with TiO<sub>2</sub> and ZnO the surface charge was the main factor influencing the attachment process where positively charged ZnO showed higher sorption results than TiO<sub>2</sub>. However with SiO<sub>2</sub> hydrophobic interaction proved to be the dominating force, resulting in sorption of two to three degrees magnitude higher than ZnO.

# Chapter 5

# MAJOR FINDINGS AND RESEARCH NEEDS

## 5.1 Major findings

The key motivation behind the current research was to understand the fate of engineered nanoparticles that flow into wastewater treatment plants. The research proves the overall fate, and transport of engineered nanoparticles under operational field conditions of wastewater treatment plants. Quantitative analysis showed the distribution characteristics of engineered nanoparticles in wastewater treatment plants. Results showed a minimum 90% particle removal throughout the plant with the majority of the particles found in the primary and secondary sludge. Concentration and mass flow profiles of TiO<sub>2</sub> and ZnO both presented fairly desirable mass balance results with both primary and secondary sediment tanks. Through the quantitative analysis, the study showed methods to accurately measure and predict the redistribution of nanoparticles once flown into a wastewater treatment plant. Seasonal variations of engineered nanoparticle inflow showed elevated quantities in the summer and winter, which was proven to agree with nanoparticle product sales and utilization. The matching trend of product sales and the amounts particles detected provided the direct relationship between the utilization and waste of nanoparticles in consumer products. The results are also reinforced with the fact that higher concentrations of TiO<sub>2</sub>, compared to ZnO, were found in the wastewater treatment plants, where TiO<sub>2</sub> is also utilized in higher concentrations and wider range of consumer products.

Alongside the quantifiable measurement of the fate and transport of nanoparticles, the mechanism behind the phenomenon was also observed through nanoparticle interaction research. Particle-particle interaction research under field conditions showed that higher concentrations of dissolved organic matter would results in higher sorption onto nanoparticles, and also leads to higher stability and lower interaction between particles. Particle-sludge interaction studies showed how the effects of various sludge conditions could impact the overall amount of nanoparticle attachment to sludge particulates. Conditions such as sludge concentration, ionic strength, and material composition of nanoparticles influenced the attachment affinity. Higher amounts of attachments were observed under lower sludge concentrations due to smaller sludge flocs with larger surface areas. And compared with primary sludge, secondary sludge also showed higher attachment due to its more complicated and looser floc structures. Under high ionic strengths, a dual layer attachment of particles where observed with a lower amount of attachment shown under higher ionic strengths. Finally, the material composition of the nanoparticles also showed different results with SiO<sub>2</sub> showing the highest amount of attachment followed by ZnO and TiO<sub>2</sub>. However among the tested conditions, the size of sludge flocs did not show significantly different results compared with the other conditions tested above. Through the current research, the mechanisms and the overall results of nanoparticle fate in wastewater treatment plants were determined with the hope that it will aid in future research and policy decisions.

### 5.2 Future research needs

Through the current research, various approaches have been made to understand the fate of engineered nanoparticles in wastewater treatment plants. As mentioned earlier, wastewater treatment plants act as a redistribution point for the fate of engineered nanoparticles. Although the amount of redistribution and its mechanisms were identified in the current research, the fate of the particles beyond the wastewater treatment plant still lacks accurate understanding. To fill the gaps of knowledge in the fate of engineered nanoparticles, additional research should be conducted on the exposure in the environment. Exposure studies in the river, sea, groundwater, as well as soil exposure and landfill accumulation are a few of the numerous studies that should be conducted to understand nanoparticle fate in the environment. And aside from the experimental aspect, additional effort can be directed toward environmental modeling to develop more accurate estimations and parameters, lowering the uncertainty in the procedure.

Throughout the distribution research, the concentration and mass flow of nanoparticles were measured and verified by the mass balance. However through mass flow analysis, unaccountable nanoparticle loss in the treatment plant was observed. This may be due to the detection error of the analysis process or unaccounted accumulation of particles in certain locations of the treatment plant. To verify the unaccounted particle loss, additional effort should be directed in the details of transport pathways in the treatment plant. Detailed sampling in the sand filtration process as well as particle sedimentation in the pipelines or weirs addition to hydraulic modeling of the particle transport process may aid in the verification process.

For transport studies the effect of natural organic matter, in concentrations and conditions identical to field operations, was tested for attachment efficiency studies. As the total body of natural organic matter (NOM) was tested, it can also be tested based on the different fractions such as hydro –philic –phobic constituents.

In the current research the physical aspects of activated sludge was the main variation in the attachment of engineered nanoparticles to sludge. Factors such as sludge particulate diameter, organic concentrations and its impact on attachment were some of the main interest. In the future, effects of the chemical aspects of sludge as well as aquatic conditions should be examined. Chemical aspects such as the number and distribution of functional groups, aromatic substances ratio, and microbial community configurations may be factors that should be further investigated.

Additionally, the variation of sludge composition under various environmental factors should also be considered. The configuration of sludge can change with external influences such as temperature, configuration of dissolved organic matter, and aeration. Factors such as the size, shape, organic and biological content are a few of the changes that are influenced by external factors. And the change in sludge configuration will influence the attachment of nanoparticles. So to better understand and predict the attachment of nanoparticles to sludge particulates it will be important to understand the change of sludge characteristics under various external influences.

As it has been proven in our research, wastewater treatment plants play a pivotal role in the fate and transport of engineered nanoparticles. However, in future research it will be important to extend the knowledge past the wastewater treatment plants and into the environment. The knowledge investigated on the detection and interaction of nanoparticles should be applied and extended not only on local level distribution points but also into the regional and global issues as well.

## REFERENCES

- 1. H. Chen, M. C. Roco, J. Son, S. Jiang, C.A. Larson, Q. Gao, "Global nanotechnology development from 1991 to 2012: patents, scientific publications, and effect of NSF funding." *J Nanopart Res*, 2013, 15:1951
- 2. Bernardo E, Colombo P, Hampshire S. "Advanced ceramics from a preceramic polymer and nano-filters" J Eur Ceram Soc, 2009, 29, 843-849
- 3. Jin LQ, Qu YC, Wang BQ, Li SD, Jiang BJ, Yang LB, Fu W, Fu HG, Sun JZ. "Review of photoluminescence performance of nano-sized semiconductor materials and its relationships with photocatalytic activity." Sol Energ Mat Sol C, 2006, 90, 1773-1787
- 4. Ai-Edresi A, Baie S. "Formulation and stability of whitening VCO-inwater nano-cream." Int J Pharm. 2009, 22, (373), 174-178
- 5. Chu PK, "Application of plasma-based technology to microelectrics and biomedical engineering." Sur Coat Tech, 2009, (203), 2793-2798
- 6. Sadurni N, Solans C, Azemar N, Garcia-celma MJ. "Studies on the formation of O/W nano-emulsions, by low-energy emulsification methods, suitable for pharmaceutical applications." Eur J Pharm Sci, 2005, 26, 438-445
- Anton N, Benoit JP, Saulnier P "Design and production of nano particles formulate from nano-emulsion templates-a review". J Control Release, 2008, 128, 185-199
- 8. Fang GY, Li H, Yang F, Liu X, Wu SM, "Preparation and characterization of nano-encapsulated n-tetrodecane asphase chang material for thermal energy stoage." Chem Eng J, 2009, 153, 217-221
- 9. Darbha GK, Ray A, Ray PC, "Gold nanoparticle-based miniaturized nanomaterial surface energy transfer probe for rapid and ultrasensitive detection of mercury in soil, water, and fish." ACS Nano, 2007, 3, 208-214

- 10. Rajesh, Tarushee A, Kumar D. "Recent progress in the development of nanostructured conducting polymers/nanocomposites for sensor applications." Sensor ActuatB-Chem, 2009, 136, 275-286
- 11. Janeway P, "Nanotechnology-It's more than size." Am Ceram. Soc. Bull, 2003, 82(4), 31-38
- M. C. Roco, C. A. Mirkin, M. C. Hersam, "Nanotechnology research directions for societal needs in 2020: summary of international study." National Science Foundation and World Technology Evaluation Center, Springer, Berlin, Germany, and Boston, MA.
- Mihail C. Roco, "The long view of nanotechnology development: the National Nanotechnology Initiative at 10 years." J Nanoparticle Research, (2011) 13, 427-445
- 14. T. M. Benn, P. Westerhoff, "Nanoparticle silver released into water from commercially available sock fabrics." 2008, *Environ. Sci. Technol*, 42, 4133-4139.
- L. Windler, C. Lorenz, N. von Goetx, K. Hungerbuhler, M. Heuberger, B. Nowack, "Release of titanium dioxide from textiles during washing." 2012, *Environ. Sci. Technol*, 46, 8181-8188
- A. Weir, P. Westerhoff, L. Fabricius, K. Hristovski, N. von Goetz, "Titanium Dioxide Nanoparticles in Food and Personal Care Products." *Environ. Sci. Technol.* 2012, 46, 2242–2250
- B. Nowack, H. F. Krug, M. Height, "120 years of nanosilver history: Implications of policy makers." *Environ. Sci. Technol*, (2011), 45, 1177-1183
- Y. Zhang, Y. R. Leu, R. J. Aitken, M, Riediker, "Inventory of nanoparticlecontaining consumer products available in the Singapore retail market and likelihood of release into the aquatic environment." 2015, Int. J. Environ. Res. Public Health, 12, 8717–8743
- L.J Loretz, A.M Api, L.M. Barraj, J. Burdick, W.E. Dressler, S.D. Gettings, H. Han Hsu, Y.H.L. Pan, T.A. Re, K.J. Renskers, "Exposure data for cosmetic products: lipstick, body lotion, and face cream." 2005, Food Chem. Toxicol., 43, 279–291

- L.J Loretz, A.M. Api, L. Babcock, L.M. Barraj, J.Burdick, K.C. Cater, G. Jarrett, S. Mann, Y.H.L. Pan, T.A. Re, "Exposure data for cosmetic products: facial cleanser, hair conditioner, and eye shadow." 2008, Food Chem. Toxicol., 46, 1516–1524.
- J.W.H Biesterbos, T. Dudzina, C.J.E. Delmaar, M.I. Bakker, F.G.M. Russel, N. von Goetz, P.T.J. Scheepers, N. Roeleveld, "Usage patterns of personal care products: important factors for exposure assessment." 2013, Food Chem. Toxicol., 55, 8–17.
- 22. A. Lazareva, A. A. Keller, "Estimating potential life cycle releases of engineered nanomaterials from wastewater treatment plants." 2014, ACS Sustainable Chem. Eng., 2, 1656–1665
- 23. A. A. Keller, A. Lazareva, "Predicted Releases of Engineered Nanomaterials: From Global to Regional to Local." *Environ. Sci. Technol. Lett.*, 2014, *1* (1), pp 65–70
- C.Hendren, M. Lowry, K. D. Grieger, E. S. Money, J. M. Johnston, M. R. Wiesner, S. M. Beaulieu, "Modeling Approaches for Characterizing and Evaluating Environmental Exposure to Engineered Nanomaterials in Support of Risk-Based Decision Making." 2013, *Environ. Sci. Technol.*, 47, 1190–1205
- 25. P. Westerhoff, A. Kiser, K. Hristovski, "Nanomaterial Removal and Transformation During Biological Wastewater Treatment." *Environmental engineering science*, 2012, 30, (3), 1-9
- 26. T. Benn, B. Cavanagh, K. Hristovski, J. D. Posner, P. Westerhoff, "The Release of Nanosilver from Consumer Products Used in the Home." 2011, *Journal of Environmental Quality*, 39,1875-1882
- C. Botta, J. Labille, M. Auffan, D. Borschneck, H. Miche, M. Cabié, A. Masion, J. Rose, J.Y. Bottero. "TiO<sub>2</sub>-based nanoparticles released in water from commercialized sunscreens in a life-cycle perspective: Structures and quantities." 2011, *Environmental Pollution*, 159, 1543-1550
- R. Kaegi, A. Ulrich, B. Sinnet, R. Vonbank, A. Wichser, S. Zuleeg, H. Simmler, S. Brunner, H. Vonmont, M. Burkhardt, M. Boller. "Synthetic TiO<sub>2</sub> nanoparticle emission from exterior facades into the aquatic environment." 2008, *Environmental Pollution*, 156, 233–239

- 29. S. Wagener, N. Dommershausen, H. Jungnickel, P. Laux, D. Mitrano, B. Nowack, G. Schneider, A. Luch, "Textile functionalization and its effects on the release of silver nanoparticles into artificial sweat." *Environ. Sci. Technol.*, 2016, *50* (11), 5927–5934
- S.E. Cross, B. Innes, M.S. Roberts, T. Tsuzuki, T.A. Robertson, P. McCormick, "Human skin penetration of sunscreen nanoparticles: In vitro assessment of a novel micronized zinc oxide formulation." 2007, Skin Pharmacol. Phys., 20, 148–154.
- R. Gehrig, M. Hill, B. Buchmann, D. Imhof, E. Weingartner, U. Baltensperger, "Separate determination of PM<sub>10</sub> emission factors of road traffic for tailpipe emissions and emissions from abrasion and resuspension processes." 2005, Int. J. Environ. Pollut., 22, 3, doi:10.1504/IJEP.2004.005549
- 32. P. Westerhoff, M.A. Kiser, T. Benn, Y. Wang, J. Perez-Rivera, K Hristovski, "Titanium nanomaterial removal and release from wastewater treatment plants" 2009, *Environ. Sci. Technol*, 43, 6757-6763
- 33. L. Li, G. Hartmann, M. Doblinger, M. Schuster, "Quantification of nanoscale silver particles removal and release from municipal wastewater treatment plants in Germany" 2013, *Environ. Sci. Technol*, 47, 7317-7323
- 34. M. A. Kiser, D. A. Ladner, K. D. Hristovski, P. K. Westerhoff. "Nanomaterial transformation and association with fresh and freeze-dried wastewater activated sludge: Implications for testing protocol and environmental fate." 2012, *Environ. Sci. Technol*, 46, 7046-7053
- R. Kaegi, A. Voegelin, B. Sinnet, S. Zuleeg, H. Hagendorfer, M. Burkhardt, H. Siegrist, "Behavior of metallic silver nanoparticle in a pilot wastewater treatment plant." 2011, *Environ. Sci. Technol*, 45, 3902-3908
- H. J. Park, H. Y. Kim, S. Cha, C. H. Ahn, J. Roh, S. Park, S. Kim, K. Choi, J. Yi, Y. Kim, J. Yoon, "Removal characteristics of engineered nanoparticles by activated sludge" 2013, *Chemosphere*, 92, 524-528
- E. Lombi, E. Donner, E. Tavakkoli, T. W. Turney, R. Naidu, B. W. Miller, K. G. Scheckel, "Fate of Zinc Oxide Nanoparticles during Anaerobic Digestion of Wastewater and Post-Treatment Processing of Sewage Sludge." 2012, *Environ. Sci. Technol.*, 46, 9089–9096

- F. Gomez-Rivera, J. A. Field, D. Brown, R. Sierra-Alvarez, "Fate of cerium dioxide nanoparticles in municipal wastewater during activated sludge treatment" 2012, *Environ. Sci. Technol*, 108, 300-304
- Y. Wnag, P. Westerhoff, K. D. Hristovsk, "Fate and biological effects of silver, titanium dioxide and C<sub>60</sub> (fullerene) nanomaterials during simulated wastewater treatment processes" 2012, *Journal of Hazardous Materials*, 201-202,16-22
- L. K. Limbach, R. Bereiter, E. Muler, R. Krebs, R. Galli, W. J. Stark, "Removal of oxide nanoparticles in a model wastewater treatment plant: Influence of Agglomeration and surfactants on clearing efficiency." 2008, *Environ. Sci. Technol*, 42, 5828-5833
- 41. K. Khosravi, M. E. Hoque, B. Dimock, H. Hintelmann, C. D. Metcalfe, "A novel approach for determining total titanium from titanium dioxide nanoparticles suspended in water and biosolids by digestion with ammonium persulfate." 2012, *Analytical chimica acta*, 713, 86-91
- 42. R. Kaegi, A. Voegelin, C. Ort, B. Sinnet, B. Thalmann, J. Krismer, H. Hagendorfer, M. Elumelu, E. Mueller, "Fate and transformation of silver nanoparticles in urban wastewater systems." 2013, *Waster Research*, 47, 3866-3877
- 43. R. Ma, C. Levard, J. D. Judy, J. M. Unrine, M. Durenkamp, B. Martin, B. Jefferson, G. V. Lowry. "Fate of Zinc oxide and silver nanoparticles in a pilot wastewater treatment plant and in processed biosolids." 2014, *Environ. Sci. Technol*, 48(1), 104-112
- 44. M.R. Chang, D.J. Lee, J.Y. Lai, "Nanoparticles in wastewater for a science-based industrial park-Coagulation using polyaluminum chloride" 2007, *Journal of Environmental Management*, 85, 1009-1014
- 45. H. P. Jarvie, H. Al-Obaidi, S. M. King, M. J. Bowes, M. J. Lawrence, A. F. Drake, M. A. Green, P. J. Dobson, "Fate of silica nanoparticles in simulated primary wastewater treatment." 2009, *Environ. Sci. Technol*, 43, 8622-8628.
- 46. D. Zhou, A. A. Keller, "Role of morphology in the aggregation kinetics of ZnO nanoparticles." 2010, *Waster Research*, 44, 2948-2956
- 47. E.F.C. Chauque, J.N. Zvimba, J.C. Ngila, N. Musee. "Stability studies of commercial ZnO engineered nanoparticles in domestic wastewater." 2014, *Physics and chemistry of the Earth*, 67-69, 140-144

- 48. [31] J. Liu, J. Chao, R. Liu, Z. Tan, Y. Yin, Y. Wu, G. Jiang, "Cloud point extraction as an advantageous preconcentration approach for analysis of trace silver nanoparticles in environmental waters." 2009, *Anal. Chem.*, 81, 6496–6502
- 49. R. M. DiSalvo, Jr., G. R. McCollum, A. Whelton, "Evaluating the impact of nanoparticles on wastewater collection and treatment systems in verginia." Water jam 2008, Virginia Beach, Virginia, September 7 September 11, 2008
- 50. D. Perret, M. E. Newman, J.C. Nerge, Y. Chen, J. Buffle., "Submicron particles in the Rhine river I physico-chemical characterization." 1994, *Wat. Res.*, 28,1, 91-106
- 51. S. Dubascoux, F. Von Der Kammer, I. Le Hécho, M. P. Gautier, G. Lespes, "Optimisation of asymmetrical flow field flow fractionation for environmental nanoparticles separation." *Journal of Chromatography A*, 1206, (2), 160-165
- 52. F. von der Kammer<sup>,</sup>, S. Legros, T. Hofmann, E. H. Larsen, K. Loeschner, "Separation and characterization of nanoparticles in complex food and environmental samples by field-flow fractionation." *TrAC Trends in Analytical Chemistry*. 30, (3), 425–436
- 53. M. Baalousha, B. Stolpe, J.R. Lead, "Flow field-flow fractionation for the analysis and characterization of natural colloids and manufactured nanoparticles in environmental systems: A critical review." *Journal of Chromatography A*, 1218, (27), 4078–4103
- 54. C. Contado, A. Pangnoni, "TiO<sub>2</sub> nano- and micro-particles in commercial foundation creams: Field-flow-fractionation techniques together with ICP-AES and SQW voltammetry for their characterization." 2010, *Anal. Methods.*, 2, 1112-1124
- 55. K. L. Plathe, F. von der Kammer, M. Hassellöv, J. Moore, M. Murayama, T. Hofmann, M. F. Hochella Jr., "Using FIFFF and aTEM to determine trace metal-nanoparticle associations in riverbed sediment." 2010, *Environ. Chem.*, 7, 82–93.
- 56. A Samontha, J. Shiowatana, A. Siripinyanond, "Particle size characterization of titanium dioxide in sunscreen products using sedimentation field-flow fractionation-inductively coupled plasma-mass spectrometry." 2011, Anal Bioanal Chem., 399, 973–978

- 57. Fang, H. H. P., Jia, X.-S. "Soluble microbial products (SMP) of Acetotrophic Methanogenesis." 1988, *Bioresour. Technol.*, 66, 235–239.
- 58. Y. Zhang, Y. Chen, P. Westerhoff, J. Crittenden, "Impact of natural organic matter and divalent cations on the stability of aqueous nanoparticles." *Water Research*, 2009, 43, 4249-4257
- 59. I. Chowdhury, D. M. Cwiertny, S. L. Walker, "Combined Factors Influencing the Aggregation and Deposition of nano-TiO2 in the Presence of Humic Acid and Bacteria." 2012, *Environ. Sci. Technol.*, 46, 6968–6976
- 60. Y. Yin, X. Zhang, J. Graham, L. Luongo, "Examination of purified singlewalled carbon nanotubes on activated sludge process using batch reactors." 2009, *Journal of Environmental Science and Health Part A*, 44, 661–665
- 61. P. Nguyen, N. P. Hankins, N. Hilal, "A comparative study of the flocculation behavior and final properties of synthetic and activated sludge in wastewater treatment." 2007, *Desalination*, 204, 277-295
- 62. Perret. D, Newman. M, Negre. J.C., Chen. Y, Buffle. J, "Submicron particle in the Rhine river I. Physic-chemical characterization" *Wat. Res.*, 1994, 28(1), 91-106
- R. Kaegi, A. Ulrich , B. Sinnet, R. Vonbank, A. Wichser, S. Zuleeg, H. Simmler, S. Brunner, H. Vonmont, M. Burkhardt, M. Boller "Synthetic TiO<sub>2</sub> nanoparticle emission from exterior facades into the aquatic environment" *Environmental Pollution*, 2008, 156, 233–239
- 64. M.R. Chang, D.J. Lee, J.Y. Lai, "Nanoparticles in wastewater from a science-basec industrial park-Coagulation using polyaluminum chloride" *Journal of Environmental Management*, 2007, 85, 1009–1014
- 65. M.-K.H. Winkler, J.P. Bassin, R. Kleerebezem, R.G.J.M. van der Lans, M.C.M. van Loosdrecht, "Temperature and salt effects on settling velocity in granular sludge technology." Water Research, 2012, 46, 3897-3902
- M. C. E. Lomer, R. P. H. Thompson, J. Commisso, C. L. Keenb, J. J. Powell, "Determination of titanium dioxide in foods using inductively coupled plasma optical emission spectrometry." Analyst, 2000, 125, 2339– 2343
- 67. Kai Loon Chen, Steven E. Mylon, Menachem Elimelech, "Aggregation kinetics of alginate-coated hematite nanoparticles in monovalent and divalent electrolytes."Environ. Sci. Technol. 2006, 40, 1516-1523

- 68. Kai Loon Chen, Menachem Elimelech, "Influence of humic acid on the aggregation kinetics of fullerene (C<sub>60</sub>) nanoparticles in monovalent and divalent electrolyte solutions." Journal of Colloid and Interface Science, 2007, 309, 126–134
- 69. Mohamed Erhayem, Mary Sohn, "Stability studies for titanium dioxide nanoparticles upon adsorption of Suwannee River humic and fulvic acids and natural organic matter." Science of the Total Environment, 2014, 468–469, 249–257
- E. Illés, E. Tombácz, "The effect of humic acid adsorption on pHdependent surface charging and aggregation of magnetite nanoparticles." Journal of Colloid and Interface Science, 2006, 295, 115–123
- 71. M. Li, C.P. Hunag, "Stability of oxidized single-wlled carbon nanotubes in the presence of simple electrolytes and humic acid." Carbon, 2010, 48, 4527-4534
- 72. Yi Jiang, Ramesh Raliya, John D. Fortner, Pratim Biswas, "Graphene Oxides in Water: Correlating Morphology and Surface Chemistry with Aggregation Behavior." *Environ. Sci. Technol.*, **2016**, *50* (13), 6964–6973
- 73. Duch, M. C., Budinger, G. R. S., Liang, Y. T., Soberanes, S., Urich, D., Chiarella, S. E., Campochiaro, L. A., Gonzalez, A., Chandel, N. S., Hersam, M. C., Mutlu, G. M. "Minimizing oxidation and stable nanoscale dispersion improves the biocompatibility of graphene in the lung." Nano Lett. 2011, 11 (12), 5201–5207.
- 74. Hirsch, A.; Vostrowsky, O. "In Functional Organic Materials: Syntheses, Strategies and Applications; Mu<sup>"</sup> ller, T. J. J., Bunz, U. H. F., Eds.; Wiley-VCH: Weinheim, 2005; pp 193-237.
- 75. Indranil Chowdhury, Matthew C. Duch, Nikhita D. Mansukhani, Mark C. Hersam, Dermont Bouchard "Colloidal Properties and Stability of Graphene Oxide Nanomaterials in the Aquatic Environment." Environ. Sci. Technol., 2013, 47, 6288–6296
- 76. Dongxu Zhou, Arturo A. Keller, "Role of morphology in the aggregation kinetics of ZnO nanoparticles." Water Research, 2010, 44, 2948–2956
- 77. Paul Hiemenz C, Rajagopalan R, "Principles of colloidal and surface chemistry." 3<sup>rd</sup> ed. New York: Marcel Dekker; 1997.
- 78. Waldemar Nowicki, Grazyna Nowicka "Verification of the Schulze-Hardy Rule" *Journal of Chemical education*, 1994, 71 (7), 624-626
- 79. Hunter RJ. "Foundations of Colloid Science." 2<sup>nd</sup> ed. USA: Oxford University Press; 2001.
- 80. H. Holthoff, S. U. Egelhaaf, M. Borkovec, P. Schurtenberger, H. Sticher, "Coagulation rate measurements of colloidal particles by simultaneous static and dynamic light scattering." Langmuir 1996, 12, 5541-5549
- 81. J. H. Van Zanten, M. Elimelech, "Determination of absolute coagulation rate constants by multiangle light scattering." Journal of Colloid and interface Science, 1992, 154, 1-7
- Yang-Hsin Shih, Wei-Szu Liu, Yuh-Fan Su, "Aggregation of stabilized TiO<sub>2</sub> nanoparticle suspension in the presence of inorganic ions." Environmental Toxicology and Chemistry, 2012, 31, 8, 1693–1698
- 83. Thio BJR, Zhou DX, Keller AA. "Influence of natural organic matter on the aggregation and deposition of titanium dioxide nanoparticles." Journal of Hazard Materials, 2011, 189, 556–563.
- 84. Liu XY, Chen GX, Su CM. 2011. "Effects of material properties on sedimentation and aggregation of titanium dioxide nanoparticles of anatase and rutile in the aqueous phase." Journal of Colloid Interface Science, 2011, 363, 84–91
- 85. Bae HS, Lee MK, Kim WW, Rhee CK. "Dispersion properties of TiO<sub>2</sub> nano-powder synthesized by homogeneous precipitation process at low temperatures." Colloid Surf A-Physicochem Eng Asp, 2003, 220,169–177.
- 86. Shih YH. "The transformation and fate of nanoparticle powders in aquatic environment." Taiwan EPA report, 2008, EPA 97-U1U1-02-104. Environmental Protection Agency, Taiwan, Republic of China.
- 87. Shih YH. "The transformation and fate of self-synthesized nanoparticles in aquatic environment, Taiwan EPA report. 2009, EPA 98-U1U1-02-102. Environmental Protection Agency, Taiwan, Republic of China.
- 88. Mylon SE, Chen KL, Elimelech M. Influence of natural organic matter and ionic composition on the kinetics and structure of hematite colloid aggregation: Implications to iron depletion in estuaries. Langmuir, 2004, 20(21), 9000–6.

- 89. Paul Hiemenz C, Rajagopalan R. Principles of colloid and surface chemistry. 3rd ed. New York: Marcel Dekker; 1997
- 90. Baoling Yuan, Mai Pham, Thanh H. Nguyen, "Deposition Kinetics of Bacteriophage MS2 on a Silica Surface Coated with Natural Organic Matter in a Radial Stagnation Point Flow Cell." Environ. Sci. Technol., 2008, 42 (20), 7628–7633
- 91. Wang Y, Combe C, Clark MM. "The effects of pH and calcium on the diffusion coefficient of humic acid." J Membr Sci, 2001, 183, 49–60.
- 92. R. Kaegi, A. Voegelin, C. Ort, B. Sinnet, B. Thalmann, J. Krismer, H. Hagendorfer, M. Elumelu, E. Mueller," Fate and transformation of silver nanoparticles in urban wastewater systems." Water Research, 2013, 47, 3866-3877
- 93. E. Lombi, E. Donner, E. Tavakkoli, T. W. Turney, R. Naidu, B. W. Miller, K. G. Scheckel, "Fate of zinc oxide nanoparticles during anaerobic digestion of wastewater and post-treatment processing of sewage sludge." 2012, Environmental Science and Technology, 46, 9089–9096
- 94. R Ma, C Levard, J. D. Judy, J. M. Unrine, M. Durenkamp, B. Martin, B. Jefferson, G. V. Lowry, "Fate of zinc oxide and silver nanoparticles in a pilot wastewater treatment plant and in processed biosolids." *Environ. Sci. Technol.*, 2014, 48 (1), 104–112
- 95. B. Kim, C.S. Park, M. Murayama, M.F. Hochella, "Discovery and characterization of silver sulfide nanoparticle in final sewage sludge products." 2010, Environmental Science and Technology, 44, 7509–7514
- 96. Y. Yue R. Behra, L. Sigg, P. Ferna'ndez Freire, S. Pillai, K. Schirmer, "Toxicity of silver nanoparticles to a fish gill cell line: Role of medium composition." Nanotoxicology, 2014, 9 (1), 54-63
- 97. Y. Yue R. Behra, L. Sigg, P. Ferna ndez Freire, S. Pillai, K. Schirmer, "Toxicity of silver nanoparticles to a fish gill cell line: Role of medium composition." Nanotoxicology, 2014, 9 (1), 54-63
- 98. North East Biosolids and Residuals Association (NEBRA). National Biosolids Regulation, Quality, End Use and Disposal Survey Preliminary Report. http://www.nebiosolids.org/uploads/pdf/NtlBiosolidsReport-20July07.pdf (accessed 07/25/13).

- Y. Ge, J. P. Schimel, P. A. Holden, "Evidence for negative effects of TiO2 and ZnO nanoparticles on soil bacterial communities" Environ. Sci. Technol. 2011, 45, 1659–1664
- 100. Changwoo Kim, Jaesang Lee, Seunghak Lee, "TiO<sub>2</sub> nanoparticle sorption to sand in the presence of natural organic matter."Environmental Earth Sciences, 2015, 73, 9, 5585–5591
- 101. Sondra Klitzke, George Metreveli, Andre Peters, Gabriele E. Schaumann, Friederike Lang, "The fate of silver nanoparticles in soil solution — Sorption of solutes and aggregation." Science of The Total Environment, 535, 1 2015, 54–60
- J. Brant, H. Lecoanet, M. R. Wiesner, "Aggregation and deposition characteristics of fullerene nanoparticles in aqueous systems." J. Nanopart. Res. 2005, 7 (4-5), 545–553.
- 103. Y. Wang, Y. Li, K. D. Pennell, "Influence of electrolyte species and concentration on the aggregation and transport of fullerene nanoparticles in quartz sands." Environ. Toxicol. Chem. 2008, 27 (9), 1860–1867.
- X. Cheng, A. T. Kan, M. B. Tomson, "Study of C60 transport in porous media and the effect of sorbed C60 on naphthalene transport." J. Mater. Res. 2005, 20 (12), 3244–3254.
- 105. Y. Li, Y. Wang, K. D. Pennell, L. M. Abriola, "Investigation of the transport and deposition of fullerene (C60) nanoparticles in quartz sands under varying flow conditions." Environ. Sci. Technol. 2008, 42 (19), 7174–7180.
- 106. K. L. Chen, M. Elimelech, "Aggregation and deposition kinetics of fullerene (C60) nanoparticles." Langmuir 2006, 22, 10994-11001
- 107. K. L. Chen, M. Elimelech, "Interaction of fullerene (C60) nanoparticles with humic acid and alginate coated silica surfaces: measurements, mechanisms, and environmental implications." Environ. Sci. Technol. 2008, 42, 7607–7614
- X. Jiang, M. Tong, H. Li, K. Yang, "Deposition kinetics of zinc oxide nanoparticles on natural organic matter coated silica surfaces." Journal of Colloid and Interface Science, 2010, 350, 427–434

- Nicolosi, V., D. Vrbanic, "Solubility of Mo6S4.5I4.5 nanowires in common solvents: A sedimentation study." Journal of Physical Chemistry B, 2005, 109(15), 7124-7133
- 110. Weber, W. J., P. M. Micginley, "Sorption phenomena in subsurface systems: Concepts, models and effects on contaminant fate and transport." Water Research, 1991, 25(5), 499-528.
- 111. A.R. Petosa, S.J. Brennan, F Rajput, N. Tufenkji, "Transport of two metal oxide nanoparticles in saturated granular porous media: Role of water chemistry and particulate coating." Water Research, 2012, 46, 1273-1285
- 112. E. Tipping, D.C. Higgins, "The effect of adsorbed humic substances on the colloid stability of heamatite particles." Colloids Surf. 1982, 5, 85–92.
- 113. X.H. Zhou, B.C. Huang, T. Zhou, Y.C. Liu, H.C. Shi, "Aggregation behavior of engineered nanoparticles and their impact on activated sludge in wastewater treatment." Chemosphere, 2015, 119, 568–576
- 114. M. Lin, Y. H. Tseng, C.P. Huang, "Interactions between nano-TiO2 particles and algal cells at moderate particle concentration." Frontiers of Chemical Science and Engineering, 2015, 9, 2, 242–257
- 115. M. Sezgin, D. Jenkins, D.S. Parker, "A unified theory of filamentous activated sludge bulking." J. Wat. Pollut. Control Fed., 1978, 50, 362-381
- 116. Lindahl M, Faris A, Wadstrom T and HjertCn S. "A new test based on 'salting out' to measure relative surface hydrophobicity of bacterial cells." Biochim. Biophys. Acta 1981, 677, 471-476
- 117. C.P. Cousin, J.J. Ganczarczyk, "Effect of salinity on physical characteristics of activated sludge flocs." Water Quality Research. J. Canada, 1998, 33, 4, 565-587
- M. Bostrom, D.R.M. Williams, B. W. Ninham, "Specific ion effects: Why DLVO theory fails for biology and colloidal systems." Physical review letters, 2001, 87, 16, 168103-1-4
- 119. F Dilek Sanin, "Effect of solution physical chemistry on the rheological properties of activated sludge." Water SA, 2002, 28, 2, 207-212
- 120. Forster, C.F., "Bound water in sewage sludge and its relationship to sludge surfaces and sludge viscosities." J. Chem. Tech. Biol., 1983, 33B, 76-84

- 121. M.B. Romanello, M. M. Fidalgo de Cortalezzi, "An experimental study on the aggregation of TiO2 nanoparticles under environmentally relevant conditions." Waster Research, 2013, 47, 3887-3898
- Da-hong Li, Jerzy J. Ganczarczyk, "Structure of activated sludge flocs." Biotechnology and Bioengineering, 1990, 35, 57-65
- 123. J. Xu, X.Y. Li, "Investigation of the effect of nanoparticle exposure on the flocculability of activated sludge using particle image velocimetry incombination with the extended DLVO analysis." Colloids and Surfaces B: Biointerfaces, 2016, 143, 382-389
- 124. T.P. Nguyen, N.P. Hankins, N. Hilal, "A comparative study of the flocculation behavior and final properties of synthetic and activated sludge in wastewater treatment." Desalination, 2007, 204, 277-295
- 125. B. Ormeci, P.A. Vesilind, "Development of an improved synthetic sludge: a possible surrogate for studying activated sludge dewatering characteristics." Water Research, 2000, 34, 4, 1069-1078
- 126. R. Williams, A. M. Goodman, "Wetting of thin layers of Si0<sub>2</sub> by water." Appl. Phys. Lett., 1974, 25, 10, 531-532

## Appendix A

#### SCHEMATIC OF DIALYSIS SYSTEM



Figure A1. Schematic outlay of dialysis test unit. Deionized (DI) water was pumped into a stirred reservoir (2 L) for dialysis. A dialysis tube (DT) containing wastewater samples were submerged in the reservoir for salt removal. The water was pumped into the reservoir at a flow rate of 0.1 mL/h.

## Appendix B

# CONDUCTIVITY OF DESALTED SLUDGE WITH AND WITHOUT WATER CIRCULATION IN SUBMERGED TANK

Hour	w/circulation	w/o circulation
0	3.70	3.68
2	2.10	2.22
4	1.26	1.38
6	.78	1.00
8	.54	.76
10.5	.34	.62
12	.26	.52

Table B1. Conductivity of sludge in dialysis tubes with and without water circulation in the water tank. (Data for Fig. 2.2)

# Appendix C

#### **OXIDATION OF SLUDGE BY WET PEROXIDE METHOD**

$[H_2O_2]$	1 <sup>st</sup> cycle	2 <sup>nd</sup> cycle
2.5	8531.64	5530.94
5	6899.35	3918.69
7.5	5301.66	3246.45
10	3266.92	2597.19
15	418.58	228.91
20	408.37	181.66
25	495.80	124.80

Table C1. Removal of organic content in sludge sample with wet peroxide method. (Data for Fig. 2.3)

# Appendix D

Z	n	Zn (A	AD)	Т	i	Ti (A	AD)
Added	Detected	Added	Detected	Added	Detected	Added	Detected
0.0802	0.0486	0.0802	0.114	0.06	0.028	0.06	0.127
0.201	0.1301	0.201	0.261	0.15	0.077	0.15	0.186
0.401	0.278			0.3	0.151		
0.602	0.440	0.602	0.523	0.45	0.222	0.45	0.482
0.803	0.621	0.803	0.791	0.6	0.297	0.6	0.63

## **EFFECT OF ACID DIGESTION**

Table D1. The effect of acid digestion (AD) on the recovery of engineered nanoparticles. (Data for Fig. 2.4)

					•	
	n	n	nn	1	87	- H.
-						<b>י</b> י
4.		· •	~	-		_
	_					

## SPATIAL DISTRIBUTION OF ENGINEERED NANOPARTICLES IN WASTEWATER TREATMENT PLANT B

		Concen	tration			ENP/Dr	y sludge			Mass Fl	ow Rate	
	Ti		Zn		Ti		Zn		Ti		Zn	
	μg/L	St.dev	µg/L	St.dev	g/kg	St.dev	g/kg	St.dev	kg/day	St.dev	kg/day	St.dev
1-1	149.24	42.52	102.07	8.89	1.35	0.38	0.924	0.080	86.66	21.13	59.27	4.42
1-2	125.10	29.36	57.78	2.24	2.61	0.61	1.204	0.047	70.84	14.20	32.72	1.08
1-3	1065.21	363.17	1424.59	214.77	0.59	0.20	0.788	0.119	15.65	4.84	20.92	2.86
2-1	114.42	33.88	32.73	3.40	0.62	0.18	0.177	0.018	79.48	23.58	22.74	2.36
2-2	47.60	15.37	25.05	3.34	0.79	0.25	0.414	0.055	23.33	7.64	12.28	1.66
2-3	4709.94	1021.85	703.71	39.50	0.50	0.11	0.075	0.004	928.89	73.36	138.79	4.44
3	30.02	15.39	17.88	0.72	0.66	0.34	0.392	0.016	14.90	7.44	8.87	0.35

Table E1. Spatial distribution of engineered nanoparticles in wastewater treatment plant B. (Data for Fig. 2.7 (a), (b), (c))

#### Appendix F

#### SPATIAL DISTRIBUTION OF ENGINEERED NANOPARTICLES IN WASTEWATER TREATMENT PLANT W

	Cor	ncentration	Mass	Flow Rate	ENP/E	Dry sludge
	Ti	Zn	Ti	Zn	Ti	Zn
1-1	106.04	46.59	106.78	46.92	0.089	0.039
1-2	112.59	30.88	113.38	31.09	0.218	0.060
1-3	246.46	66.36	7.64	2.06	0.077	0.021
2-1	457.58	43.79	781.14	74.75	0.173	0.017
2-2	30.41	3.89	29.68	3.80	0.051	0.006
2-3	8833.00	811.18	6457.99	593.07	1.298	0.119
3-1	889.97	144.46	1855.77	301.24	0.529	0.086
3-2	37.09	22.38	36.20	21.85	0.160	0.096
3-3	2610.22	443.97	2895.31	492.46	0.740	0.126
4	0.56	0.00	0.55	0.00	0.002	0.000

Table F1. Concentration, Mass flow, and nanoparticle concentration per weight of dry sludge in wastewater treatment plant W. The units of the results are in µg/L, kg/day, and g/kg, respectively (Data for Fig. 2.8 (a), (b), (c), (d), (e))

Sample	Al	Ti	Fe	Zn
1	2.741	0.807	62.646	25.144
2	-1.228	0.992	45.364	12.278
3	0.933	5.176	75.260	8.569
4	-2.541	-0.903	67.296	-4.276
5	-2.737	-1.151	80.943	-7.547
6	-2.319	0.511	127.773	-4.397
7	-0.264	5.622	158.953	-3.957
8	6.728	11.736	226.145	3.792
9	2.908	0.825	161.724	2.611
10	1.258	1.700	128.932	0.172

# ENGINEERED NANOPARTICLE CONCENTRATION IN SIAO LI RIVER

Appendix G

Table G1. Nanoparticle concentrations of various sample positions in the Sio Li river. (Data for Fig. 2.11)

Sample	Al	Ti	Fe	Zn
1-1	0.11	-0.53	20.03	13.47
1-2	39.61	2.98	272.80	110.90
1-3	-1.10	-1.15	10.09	8.08
1-4	4.12	-0.69	22.91	19.16
1-5	-1.06	-2.43	4.73	14.13

Table G2. Nanoparticle analysis from samples collected at the New Fulong tea factory. (Data for Fig. 2.12(a))

Sample	Al	Ti	Fe	Zn
2-1	12.35	22.02	130.11	40.69
2-2	23.44	41.41	198.26	39.31
2-3	11.67	37.57	242.02	45.56
2-4	10.69	26.64	169.43	44.78
2-5	8.21	29.75	163.97	107.26

Table G3. Nanoparticle analysis from samples collected at He Sing bridge (Data for Fig. 2.12 (b))

Sample	Al	Ti	Fe	Zn
3-1	23.69	8.38	107.71	16.16
3-2	38.93	8.22	99.62	25.25
3-3	16.87	14.05	151.41	18.28
3-4	23.54	17.01	132.70	78.92
3-5	11.19	15.51	120.16	75.79

Table G4. Nanoparticle analysis from samples collected at Long Sing bridge (Data for Fig. 2.12 (c))

Sample	Al	Ti	Fe	Zn
4-1	33.64	12.77	217.93	13.55
4-2	90.27	18.54	419.03	19.69
4-3	17.97	13.22	121.42	12.69
4-4	11.60	8.08	67.02	20.58
4-5	4.32	12.01	78.37	23.95

Table G5. Nanoparticle analysis from samples collected at Siao Li bridge (Data for Fig. 2.12 (d))

Sample	Al	Ti	Fe	Zn
5-1	36.17	8.72	164.66	9.58
5-2	74.06	17.77	241.57	14.99
5-3	49.70	5.22	73.01	8.38
5-4	0.13	2.67	48.82	25.63
5-5	0.63	8.98	95.16	12.14

Table G6. Nanoparticle analysis from samples collected at Bao Shih bridge (Data for Fig. 2.12 (e))

Sample	Al	Ti	Fe	Zn
6-1	38.06	10.66	136.20	9.07
6-2	35.02	11.12	158.74	12.76
6-3	21.55	24.40	132.55	9.49
6-4	4.51	7.82	78.95	6.09
6-5	1.58	26.79	96.02	6.01

Table G7. Nanoparticle analysis from samples collected at Fong Hunag bridge (Data for Fig. 2.13 (a))

Sample	Al	Ti	Fe	Zn
7-1	55.83	13.64	166.45	8.91
7-2	265.58	25.12	507.21	14.52
7-3	40.46	23.32	189.29	7.55
7-4	279.88	9.38	108.16	9.72
7-5	5.04	12.41	56.75	9.00

Table G8. Nanoparticle analysis from samples collected at Jhong Sing bridge (Data for Fig. 2.13 (b))

Sample	Al	Ti	Fe	Zn
8-1	45.03	18.67	113.81	19.81
8-2	101.74	45.65	127.54	16.30
8-3	59.61	5.96	72.19	16.09
8-4	19.84	0.12	36.65	23.54
8-5	19.71	1.69	51.46	24.18

Table G9. Nanoparticle analysis from samples collected at Jhen Sing bridge (Data for Fig. 2.13 (c))

Sample	Al	Ti	Fe	Zn
9-1	33.68	3.92	75.16	12.55
9-2	48.74	8.84	123.36	18.24
9-3	8.44	7.98	55.49	11.56
9-4	15.79	9.93	69.69	14.21
9-5	15.47	5.08	74.04	17.59

Table G10. Nanoparticle analysis from samples collected at Tian Gong monastery bridge (Data for Fig. 2.13 (d))

Sample	Al	Ti	Fe	Zn
10-1	47.59	41.91	240.48	164.29
10-2	67.49	18.35	247.13	23.03
10-3	72.92	23.18	214.29	27.25
10-4	19.67	21.43	152.81	21.22
10-5	17.37	11.96	147.25	21.63

Table G11. Nanoparticle analysis from samples collected at Niou Be bridge bridge (Data for Fig. 2.13 (e))

Α	pp	end	lix	Η
	PP			

#### U.S. MONTHLY SALES OF HEALTH AND PERSONAL CARE PRODUCTS

Period	Value (\$)						
Jan-12	22863	Jan-13	23520	Jan-14	24385	Jan-15	25865
Feb-12	22740	Feb-13	21971	Feb-14	22997	Feb-15	24257
Mar-12	23987	Mar-13	23788	Mar-14	24863	Mar-15	26446
Apr-12	22748	Apr-13	23104	Apr-14	24658	Apr-15	25742
May-12	23515	May-13	23853	May-14	25398	May-15	25665
Jun-12	22170	Jun-13	22426	Jun-14	24669	Jun-15	25898
Jul-12	22305	Jul-13	23389	Jul-14	25053	Jul-15	26084
Aug-12	23128	Aug-13	23692	Aug-14	25107	Aug-15	26105
Sep-12	21517	Sep-13	23084	Sep-14	25077	Sep-15	25955
Oct-12	22992	Oct-13	24440	Oct-14	25971	Oct-15	26904
Nov-12	22479	Nov-13	23510	Nov-14	24518	Nov-15	25937
Dec-12	25355	Dec-13	26984	Dec-14	29448	Dec-15	30511

Table H1. U.S. monthly sales of health and personal care products. Source: Advanced monthly retail trade report, U.S. census bureau. (Data for Fig. 2.15)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Nov	Dec
1-1	275.9	47.4	57.3	38.2	81.3	108.9	117.2	391.7	155.9	21.6	149.2
1-2	95.4	65.9	37.4	28.1	95.3	49.2	61.4	118.1	74.1	31.5	125.1
1-3	1955.0	2319.5	23.1	295.7	713.9	1945.5	248.3	4458.0	1878.0	7.9	1065.2
2-1	149.0	2126.7	86.5	309.5	255.1	32.0	124.8	1457.9	1005.3	16.2	114.4
2-2	14.9	16.6	14.9	32.5	32.4	101.4	9.8	36.0	7.8	0.0	47.6
2-3	12297.9	7575.2	3391.1	702.1	994.1	3972.0	7624.5	4404.0	1717.5	121.9	4709.9
3	16.4	11.1	20.0	38.7	13.4	13.4	11.1	33.3	8.7	0.0	30.0

Table I1. Concentration of titanium in wastewater treatment plant B. The concentration of the titanium is in  $\mu g/L$ . Sampling locations are sited at the far left of the table based on the numbering designation of Fig. 2.1. (Data for Fig. 2.17)

CONCENTRATION OF TITANIUM IN WASTEWATER TREATMENT PLANT B

Appendix I

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Nov	Dec
1-1	166.3	93.9	165.4	39.9	15.0	74.1	79.5	221.3	43.5	20.7	102.1
1-2	59.4	25.4	130.7	19.6	17.4	33.9	58.2	54.8	97.2	4.4	57.8
1-3	1212.2	481.7	156.8	303.3	245.9	2347.5	322.4	3336.0	1113.6	25.2	1424.6
2-1	22.1	592.9	71.5	22.1	4.9	33.0	49.5	234.9	302.4	8.5	32.7
2-2	0.0	0.0	55.1	10.4	2.3	81.6	23.3	25.4	11.3	0.0	25.0
2-3	1420.8	1482.5	1288.7	595.2	579.6	1333.4	1875.0	1446.2	2254.5	235.5	703.7
3	0.0	50.9	61.4	15.0	6.2	71.7	52.7	167.4	21.3	0.0	17.9

Table I2. Concentration of zinc in wastewater treatment plant B. The concentration of the zinc is in µg/L. Sampling locations are sited at the far left of the table based on the numbering designation of Fig 2.1. (Data for Fig. 2.18)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Nov	Dec
1-1	178.4	29.0	34.9	18.6	41.1	56.0	60.5	208.7	80.5	14.0	86.7
1-2	60.3	39.3	22.2	13.4	47.0	24.7	30.9	61.2	37.2	19.9	70.8
1-3	27.7	32.8	0.3	3.9	8.1	22.7	3.3	63.6	26.1	0.1	15.6
2-1	122.7	1664.8	68.2	216.3	172.7	22.9	86.7	1029.8	702.9	11.6	79.5
2-2	8.6	9.1	8.2	15.8	16.1	51.0	4.8	18.1	3.8	0.0	23.3
2-3 (1)	2890.9	1717.6	780.5	144.3	208.1	819.4	1500.8	880.2	344.6	24.7	928.9
2-3 (2)	95.4	67.7	27.9	6.0	10.2	31.9	42.4	23.5	10.0	0.7	32.8
3	10.8	7.4	12.0	19.8	6.6	6.5	5.1	15.8	4.1	0.0	14.9

Table I3. Mass flow of titanium in wastewater treatment plant B. The concentration of the titanium is in kg/day. Samplinglocations are sited at the far left of the table based on the numbering designation of Fig 2.1. (Data for Fig. 2.19)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Nov	Dec
1-1	107.5	57.4	100.7	19.5	7.6	38.1	41.0	117.9	22.5	13.4	59.3
1-2	37.6	15.2	77.6	9.3	8.6	17.0	29.3	28.4	48.8	2.8	32.7
1-3	17.2	6.8	2.3	4.0	2.8	27.4	4.3	47.6	15.5	0.4	20.9
2-1	18.2	464.2	56.4	15.5	3.3	23.7	34.4	165.9	211.4	6.1	22.7
2-2	0.0	0.0	30.3	5.1	1.1	41.1	11.5	12.7	5.5	0.0	12.3
2-3 (1)	334.0	336.1	296.6	122.3	121.3	275.1	369.1	289.0	452.3	47.7	138.8
2-3 (2)	11.0	13.2	10.6	5.1	6.0	10.7	10.4	7.7	13.1	1.4	4.9
3	0.0	33.8	36.9	7.7	3.0	35.1	24.1	79.4	10.1	0.0	8.9

Table I4. Mass flow of zinc in wastewater treatment plant B. The concentration of the zinc is in kg/day. Sampling locationsare sited at the far left of the table based on the numbering designation of Fig 2.1. (Data for Fig. 2.20)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Nov	Dec
1-1	1.93	0.59	0.27	0.35	0.57	0.77	0.83	2.78	1.11	0.20	1.35
1-2	0.73	1.37	0.29	0.59	0.73	0.38	0.47	0.90	0.57	0.66	2.61
1-3	1.05	2.02	0.02	0.16	0.38	2.04	0.26	4.67	1.97	0.00	0.59
2-1	0.82	6.22	0.43	1.67	1.41	0.16	0.61	7.18	4.95	0.09	0.62
2-2	0.08	0.07	0.06	0.54	0.17	0.39	0.04	0.14	0.03	0.00	0.79
2-3	1.28	0.81	0.36	0.07	0.10	0.43	0.82	0.47	0.18	0.01	0.50
3	0.37	0.36	0.32	0.85	0.31	0.22	0.18	0.54	0.14	0.00	0.66

Table I5. Titanium concentration per weight of dry sludge in wastewater treatment plant B. The units of the results are in g/kg. Sampling locations are sited at the far left of the table based on the numbering designation of Fig 2.1. (Data for Fig. 2.21)

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Nov	Dec
1-1	1.16	1.17	0.78	0.36	0.07	0.53	0.56	1.57	0.31	0.19	0.92
1-2	0.46	0.53	1.00	0.41	0.13	0.26	0.45	0.42	0.74	0.09	1.20
1-3	0.65	0.42	0.16	0.17	0.26	2.46	0.34	3.49	1.17	0.01	0.79
2-1	0.12	1.73	0.35	0.12	0.02	0.16	0.24	1.16	1.49	0.05	0.18
2-2	0.00	0.00	0.21	0.17	0.01	0.31	0.09	0.10	0.04	0.00	0.41
2-3	0.15	0.16	0.14	0.06	0.06	0.14	0.20	0.16	0.24	0.02	0.07
3	0.00	1.64	0.99	0.33	0.10	1.16	0.85	0.27	0.34	0.00	0.39

Table I6. Zinc concentration per weight of dry sludge in wastewater treatment plant B. The units of the results are in g/kg. Sampling locations are sited at the far left of the table based on the numbering designation of Fig 2.1. (Data for Fig. 2.22)

pH	3	pH	3	pH	6	pH	8
NaCl	AE	$Na_2SO_4$	AE	NaCl	AE	NaCl	AE
100	0.992	50	0.839	6.0	1.051	100	1.140
70	0.962	30	1.114	1.0	0.949	50	0.943
50	1.008	10	1.021	0.6	0.988	30	0.862
30	0.573	5	1.060	0.3	1.139	20	0.701
14	0.449	1	0.966	0.1	1.113	15	0.200
10	0.699	0.1	0.242	0.06	0.907	10	0.080
5	0.164	0.5	0.832	0.03	0.362	5	0.041
2	0.095	0.2	0.188	0.02	0.097		
1	0.093	0.02	0.024	0.01	0.057		
0.5	0.038	0.05	0.068	0.005	0.031		
0.2	0.018						

# ATTACHMENT EFFICIENCY STUDIES OF ENGINEERED NANOPARTICLES

Appendix J

Table J1. Attachment efficiency (AE) of TiO<sub>2</sub> under various pH and electrolyte conditions. (Data for Fig. 3.2, 3.3)

Ti	O <sub>2</sub>	TiO <sub>2</sub> +10	% DOM	TiO <sub>2</sub> +50	% DOM	TiO <sub>2</sub> +	DOM
NaCl	AE	NaCl	AE	NaCl	AE	NaCl	AE
5.00E-							
03	0.031	100	0.9435	100	0.9823	7	0.0205
0.01	0.0571	50	1.0414	50	0.9105	10	0.0401
0.02	0.0968	20	1.0152	20	1.1072	20	0.1562
0.03	0.3621	10	0.344	10	0.6893	40	0.4749
0.06	0.9071	6	0.1675	6	0.7795	60	0.9202
0.1	1.1133	3	0.0583	4	0.3432	100	1
0.3	1.1394	2	0.0267	2	0.0357		
0.6	0.9884						
1	0.9487						
6	1.0513						

Table J2. Attachment efficiency (AE) of  $TiO_2$  under various dissolved organic matter (DOM) concentrations. (Data for Fig. 3.4(a))

ZnO		ZnO +D0	DM
NaCl	AE	NaCl	AE
0.1	7.03E-03	8	6.08E-03
0.6	0.0461	20	0.0676
1	0.1422	60	0.5993
2	0.3117	100	1.1014
6	0.6773	200	0.8986
10	0.8508		
20	0.9977		
60	1.0023		

Table J3. Attachment efficiency (AE) of  $TiO_2$  under various dissolved organic matter (DOM) concentrations. (Data for Fig. 3.4(b))

A	ppendix	K
11	ррспал	

	ZnO + H	Primary		ZnO + Secondary						
d < 100nm		100nm <	d <1um	d < 10	00nm	100nm < d <1um				
ln(C <sub>e</sub> )	$\ln(\Gamma)$	ln(C <sub>e</sub> )	$\ln(\Gamma)$	ln(Ce)	$\ln(\Gamma)$	ln(C <sub>e</sub> )	$\ln(\Gamma)$			
1.83	1.62	2.06	0.56	0.59	2.14	1.48	2.44			
2.84	1.33	2.43	1.94	1.24	2.84	2.10	3.15			
3.25	2.95	2.65	3.04	2.12	3.49	2.60	3.53			
3.70	3.28	3.28	3.30	2.73	3.83	3.05	3.77			
4.07	3.36			2.90	4.16					

Table K1. Attachment of primary and secondary organic matter to ZnO nanoparticles (Data for Fig. 3.5)

## Appendix L

## SEDIMENT CURVE AND ANALYSIS OF ENGINEERED NANOPARTICLE TO SLUDGE

Time (min)	TS	St. dev
0	1.64	0.02
10	1.25	0.03
20	1.13	0.03
30	1.05	0.04
40	0.95	0.07
50	0.90	0.03
60	0.83	0.08
80	0.77	0.04
100	0.70	0.07
120	0.67	0.03
150	0.61	0.03
180	0.57	0.03
210	0.52	0.05
240	0.50	0.03
270	0.48	0.04

Table L1. Sedimentation cure and analysis of engineered nanoparticle to sludge. (Data for Fig. 4.1)

#### Appendix M

#### SORPTION OF VARIOUS ENGINEERED NANOPARTICLES TO SECONDARY SLUDGE FROM WASTEWATER TREATMENT PLANTS

Textile	#1	Textile	#2	Textile	#3	TiO <sub>2</sub> in reacte	lab or	TiO <sub>2</sub> - Slu	dge (1)	TiO <sub>2</sub> - Slu	dge (2)	ZnO - Sl	udge	TiO <sub>2</sub> - Slu	dge (3)
$ln(C_{free})$	$\ln(\Gamma)$	ln(C <sub>free</sub> )	$\ln(\Gamma)$	ln(C <sub>free</sub> )	$\ln(\Gamma)$	ln(C <sub>free</sub> )	$\ln(\Gamma)$	$ln(C_{free})$	$\ln(\Gamma)$	$ln(C_{free})$	$\ln(\Gamma)$	$ln(C_{free})$	$\ln(\Gamma)$	$ln(C_{free})$	$\ln(\Gamma)$
2.20	2.19	1.48	2.13	1.03	1.43	2.00	-3.57	4.19	0.85	4.16	0.73	4.19	1.59	4.49	0.75
2.30	2.20	1.63	2.22	1.17	1.49	2.08	-3.53	4.52	1.04	4.52	0.63	4.67	1.72	4.81	0.99
2.41	2.19	1.86	2.31	1.20	1.62	2.02	-3.48	4.70	1.18	4.70	0.80	4.81	1.95	4.94	1.32
2.47	2.24	2.04	2.49	1.32	1.75	2.04	-3.45	4.83	1.30	4.61	1.40	5.13	1.74	5.04	1.51
2.52	2.30	2.15	2.65	1.55	1.84	2.09	-3.42	4.95	1.26	4.87	1.23	5.18	1.88	5.19	1.47
2.58	2.35			1.63	1.81	2.27	-3.41	5.01	1.46	4.91	1.39	5.28	1.91	5.25	1.61
2.63	2.34			1.68	1.86	2.28	-3.40	5.06	1.62	4.92	1.55	5.30	1.97	5.34	1.42
2.65	2.65			1.73	1.88	2.33	-3.33	5.08	1.79	5.03	1.53	5.36	1.92	5.36	1.51
						2.46	-3.32	5.15	1.80	5.08	1.58			5.40	1.48
						2.53	-3.23								

Table L1. Sorption of various engineered nanoparticles to secondary sludge from wastewater treatment plant. Textile #1,2,3[14], TiO2 in lab reactor [15], TiO2-sludge and ZnO-sludge are each from the current research. (Data for Fig. 4.2)

An	$\mathbf{n} \mathbf{n} \mathbf{n}$	
AU		
<b>****</b>		- <b>1</b>

SEDIMENTATION P	<b>ROFILE O</b>	F NANOPARTI	CLES TO SLUDGE

	0 1	mg/L	10	mg/L	20	mg/L	40	mg/L	60	mg/L	80	mg/L
Time (min)	TS	St. dev	TS	St. dev	TS	St. dev						
0	-3.15	1.35	-1.41	0.49	-0.89	0.33	-0.24	0.18	0.27	0.20	0.19	0.26
10	-3.22	1.42	-1.40	0.52	-0.90	0.34	-0.24	0.18	0.28	0.20	0.11	0.33
20	-3.20	1.41	-1.42	0.53	-0.90	0.35	-0.25	0.16	0.27	0.20	0.09	0.35
30	-3.28	1.49	-1.41	0.51	-0.90	0.34	-0.25	0.18	0.27	0.20	0.06	0.37
40	-3.29	1.51	-1.42	0.52	-0.91	0.35	-0.25	0.18	0.26	0.20	0.04	0.39
50	-3.29	1.50	-1.43	0.54	-0.90	0.35	-0.26	0.19	0.26	0.20	0.02	0.41
60	-3.26	1.46	-1.44	0.55	-0.91	0.35	-0.26	0.18	0.21	0.25	-0.03	0.46
80	-3.29	1.50	-1.43	0.55	-0.92	0.36	-0.27	0.19	0.25	0.21	-0.09	0.50
100	-3.29	1.49	-1.44	0.55	-0.93	0.36	-0.27	0.19	0.25	0.22	-0.11	0.52
120	-3.32	1.54	-1.44	0.54	-0.91	0.35	-0.28	0.20	0.24	0.22	-0.14	0.55
150	-3.33	1.55	-1.46	0.56	-0.94	0.36	-0.29	0.19	0.23	0.22	-0.16	0.55
180	-3.30	1.51	-1.46	0.55	-0.93	0.36	-0.30	0.20	0.22	0.23	-0.18	0.57
210	-3.25	1.49	-1.48	0.56	-0.94	0.37	-0.19	0.28	0.22	0.24	-0.21	0.59
240	-3.29	1.51	-1.48	0.56	-0.97	0.38	-0.33	0.20	0.21	0.24	-0.24	0.60
270	-3.31	1.54	-1.49	0.57	-0.97	0.38	-0.34	0.20	0.20	0.24	-0.26	0.61

Table N1. Sedimentation profile of  $TiO_2$  with dissolved organic matter from the primary sediment tank. (Data for Fig. 4.3(a))
	0 1	ng/L	10	mg/L	20	mg/L	40	mg/L	60	mg/L	80	mg/L
Time (min)	TS	St. dev	TS	St. dev	TS	St. dev						
0	-2.55	1.01	-1.31	0.47	-0.75	0.34	-0.20	0.24	0.17	0.21	0.43	0.14
10	-2.53	1.02	-1.31	0.50	-0.75	0.36	-0.20	0.25	0.17	0.19	0.43	0.15
20	-2.53	1.01	-1.30	0.50	-0.75	0.35	-0.20	0.25	0.17	0.21	0.43	0.15
30	-2.53	1.01	-1.31	0.51	-0.76	0.36	-0.20	0.24	0.16	0.21	0.43	0.15
40	-2.54	0.99	-1.32	0.50	-0.75	0.35	-0.21	0.25	0.16	0.21	0.43	0.15
50	-2.52	0.99	-1.32	0.51	-0.76	0.36	-0.20	0.26	0.15	0.21	0.42	0.15
60	-2.56	1.01	-1.32	0.50	-0.76	0.36	-0.21	0.26	0.15	0.20	0.42	0.14
80	-2.51	1.01	-1.32	0.51	-0.76	0.36	-0.21	0.26	0.14	0.21	0.41	0.15
100	-2.53	1.00	-1.31	0.49	-0.77	0.36	-0.21	0.25	0.14	0.20	0.40	0.15
120	-2.49	0.99	-1.32	0.50	-0.80	0.39	-0.22	0.26	0.13	0.21	0.39	0.15
150	-2.52	1.00	-1.32	0.50	-0.79	0.38	-0.23	0.26	0.12	0.21	0.38	0.16
180	-2.50	0.97	-1.32	0.48	-0.79	0.37	-0.24	0.26	0.11	0.21	0.37	0.16
210	-2.49	0.96	-1.33	0.49	-0.80	0.37	-0.25	0.25	0.10	0.21	0.36	0.16
240	-2.47	0.95	-1.34	0.50	-0.80	0.38	-0.26	0.26	0.09	0.22	0.35	0.16
270	-2.46	0.95	-1.34	0.50	-0.81	0.38	-0.27	0.26	0.08	0.21	0.34	0.17

Table N2. Sedimentation profile of TiO<sub>2</sub> with dissolved organic matter from the secondary sediment tank. (Data for Fig 4.2 (b))

	0 r	ng/L	10	mg/L	20 :	mg/L	40	mg/L	60	mg/L	80 :	mg/L
Time (min)	TS	St. dev										
0	-1.94	0.86	-1.66	0.30	-1.50	0.58	-1.34	0.43	-1.09	0.32	-0.86	0.26
10	-1.98	0.87	-1.66	0.27	-1.50	0.54	-1.31	0.40	-1.10	0.33	-0.86	0.26
20	-1.89	0.91	-1.60	0.18	-1.43	0.46	-1.25	0.31	-1.07	0.27	-0.83	0.20
30	-1.76	0.75	-1.56	0.14	-1.42	0.47	-1.25	0.32	-1.06	0.27	-0.82	0.21
40	-1.86	0.63	-1.54	0.14	-1.42	0.47	-1.24	0.33	-1.05	0.27	-0.83	0.22
50	-1.86	0.68	-1.56	0.16	-1.42	0.48	-1.24	0.32	-1.05	0.27	-0.81	0.21
60	-1.89	0.78	-1.55	0.16	-1.40	0.47	-1.24	0.32	-1.04	0.27	-0.81	0.22
80	-1.79	0.73	-1.54	0.18	-1.40	0.49	-1.24	0.33	-1.05	0.28	-0.82	0.23
100	-1.72	0.61	-1.51	0.17	-1.41	0.48	-1.24	0.33	-1.04	0.29	-0.81	0.21
120	-1.70	0.60	-1.53	0.18	-1.43	0.49	-1.24	0.34	-1.04	0.29	-0.83	0.20
150	-1.72	0.62	-1.51	0.18	-1.40	0.50	-1.24	0.34	-1.04	0.29	-0.81	0.21
180	-1.70	0.65	-1.53	0.16	-1.40	0.50	-1.23	0.34	-1.04	0.28	-0.81	0.21
210	-1.68	0.60	-1.53	0.17	-1.40	0.50	-1.22	0.34	-1.03	0.29	-0.81	0.21
240	-1.65	0.63	-1.51	0.17	-1.39	0.53	-1.22	0.36	-1.03	0.31	-0.80	0.24
270	-1.64	0.62	-1.50	0.17	-1.38	0.49	-1.21	0.35	-1.04	0.35	-0.81	0.22

196

Table N3. Sedimentation profile of ZnO with dissolved organic matter from the primary sediment tank. (Data for Fig 4.2 (c))

	0	mg/L	10	mg/L	20	mg/L	40	mg/L	60	mg/L	80	mg/L
Time (min)	TS	St. dev										
0	-2.07	1.27	-1.60	0.84	-1.44	0.67	-1.15	0.53	-0.89	0.40	-0.77	0.29
10	-2.01	1.22	-1.53	0.87	-1.37	0.68	-1.10	0.50	-0.84	0.42	-0.73	0.31
20	-1.81	0.97	-1.42	0.76	-1.30	0.55	-1.03	0.46	-0.81	0.34	-0.69	0.27
30	-1.78	0.98	-1.43	0.75	-1.29	0.59	-1.05	0.42	-0.81	0.34	-0.69	0.26
40	-1.76	0.97	-1.42	0.74	-1.30	0.54	-1.03	0.43	-0.80	0.36	-0.68	0.26
50	-1.75	0.94	-1.41	0.71	-1.28	0.55	-1.02	0.42	-0.80	0.33	-0.69	0.24
60	-1.70	0.90	-1.38	0.71	-1.27	0.52	-1.01	0.44	-0.79	0.34	-0.69	0.24
80	-1.70	0.87	-1.37	0.69	-1.24	0.50	-1.01	0.43	-0.79	0.33	-0.68	0.25
100	-1.64	0.78	-1.39	0.68	-1.25	0.50	-1.00	0.41	-0.79	0.32	-0.69	0.21
120	-1.66	0.79	-1.42	0.66	-1.27	0.50	-1.02	0.42	-0.81	0.30	-0.68	0.24
150	-1.65	0.77	-1.39	0.65	-1.28	0.51	-1.02	0.41	-0.80	0.31	-0.71	0.20
180	-1.68	0.81	-1.37	0.61	-1.24	0.40	-1.02	0.39	-0.80	0.31	-0.69	0.23
210	-1.68	0.81	-1.37	0.60	-1.25	0.47	-1.02	0.39	-0.80	0.30	-0.71	0.23
240	-1.70	0.84	-1.38	0.62	-1.26	0.52	-1.02	0.40	-0.81	0.29	-0.73	0.19
270	-1.67	0.78	-1.38	0.60	-1.25	0.46	-1.02	0.38	-0.81	0.28	-0.70	0.21

197

Table N4. Sedimentation profile of ZnO with dissolved organic matter from the secondary sediment tank. (Data for Fig 4.2 (d))

	0 :	mg/L	10	mg/L	20	mg/L	40	mg/L	60	mg/L	80	mg/L
Time (min)	TS	St. dev	TS	St. dev	TS	St. dev	TS	St. dev	TS	St. dev	TS	St. dev
0	0.15	0.58	0.33	0.45	0.45	0.37	0.61	0.24	0.76	0.21	0.90	0.17
10	0.07	0.57	0.24	0.44	0.37	0.37	0.56	0.25	0.72	0.21	0.86	0.18
20	0.05	0.56	0.21	0.42	0.34	0.37	0.54	0.24	0.69	0.21	0.82	0.18
30	0.01	0.56	0.19	0.44	0.31	0.37	0.51	0.25	0.66	0.22	0.79	0.20
40	-0.03	0.55	0.15	0.43	0.28	0.36	0.47	0.25	0.62	0.22	0.75	0.21
50	-0.07	0.52	0.09	0.43	0.25	0.36	0.45	0.25	0.58	0.23	0.70	0.21
60	-0.12	0.54	0.06	0.44	0.23	0.35	0.42	0.24	0.55	0.23	0.67	0.22
80	-0.18	0.54	0.02	0.43	0.17	0.34	0.36	0.27	0.51	0.24	0.62	0.23
100	-0.21	0.54	-0.01	0.44	0.14	0.35	0.34	0.25	0.47	0.24	0.59	0.23
120	-0.25	0.53	-0.04	0.45	0.07	0.31	0.32	0.25	0.44	0.25	0.56	0.23
150	-0.27	0.52	-0.07	0.44	0.08	0.34	0.28	0.26	0.38	0.27	0.52	0.24
180	-0.30	0.52	-0.10	0.44	0.05	0.34	0.25	0.26	0.37	0.26	0.47	0.25
210	-0.33	0.52	-0.13	0.45	0.03	0.35	0.23	0.26	0.34	0.26	0.43	0.26
240	-0.39	0.50	-0.15	0.45	0.02	0.35	0.21	0.26	0.31	0.27	0.41	0.27
270	-0.39	0.51	-0.17	0.45	0.00	0.35	0.19	0.27	0.28	0.28	0.38	0.27

N5. Sedimentation profile of TiO<sub>2</sub> with organic matter with diameter  $100 \text{ mm} < d < 1 \text{ } \mu\text{m}$  from the primary sediment tank. (Data for Fig. 4.4 (a))

	0 n	ng/L	10 :	mg/L	20	mg/L	40	mg/L	60	mg/L	80	mg/L
Time (min)	TS	St. dev	TS	St. dev	TS	St. dev	TS	St. dev	TS	St. dev	TS	St. dev
0	0.411	0.230	0.522	0.225	0.640	0.190	0.793	0.154	0.920	0.132	1.038	0.084
10	0.312	0.286	0.426	0.257	0.554	0.215	0.717	0.173	0.842	0.149	0.972	0.133
20	0.198	0.329	0.325	0.285	0.467	0.256	0.637	0.220	0.784	0.189	0.913	0.160
30	0.125	0.403	0.263	0.366	0.401	0.312	0.588	0.263	0.743	0.229	0.884	0.198
40	0.104	0.440	0.256	0.391	0.381	0.327	0.576	0.275	0.724	0.248	0.871	0.213
50	0.043	0.448	0.232	0.410	0.384	0.337	0.560	0.294	0.719	0.257	0.866	0.226
60	-0.002	0.492	0.190	0.421	0.341	0.342	0.535	0.302	0.700	0.259	0.842	0.223
80	-0.045	0.517	0.127	0.448	0.327	0.361	0.515	0.308	0.685	0.267	0.813	0.235
100	-0.058	0.531	0.112	0.440	0.299	0.370	0.503	0.314	0.666	0.275	0.810	0.238
120	-0.085	0.542	0.109	0.462	0.274	0.387	0.483	0.322	0.649	0.274	0.803	0.245
150	-0.121	0.544	0.080	0.471	0.244	0.393	0.470	0.316	0.621	0.278	0.786	0.243
180	-0.144	0.544	0.051	0.469	0.217	0.375	0.459	0.316	0.620	0.277	0.762	0.239
210	-0.147	0.492	0.038	0.470	0.225	0.375	0.442	0.314	0.613	0.275	0.754	0.268
240	-0.160	0.539	0.033	0.458	0.207	0.378	0.431	0.303	0.603	0.304	0.750	0.258
270	-0.183	0.528	0.012	0.466	0.190	0.388	0.405	0.290	0.592	0.302	0.729	0.256

Table N6. Sedimentation profile of TiO<sub>2</sub> with diameter 100nm  $< d < 1 \mu$ m from the secondary sediment tank. (Data for Fig. 4.4 (b))

	0	mg/L	10	mg/L	20	mg/L	40	mg/L	60	mg/L	80	mg/L
Time (min)	TS	St. dev										
0	0.23	0.27	0.26	0.26	0.30	0.26	0.32	0.21	0.35	0.20	0.42	0.19
10	0.24	0.27	0.28	0.24	0.30	0.23	0.34	0.21	0.37	0.19	0.43	0.19
20	0.25	0.26	0.28	0.23	0.30	0.22	0.34	0.19	0.38	0.18	0.44	0.17
30	0.23	0.24	0.28	0.23	0.29	0.21	0.33	0.19	0.37	0.18	0.44	0.17
40	0.22	0.24	0.28	0.23	0.29	0.22	0.33	0.19	0.37	0.18	0.43	0.17
50	0.53	0.30	0.27	0.23	0.28	0.21	0.32	0.18	0.36	0.18	0.42	0.15
60	0.21	0.24	0.26	0.22	0.25	0.18	0.31	0.18	0.36	0.17	0.39	0.14
80	0.19	0.24	0.25	0.23	0.25	0.20	0.30	0.17	0.33	0.17	0.39	0.15
100	0.17	0.24	0.23	0.22	0.21	0.19	0.27	0.16	0.30	0.18	0.37	0.14
120	0.14	0.24	0.21	0.22	0.22	0.19	0.26	0.17	0.29	0.15	0.35	0.14
150	0.12	0.21	0.18	0.21	0.19	0.18	0.23	0.15	0.26	0.14	0.32	0.12
180	0.08	0.21	0.15	0.20	0.16	0.17	0.19	0.14	0.23	0.13	0.30	0.11
210	0.05	0.20	0.08	0.24	0.14	0.16	0.17	0.14	0.17	0.15	0.27	0.11
240	0.01	0.18	0.10	0.18	0.12	0.15	0.15	0.13	0.19	0.12	0.23	0.10
270	0.00	0.18	0.08	0.18	0.10	0.15	0.13	0.12	0.17	0.11	0.24	0.10

Table N7. Sedimentation profile of ZnO with diameter 100nm  $< d < 1 \mu$ m from the primary sediment tank. (Data for Fig. 4.4 (c))

	0 :	mg/L	10	mg/L	20	mg/L	40	mg/L	60	mg/L	80	mg/L
Time (min)	TS	St. dev	TS	St. dev	TS	St. dev						
0	0.35	0.03	0.38	0.03	0.44	0.41	0.43	0.02	0.47	0.04	0.50	0.03
10	0.26	0.05	0.30	0.01	0.26	0.32	0.33	0.06	0.38	0.03	0.43	0.02
20	0.19	0.04	0.19	0.02	0.15	0.24	0.26	0.02	0.31	0.02	0.34	0.03
30	0.12	0.04	0.15	0.04	0.12	0.20	0.20	0.03	0.26	0.01	0.31	0.03
40	0.07	0.04	0.09	0.03	0.08	0.16	0.17	0.03	0.21	0.03	0.28	0.02
50	0.03	0.04	0.06	0.04	0.06	0.13	0.14	0.03	0.19	0.05	0.26	0.02
60	-0.01	0.05	0.04	0.04	0.01	0.11	0.13	0.03	0.18	0.02	0.24	0.02
80	-0.05	0.04	0.01	0.04	-0.02	0.08	0.09	0.03	0.15	0.02	0.21	0.02
100	-0.09	0.04	-0.01	0.04	-0.04	0.05	0.06	0.03	0.13	0.03	0.15	0.03
120	-0.12	0.03	-0.04	0.03	-0.06	0.02	0.05	0.03	0.11	0.04	0.17	0.01
150	-0.16	0.03	-0.06	0.04	-0.08	0.00	0.03	0.02	0.09	0.03	0.16	0.02
180	-0.17	0.02	-0.10	0.03	-0.09	-0.02	0.01	0.01	0.09	0.02	0.15	0.01
210	-0.19	0.02	-0.11	0.01	-0.09	-0.04	0.01	0.03	0.07	0.02	0.13	0.01
240	-0.21	0.03	-0.12	0.01	-0.11	-0.07	-0.02	0.02	0.06	0.01	0.13	0.01
270	-0.23	0.04	-0.12	0.01	-0.15	-0.09	-0.03	0.02	0.06	0.01	0.09	0.02

Table N8. Sedimentation profile of ZnO with diameter 100nm  $< d < 1 \mu$ m from the secondary sediment tank. (Data for Fig. 4.4 (d))

	0 1	mg/L	10	mg/L	20	mg/L	40	mg/L	60	mg/L	80	mg/L
Time (min)	TS	St. dev	TS	St. dev	TS	St. dev						
0	1.00	0.07	1.12	0.10	1.08	0.02	1.16	0.05	1.23	0.03	1.28	0.01
10	0.54	0.12	0.65	0.13	0.68	0.11	0.75	0.02	0.80	0.05	0.84	0.10
20	0.32	0.02	0.42	0.04	0.51	0.02	0.58	0.09	0.65	0.08	0.71	0.14
30	0.16	0.07	0.30	0.07	0.39	0.08	0.47	0.13	0.57	0.14	0.64	0.15
40	0.02	0.16	0.18	0.14	0.30	0.10	0.39	0.16	0.50	0.16	0.58	0.17
50	-0.09	0.22	0.11	0.17	0.22	0.13	0.33	0.17	0.44	0.17	0.53	0.17
60	-0.20	0.25	0.03	0.18	0.16	0.13	0.27	0.19	0.39	0.17	0.47	0.16
80	-0.32	0.25	-0.07	0.19	0.07	0.14	0.19	0.19	0.33	0.17	0.44	0.16
100	-0.42	0.23	-0.16	0.19	-0.01	0.14	0.13	0.19	0.28	0.17	0.40	0.15
120	-0.54	0.31	-0.24	0.22	-0.12	0.22	0.08	0.19	0.23	0.16	0.36	0.14
150	-0.61	0.28	-0.32	0.22	-0.17	0.19	0.03	0.18	0.19	0.16	0.32	0.14
180	-0.70	0.24	-0.40	0.22	-0.23	0.18	-0.02	0.17	0.15	0.16	0.29	0.14
210	-0.76	0.23	-0.44	0.21	-0.28	0.18	-0.06	0.17	0.12	0.16	0.26	0.14
240	-0.82	0.22	-0.49	0.22	-0.32	0.17	-0.09	0.17	0.10	0.16	0.24	0.14
270	-0.86	0.20	-0.52	0.21	-0.35	0.17	-0.11	0.17	0.08	0.16	0.22	0.15

Table N9. Sedimentation profile of TiO<sub>2</sub> with diameter  $d > 1 \mu m$  from the primary sediment tank. (Data for Fig. 4.5 (a))

	0 n	ng/L	10 r	ng/L	20 r	ng/L	40 r	ng/L	60	mg/L	80	mg/L
Time (min)	TS	St. dev	TS	St. dev	TS	St. dev						
0	1.686	0.066	1.762	0.112	1.712	0.046	1.765	0.059	1.782	0.039	1.862	0.061
10	1.897	0.061	1.862	0.061	1.897	0.061	1.933	0.000	1.933	0.000	1.933	0.000
20	1.191	0.649	1.377	0.786	1.189	0.647	1.302	0.555	1.334	0.522	1.503	0.376
30	0.545	0.481	1.110	0.666	0.683	0.449	0.768	0.306	0.843	0.254	0.967	0.145
40	0.148	0.307	0.480	0.317	0.328	0.271	0.490	0.178	0.612	0.130	0.766	0.056
50	-0.061	0.294	0.256	0.258	0.174	0.222	0.357	0.132	0.489	0.098	0.654	0.055
60	-0.237	0.339	0.100	0.240	0.044	0.210	0.260	0.119	0.429	0.077	0.574	0.060
80	-0.420	0.343	-0.044	0.249	-0.077	0.210	0.166	0.112	0.340	0.077	0.492	0.006
100	-0.575	0.383	-0.164	0.269	-0.187	0.237	0.087	0.112	0.280	0.075	0.453	0.025
120	-0.708	0.414	-0.284	0.290	-0.239	0.224	0.025	0.106	0.232	0.070	0.413	0.018
150	-0.824	0.426	-0.360	0.284	-0.311	0.216	-0.038	0.104	0.192	0.059	0.373	0.039
180	-0.902	0.429	-0.424	0.308	-0.374	0.216	-0.071	0.081	0.151	0.055	0.344	0.044
210	-1.016	0.479	-0.477	0.301	-0.414	0.214	-0.117	0.078	0.103	0.014	0.320	0.052
240	-1.070	0.450	-0.512	0.305	-0.464	0.212	-0.155	0.083	0.103	0.034	0.294	0.064
270	-1.133	0.486	-0.549	0.258	-0.493	0.204	-0.178	0.058	0.075	0.024	0.277	0.068

Table N10. Sedimentation profile of TiO<sub>2</sub> with diameter  $d > 1 \mu m$  from the secondary sediment tank. (Data for Fig. 4.5 (b))

	0 1	mg/L	10	mg/L	20	mg/L	40	mg/L	60	mg/L	80	mg/L
Time (min)	TS	St. dev										
0	0.91	0.29	0.88	0.32	0.88	0.32	0.94	0.22	0.95	0.24	0.94	0.23
10	0.37	0.10	0.30	0.14	0.25	0.07	0.23	0.05	0.23	0.08	0.26	0.13
20	0.24	0.10	0.14	0.08	0.11	0.05	0.09	0.01	0.04	0.07	0.10	0.09
30	0.13	0.08	0.05	0.10	-0.02	0.01	0.00	0.02	-0.01	0.02	-0.01	0.08
40	-0.04	0.15	-0.04	0.12	-0.10	0.05	-0.09	0.02	-0.09	0.01	-0.08	0.10
50	-0.05	0.06	-0.11	0.11	-0.17	0.05	-0.16	0.03	-0.19	0.06	-0.15	0.10
60	-0.11	0.06	-0.18	0.13	-0.24	0.06	-0.22	0.04	-0.25	0.07	-0.21	0.11
80	-0.23	0.07	-0.29	0.14	-0.36	0.07	-0.33	0.06	-0.31	0.03	-0.30	0.12
100	-0.33	0.06	-0.39	0.17	-0.44	0.09	-0.40	0.07	-0.39	0.07	-0.37	0.15
120	-0.41	0.05	-0.46	0.15	-0.50	0.10	-0.46	0.07	-0.46		-0.43	0.15
150	-0.51	0.05	-0.54	0.14	-0.59	0.12	-0.53	0.08	-0.49	0.07	-0.47	0.14
180	-0.59	0.05	-0.61	0.16	-0.68	0.09	-0.59	0.10	-0.56	0.05	-0.52	0.14
210	-0.66	0.06	-0.67	0.16	-0.72	0.15	-0.64	0.11	-0.58	0.08	-0.56	0.15
240	-0.72	0.04	-0.73	0.17	-0.79	0.10	-0.68	0.11	-0.62	0.08	-0.59	0.14
270	-0.77	0.06	-0.79	0.18	-0.82	0.11	-0.71	0.11	-0.64	0.08	-0.62	0.16

Table N11. Sedimentation profile of ZnO with diameter  $d > 1 \mu m$  from the primary sediment tank. (Data for Fig. 4.5 (c))

	0	mg/L	10	mg/L	20	mg/L	40	mg/L	60	mg/L	80	mg/L
Time (min)	TS	St. dev										
0												
10												
20												
30												
40	0.62	0.93	0.38	0.81	0.39	0.77	0.44	0.86	0.53	0.80	0.59	0.78
50	0.26	0.75	0.13	0.65	0.10	0.64	0.13	0.68	0.26	0.68	0.35	0.63
60	0.07	0.68	-0.03	0.60	-0.06	0.62	-0.01	0.66	0.12	0.67	0.22	0.62
80	-0.11	0.75	-0.15	0.64	-0.22	0.64	-0.17	0.64	-0.04	0.63	0.07	0.60
100	-0.26	0.79	-0.27	0.63	-0.32	0.66	-0.27	0.65	-0.14	0.65	-0.05	0.60
120	-0.37	0.82	-0.35	0.66	-0.41	0.68	-0.36	0.69	-0.23	0.66	-0.10	0.62
150	-0.52	0.91	-0.44	0.73	-0.49	0.74	-0.45	0.70	-0.32	0.67	-0.19	0.64
180	-0.62	0.94	-0.53	0.76	-0.41	0.94	-0.51	0.75	-0.36	0.72	-0.26	0.65
210	-0.72	1.02	-0.60	0.80	-0.47	0.94	-0.58	0.77	-0.43	0.75	-0.32	0.67
240	-0.77	1.02	-0.65	0.76	-0.54	0.97	-0.61	0.76	-0.47	0.75	-0.34	0.68
270	-0.82	1.08	-0.73	0.85	-0.58	0.97	-0.67	0.81	-0.49	0.74	-0.36	0.72

Table N12. Sedimentation profile of ZnO with diameter  $d > 1 \mu m$  from the secondary sediment tank. (Data for Fig. 4.5 (d))

	0	mg/L	10	mg/L	20	mg/L	40	mg/L	60	mg/L	80	mg/L
Time (min)	TS	St. dev										
0	1.64	0.16	1.70	0.18	1.70	0.07	1.75	0.09	1.73	0.06	1.79	0.04
10	1.22	0.23	1.31	0.25	1.28	0.10	1.38	0.04	1.34	0.11	1.40	0.09
20	1.08	0.35	1.15	0.35	1.15	0.08	1.24	0.06	1.20	0.13	1.23	0.11
30	0.96	0.40	1.04	0.41	1.03	0.12	1.12	0.07	1.08	0.14	1.13	0.11
40	0.84	0.45	0.94	0.41	0.95	0.10	1.03	0.08	1.01	0.14	1.05	0.12
50	0.79	0.43	0.86	0.40	0.87	0.12	0.96	0.08	0.94	0.14	0.99	0.12
60	0.72	0.41	0.80	0.45	0.81	0.11	0.90	0.09	0.89	0.14	0.93	0.13
80	0.64	0.39	0.72	0.45	0.74	0.13	0.82	0.10	0.82	0.13	0.85	0.13
100	0.57	0.46	0.65	0.44	0.67	0.13	0.76	0.10	0.75	0.14	0.79	0.13
120	0.51	0.48	0.60	0.42	0.62	0.13	0.70	0.10	0.68	0.17	0.74	0.11
150	0.44	0.46	0.53	0.45	0.56	0.12	0.64	0.10	0.65	0.14	0.69	0.12
180	0.39	0.46	0.48	0.46	0.51	0.12	0.59	0.10	0.60	0.14	0.64	0.12
210	0.35	0.42	0.44	0.43	0.48	0.11	0.55	0.10	0.56	0.14	0.60	0.12
240	0.32	0.46	0.41	0.45	0.44	0.12	0.52	0.11	0.53	0.14	0.56	0.11
270	0.29	0.42	0.37	0.33	0.41	0.12	0.49	0.11	0.50	0.14	0.53	0.12

Table N13. Sedimentation profile of  $TiO_2$  in sludge from the primary sediment tank. (Data for Fig. 4.6 (a))

	0	mg/L	10	mg/L	20	mg/L	40	mg/L	60	mg/L	80	mg/L
Time (min)	TS	St. dev										
0												<u> </u>
10												
20												
30												
40	1.49	0.31			1.48	0.33			1.51	0.43		
50	1.12	0.19	1.28	0.13	1.16	0.25	1.37	0.32	1.26	0.34	1.36	0.26
60	1.00	0.19	1.11	0.15	1.03	0.27	1.18	0.26	1.15	0.32	1.26	0.23
80	0.87	0.20	0.98	0.17	0.94	0.27	1.09	0.21	1.07	0.29	1.17	0.19
100	0.78	0.22	0.89	0.20	0.89	0.26	1.02	0.21	1.04	0.27	1.12	0.17
120	0.72	0.24	0.84	0.20	0.84	0.30	0.97	0.21	0.99	0.28	1.08	0.18
150	0.70	0.21	0.79	0.21	0.80	0.29	0.92	0.19	0.94	0.30	1.04	0.17
180	0.63	0.28	0.76	0.21	0.77	0.31	0.89	0.22	0.92	0.29	1.01	0.18
210	0.60	0.27	0.73	0.22	0.72	0.32	0.84	0.23	0.89	0.28	0.98	0.18
240	0.69	0.25	0.70	0.24	0.73	0.31	0.85	0.21	0.85	0.31	0.98	0.20
270	0.55	0.28	0.73	0.22	0.70	0.32	0.83	0.22	0.85	0.31	0.97	0.19

Table N14. Sedimentation profile of  $TiO_2$  in sludge from the secondary sediment tank. (Data for Fig. 4.6 (b))

	0	mg/L	10	mg/L	20	20 mg/L		mg/L	60	mg/L	80 mg/L	
Time (min)	TS	St. dev	TS	St. dev								
0	1.62	0.02	1.62	0.04	1.61	0.02	1.62	0.02	1.63	0.03	1.64	0.02
10	1.24	0.03	1.23	0.04	1.26	0.02	1.26	0.04	1.25	0.05	1.25	0.03
20	1.10	0.05	1.10	0.05	1.11	0.03	1.12	0.05	1.12	0.04	1.13	0.03
30	1.01	0.05	1.01	0.05	1.05	0.02	1.02	0.03	1.03	0.03	1.05	0.04
40	0.92	0.05	0.88	0.04	0.95	0.05	0.95	0.04	0.95	0.03	0.95	0.07
50	0.86	0.05	0.85	0.03	0.91	0.01	0.88	0.04	0.87	0.04	0.90	0.03
60	0.80	0.05	0.79	0.05	0.85	0.01	0.84	0.05	0.83	0.03	0.83	0.08
80	0.71	0.05	0.72	0.04	0.76	0.02	0.76	0.04	0.76	0.02	0.77	0.04
100	0.64	0.04	0.66	0.04	0.69	0.02	0.70	0.04	0.70	0.02	0.70	0.07
120	0.59	0.04	0.61	0.03	0.64	0.02	0.65	0.03	0.65	0.02	0.67	0.03
150	0.52	0.03	0.52	0.05	0.58	0.02	0.57	0.06	0.60	0.01	0.61	0.03
180	0.46	0.04	0.49	0.02	0.54	0.02	0.54	0.03	0.55	0.02	0.57	0.03
210	0.42	0.03	0.45	0.02	0.49	0.03	0.50	0.03	0.51	0.02	0.52	0.05
240	0.38	0.02	0.41	0.02	0.47	0.02	0.47	0.02	0.48	0.01	0.50	0.03
270	0.32	0.06	0.37	0.03	0.44	0.02	0.45	0.02	0.46	0.01	0.48	0.04

N15. Sedimentation profile of ZnO in sludge from the primary sediment tank. (Data for Fig. 4.6 (c))

	0	mg/L	10	mg/L	20	mg/L	40	mg/L	60	mg/L	80	mg/L
Time (min)	TS	St. dev										
0												
10												
20												
30												
40	1.50	0.06	1.50	0.27	1.44	0.29	1.30	0.34	1.27	0.27	1.39	0.40
50	1.11	0.03	1.06	0.13	1.04	0.13	1.01	0.13	1.02	0.12	1.07	0.13
60	0.99	0.03	0.91	0.10	0.91	0.11	0.92	0.07	0.89	0.10	0.96	0.10
80	0.84	0.02	0.81	0.06	0.80	0.07	0.82	0.04	0.84	0.05	0.88	0.07
100	0.76	0.02	0.74	0.06	0.72	0.08	0.76	0.02	0.76	0.07	0.83	0.04
120	0.71	0.02	0.69	0.06	0.68	0.07	0.72	0.02	0.73	0.07	0.79	0.03
150	0.66	0.03	0.64	0.05	0.63	0.09	0.68	0.03	0.70	0.06	0.73	0.04
180	0.63	0.03	0.61	0.06	0.60	0.09	0.65	0.02	0.67	0.07	0.72	0.03
210	0.60	0.03	0.58	0.07	0.57	0.08	0.62	0.04	0.64	0.07	0.68	0.06
240	0.58	0.04	0.56	0.06	0.55	0.08	0.60	0.03	0.61	0.09	0.67	0.04
270	0.52	0.02	0.53	0.07	0.53	0.09	0.59	0.03	0.61	0.07	0.66	0.03

Table N16. Sedimentation profile of ZnO in sludge from the secondary sediment tank. (Data for Fig. 4.6 (d))

100 nm < a	<i>l</i> < 1µ	$d > 1\mu$		Total sludge		
ln(Ce)	$\ln(\Gamma)$	ln(C <sub>e</sub> )	$\ln(\Gamma)$	ln(C <sub>e</sub> )	$\ln(\Gamma)$	
2.47	1.44	3.24	1.78	2.67	1.63	
3.07	2.26	3.69	2.27	3.52	2.26	
3.74	2.22	4.01	2.56	3.88	2.83	
3.97	2.66	4.32	2.36	4.17	3.16	
4.20	2.87	4.57	2.51	4.36	3.50	
4.47	2.85	4.69	2.68	4.55	3.61	
4.49	3.28	4.79	3.04	4.64	4.03	
4.58	3.48	4.87	3.27	4.67	4.44	
4.75	3.53			4.75	4.60	

#### EFFECT OF SLUDGE PARTICULATE SIZE ON NANOPARTICLE ATTACHMENT

Appendix O

Table O1. Effect of sludge particulate size on the attachment on  $TiO_2$ . The data is shown in a graphical from in Fig. 4.7. (Data for Fig. 4.7)

100 nm < 6	$d < 1\mu$	d > 1	μ	Total slud	ge
ln(Ce)	$\ln(\Gamma)$	ln(C <sub>e</sub> )	$\ln(\Gamma)$	ln(C <sub>e</sub> )	$\ln(\Gamma)$
2.06	0.56	2.65	2.47	3.50	2.65
2.43	1.94	2.81	3.35	4.22	2.80
2.65	3.04	3.48	3.82	4.50	4.04
3.28	3.30	4.13	3.87	4.75	4.51
3.91	3.19	4.30	4.18	4.97	4.79
		4.67	4.24	5.13	5.02
		4.74	4.33	5.18	5.15
		4.83	4.40	5.30	5.11
		4.89	4.48	5.36	5.22

Table O2. Effect of sludge particulate size on the attachment on ZnO. The data is shown in a graphical from in Fig. 4.8. (Data for Fig. 4.8)

280 n	nM	480 m	М	680 ml	М	830 ml	М
Ce	Γ	Ce	Γ	Ce	Γ	Ce	Γ
1.81	8.32	11.65	3.82	3.05	0.99	13.59	2.93
15.19	11.35	17.89	10.11	13.04	12.33	26.72	6.08
30.35	13.56	37.19	10.43	38.08	10.03	40.92	8.73
41.60	17.56	45.62	15.73	40.01	18.29	44.19	16.38
39.62	27.62	51.72	22.08	59.75	18.41	59.19	18.67
36.12	38.37	50.84	31.63	62.90	26.12	79.40	18.57
50.09	40.76	65.28	34.18	72.13	31.04	88.63	23.50
46.75	51.80	77.36	37.80	85.10	34.26	94.26	30.07
62.04	53.95	80.83	45.36			102.28	35.55

## Appendix P

## EFFECT OF IONIC STRENGTH ON THE ATTACHMENT OF TIO2 ON SLUDGE PARTICULATES

Table P1. Effect of ionic strength on the attachment of TiO<sub>2</sub> onto sludge particulates. Units:  $C_e = mg/L$ ,  $\Gamma = \mu g$ -ENP/mg-dry sludge. (Data for Fig. 4.10, 11)

Appendix	0
	×

#### EFFECT OF MLSS ON NANOPARTICLE ATTACHMENT

200 m	ng/L	314 n	ng/L	470 n	ng/L	627 n	ng/L	784 n	ng/L	1569 1	ng/L	2352 1	mg/L
ln(C <sub>e</sub> )	$\ln(\Gamma)$												
8.98	2.34	1.58	2.80	2.51	0.21	2.40	0.54	2.90	0.09	0.35	0.31	1.46	1.20
21.59	3.91	1.62	3.81	3.34	0.73	3.38	0.75	3.92	1.81	2.01	0.25	3.28	1.04
47.86	2.58	3.09	3.34	3.92	0.90	3.70	1.41	4.29	1.36	2.77	1.30	3.30	1.63
62.42	3.74	3.61	2.77	4.12	1.33	3.99	1.70	3.97	3.11	3.64	0.90	3.82	1.99
71.53	6.05	3.69	4.16	4.38	1.44	4.19	1.97	4.63	2.28	3.68	1.85	4.17	1.96
93.54	5.62	3.85	4.14	4.59	1.51	4.37	2.12	4.54	3.34	3.78	2.18	4.15	2.44
99.71	8.56	4.02	4.35	4.66	1.99	4.48	2.40	4.77	3.26	3.84	2.56	4.15	2.91
123.27	12.06	4.01	4.94	4.67	2.43	4.50	2.70	4.85	3.50	4.02	2.76	4.38	2.89
				4.82	2.47	4.58	2.84			4.31	2.97	4.45	3.13

Table Q1. Attachment of TiO<sub>2</sub> to primary waste activated sludge of various concentrations. The graph of the following table is shown in Fig. 4.12 (a)

-	157 mg	g/L	200 mg	g/L	627 mg	g/L	784 m	g/L
_	ln(Ce)	$\ln(\Gamma)$	ln(C <sub>e</sub> )	$\ln(\Gamma)$	ln(C <sub>e</sub> )	$\ln(\Gamma)$	ln(C <sub>e</sub> )	$\ln(\Gamma)$
-	1.84	3.76	1.55	2.06	2.46	3.27	1.92	3.56
	2.83	4.28	2.71	2.27	3.31	3.66	3.31	3.64
	3.60	4.30	2.81	3.01	3.77	3.97	3.75	3.95
	3.91	4.56	3.05	3.36	4.07	4.21	3.80	4.08
	4.16	4.75	3.23	3.63	4.35	4.27	4.16	4.73
	4.19	5.15	3.36	3.86	4.50	4.56	4.24	4.99
	4.30	5.36	3.44	4.09	4.61	4.83	4.28	5.34
	4.39	5.53	3.55	4.25	4.67	5.11	4.54	5.34
_	4.47	5.69	3.74	4.49	4.89	4.94	4.66	5.45

Table Q2. Attachment of TiO<sub>2</sub> to secondary waste activated sludge of various concentrations. The graph of the following table is shown in Fig. 4.12 (b)

200 r	ng/L	314 n	ng/L	470 r	ng/L	627 r	ng/L	784 r	ng/L	1569	mg/L	2352	mg/L
ln(C <sub>e</sub> )	$\ln(\Gamma)$												
1.99	4.71	2.90	1.23	3.50	0.35	3.94	1.78	1.07	3.02	3.40	1.97	4.59	1.26
3.12	5.16	3.94	1.79	4.22	0.50	4.51	1.95	3.59	3.17	3.82	2.12	4.96	0.90
3.34	5.69	4.33	2.21	4.50	1.73	4.75	2.17	4.29	3.38	3.98	2.34	5.10	2.00
3.23	6.16	4.58	2.58	4.75	2.21	4.89	2.62	3.83	4.28	4.16	2.43	5.32	1.83
3.31	6.37	4.90	2.63	4.97	2.49	5.07	2.85	4.52	4.52	4.25	2.65	5.35	2.33
3.82	6.30	5.05	2.88	5.13	2.71	5.12	2.98	4.38	4.72	4.42	2.87	5.43	2.35
4.39	6.22	5.18	2.86	5.18	2.85	5.20	3.05	4.64	4.64	4.72	2.92	5.49	2.55
4.58	6.26	5.05	3.27	5.26	2.91	5.24	3.16	4.73	4.64				
4.72	6.33	5.26	3.12	5.38	2.88			4.94	4.58				

Table Q3. Attachment of ZnO to primary waste activated sludge of various concentrations. The graph of the following table is shown in Fig. 4.13 (a)

157 r	ng/L	200 1	ng/L	627 1	ng/L	784	mg/L
ln(C <sub>e</sub> )	$\ln(\Gamma)$						
0.39	4.50	1.52	4.72	2.48	4.45	3.22	3.86
2.51	4.98	2.91	5.28	3.62	4.91	3.92	4.46
2.13	5.51	4.04	5.30	3.81	4.94	4.16	5.01
2.73	5.78	4.54	5.34	4.58	5.26	4.45	5.46
2.36	6.05	4.82	5.49	4.78	5.54	4.79	5.53
3.02	6.14	5.05	5.58	5.00	5.67	4.92	5.78
3.35	6.23	5.13	5.67	5.06	5.79	5.14	5.64
3.68	6.28	5.24	5.67	5.21	5.73	5.19	5.77
3.13	6.53	5.29	5.75	5.34	5.67	5.29	5.79

Table Q4. Attachment of ZnO to secondary waste activated sludge of various concentrations. The graph of the following table is shown in Fig. 4.13 (b)

ZETA POTENTIAL VS. pH OF ENGINEERED NANOPARTICLES

TiO <sub>2</sub>		ZnO		$SiO_2$		Alginate		Humic Acid	
pН	Zeta	pН	Zeta	pН	Zeta	pН	Zeta	pН	Zeta
2.2	42.5	6.04	10.7	2.13	-0.118	3	-13.1	2.94	-30
2.47	43.4	7	4.66	2.77	1.01	3.97	-14.3	3.57	-37
3.12	40.9	7.44	3.87	3.38	0.0915	4.78	-17.1	4.59	-42.3
4.1	38.2	8.08	3.58	3.86	-1.28	5.24	-23.9	5.45	-47.9
4.99	35.5	8.4	0.589	4.42	-4.25	5.76	-28.7	6.45	-50.6
5.5	15.5	8.97	-1.62	5.05	-6.99	6.22	-28.9	7.32	-61.3
6.02	5.58	9.51	-4.41	5.43	-11.1	6.89	-29.7	8.07	-62.8
7.04	-21.3	9.94	-11.9	5.88	-13.2	7.62	-30.5	9.79	-62.8
7.18	-22.5	10.6	-16.7	6.43	-15.9	9.73	-32	11.1	-68.9
7.49	-30			6.8	-18	10.8	-32.1		
7.83	-39.7			7.45	-19.4				
9.37	-44.6			7.89	-20.1				
10.3	-43.7			8.41	-23.3				
11.4	-44.6			8.82	-26.6				
				9.35	-27				
				9.77	-29.3				

Table R1. Zeta potential vs. pH of ENP particles and organic material. (Data for Fig. 4.14)

# Appendix S

# ATTACHMENT OF VARIOUS ENGINEERED NANOPARTICLES TO SLUDGE

TiO <sub>2</sub>		Zn	Si	SiO <sub>2</sub>	
ln(C <sub>e</sub> )	$\ln(\Gamma)$	ln(C <sub>e</sub> )	$\ln(\Gamma)$	ln(C <sub>e</sub> )	$\ln(\Gamma)$
3.24	1.78	2.65	2.47	1.55	4.64
3.69	2.27	2.81	3.35	2.72	5.32
4.01	2.56	3.48	3.82	3.46	5.71
4.32	2.36	4.13	3.87	3.44	6.01
4.57	2.51	4.30	4.18	3.94	6.23
4.69	2.68	4.67	4.24	4.26	6.4
4.79	3.04	4.74	4.33	4.28	6.49
4.87	3.27	4.83	4.40	4.2	6.57
		4.89	4.48	4.36	6.63

Table S1. Attachment of various engineered nanoparticles composed of different materials. (Data for Fig. 4.15)