

Effect of Process Intensification Techniques on Biosolids Management

Dian Zhang

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Zhiwu Wang, Chair

Amy Pruden-Bagchi,

Danmeng Shuai,

John T. Novak

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Abstract

This study is aimed to provide comprehensive evaluation and mechanistic understanding of the impact of process intensification techniques applied in main and side stream wastewater treatment on biosolids management in terms of anaerobic digestion enhancement, dewaterability improvement, odor mitigation, as well as phosphorus and nitrogen removal. The first part of this study was conducted to understand the effect of anaerobic digester solids retention time (SRT) on odor emission from biosolids. A kinetic model and inhibitory studies showed the emission of methanethiol (MT), a representative odor compound, was primarily determined by the dynamic concurrence of MT production from amino acid and utilization by methanogens in the course of anaerobic digestion. MT emission pattern follows a bell-shape curve with SRT in anaerobic digesters. However, for digested and dewatered biosolids, SRT ranging from 15 to 50 days in anaerobic digesters demonstrated insignificant effect on the odor emission from biosolids. In contrast, the peak odor emission was found to exponentially increase with both shear intensity and polymer dose applied during dewatering.

The second part of this study investigated the impact of process intensification practices on sludge dewatering performance. The integration of high-rate activated sludge process and anaerobic digestion elevated the sludge orthophosphate level, leading to struvite scaling and dewaterability deterioration. Superior orthophosphate removal, significant improvement of sludge dewaterability, and favorable economics were achieved through sludge conditioning by cerium chloride.

Continuous flow aerobic granulation technology offered significant process intensification of mainstream treatment trains. However, its impact on biosolids management was not studied. This study showed that there was little dewaterability difference between aerobic granular sludge and activated sludge when polymer was not added. However, about 75% polymer saving and improved dewatering performance were observed with polymer addition. When subjected to high shear, a greater dewaterability deterioration was observed for granular sludge than activated sludge.

The last part of this study is focused on the impact of anaerobic digestion process intensification through thermal treatment including pre-pasteurization, thermophilic anaerobic digestion,

temperature phased anaerobic digestion, and thermal hydrolysis pretreatment. Improved methane production, pathogen reduction, dewatering performance, and odor mitigation were observed with the involvement of these high-temperature processes. However, special cautions and measure should be taken during the start-up of these high rate processes as they are more liable to digester souring. In addition, the in-depth understanding of the mechanism of recalcitrant dissolved organic nitrogen formation during sludge thermal pretreatment was provided.

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General Audience Abstract

Globally, rapid urbanization results in dramatic increase of wastewater loading, the excessive sludge production, and the limited land for facility expansion. One of the best choices for urban wastewater treatment plants to cope with these challenges is to seek process intensification techniques to accommodate the increased treatment loading within their existing infrastructure without significant footprint expansion and additional capital investment. This study is aimed to provide comprehensive evaluation and mechanistic understanding of the impact of process intensification techniques applied in main and side stream wastewater treatment on biosolids management in terms of anaerobic digestion enhancement, dewaterability improvement, odor mitigation, as well as phosphorus and nitrogen removal.

Mainstream process intensification techniques such as high-rate activated sludge process and continuous flow aerobic granulation can potentially impact the downstream dewatering process. The integration of high-rate activated sludge process and anaerobic digestion elevated the sludge orthophosphate level, leading to struvite scaling and dewaterability deterioration. To prevent struvite scalling, cerium chloride was evaluated as a superior orthophosphate precipitate which also significantly improved sludge dewaterability. Continuous flow aerobic granulation technology offered sludge settelability improvemnt. However, this study showed that there was little dewaterability difference between the thickened aerobic granular sludge and activated sludge when polymer was not added. With polymer addition, 75% polymer saving and improved dewatering performance were observed with granular sludge. In addition, granular sludge was more sensitive to high shear dewatering method than activated sludge.

Decreasing digester solids retention time (SRT) is a common way to intensify the sidestream sludge anaerobic digestion. A kinetic model and inhibitory studies were conducted to understand the effect of SRT ranging from 15 to 50 days on the emission of methanethiol (MT), a representative odor compound. MT emission pattern was found to follow a bell-shape curve with SRT in anaerobic digesters. However, for digested and dewatered biosolids, shear intensity and polymer dose applied during dewatering demonstrated more significant effects than SRT on the odor emission from

biosolids. Anaerobic digestion process can be also intensified through thermal treatment including pre-pasteurization, thermophilic anaerobic digestion, temperature phased anaerobic digestion, and thermal hydrolysis pretreatment. Improved digestion performances were observed with the involvement of these high-temperature processes. However, a major drawback of recalcitrant dissolved organic nitrogen formation was also identified during sludge thermal pretreatment.

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Chapter 1 : Introduction

Biosolids are the nutrient-rich organic materials resulting from the treatment of domestic sewage in a wastewater treatment facility (i.e., treated sewage sludge). However, increasing amounts of biosolids was produced as residual along with the application of a number of mainstream and sidestream process intensification techniques for accommodating the ever increasing influent loading rate. The treatment capacity of urban wastewater treatment plants (WWTPs) is prevalently constrained by the increasing conflict between the land availability and the rapid population growth. Globally, rapid urbanization results in dramatic increase of wastewater loading and thus the excessive sludge production. By 2050, 66% of the world's population is projected to live in urban area with an urban population increase of another 2.5 billion (United Nations 2014), which will add 58 million ton sludge production per year based on the current sludge production of 23 kg per capita in the U.S. (Peccia and Westerhoff 2015). In addition to the limited land availability for future capacity increase, the urban expansion also brings challenges to the sewage sludge and biosolids management by raising biosolids transportation and disposal cost, strengthening regulations for biosolids reuse or disposal, and increasing odor nuisance complaints as community development expands towards sludge treatment facilities (Adams and Witherspoon 2004, LueHing et al. 1996, Tyagi et al. 2009). One of the best choices for urban WWTPs to cope with these challenges is to seek process intensification, namely 'achieving more with less', or more specifically, as methodologies for enhancement of process performance at reduced equipment size, energy consumption, and/or waste generation.

As a well-established sludge stabilization technology, anaerobic digestion has been used for quite some time. One major limitation of anaerobic digestion lies in the slow growth rate of anaerobes and their high sensitivity to environmental stress (Ahring et al. 2001, Appels et al. 2008, Chen et al. 2008). A solids retention time (SRT) of more than 15 days is often required by regulatory authorities for conventional mesophilic anaerobic digestion processes, but it is not uncommon to find full-scale digesters operating at a SRT longer than 25 days for stable performance (Adams and Witherspoon 2004). In an effort of anaerobic digestion process intensification, technologies associated with high temperature and short SRT have gained considerable interests. For example, the thermophilic process typical at 55 °C and with a much shorter SRT of 4 - 15 days has been utilized in single stage or co-phase with mesophilic process to improve digestion rate through

enhanced hydrolytic activity of thermophilic bacterial populations (Ferrer et al. 2010, Oles et al. 1997, Ponsá et al. 2008, Roberts et al. 1999). Similarly, using high temperatures (130 – 170 °C) and pressures (2 – 10 bar), thermal hydrolysis pretreatment (THP) is able to solubilize complex organic contents of sludge to achieve better biodegradability, at much higher solids content e.g. 10% total solids (TS), and with shortened SRT e.g. 15 days, for anaerobic digestion (Barber 2016).

Despite the well-recognized digestion efficiency improvement that can be achieved by anaerobic digestion process intensification in the sidestream, it should be also noted that the selection of anaerobic digestion process intensification technique often needs to be well suited to the unique demands of a particular WWPT. These demands include but not limited to odor mitigation, sludge dewatering cost reduction, and quality requirements for biosolids and anaerobic digestion return liquor. For these reasons, one of the aims of this dissertation is to shed light on the effects of temperature and SRT on anaerobic digestion performance in terms of biosolids odor, sludge dewaterability, recalcitrant dissolved organic nitrogen (rDON) formation, etc., in order to provide a comprehensive understanding of the impact of the sidestream process intensification technologies on urban sludge management practices.

Odor emission from biosolids has been identified as a main complaint received from the public (National Research Council 2002). A national wide study conducted by Water Environmental Research Foundation at 11 WWTPs across North America with capacities from 13 to 350 million gallons per day revealed that biosolids odors, mainly volatile sulfur compounds (VSCs) including hydrogen sulfide (HS), methanethiol (MT), and dimethyl sulfides (DMS), were highly correlated to the amount of bio-available proteins remained in biosolids after anaerobic digestion (Adams and Witherspoon 2004, Novak et al. 2006). Further research confirmed that microbial regrowth may occur in biosolids during the storage phase in which the residual sulfur-containing proteins from incomplete anaerobic digestion were metabolized to MT and DMS which were subsequently utilized by the methylotrophic methanogens as substrates (Chen et al. 2011, Higgins et al. 2006, Qi et al. 2007). Given the essential role of proteins and microbial activity involved in biosolids odor emission, the efficiency of anaerobic digestion may potentially affect the odor emission because it simultaneously regulates the protein reduction and the microbial population in the anaerobic digestion effluent (Shin et al. 2010, Vanwonterghem et al. 2015, Zhang and Tatsuya 1994). In addition to anaerobic digestion efficiency, field observations indicated that biosolids

handling after anaerobic digestion such as sludge conditioning, dewatering centrifugation, biosolids pumping, and cake storage may also affect the biosolids odor emission (Adams and Witherspoon 2004).

Another major consideration when evaluating the sidestream intensification technologies is their impacts on downstream sludge dewatering and the quality of return centrate. Sludge digested by conventional anaerobic digestion is typically considered to have a poorer dewaterability than activated sludge due to the release of biopolymer and ion such as divalent cations and phosphate in anaerobic digestion (Higgins et al. 2014, Mikkelsen and Keiding 2002, Novak et al. 2003). A few studies have indicated that thermophilic anaerobic digestion resulted in greater biopolymer release and poorer sludge dewaterability (Bivins and Novak 2001). On the contrary, THP has been demonstrated to significantly improve sludge dewaterability in comparison with conventional anaerobic digestion (Oosterhuis et al. 2014, Phothilangka et al. 2008).

Although THP is well-recognized as a process intensification technique for anaerobic digestion, its application is challenged by many drawbacks, among which the production of substances with high color, UV-quenching ability, and rDON content is a particular concern due to its negative impact on the efficiency of UV-disinfection and biological nitrogen removal in WWTPs. Penaud et al. (2000) compared the refractory, soluble compounds produced from activated sludge THP and the Maillard reaction in terms of color, molecular weight, and the influence of acid precipitation, and concluded that they are very similar. More recently, increased color, decreased ultraviolet transmission, and increased dissolved organic nitrogen (DON) concentration in plant effluent after the implementation of sludge THP was also reported in a full-scale biological nutrient removal system, and the Maillard was again suspected to be responsible (Dwyer et al. 2008). The Maillard reaction is a non-enzymatic browning reaction that occurs between reducing sugar and amino groups and forms highly UV-quenching and hardly biodegradable polymers (Hodge 1953, Maillard 1912, Reynolds 1965). Although Maillard reaction was discovered one hundred years ago, it was not until recently that its impact on the THP of municipal sludge was realized. Yet, a clear correlation between Maillard reaction and rDON formation remains to be established.

Besides the aforementioned sidestream process intensification techniques, the impact of mainstream process intensification on biosolids management is also important to be investigated. , reduce the energy demand of wastewater treatment, and to capture organic matter for valorizing

through anaerobic digestion (Jimenez et al. 2015) . However, the anaerobic condition in HRAS can lead to unintentional growth of phosphorus accumulation organisms (PAO) and thus the phosphorus enrichment in the wasted activated sludge (WAS). The release of soluble orthophosphate (OP) from the processes, e.g. anaerobic digestion, handling such a high phosphorus content sludge often leads to the formation of crystalline phosphate scale on the internal walls of pipes, pumps, and valves used in the post digestion processes, a problem that increases the maintenance cost and reduces the hydraulic capacity of pipes (Doyle and Parsons 2002). This problem becomes even more prominent when centrifuges are used for sludge dewatering in that it results in carbon dioxide degassing and thus an increase in the pH to above 7. Therefore, an economical countermeasure to this HRAS-caused problem in dewatering system should be developed.

Another promising mainstream process intensification technique is the continuous flow aerobic granulation because it holds promise to intensify the capacity of biological treatment trains by greater biomass retention and faster sludge sedimentation with minor modification of the existing infrastructure in WWTPs (Kent et al. 2018, Sun et al. 2019). Yet, the dewaterability of aerobic granular sludge formed within the continuous flow system by using dewatering methods commonly applied in WWTPs is still unknown. Therefore, one of the objectives of this dissertation is to provide a comprehensive examination of the dewaterability of continuous flow aerobic granular sludge by measuring the capillary suction time (CST), cake dryness, specific resistance to filtration (SRF), and compressibility in response to the thickening, polymer dose, and shear force effects commonly encountered in WWTP dewatering processes. The outcome from this study will hopefully provide additional insights into granular sludge dewaterability and offer helpful guidance to dewatering process optimization and equipment selections in WWTPs considering the adoption of a continuous flow aerobic granulation technique.

In short, this dissertation is aimed to address the impact of mainstream and sidestream process intensification techniques on biosolids management in consideration of particular demands of WWTPs on odor mitigation, dewaterability improvement, and quality requirements for biosolids and return liquor. Specific objectives associated with their application background in each chapter are summarized below:

- Chapter 2: Kinetic modeling of the effect of solids retention time on methanethiol dynamics in anaerobic digestion.** The WWTP in this study is located at highly urbanized area where is particularly sensitive to odor emission. To understand and predict the effect of SRT on odor emission during mesophilic anaerobic digestion, mathematical model was developed to describe MT formation and utilization as a function of SRT based on the growth kinetics of relevant microbial communities in lab-scale digesters.
- Chapter 3: Effects of solids retention time, shear intensity, and polymer doses on the odor emission of anaerobically digested sludge.** As a follow-up study of Chapter 2, this study focused on the odor emission from digested and dewatered biosolids. In particular, the effects of SRT, anaerobic storage, shear intensity, and polymer doses on odor emission were investigated.
- Chapter 4: Using cerium chloride to control soluble orthophosphate concentration and improve the dewaterability of sludge: Part I. Mechanistic understanding.** The WWTP in this study suffered from deteriorated sludge dewaterability and severe struvite scaling due to the anaerobic digestion of phosphorus-enriched sludge. A novel precipitant was evaluated as a potential solution for the issue. Insight into the mechanism of the sludge dewaterability improvement through phosphate removal was also provided.
- Chapter 5: Using cerium chloride to control soluble orthophosphate concentration and improve the dewaterability of sludge: Part II. Case study.** As a follow-up case study of Chapter 4, this study identified the cause of the problem and provided economic analysis of using CeCl_3 for phosphate removal and dewaterability enhancement based on the historical data obtained from the WWTP.
- Chapter 6: The dewaterability of aerobic granular sludge cultivated in continuous flow bioreactors fed with real domestic wastewater.** Sludge granulation in either aerobic or anaerobic environment holds promise to intensify existing biological wastewater treatment processes. Following the success in continuous flow granulation conducted by the WWTP on the pilot scale, this study was aimed to fill the knowledge gap of the impact of continuous flow aerobic granulation on the downstream dewatering process.

Chapter 7: Effect of temperature-phased anaerobic digestion and thermal hydrolysis

pretreatment on the process intensification of anaerobic digestion. The two advanced anaerobic digestion technologies are under consideration for the sidestream process intensification of modern WWTPs. Performances including methane production, organic reduction, system stability, sludge dewaterability, pathogen destruction, and odor emission were evaluated for these two potential sidestream process intensification technologies.

Chapter 8: Recalcitrant dissolved organic nitrogen formation in thermal hydrolysis

pretreatment of municipal sludge. The formation of rDON during THP is drawing attentions, as a growing number of WWTPs starting to install THP. This interdisciplinary literature review presented in-depth understanding of rDON formation mechanism during THP, identified future research needs, and provided technical guidance for rDON control.

Chapter 9: Impact of Thermal Hydrolysis Pretreatment and Anaerobic Digestion on the Formation of Dissolved Organic Nitrogen Recalcitrant to Aerobic Treatment.

This study tracked the turnover of DON through sludge THP, THP-anaerobic digestion, and aerobic treatment. The Maillard reaction well explained the rDON formation in the THP effluent, but not in the effluent of THP-anaerobic digestion, as the rDON reduction and reformation occurred during anaerobic digestion.

Attribution

Each coauthor is credited for his or her contribution to this work in their sharing of technical expertise:

Zhiwu Wang, Ph.D., Professor of Civil and Environmental Engineering (Principal Investigator)

Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State University, Manassas, VA 20110

Coauthor of Chapter 2, 3, 4, 5, 6, 7, 8, and 9

John Novak, Ph.D., Professor of Civil and Environmental Engineering

Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Coauthor of Chapter 2, 3, and 7

Mary Strawn, Chief Engineer

Arlington County Water Pollution Control Bureau, 3402 S Glebe Rd, Arlington, VA 22202

Coauthor of Chapter 2,3, 7, and 9

Tom Broderick, Chief

Arlington County Water Pollution Control Bureau, 3402 S Glebe Rd, Arlington, VA 22202

Coauthor of Chapter 3 and 7

Bob Angelotti, Deputy Executive Director of Technical Services

Upper Occoquan Service Authority, 14631 Compton Rd, Centreville, VA 20121

Coauthor of Chapter 4,5, and 6

Erick Schlosser, Lead Process Control Engineer

Upper Occoquan Service Authority, 14631 Compton Rd, Centreville, VA 20121

Coauthor of Chapter 4 and 5

Hari Santha, Process Manager

Alexandria Renew Enterprises, 1800 Limerick Street, Alexandria, VA 22314

Coauthor of Chapter 7

Haibo Huang, Ph.D., Professor of Food Science and Technology

Department of Food Science and Technology, Virginia Polytechnic Institute and State University,
Blacksburg, VA 24061

Coauthor of Chapter 8

Yiming Feng, Ph.D., Postdoctoral Research Associate of Food Science and Technology

Department of Food Science and Technology, Virginia Polytechnic Institute and State University,
Blacksburg, VA 24061

Coauthor of Chapter 8

Wendell Khunjar, Ph.D., Director of Wastewater Innovation

Hazen and Sawyer, 4035 Ridge Top Road, Suite 400, Fairfax VA 22030

Coauthor of Chapter 8 and 9

Yewei Sun, Graduate student of Civil and Environmental Engineering

Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State
University, Manassas, VA 20110

Coauthor of Chapter 6

Zhaohui An, Graduate student of Civil and Environmental Engineering

Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State
University, Manassas, VA 20110

Coauthor of Chapter 9

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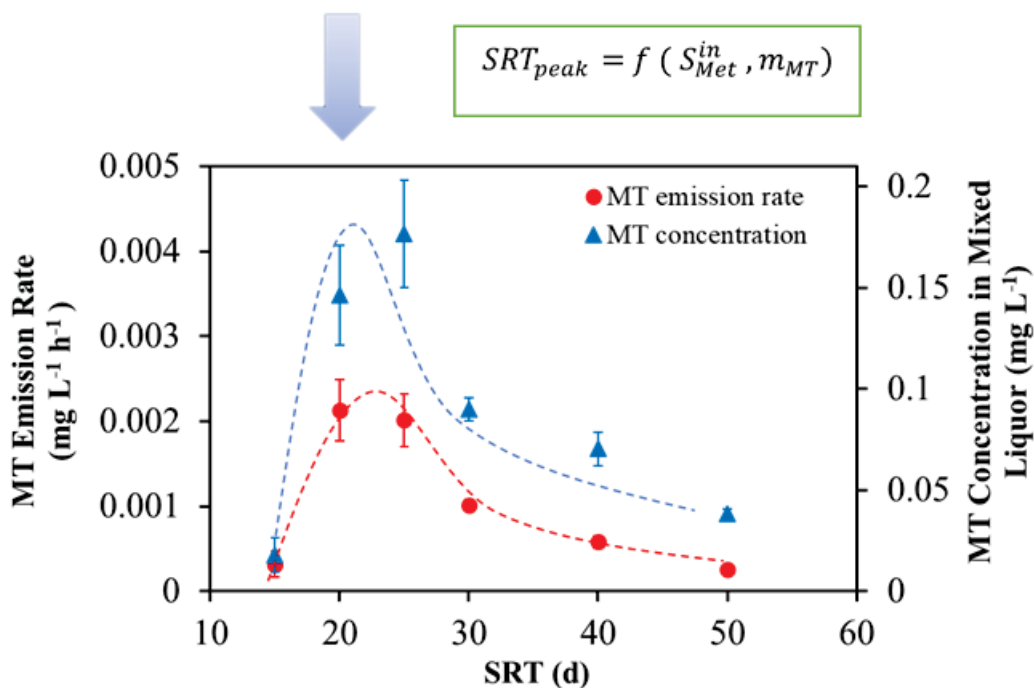
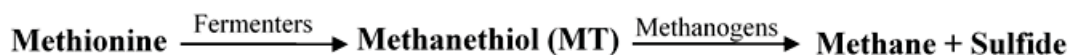
Chapter 2 : Kinetic modeling of the effect of solids retention time on methanethiol dynamics in anaerobic digestion

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2.1 Abstract

The highly volatile MT with an extremely low odor threshold and distinctive putrid smell is often identified as a major odorous compound generated under anaerobic conditions. As an intermediate compound in the course of anaerobic digestion, the extent of MT emission is closely related to the time of anaerobic reaction. In this study, lab-scale anaerobic digesters were operated at SRTs of 15, 20, 25, 30, 40 and 50 days to investigate the effect of SRT on MT emission. The experimental results demonstrated a bell-shaped curve of MT emission versus SRT with a peak around 20 days SRT. In order to understand this SRT effect, a kinetic model was developed to describe MT production and utilization dynamics in the course of anaerobic digestion and calibrated with the experimental results collected from this study. The model outcome revealed that the high protein content in the feed sludge together with the large maintenance coefficient of MT fermenters are responsible for the peak MT emission emergence in the range of typical SRT used for anaerobic digestion. A further analysis of the kinetic model shows that it can be extensively simplified with reasonable approximation to a form that anaerobic digestion practitioners could easily use to predict the MT and SRT relationship.

2.2 Graphical abstract



2.3 Keywords:

Anaerobic digestion; Mathematical model; Methanethiol; Odor

2.4 Introduction

Volatile organic sulfur compounds (VOSCs) such as MT, DMS, and dimethyl disulfide are major odorous compounds identified in wastewater treatment practices associated with anaerobic conditions, including sewage conveyance, sludge composting, anaerobic digestion, biosolids dewatering and land application (Chen et al. 2005, Hort et al. 2013, Park and Novak 2013, Wang et al. 2015). Among these VOSCs, MT is regarded as the most important odorous compound because of its relatively low odor threshold as well as high volatility (Feilberg et al. 2010, J. De Bruyn et al. 1995). Indeed, MT is the only VOSC regulated by Occupational Safety and Health Administration standard for its adverse health effects even at a very low exposure limit, e.g. 10 ppm (OSHA 2012). MT is also the only VOSC directly formed from the microbial fermentation of sulfur-containing proteins, more specifically, the amino acid methionine (Chen et al. 2005, Higgins et al. 2006). It was recognized that the presence of methanogens potentially leads to a

lower net MT emission for their utilization of MT. The utilization mechanism has been largely attributed to the MT metabolism by methanogens, particularly methylotrophic methanogens isolated from freshwater (Kiene et al. 1986), marine and salt lake sediments (Finster et al. 1992), as well as anaerobic digesters (De Bok et al. 2006). Since MT is an intermediate compound serving as both the product and substrate of the microbial community in anaerobic digesters, it can be hypothesized that the extent of MT emission can be controlled through the optimization of anaerobic reaction kinetics.

For anaerobic digestion systems, SRT is one of the most fundamental design and operating parameters. It determines the population, activity, as well as the structure of the microbial community in anaerobic digesters, and hence the formation and utilization of MT (Jang et al. 2014, Shin et al. 2010, Tian Cheng and Noike 1994, Vanwonterghem et al. 2015). With regard to the influence of SRT on microbial growth, it is well known that increasing the SRT can reduce microbial growth rates and increase the fraction of substrate allocated for non-growth related maintenance activities such as maintenance of cell integrity and transmembrane gradients of the solute, and also the turnover of proteins (Ghosh and Pohland 1974, Low and Chase 1999, Wei et al. 2003). Significantly higher maintenance related substrate consumption has been observed for many fermentative bacteria at relatively long SRTs (Boender et al. 2009, Ercan et al. 2013, Goffin et al. 2010, Overkamp et al. 2015). Since methionine is biologically converted to the odorous compound MT, understanding the effect of SRT on maintenance energy allocation and methionine utilization efficiency can potentially lead to the development of MT odor control strategies.

Mathematical modeling is an effective tool to give insights into kinetic behaviors and interpret mechanistic unknowns. Currently, there is very limited kinetic information about MT formation and degradation. Out of the two existing literatures about MT kinetics in wastewater treatment, Du and Parker (2012) simplified MT turnover in anaerobic Met incubation as a second order kinetics. The study by Sun et al. (2015) modeled MT degradation but not production in sewers biofilms. To our knowledge, there is still no theoretical kinetic modeling framework available for addressing the effect of SRT on MT turnover in anaerobic digestion. Therefore, the aim of this study was to evaluate the influence of SRT on MT emissions under anaerobic conditions through kinetic modeling. To achieve this goal, we operated six lab-scale anaerobic digesters in semi-continuous mode at SRTs of 15, 20, 25, 30, 40 and 50 days, respectively. A mathematical model was

developed to describe MT formation and utilization as a function of SRT based on the growth kinetics of relevant microbial communities. The model was calibrated with experimental results, and the maintenance energy requirement was evaluated within the model. Comparative experiments with inhibitory compound addition were also carried out with the aim of examining the possible methionine catabolism pathways.

2.5 Materials and methods

2.5.1 Anaerobic digester setup

Six lab-scale anaerobic digesters, each with a constant liquid volume of 10 liters and a headspace of 20 liters, were operated in semi-continuous mode under mesophilic conditions (35.5 ± 0.3 °C). The digesters were mixed by recirculating biogas from the headspace to the bottom of the reactor with a gas flow rate around 1 L min^{-1} . The biogas production was monitored using tipping-bucket meters (Archae Press, Nashville, USA) coupled with automatic data loggers. The digesters were inoculated with digested sludge from a full-scale mesophilic anaerobic digester from a local wastewater treatment plant. Each digester was continuously operated for 7 months with SRTs of 15, 20, 25, 30, 40, and 50 days. The feed sludge consists of thickened primary and secondary sludge from a local treatment plant, blended with a dry mass ratio of 62:38.

2.5.2 Chemical analysis

Gas samples collected from the headspaces of either digesters or serum bottles used in this study were measured for methane and MT contents using a gas chromatograph (GC) equipped with a flame ionization detector (Hewlett-Packard 5890 Series II, Palo Alto, California) and a 60 m long capillary column with a 0.53 mm diameter (Agilent, DB-1, Santa Clara, California). Headspace gas samples were collected using a 1 mL gas-tight syringe (Hamilton, Reno, Nevada). Standard gases (Airgas, Inc., Radnor, Pennsylvania) were used to develop calibration curves and quantify methane and MT. The volatile solids (VS) and volatile fatty acids (VFAs) were determined according to standard methods (APHA 2012). The VS content in the blended sludge was relatively stable ($2.8 \pm 0.25\%$) during the entire study period. The protein content in feed sludge ($11.85 \pm 0.25 \text{ g L}^{-1}$) and mixed liquor was determined using a modified Lowry protein assay kit (Pierce^{MT}, Thermo Scientific, Massachusetts).

2.5.3 Inhibition experiment

In order to give insight into the MT turnover pathways, two inhibitory compounds were employed in this experiment to block either MT production or MT utilization pathway so as to investigate one of the two pathways at a time. 2-Bromoethanesulfonate (BES) is an inhibitor that only selectively inhibits the activity of methanogen but not other anaerobes (Zinder et al. 1984). We used BES to block the methanogenic utilization of MT. Briefly, 15 mM BES was added into serum bottles to inhibit MT-to-methane conversion. The over 100 times higher MT emission and negligible methane production measured following the BES addition were used as an indicator of the successful inhibition of methanogenic utilization of MT by BES.

As for the blockage of MT production, it is known that there are mainly two types of metabolism employed by anaerobes to convert methionine to MT, namely the Stickland reaction or the single amino acid degradation (Barker 1981). A major difference between the two types of metabolism is that the Stickland reaction is dependent on coupled amino acids acting as both hydrogen donors and acceptors, while the single amino acid degradation requires the presence of alternative hydrogen-acceptors such as hydrogenotrophic methanogens (Nagase and Matsuo 1982). Single amino acid degradation can be carried out either by the lyase- or the transaminase- catalyzed reactions (Wolle et al. 2006). It should be pointed out that pyridoxal-5'-phosphate (PLP) is a coenzyme required by both lyases and transaminases to produce MT from methionine (Wolle et al. 2006). Aminooxyacetic acid (AOA) is an inhibitor of PLP-dependent enzymes, functioning by attacking the Schiff linkage between PLP and the enzymes (Beeler and Churchich 1976). It should be noted that PLP is not an essential cofactor for the Stickland reaction in which the amino group of methionine is removed through dehydrogenase (Nisman 1954). Consequently, adding AOA is supposed to only block MT formation from transaminase- or lyase-catalyzed degradation of methionine but not from the coupled Stickland reaction. Hence, 2 mM AOA was added in addition to 15 mM BES to check whether MT production can be blocked. Again, only single amino acid degradation, but not the Stickland reaction, can be blocked by AOA. So, the use of AOA will help us verify the MT production pathway. The significant drop of MT production following AOA and BES addition was used as an indicator of the effectiveness of AOA inhibition. Both inhibition studies were carried out in 300 mL serum bottles containing 100 mL sludge collected from corresponding digesters. These serum bottles were purged with nitrogen gas and then incubated in

an incubator shaker (MaxQ SHKE420 HP, Thermal Thermo Scientific, Massachusetts) for 24 hours at 35 °C.

2.6 Results and discussion

2.6.1 Effect of SRT on MT concentration and emission

Anaerobic digestion of blended municipal sludge was carried out in six completely mixed anaerobic digesters for 7 months at SRTs of 15, 20, 25, 30, 40, and 50 days. As shown in Figure 2.1, both MT concentrations and MT emission rates demonstrated a bell-shape pattern with SRTs. The MT concentration and emission rate increased along with SRT from 15 to around 20-25 days when they peaked at 0.18 mg L⁻¹ and 0.0023 mg L⁻¹ h⁻¹, respectively. After then, both MT concentration and the emission rate decreased as the SRT further increased. It should be noted that the peak MT emission rate was four to eight times the values at lower (15 days) and upper (50 days) boundary of the SRT range investigated in this study (Figure 2.1), suggesting that MT from anaerobic digesters running at SRT 20-25 days are likely to be more problematic.

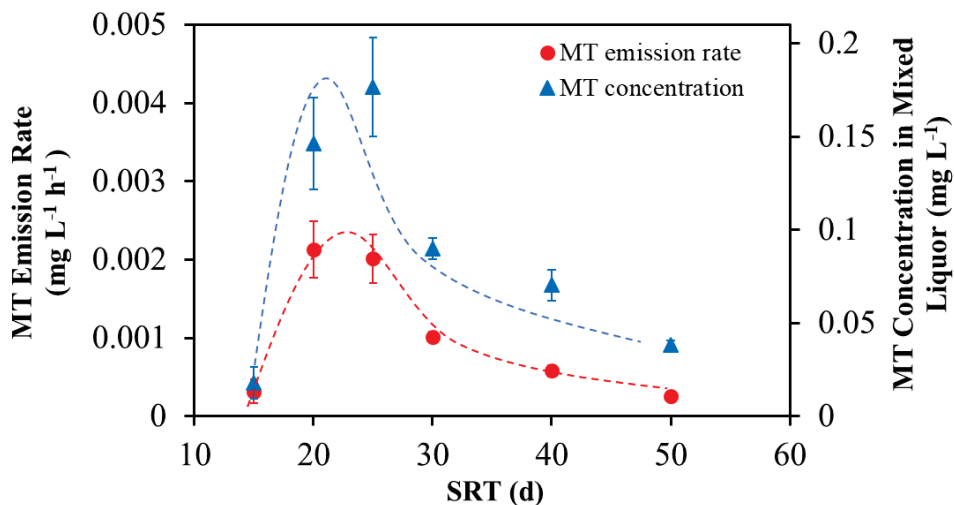


Figure 2.1 Effect of SRT on steady-state MT emission rate and MT concentration in mixed liquor (Dashed lines indicate the overall trends of the experimental data)

2.6.2 Effect of SRT on digester performance

As shown in Figure 2.2a and b, the methane content and the VS reduction varied very little under different SRTs within the ranges of 64.5% - 67.6% and 52.8% - 55.4%, respectively. The protein concentration decreased from 7.8 to 6.3 g L⁻¹ with the increase in SRT (Figure 2.2b). The profiles of acetate concentration as well as methane and biogas emission rates monotonously decreased with an increase in SRT, i.e., as the SRT increased from 15 to 50 d, the methane and biogas production rates declined from 62.7 to 23.3 mg L⁻¹ h⁻¹, and 0.09 to 0.03 L L⁻¹ h⁻¹, respectively, and the acetate concentration dropped from 336 mg L⁻¹ to 115 mg L⁻¹ over the same time period (Figure 2.2a and b). The similar trend between the profiles of biogas, methane and acetate indicates the predominant role of acetate in driving the production of methane which is a major content of biogas.

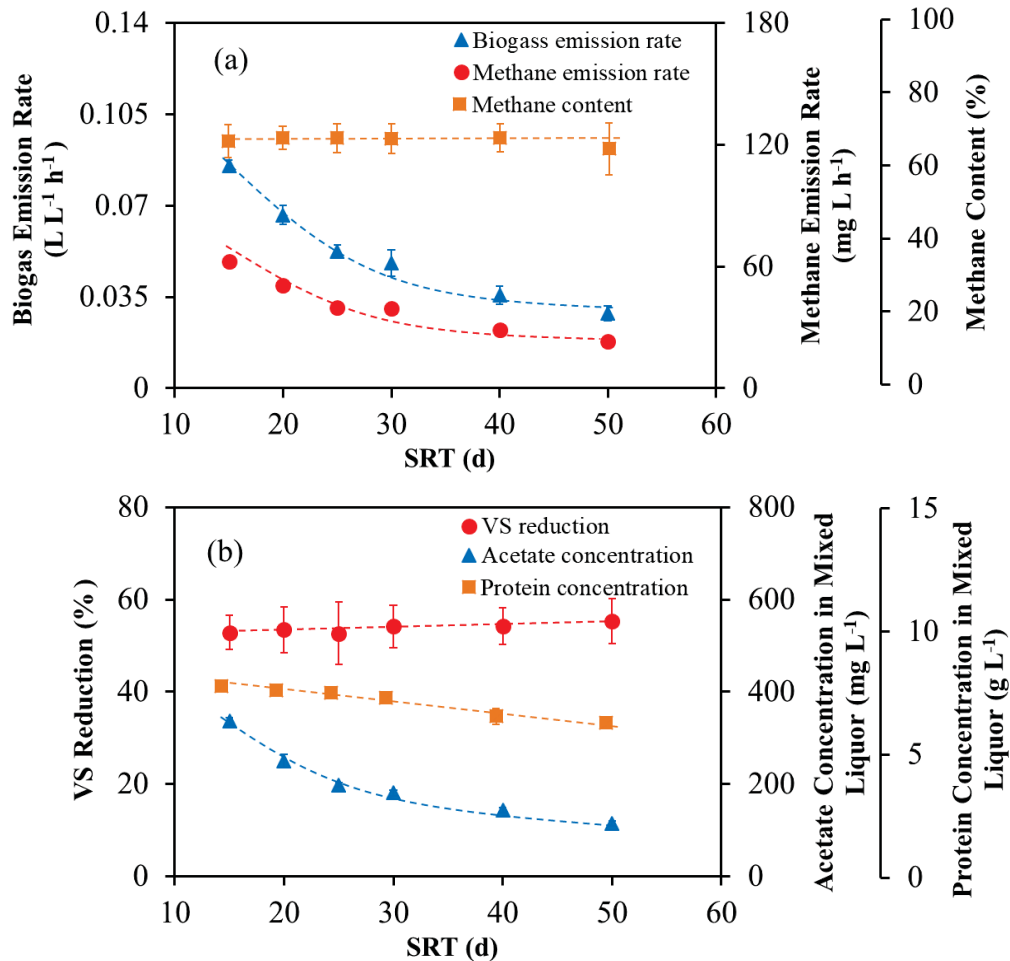


Figure 2.2 Digester performance: a) effect of SRT on steady-state methane and biogas emission rates as well as the methane content; b) effect of SRT on steady-state VS reduction rate and concentrations of acetate and protein

2.6.3 MT production and utilization

Since MT is an intermediate substrate, its concentration measured from anaerobic digesters as shown in Figure 2.1 is the net result of concurrent production and utilization. The two processes have to be separated to investigate the respective kinetics. The MT production rate was investigated by inhibiting methanogenesis using BES. As shown in Figure 2.3a, the methane production was almost completely blocked as compared to the control without BES addition. Due to the lack of MT utilization by active methanogens in this scenario, the MT emission rate in Figure 2.3b became two orders of magnitude greater than that in Figure 2.1 without BES addition, even though the trends of the two bell-shape profiles look similar in relation to SRT. It is noteworthy that MT degradation by methanogens is not the only pathway of MT utilization in anaerobic digestion. According to Higgins (2006), MT methylation to form dimethyl sulfide might be an alternative. The two-magnitude higher MT emission rate observed in Figure 2.3b as a result of methanogen inhibition implies that methanogens were indeed the primary MT utilizers in these anaerobic digesters. As a matter of fact, similar observation was also reported in previous studies, i.e., the rate of methylotrophic metabolism of MT by methanogens was magnitudes higher than that of the MT methylation (Du and Parker 2012, Sun et al. 2015). The rapid and dramatic increase of MT emission rate in response to the inactivation of methanogens implies that MT emission can be used as a sensitive indicator to show early warning of possible methanogenesis inhibition in anaerobic digestion.

In a separate batch experiment, AOA was added in addition to BES to inhibit PLP, a coenzyme essential to catalyze transaminase- or lyase-based degradation of methionine (Wolle et al. 2006). There was little MT produced following AOA addition (Figure 2.3b), which indicates that most of the MT was produced from either the transaminase or lyase reactions, but not from the Stickland reaction that does not need PLP (Nisman 1954).

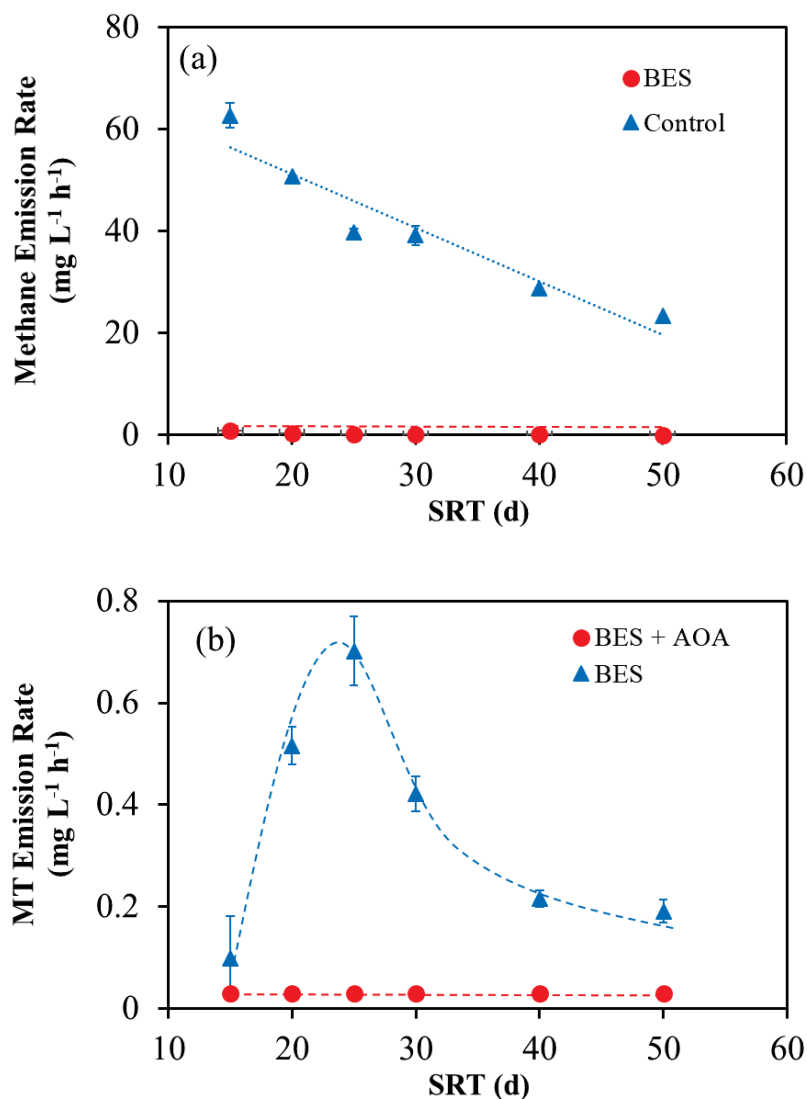


Figure 2.3 a) Effect of SRT on methane emission rate with and without BES addition; b) Effect of SRT on MT emission rate with the addition of BES or BES + AOA

2.6.4 Model development

2.6.4.1 Model assumptions

Following assumptions were made in the model development:

(1) As indicated by the experimental results shown in Figures 2.1 and 2.3, MT was produced mainly by microbial fermentation of methionine and then utilized by methanogens. Thus, these two processes are considered as the dominant MT turnover pathways in the model development.

(2) To reduce model complexity, acetotrophic methanogenesis is considered as a predominate methane production pathway as was commonly assumed in other studies (Bernard et al. 2001, Koch et al. 2010, Lopez and Borzacconi 2009, Müller et al. 2002, Noykova and Gyllenberg 2000).

(3) The property of feed sludge was assumed to be stable as indicated by the relatively small variations of VS ($2.8 \pm 0.25\%$) and protein ($11.85 \pm 0.25 \text{ g L}^{-1}$) contents measured throughout the study period.

2.6.4.2 Model framework

Based on the experimental results shown in Figures 2.1 and 2.3, MT was produced by microbial fermentation of methionine and then utilized by methanogens. The kinetic rates and stoichiometric coefficients of the processes considered in current model framework are summarized in Table 2.1.

Table 2.1 Model stoichiometric and kinetic rate matrix

Component:	S_{Met}	S_{MT}	S_{CH_4}	S_{AC}	X_{MT}	X_{CH_4}	Growth rate
Process ↓	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹ h ⁻¹)
Uptake of Met	$-\frac{1}{Y_{X_{MT}/Met}}$	$Y_{MT/X_{MT}}$			1		$\mu_{MT} \cdot X_{MT}$
Uptake of MT		$-\frac{1}{Y_{X_{CH_4}/MT}}$				1	$\mu_{CH_4,MT} \cdot X_{CH_4}$
Uptake of AC			$Y_{CH_4/X_{CH_4}}$	$-\frac{1}{Y_{X_{CH_4}/AC}}$		1	$\mu_{CH_4} \cdot X_{CH_4}$
				Maintenance factors	$\mu_{MT} = \frac{1}{\theta} + m_{MT} \cdot Y_{X_{MT}/Met}$		
				(Eqs. 11 & 12) :	$\mu_{CH_4} = \frac{1}{\theta} + m_{CH_4} \cdot Y_{X_{CH_4}/AC}$		

Following two kinetic equations are employed to describe the MT production and utilization kinetics, respectively:

$$v_{Met} = \frac{\mu_{MT} \cdot X_{MT}}{Y_{X_{MT}/Met}} \quad (2.1)$$

$$v_{MT} = \frac{\mu_{CH_4,MT} \cdot X_{CH_4}}{Y_{X_{CH_4}/MT}} \quad (2.2)$$

in which v_{Met} and v_{MT} are the microbial uptake rates of methionine and MT; μ_{MT} and $\mu_{CH_4,MT}$ are the theoretical specific growth rates of MT producers (MT fermenters) and utilizers (methanogens); X_{MT} and X_{CH_4} represent the concentrations of MT fermenters and methanogens; $Y_{X_{MT}/Met}$ and $Y_{X_{CH_4}/MT}$ are their respective theoretical growth yields. To calculate the specific growth rates of the MT producers and utilizers, Monod equations are applied in Eqs. (2.3) and (2.4),

$$\mu_{MT} = \frac{\hat{\mu}_{MT} \cdot S_{Met}}{K_{Met} + S_{Met}} \quad (2.3)$$

$$\mu_{CH_4,MT} = \frac{\hat{\mu}_{CH_4,MT} \cdot S_{MT}}{K_{MT} + S_{MT}} \quad (2.4)$$

in which S_{Met} and S_{MT} represent the concentrations of methionine and MT, respectively. $\hat{\mu}_{MT}$ and $\hat{\mu}_{CH_4,MT}$ are the theoretical maximum specific growth rates of MT producers and utilizers, respectively. Likewise, K_{Met} and K_{MT} are their respective half-saturated constants. Accordingly, the dynamic turnover of methionine, MT, and methane in anaerobic digesters can be described by:

$$\frac{dS_{Met}}{dt} = -v_{Met} + \frac{(S_{Met}^{in} - S_{Met})}{\theta} \quad (2.5)$$

$$\frac{dS_{MT}}{dt} = \frac{S_{MT}^{in}}{\theta} + Y_{MT/X_{MT}} \cdot Y_{X_{MT}/Met} \cdot v_{Met} - v_{MT} - \frac{S_{MT}}{\theta} - \frac{v_G \cdot S_{MT}}{H^{cc}} \quad (2.6)$$

$$\frac{dS_{CH_4}}{dt} = \frac{S_{CH_4}^{in}}{\theta} + Y_{CH_4/X_{CH_4}} \cdot \mu_{CH_4} \cdot X_{CH_4} - \frac{S_{CH_4}}{\theta} - v_{CH_4} \quad (2.7)$$

in which t represents time; θ is the SRT of the completely mixed anaerobic digesters; S_{CH_4} represent the concentrations of methane; the influent methionine, MT, and methane concentrations, if any, are represented by S_{Met}^{in} , S_{MT}^{in} and $S_{CH_4}^{in}$; H^{cc} is the dimensionless Henry's constant for MT; $Y_{MT/X_{MT}}$ and $Y_{CH_4/X_{CH_4}}$ are the product yields defined as the amount of MT and methane formed along with unit biomass growth; v_G and v_{CH_4} represent the biogas and methane production rates from unit mixed liquor. μ_{CH_4} is the specific growth rate of methanogens on acetate. Given MT and methane are only formed in anaerobic conditions, we can assume their concentrations in the fresh

feed sludge were extremely low, i.e., $S_{MT}^{in} \approx 0$ and $S_{CH_4}^{in} \approx 0$. Also, the dissolved methane in digester effluent should be negligible as compared to those vaporized for their extremely low solubility (Wiesenburg and Guinasso 2002), i.e., $S_{CH_4} \approx 0$. Then, at the steady-state condition when all concentrations were stabilized in anaerobic digesters, Eqs. (2.5) to (2.7) can be simplified as:

$$v_{Met} = \frac{(S_{Met}^{in} - S_{Met})}{\theta} \quad (2.8)$$

$$\frac{S_{MT}}{\theta} + \frac{v_G \cdot S_{MT}}{H^{cc}} = Y_{MT/X_{MT}} \cdot Y_{X_{MT}/Met} \cdot v_{Met} - v_{MT} \quad (2.9)$$

$$v_{CH_4} = Y_{CH_4/X_{CH_4}} \cdot \mu_{CH_4} \cdot X_{CH_4} \quad (2.10)$$

In completely mixed anaerobic digesters used in this study, the apparent specific growth rates of all anaerobes should be equal to the reciprocal of SRT, namely θ^{-1} . Therefore, the apparent specific growth rates in digesters with SRT ranging from 15 to 50 days used in this study should fall within the low range of 0.0008 to 0.0028 h^{-1} , which are indeed much lower than the typical theoretical maximum growth rates of amino acid fermenters (0.1 to 0.7 h^{-1}) and methanogens (0.004 to 0.33 h^{-1}) reported in literature (Batstone et al. 2002). Under such slow growth conditions, the impact of microbial maintenance energy should become important and must be taken into consideration because a significant portion of the substrate energy has to be allocated for microbial maintenance in this case. Thus, according to Pirt (1965), the theoretical specific growth rate can be described as the sum of the apparent specific growth rate and the growth independent-maintenance rate as given in Eq. (2.11),

$$\mu_{MT} = \frac{1}{\theta} + m_{MT} \cdot Y_{X_{MT}/Met} \quad (2.11)$$

in which m_{MT} is the maintenance energy coefficient of MT producers. Since most of the methane produced in anaerobic digesters is from acetate as a substrate (e.g. 70%) (Batstone et al. 2002), we tentatively assume acetate-utilizing methanogens are major MT utilizers for the sake of model simplification (the suitability of this simplification is verified in later section). A comparison of Figure 2.1 to Figure 2.2 shows that the concentrations of acetate were three orders of magnitude higher than those of the MT. Obviously, acetate was a major substrate for methanogens, and MT

was only utilized as a minor substrate. Then the theoretical specific growth rate of the methanogens can be approximated as,

$$\mu_{CH_4} = \frac{1}{\theta} + m_{CH_4} \cdot Y_{X_{CH_4}/AC} \quad (2.12)$$

in which m_{CH_4} is the maintenance energy coefficient of methanogens. $Y_{X_{CH_4}/AC}$ is the growth yield of methanogens on acetate. Accordingly, Eq. (2.13) can be derived from Eqs. (2.3) and (2.11) to calculate S_{Met} . Likewise, Eq. (2.14) can be derived from Eqs. (2.10) and (2.12) to determine X_{CH_4} ,

$$S_{Met} = \frac{(\frac{1}{\theta} + m_{MT} \cdot Y_{X_{MT}/Met}) \cdot K_{Met}}{\hat{\mu}_{MT} - (\frac{1}{\theta} + m_{MT} \cdot Y_{X_{MT}/Met})} \quad (2.13)$$

$$X_{CH_4} = \frac{v_{CH_4}}{(\frac{1}{\theta} + m_{CH_4} \cdot Y_{X_{CH_4}/AC}) \cdot Y_{CH_4/X_{CH_4}}} \quad (2.14)$$

For the determination of S_{MT} , Eq. (2.15) can be derived from Eqs. (2.2), (2.8), and (2.9),

$$S_{MT} = \frac{Y_{MT/X_{MT}} \cdot Y_{X_{MT}/Met} \cdot (S_{Met}^{in} - S_{Met})}{\theta \left(\frac{1}{\theta} + \frac{v_G}{H^{cc}} + \frac{\mu_{CH_4,MT} \cdot X_{CH_4}}{Y_{X_{CH_4}/MT} \cdot S_{MT}} \right)} \quad (2.15)$$

in which $\mu_{CH_4,MT}$ can be expressed by Eq. (2.4); S_{Met} and X_{CH_4} can be expressed by Eqs. (2.13) and (2.14), respectively. S_{Met}^{in} can be estimated from the measured influent protein content ($11.85 \pm 0.25 \text{ g L}^{-1}$) by using a typical methionine composition (46 to 120 mg g⁻¹) in sewage sludge protein (Lau 1981, Quitain et al. 2002, Subrahmanyam et al. 1960).

2.6.4.3 Determination of the maintenance coefficient of methanogens

Technically, Eq. (2.15) can be used along with Eqs. (2.13) and (2.14) for MT concentration and emission predictions. While most of the kinetic coefficients used in Eqs. (2.13) to (2.15) can be adapted from the classic Anaerobic Digestion Model No. 1 framework (Batstone et al. 2002) as listed in Table 2.2, several are rarely studied or reported.

Table 2.2 Summary of the model parameters and their 95% confidence intervals.

Model parameter	Value	Range	Reference
<i>Kinetics of MT formation from methionine</i>			
S_{Met}^{in} (mg L ⁻¹)	1114		Measured
$\hat{\mu}_{MT}$ (h ⁻¹)	0.166		Batstone et al. (2002)
$Y_{X_{MT}/Met}$ (mg mg ⁻¹)	0.170		Batstone et al. (2002)
$Y_{MT/X_{MT}}$ (mg mg ⁻¹)	1.893	[1.850, 1.912]	Regressed
K_{Met} (mg L ⁻¹)	1.900	[1.804, 1.992]	Regressed
m_{MT} (mg mg ⁻¹ h ⁻¹)	0.960	[0.959, 0.960]	Regressed
<i>Kinetics of MT utilization by methanogens</i>			
K_{MT} (mg L ⁻¹)	0.25		Sun et al. (2015)
H^{cc} (dimensionless)	6.87		Przyjazny et al. (1983)
$\hat{\mu}_{CH_4,MT} \cdot Y_{X_{CH_4}/MT}^{-1}$ (mg mg ⁻¹ h ⁻¹)	0.0025	[0.0025, 0.0028]	Regressed
<i>Kinetics of methane formation from acetate</i>			
$\hat{\mu}_{CH_4}$ (h ⁻¹)	0.006		Batstone et al. (2002)
$Y_{X_{CH_4}/AC}$ (mg mg ⁻¹)	0.038		Batstone et al. (2002)
$Y_{CH_4/X_{CH_4}}$ (mg mg ⁻¹)	25.525		Batstone et al. (2002)
K_{AC} (mg L ⁻¹)	250		Batstone et al. (2002)
m_{CH_4} (mg mg ⁻¹ h ⁻¹)	0.0282	[0.0274, 0.0294]	Regressed

The maintenance coefficient (m_{CH_4}) of methanogens in Eq. (2.14) is one of those. In order to determine m_{CH_4} , Eq. (2.12) is transformed to Eq. (2.17), and Eq. (2.16) is used to calculate μ_{CH_4} . Plotting its term $\theta \cdot \mu_{CH_4}$ versus $\theta \cdot Y_{X_{CH_4}/AC}$ in Figure 2.4a by using S_{AC} data from Figure 2.2, a linear plot ($R^2 = 0.95$) with a y-intercept of 1 as predicted by Eq. (2.17) results in a slope of $m_{CH_4} = 0.0282$ mg mg⁻¹ h⁻¹. It is noteworthy that this maintenance coefficient value is not much different from those reported for regular anaerobes (Ercan et al. 2015). The acceptable fit of experimental data in Figure 2.4a suggests that Eqs. (2.12) and (2.14) used in this study offer suitable simulation of both the growth and maintenance kinetics of methanogens. Accordingly, X_{CH_4} can be estimated

in Figure 2.4b by substituting parameters listed in Table 2.2 and the measured v_{CH_4} in Figure 2.2 into Eq. (2.14). Figure 2.4b shows that X_{CH_4} only slightly decreased along with the SRT increase.

$$\mu_{CH_4} = \frac{\hat{\mu}_{CH_4} \cdot S_{AC}}{K_{AC} + S_{AC}} \quad (2.16)$$

in which $\hat{\mu}_{CH_4}$ is the theoretical maximum specific growth rate of methanogens on acetate; S_{AC} is the acetate concentration; K_{AC} is the half-saturated constant of acetate for methanogens.

$$\theta \cdot \mu_{CH_4} = \theta \cdot m_{CH_4} \cdot Y_{X_{CH_4}/AC} + 1 \quad (2.17)$$

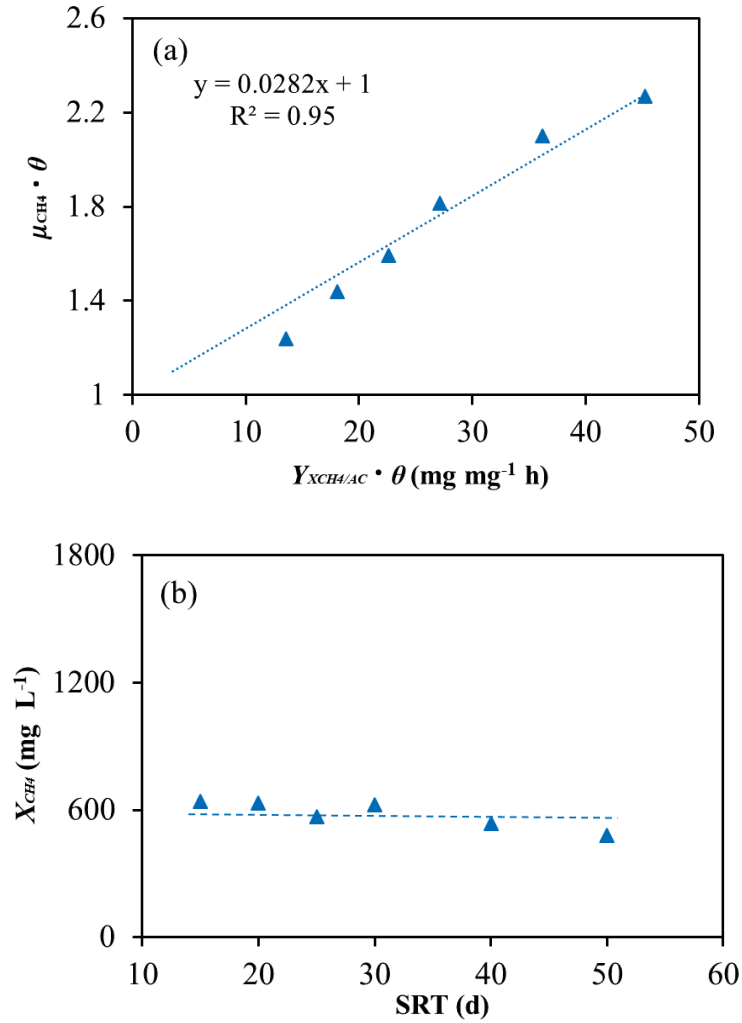


Figure 2.4 a) Model estimation of the maintenance coefficient (m_{CH_4}) using Eq. (2.17); b) Model prediction of the effect of SRT on methanogen population (X_{CH_4}) by using Eq. (2.14)

2.6.4.4 Determination of the maximum specific MT utilization rate

As for the kinetic coefficients of MT utilizers used in Eqs. (2.4) and (2.15), $K_{MT} = 0.25 \text{ mg L}^{-1}$ is probably the only one that has been reported previously (Sun et al. 2015), i.e., neither $\hat{\mu}_{CH_4,MT}$ nor $Y_{X_{CH_4}/MT}$ is known. In this regard, we treated $\frac{\hat{\mu}_{CH_4,MT}}{Y_{X_{CH_4}/MT}}$ as a single parameter and defined it as the specific maximum MT uptake rate by methanogens. Then, Eq. (2.18) can be derived from Eqs. (2.2), (2.4), and (2.9). Terms on the left hand-side of Eq. (2.18) include MT formation rate ($Y_{p,MT} \cdot Y_{MT} \cdot v_{Met}$), MT discharge rate ($\frac{S_{MT}}{\theta}$), and MT emission rate ($\frac{v_G \cdot S_{MT}}{H^{cc}}$). The net difference between these MT turnover rates is equal to the MT utilization rate calculated on the right hand-side of the Eq. (2.18). It should be noted that the only unknown in Eq. (2.18) is the term $\frac{\hat{\mu}_{CH_4,MT}}{Y_{X_{CH_4}/MT}}$ which is the slope of the trend line going through origin in Figure 2.5. The straight line with $R^2 = 0.91$ plotted with experimental data in Figure 2.5 using Eq. (2.18) implies that the MT utilization kinetic model used in this study is able to provides a good fit to the experimental data. Thereby, $\frac{\hat{\mu}_{CH_4,MT}}{Y_{X_{CH_4}/MT}} = 0.0025 \text{ mg mg}^{-1} \text{ h}^{-1}$ can be determined from Figure 2.5. Hereto, we have all the necessary coefficients for S_{MT} prediction in Eq. (2.15).

$$Y_{MT/X_{MT}} \cdot Y_{X_{MT}/Met} \cdot v_{Met} - \frac{S_{MT}}{\theta} - \frac{v_G \cdot S_{MT}}{H^{cc}} = \frac{\hat{\mu}_{CH_4,MT}}{Y_{X_{CH_4}/MT}} \cdot \frac{S_{MT} \cdot X_{CH_4}}{(S_{MT} + K_{MT})} \quad (2.18)$$

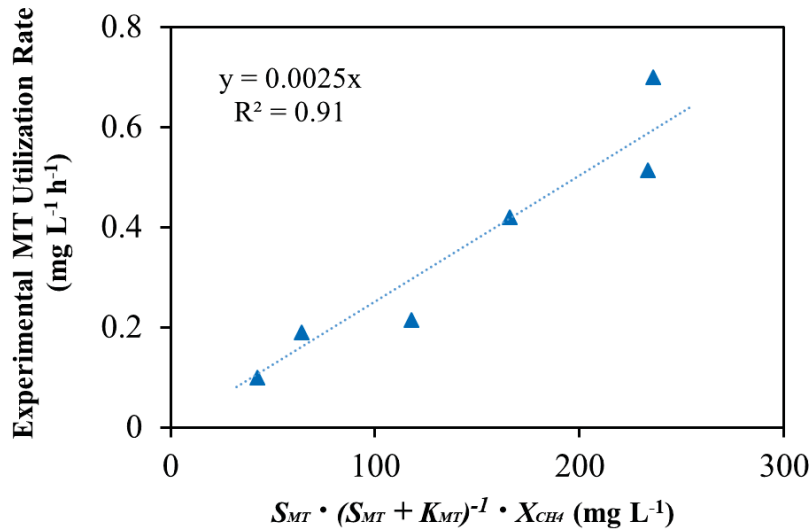


Figure 2.5 Model estimation of the specific maximum MT uptake rate using Eq. (2.18)

2.6.5 Model calibration

Based on the kinetic model developed above, three different sets of experimental data in response to SRT were used for parameter estimations. The model parameters are shown in Table 2.2, in which m_{CH_4} was estimated using the acetate concentration based on Eq. (2.17), $\frac{\hat{\mu}_{CH_4,MT}}{Y_{X_{CH_4}/MT}}$ was estimated from the MT production data obtained in the inhibition experiment based on Eq. (2.18), and $Y_{MT/X_{MT}}$, K_{Met} as well as m_{MT} were determined from the gases production rates of the digesters based on Eq. (2.15). The uncertainty evaluation of the model calibration was carried out based on the Monte Carlo approach which is particularly suitable for non-linear model with a relatively small sample size (Donoso-Bravo et al. 2011, Lopez and Borzacconi 2010, Müller et al. 2002). The theoretical model in Eq. (2.15) was used to predict the MT concentration in bulk solution of anaerobic digesters. Figure 2.6a shows that a decent agreement was reached between the theoretical prediction of Eq. (2.15) and the experimental results of the steady-state MT concentrations measured at various SRTs in Figure 2.1 with $R^2 = 0.89$, which verified the adequacy of the kinetic model developed from this study for the description of MT turnover in anaerobic digesters. The capacity of Eq. (2.15) in predicting experimental results also suggests that the assumptions and simplification made for the model development are acceptable.

2.6.6 Model simplification

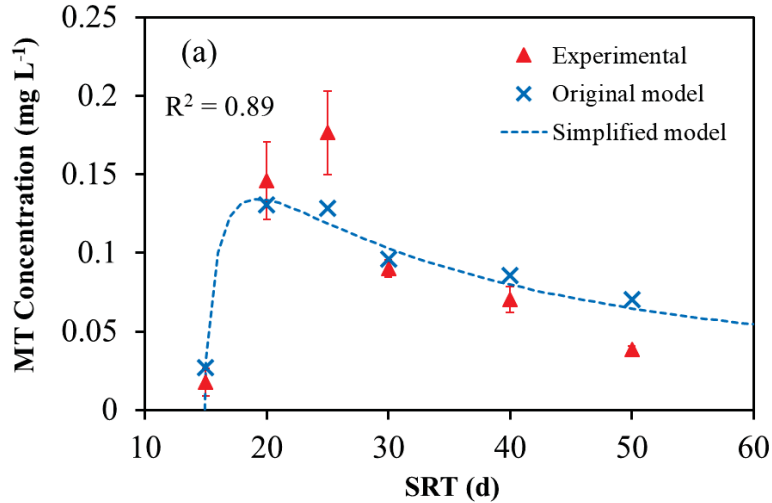
Although Eq. (2.15) has been shown capable of providing appropriate prediction of MT concentration, it appears to be too complicated to use for practitioners, so we looked into the possibility of simplifying it. As revealed in Figure 2.6b, the term $\frac{\mu_{CH_4,MT} \cdot X_{CH_4}}{Y_{X_{CH_4}/MT} \cdot S_{MT}}$ representing the specific MT utilization rate in Eq. (2.15) is almost 100 times greater than the sum of the terms representing the specific MT discharge rate ($\frac{1}{\theta}$) and emission rate ($\frac{v_G}{H^{cc}}$), indicating that the sum of the two terms ($\frac{1}{\theta} + \frac{v_G}{H^{cc}}$) is indeed negligible and can be ignored from the denominator of Eq. (2.15). Moreover, since the K_{MT} (0.25 mg L⁻¹) is much greater than the S_{MT} (0.02 - 0.18 mg L⁻¹) measured in Figure 2.1, the Monod type MT utilization kinetics in Eq. (2.4) is tentatively approximated as a first order expression, namely $\frac{\hat{\mu}_{CH_4,MT} \cdot S_{MT}}{K_{MT}}$. Additionally, as shown in Figure 2.4b, the methanogen population is relatively stable within the range of typical SRT used for anaerobic digestion. The

term representing X_{CH_4} in Eq. (2.14), namely $\frac{v_{CH_4}}{\left(\frac{1}{\theta} + m_{CH_4} \cdot Y_{X_{CH_4}/AC}\right) \cdot Y_{CH_4/X_{CH_4}}}$, can be also treated as a constant. Therefore, Eq. (2.15) can be simplified as:

$$S_{MT} = K \cdot \frac{1}{\theta} \cdot (S_{Met}^{in} - S_{Met}) \quad (2.19)$$

in which K is a constant equal to $\frac{Y_{MT/X_{MT}} \cdot Y_{X_{MT}/Met}}{\left(\frac{1}{\theta} + \frac{v_G}{H^c c} + \frac{\mu_{CH_4, MT}}{Y_{X_{CH_4}/MT} \cdot S_{MT}} \cdot X_{CH_4}\right)}$. Eq. (2.19) suggests that the steady-

state MT concentration in anaerobic digesters is primarily determined by the SRT and the influent methionine concentration. In order to determine the accuracy of this simplified model in Eq. (2.19), its prediction of S_{MT} along with SRT is compared with that of the Eq. (2.15) in Figure 2.6a. As can be seen, although the simplified model predicts a slightly lower SRT corresponding to the peak MT emission, the two predictions are almost identical, confirming the validity of the simplified model in Eq. (2.19) in providing acceptable simulation of the steady-state MT concentrations in anaerobic digesters.



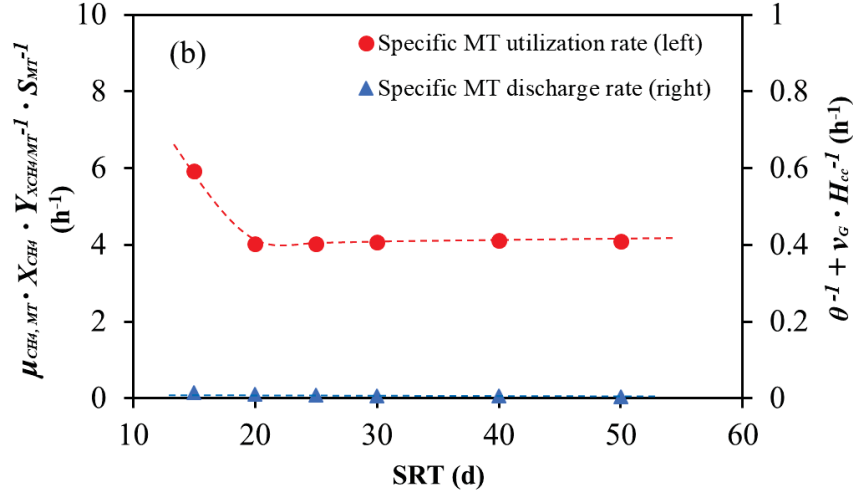


Figure 2.6 a) Comparison of experimental observation and the prediction by original model in Eq. (2.15) and simplified model in Eq. (2.19); b) Comparison of the specific rates of MT utilization ($\frac{\mu_{CH_4,MT} \cdot X_{CH_4}}{Y_{X_{CH_4}/MT} \cdot S_{MT}}$) and discharge ($\frac{1}{\theta} + \frac{v_G}{H_{cc}}$)

2.6.7 Model implications

2.6.7.1 Model prediction of the SRT that produces peak MT emission

Although odor emission from anaerobic digestion can involve many compounds, MT is often identified as the major odorous compound in wastewater treatment practices associated with anaerobic conditions (Chen et al. 2005, Du and Parker 2012, Hort et al. 2013, Novak et al. 2006, Park and Novak 2013, Wang et al. 2015). For this regard, we mainly focused on MT as a representative odorous compound in this study. Special cautions and odor control measures should be taken towards the anaerobic digesters running at the SRT that incurs peak MT emission. The peak MT emission occurs when S_{MT} is in following relation with SRT,

$$\frac{dS_{MT}}{d\theta} = 0 \quad (2.20)$$

Substituting Eqs. (2.13) and (2.19) into Eq. (2.20) gives,

$$\theta_{peak} = \frac{1}{(\hat{\mu}_{MT} - m_{MT} \cdot Y_{X_{MT}/Met}) \pm \sqrt{\left(\frac{\hat{\mu}_{MT} \cdot K_{Met}}{K_{Met} + S_{Met}^{in}} \right) \cdot (\hat{\mu}_{MT} - m_{MT} \cdot Y_{X_{MT}/Met})}} \quad (2.21)$$

in which θ_{peak} is the SRT corresponding to the peak MT emission. Since $m_{MT} \cdot Y_{X_{MT}/Met}$ is a constant specific maintenance rate, $(\hat{\mu}_{MT} - m_{MT} \cdot Y_{X_{MT}/Met})$ can be interpreted as the maximum apparent specific growth rate that can be achieved under the maintenance condition. Typically, a bioreactor will not operate at a SRT shorter than the reciprocal of maximum apparent specific growth rate in order to avoid washout, hence Eq. (2.21) can be reduced to,

$$\theta_{peak} = \frac{1}{(\hat{\mu}_{MT} - m_{MT} \cdot Y_{X_{MT}/Met}) - \sqrt{\left(\frac{\hat{\mu}_{MT} \cdot K_{Met}}{K_{Met} + S_{Met}^{in}}\right) \cdot (\hat{\mu}_{MT} - m_{MT} \cdot Y_{X_{MT}/Met})}} \quad (2.22)$$

Eq. (2.22) indicates that the value of SRT corresponding to the peak MT emission is actually influenced by S_{Met}^{in} , i.e., the higher protein content presents in influent sludge, the shorter SRT will be needed to reach the peak MT emission. The θ_{peak} in this study is around 20 d as shown in Figure 2.1.

2.6.7.2 Effect of feed sludge protein content on the SRT that produces peak MT emission

Eq. (2.22) indicates that the value of SRT corresponding to the peak MT emission (θ_{peak}) is actually influenced by S_{Met}^{in} . The higher protein content presents in influent sludge, the shorter SRT will be needed to reach the peak MT emission. When $S_{Met}^{in} \gg K_{Met}$, Eq. (2.22) can be simplified as $\theta_{peak} = (\hat{\mu}_{MT} - m_{MT} \cdot Y_{X_{MT}/Met})^{-1}$. It should be realized that the term $\hat{\mu}_{MT} - m_{MT} \cdot Y_{X_{MT}/Met}$ represents the maximum apparent specific growth rate that the MT fermenters can possibly achieve. Thus, the use of any $\theta < (\hat{\mu}_{MT} - m_{MT} \cdot Y_{X_{MT}/Met})^{-1}$ will lead to the washout of MT fermenters and in turn the plunge of S_{MT} as SRT approaches the lower boundary of the bell-shape curve shown in Figure 2.7. It should be noted that the S_{MT} corresponding to this θ_{peak} , namely $(S_{MT})_{peak}$, is indeed at the maximum value that S_{MT} can possibly reach due to the extremely high S_{Met}^{in} provided in this scenario.

As S_{Met}^{in} decreases, it is shown in Figure 2.7 that the $(S_{MT})_{peak}$ as a product will decrease accordingly, while θ_{peak} will increase. Taking a close look at the denominator of Eq. (2.22), one should easily see that S_{Met}^{in} is actually controlling the apparent specific growth rate corresponding to θ_{peak} at a

value that is $\sqrt{\left(\frac{\hat{\mu}_{MT} \cdot K_{Met}}{K_{Met} + S_{Met}^{in}}\right) \cdot (\hat{\mu}_{MT} - m_{MT} \cdot Y_{X_{MT}/Met})}$ less than the maximum apparent specific

growth rate the MT fermenters can possibly achieve, namely $(\hat{\mu}_{MT} - m_{MT} \cdot Y_{X_{MT}/Met})$. Thus can be seen, a decrease in S_{Met}^{in} will lead to a decline in apparent specific growth rate at the peak MT emission in Eq. (2.22), the reciprocal of which corresponds to the increase of θ_{peak} as shown in Figure 2.7. While, according to Eq. (2.22), there also exists a minimum $S_{Met}^{in} = K_{Met} \left(\frac{m_{MT} \cdot Y_{X_{MT}/Met}}{\hat{\mu}_{MT} - m_{MT} \cdot Y_{X_{MT}/Met}} \right)$ which is just enough to support the basic maintenance of MT fermenters. By using the kinetic parameters listed in Table 2.2, this minimum S_{Met}^{in} can be estimated to be around 100 mg L⁻¹ which is indeed significantly lower than the S_{Met}^{in} value (1114 mg L⁻¹) contained in the blended sludge used in this study. Hence, the presence of $(S_{MT})_{peak}$ at extremely long θ_{peak} is unlikely in reality. Even if this $(S_{MT})_{peak}$ was ever present at this minimum S_{Met}^{in} , it should be in such an extremely low value as shown in Figure 2.7 that does not need to be considered further. In summary, the value of θ_{peak} is mainly determined by the S_{Met}^{in} value contained in the feed sludge. One may use Eq. (2.22) to predict the θ_{peak} so as to evaluate the odor potential for digesters operated at a certain SRT.

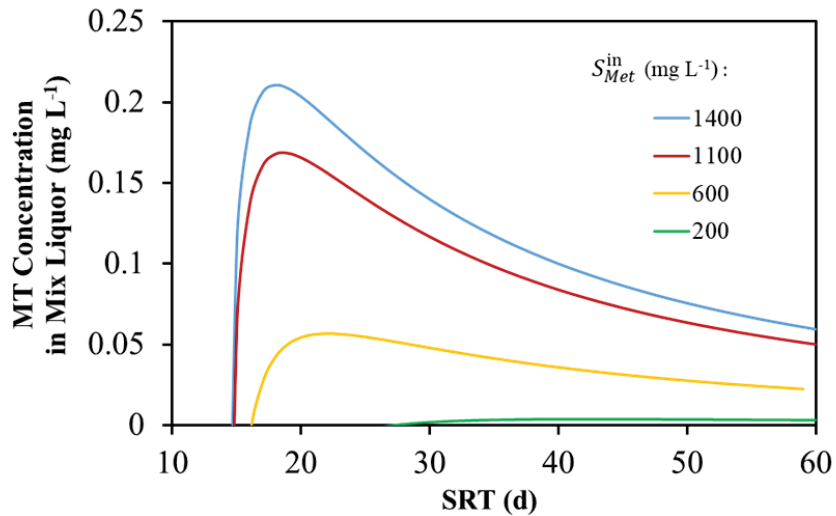


Figure 2.7 Model prediction of the effect of influent methionine concentration (S_{Met}^{in}) on steady-state MT concentration along with SRT at $K = 0.08$ h

2.6.7.3 Effect of maintenance coefficient on the SRT that produces peak MT emission

The maintenance mechanism for microbes is a trade-off between survival and growth. When under unfavorable conditions, resources are more devoted to survival of microbes rather than

reproduction (Nyström 2004). According to Eq. 11, high maintenance requirement will lead to higher theoretical specific growth rate (μ_{MT}) under a given SRT, which in turn resulted in higher methionine consumption and MT production as described in Eqs. 1 and 6. For this reason, although the maintenance coefficient is a constant but not a variable in the current model, we would like to argue that the magnitude of m_{MT} is indeed responsible for the presence of θ_{peak} within the typical SRT range used in anaerobic digestion. As simulated in Figure 2.8 using Eq. (2.19), microbes with larger maintenance coefficient (m_{MT}) tend to have a longer θ_{peak} and a lower $(S_{MT})_{peak}$. m_{MT} is indeed a burden to the growth of MT fermenters, i.e., when m_{MT} is large, more energy obtained from methionine utilization will be allocated for maintenance but not for growth, and consequently a smaller apparent specific growth rate of MT fermenters will be resulted, and so is the MT production rate. Since S_{MT} is the net result of concurrent MT production and utilization, larger m_{MT} thus tends to give lower $(S_{MT})_{peak}$ as displayed in Figure 2.8. Due to the slowdown of MT production rate by large m_{MT} burden, it is also not difficult to understand that the MT production will reach equilibrium with a slower MT utilization rate at the steady state. These slower MT production and utilization rates only occur in digesters running at a longer SRT as shown in Figure 2.8. Conversely, bacteria with a smaller m_{MT} will have a θ_{peak} so small that it will not show up in the typical SRT range of anaerobic digesters. For example, the maintenance rate ($m \cdot Y_{X/S}$) of MT fermenters estimated in this study is about 0.16 h^{-1} (Table 2.2) which is over 100 times greater than that of the acetate fermenters at 0.013 h^{-1} (Ghosh et al. 1975). As a consequence, the θ_{peak} , or in another words the bell-shaped curve, can only be observed for MT in Figure 2.1 but not for acetate in Figure 2.2b.

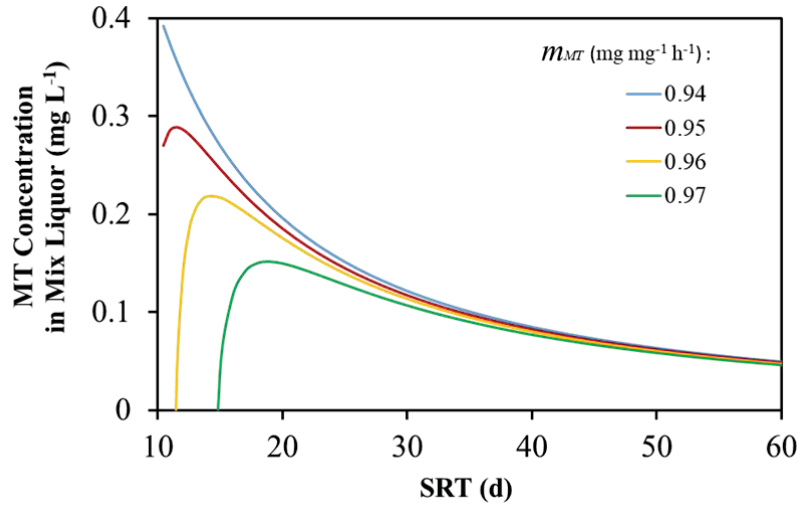


Figure 2.8 Model prediction of the effect of maintenance coefficient (m_{MT}) on steady-state MT concentration along with SRT at $K = 0.08$ h

2.6.8 Implication on MT production pathways

As summarized in Figure 2.9, there are primarily three pathways for methionine fermentation to MT: i) the Stickland reaction using coupled amino acids as both electron donors and acceptors to ferment methionine into MT along with propionate, carbon dioxide, and ammonia (Batstone et al. 2002); ii) the transamination reaction using transaminases to convert methionine to 2-oxo-4-methylthio-butanoic acid (KMBA) which is subsequently converted to MT along with α -ketobutyric acid, ammonia, and hydrogen production (Bonnarme et al. 2001); and iii) the lyase reaction which directly converts methionine to MT, α -ketobutyric acid, ammonia, and hydrogen (Soda 1987).

It should be pointed out that, unlike Stickland reaction, both transamination and lyase reactions are single amino acid degradation pathways that require PLP as a coenzyme for MT production from methionine (Wolle et al. 2006). The fact that MT production was completely stopped when using AOA to inhibit the activity of PLP in Figure 2.3a suggests that Stickland reaction is not a major pathway for MT production, leaving the possibility to either transamination or lyase reaction. The MT yield from methionine as calculated by $Y_{MT/X_{MT}} \cdot Y_{X_{MT}/Met}$ in Table 2.2 is around $0.3218 \text{ mg mg}^{-1}$ which is very close to the stoichiometric MT yield from methionine, assuming MT is the only sulfurous product, namely $0.3224 \text{ mg mg}^{-1}$. Such a complete conversion of methionine to MT just indicates that there was almost no KMBA accumulation during the anaerobic digestion. In view

of the fact that transamination reaction always converts part of the methionine to KMBA as shown in Figure 2.9, it is our inference that lyase reaction might be the major pathway for methionine to MT conversion in anaerobic digesters.

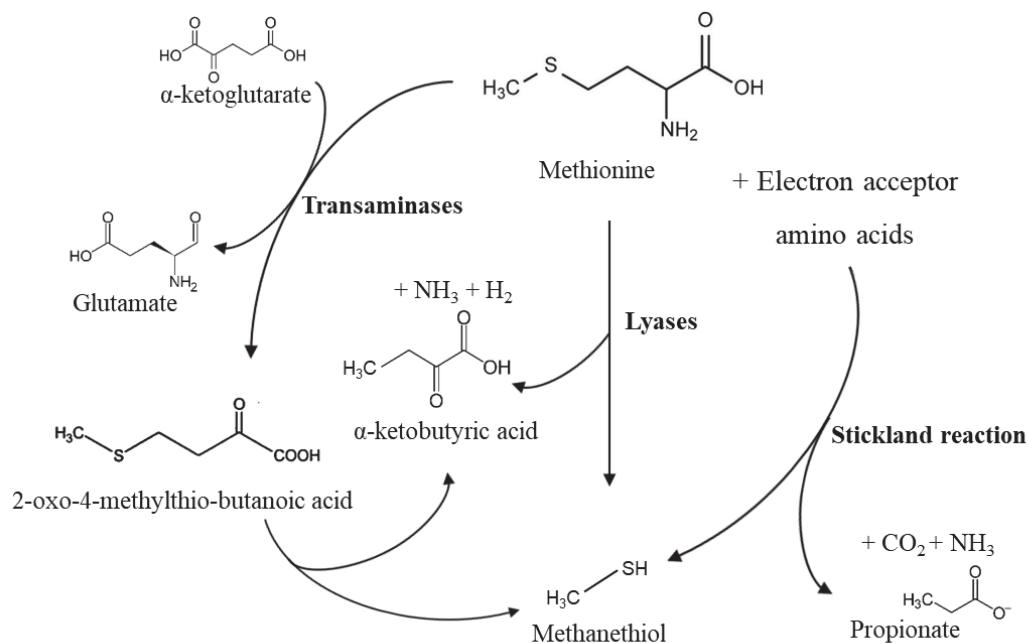


Figure 2.9 Schematic illustration of the catabolic pathways available for converting methionine to MT (Adapted from the study by Wolle et al. 2006)

2.7 Conclusions

This study found that the MT emission rate follows a bell-shape pattern with SRT in anaerobic digesters. The subsequent inhibitory experiment showed that net MT emission is primarily determined by the dynamic concurrence of MT production and utilization in the course of anaerobic digestion. Further kinetic analysis of the MT dynamics revealed that the SRT that produces this peak MT emission is likely controlled by the feed sludge protein content, and the large maintenance coefficient of MT fermenters is an intrinsic cause of the peak emission in the typical range of SRT used for anaerobic digestion. A simplified kinetic model was derived and verified from this study for the prediction of MT concentration, as well as the SRT leading to peak MT emission. The possible pathways responsible for MT production were also inferred, and lyase-

catalyzed reaction appears to be responsible for methionine-to-MT conversion in anaerobic digesters operated in this study.

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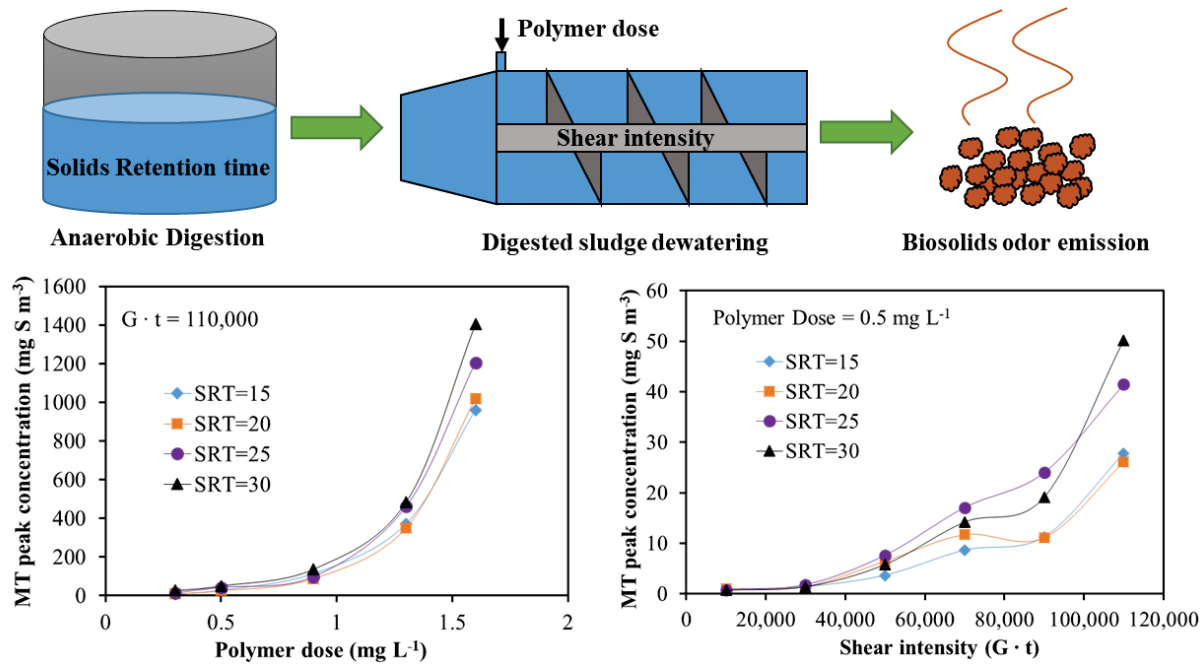
Chapter 3 : Effects of anaerobic digester solids retention time on odor emission and dewaterability of biosolids subjected to various shear intensity, polymer doses, and storage duration

(This chapter has been submitted for publication as “Zhang D., Strawn M., Broderick T., Novak J.T., and Wang Z.W. (2019) Effects of anaerobic digester solids retention time on odor emission and dewaterability of biosolids subjected to various shear intensity, polymer doses, and storage duration, Water Research & Technology”)

3.1 Abstract

Odor emitted during biosolids handling is a major issue for utilities and may pose serious nuisance to surrounding communities. The purpose of this study is to assess the effects of SRTs of mesophilic anaerobic digesters on the odor emission from biosolids dewatered under various shear intensity and polymer doses. It was found that the digester SRT varying from 15 to 50 days had a very limited effect on the odor precursors reduction in biosolids. Hence, no significant variance was observed in odor emission when SRT was evaluated alone. In contrast, the polymer dose and shear intensity used for dewatering showed more impacts than SRT on odor emission and biosolids dewaterability. In general, biosolids dewatered at higher shear intensity tended to consume more polymer to achieve desired cake dryness, and the peak odor emission was found to increase with both the shear intensity and the polymer doses likely due to the increased protein capture and exposure by polymer and shear used for dewatering.

3.2 Graphical abstract



3.3 Keywords

Anaerobic digestion; Solids retention time; Dewaterability; Odor; Shear intensity; Polymer dose

3.4 Introduction

Globally, rapid urbanization results in dramatic increase of biosolids production. As a consequence, urban anaerobic digesters undertaking sludge treatment are often overloaded and thus subjected to the risk of incomplete anaerobic digestion (Chen et al. 2012, Parkin and Owen 1986). The situation is getting worse as the sludge handling demands continuously grow. By 2050, 66% of the world's population is projected to live in urban area with an urban population increase of another 2.5 billion (United Nations 2014), which will add 58-million-ton sludge production per year based on the current sludge production of 23 kg per capita in the U.S. (Peccia and Westerhoff 2015). The potential odor emission from the incompletely digested sludge may cause serious public nuisances, especially in densely populated neighborhood of urban WWTPs. Hence, whether the compromised SRTs of anaerobic digestion can directly impact the odor emission from digested sludge is a reasonable question to ask.

Odor emission from the digested and dewatered biosolids has been identified as a main complaint received by biosolids handling facilities from the public (National Research Council 2002). Various odor-causing compounds such as HS, ammonia, trimethylamine, MT and DMS may present in odorous gas emitted from biosolids. For anaerobically digested sludge, VOSCs including MT and DMS have been identified as the key odor contributors (Adams and Witherspoon 2004, Higgins et al. 2006, Novak et al. 2006). However, nitrogen-containing compounds such as ammonia and trimethylamine are mainly produced from biosolids with pH above 9, e.g. lime stabilized biosolids (Krach et al. 2008). HS can also be of less concern for certain anaerobically digested sludge including the one used in this study, as sulfide will precipitate as ferrous sulfide for WWTPs choosing iron salt as coagulant for sludge flocculation and thickening (Novak et al. 2006). A national wide study conducted by Water Environmental Research Foundation at 11 WWTPs across North America with capacities from 13 to 350 million gallons per day revealed that biosolids odors, mainly VOSCs including MT and DMS, were highly correlated to the amount of bio-available proteins remained in biosolids after anaerobic digestion (Adams and Witherspoon 2004, Novak et al. 2006). Further microbial regrowth may occur during post digestion biosolids storage. The residual sulfur-containing proteins from incomplete anaerobic digestion were metabolized to MT and DMS, and these compounds were subsequently utilized by methanogens as substrates (Chen et al. 2011b, Higgins et al. 2006, Qi et al. 2007). Given the essential role of proteins and microbial activity involved in biosolids odor emission, the SRT of anaerobic digestion may affect the odor emission because it simultaneously regulates the protein reduction and the microbial population in the anaerobic digestion effluent (Shin et al. 2010, Vanwonterghem et al. 2015, Zhang and Tatsuya 1994). Previous research has demonstrated that SRT was a fundamental parameter determining the MT content in the biogas produced from anaerobic digesters (Zhang et al. 2018). However, the SRT effect of anaerobic digestion on odor emission from biosolids following the dewatering process remains largely unknown.

Besides SRT, previous studies also indicated that post-anaerobic digestion solids dewatering and conveyance processes such as dewatering centrifugation, biosolids pumping, and cake storage may also affect the biosolids odor emission (Adams and Witherspoon 2004, Murthy et al. 2003, Ross et al. 2002). For example, shearing of biosolids by dewatering equipment has been reported to release bioavailable proteins resulting in elevated odor emission (Chen et al. 2011a). In addition, sludge conditioning by inorganic or organic polymers is a typical practice to improve sludge

dewaterability, in which colloidal materials including proteins will be coagulated and retained in dewatered cake (Novak 2006, Zhang et al. 2014). Based on these observations, the impacts of key biosolids handling parameters including biosolids storage time, equipment shear, and polymer dose should be also taken into consideration in the evaluation of the SRT effects on odor emission.

Therefore, it is important to understand the relationship between SRT and the odor emission in conjunction with biosolids handling parameters, specifically whether a higher digester SRT can be used to reduce odors and increase dewatered cake solids. It is our desire that the digester and sludge dewatering procedures can be strategically optimized to minimize odor emission without compromising the digester performance and the biosolids quality. The outcome from this study should be appealing to both practitioners and researchers interested in the biosolids odor mitigation.

3.5 Materials and methods

3.5.1 Anaerobic digester setup

Six anaerobic digesters, each with working volume of 10 liters and headspace of 20 liters, were operated under completely mixed semi-continuous mode, i.e. the fresh blended sludge was added and the digested sludge was discharged according to the designed SRTs on a daily basis. SRTs of 15, 20, 25, 30, 40, and 50 days were set on the six digesters, respectively. The hydraulic retention time was considered to be equal to SRT in completely mixed digesters used in this study (Hassan et al. 2013). The digester configuration can be seen in Figure 3.1. Briefly, a constant temperature at 35.5 ± 0.3 °C was maintained with heating blankets, feedback temperature controllers, and insulation barriers consisting of a reflective foil layer under a 5 cm cotton layer as shown in Figure 3.1b.

The reactors were well-mixed via biogas recirculation at the flow rate of 1 L min^{-1} from the headspace to the conical bottom of the reactors as shown in Figure 3.1a. The biogas production was monitored using calibrated tipping-bucket meters coupled with automatic data loggers (Archae Press, Nashville, USA). The digesters were fed with blended primary and waste activated sludge with a dry mass ratio of 62:38 as collected from an urban WWTP, namely Arlington Water Pollution Control Plant (Arlington, VA). The blended sludge has an average TS of $3.6 \pm 0.06\%$ and VS of $79.1 \pm 0.4\%$. The digested sludge was then dewatered under various shear intensity and

polymer doses, and the dewatered cakes were monitored for odor production as illustrated in Figure 3.1c.

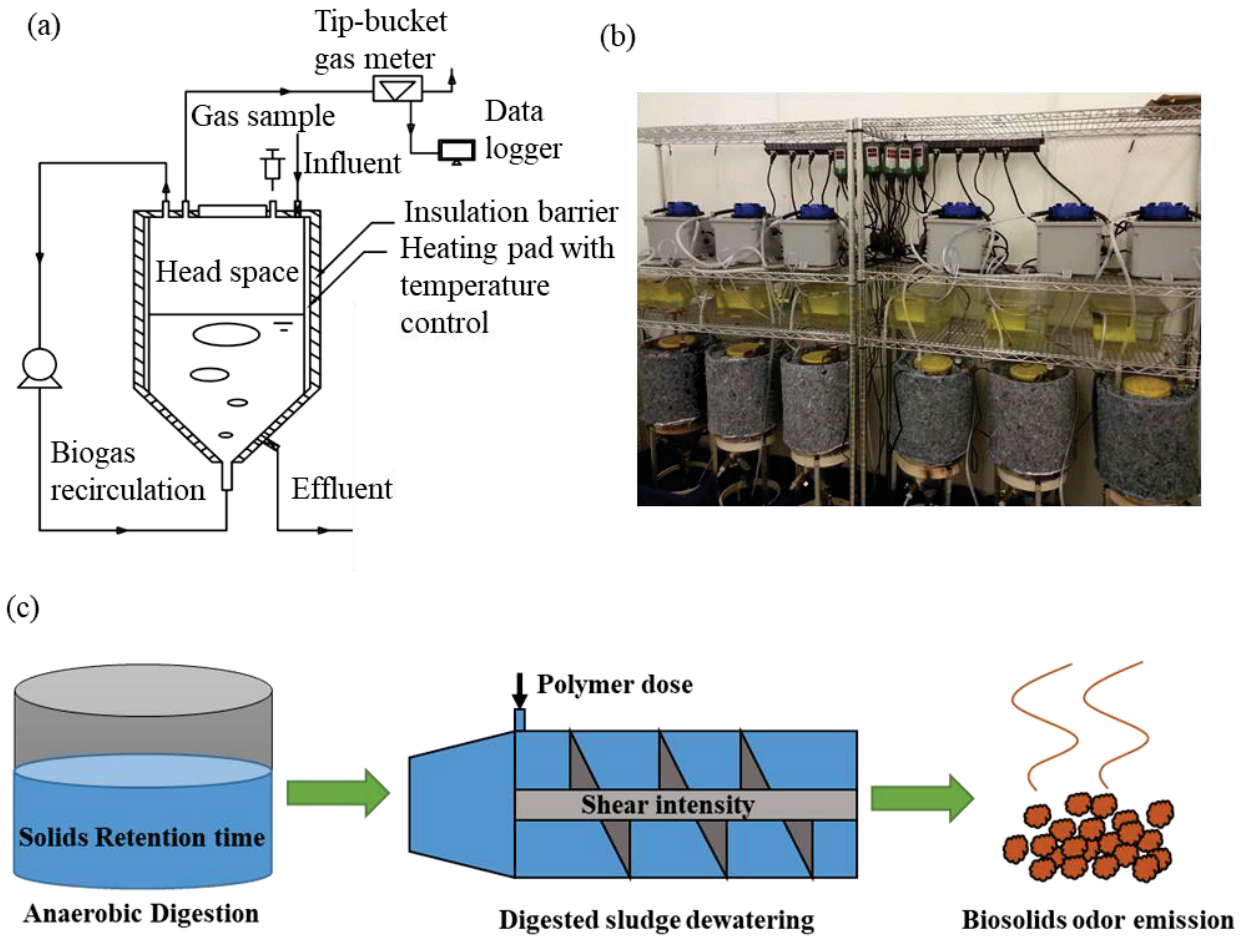


Figure 3.1 a) Schematic diagram of the lab-scale anaerobic digestion system; b) Photograph of the lab-scale anaerobic digestion system; c) schematic diagram of the digested sludge conditioning and centrifuge dewatering processes and odor emission from dewatered biosolids.

3.5.2 Dewatering system setup

A lab-scale sludge conditioning and dewatering system was used to dewater digested sludge by mimicking the mechanism of industrial dewatering methods such as belt press, plate and frame press, and centrifuge (Zhang et al. 2019a, Zhang et al. 2019b). Briefly, digested sludge samples were first mixed with polymer and sheared in a high-power commercial blender (Waring MX1200XTXP, Stamford, CT) under the controlled shear intensity ($G \cdot t$). Then the sheared sludge

was preliminarily dewatered with a lab centrifuge (Sorvall Legend X1R, Fisher Scientific, Waltham, MA) under constant centrifugal acceleration of $5000 \times g$ for 20 min, followed by further mechanical compression to produce the dewatered cake using a piston press under controlled pressure of 448 kPa for 30 min. Novak (2006) reported that belt press, plate and frame press, and centrifuge dewatering process provide shear intensity ($G \cdot t$) approximately equal to 1×10^4 , 3×10^4 , and 11×10^4 , respectively. Therefore, selected shear intensity ranging from 1×10^4 to 11×10^4 were applied to mimic the typical shear intensity of industrial dewatering methods. The polymer used in the present study was the same high molecular weight cationic polymer (SE-1045-A, Polydyne, Inc.) used in the existing centrifuge dewatering process of the WWTP.

3.5.3 Monitoring of odor emission from dewatered cake during storage

A 300 ml serum bottle was purged with nitrogen gas to remove oxygen, and then loaded with 20 g dewatered cake sample and sealed with a rubber stopper (GL45, Wenoote, Changshu, China) (Sun et al. 2014). The sludge digested under SRTs ranging from 15 to 30 days was stored for 6 days in the serum bottle to study the extent of odor emission during storage. Gas samples were taken from the bottle headspace on a daily basis to monitor HS, MT, and DMS emission profiles. However, the HS concentrations were below or near the detection limit in all gas sample. This was likely because the sulfide turned to precipitate with iron as the sludge was subjected to ferric chloride addition in the dissolved air flotation unit of the WWTP (Novak et al. 2006). The peak MT and DMS concentrations during storage were presented as a function of shear intensity and polymer dose, respectively.

3.5.4 Chemical analysis

Gas samples from the headspace of the digesters and the serum bottles were measured for methane, MT, and DMS contents using a GC equipped with a flame ionization detector (Hewlett-Packard 5890 Series II, Palo Alto, California) and a 60 m long capillary column with a 0.53 mm diameter (Agilent, DB-1, Santa Clara, California). HS was measured using a GC equipped with a flame photometric detector (GC-2014, Shimadzu, Columbia, MD). Headspace gas samples were collected using a 1 mL gas-tight syringe (Hamilton, Reno, Nevada) and were manually injected to the GC. Standard gases (Airgas, Inc., Radnor, Pennsylvania) were used to develop calibration curves and quantify methane, HS, MT, and DMS. TS, VS, pH, alkalinity, and VFA were measured for the digestate according to standard methods (APHA et al. 2012). The protein content in digester

mixed liquor was determined using a modified Lowry protein assay kit (PierceMT, Thermo Scientific, Massachusetts).

3.5.5 Statistical analysis

Two-sample t-tests at a significant level of $\alpha = 0.05$ were used to determine whether there was a significant difference in VS reduction and protein concentration between samples processed at different SRTs. Analysis of variance (two-way ANOVA without replication) tests at a significant level of $\alpha = 0.05$ were performed on relevant data sets to determine the influence and significance of SRT, shear, and polymer dose on cake dryness and peak odor emission.

3.6 Results and discussion

3.6.1 Anaerobic digester performance

AD of blended primary and waste activated sludge was carried out in six digesters for 186 days at SRTs of 15, 20, 25, 30, 40, and 50 days, respectively. The steady state was reached in all digesters within 120 days of operation as indicated by the stable methane production for a duration at least two times SRT from day 120 to day 186 in Figure 3.2a. Figure 3.2a shows the effect of SRT on accumulated methane production in the course of 186 days of anaerobic digester operation. Judging from the slope of these profiles, it is obvious to see that SRT is inversely related to the methane production rate, i.e., shorter SRTs tend to produce more methane over the same experimental timeframe. This observation is not surprising because shorter SRTs give rise to higher organic loading rates when the same concentration of feedstock was fed, which in turn fueled fast anaerobic digestion rates. Figure 3.2b shows that the steady state volumetric methane production rate is almost linearly correlated to the SRT, i.e., the methane production rate at SRT of 15 days is almost 2.4 times that at the SRT of 50 days.

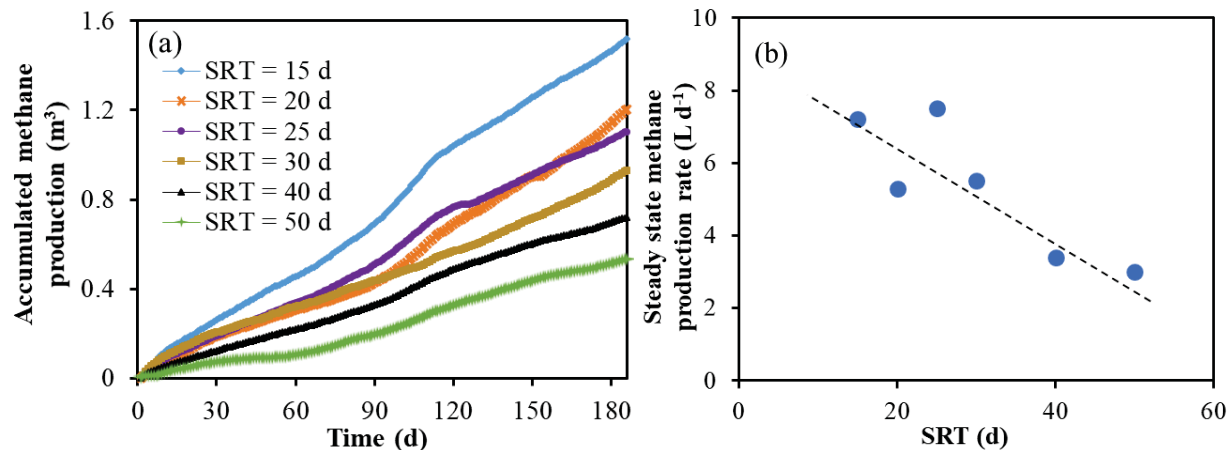


Figure 3.2 Effect of SRT on a) accumulated methane production during the 186-day operation; and b) the steady state methane production rate averaged over the day 120 to 186.

Yet, the higher rates of anaerobic digestion at shorter SRTs came with a price of lower anaerobic digestion efficiency. By normalizing daily methane production to the mass of fed VS, Figure 3.3a shows the profiles of the specific methane production rates along with the 186 days of experiment. In spite of the radical fluctuation during the digester startup, all six digesters entered their steady state phase after 120 days of operation (Figure 3.3a). It can be seen that the digesters with shorter SRTs, e.g. 15 and 20 days, tended to be stabilized around much lower specific methane production rates, indicating incomplete digestion of the fed VS. Such a positive correlation between SRT and specific methane production rates is even more evident in Figure 3.3b. It shows that a maximum anaerobic digestion efficiency in terms of specific methane production rate can be achieved when SRT is greater than 25 days, below which the efficiency will drop with SRT. Similar observation was also made in other studies (Appels et al. 2008, Lee et al. 2011, Nges and Liu 2010). Calculating the methane yield from unit VS reduced in Figure 3.3c shows that it follows the same trend as that of the specific methane production rate in Figure 3.3b. The reduced methane yield at SRT shorter than 25 days indicates that there might be accumulation of soluble intermediate products such as VFAs.

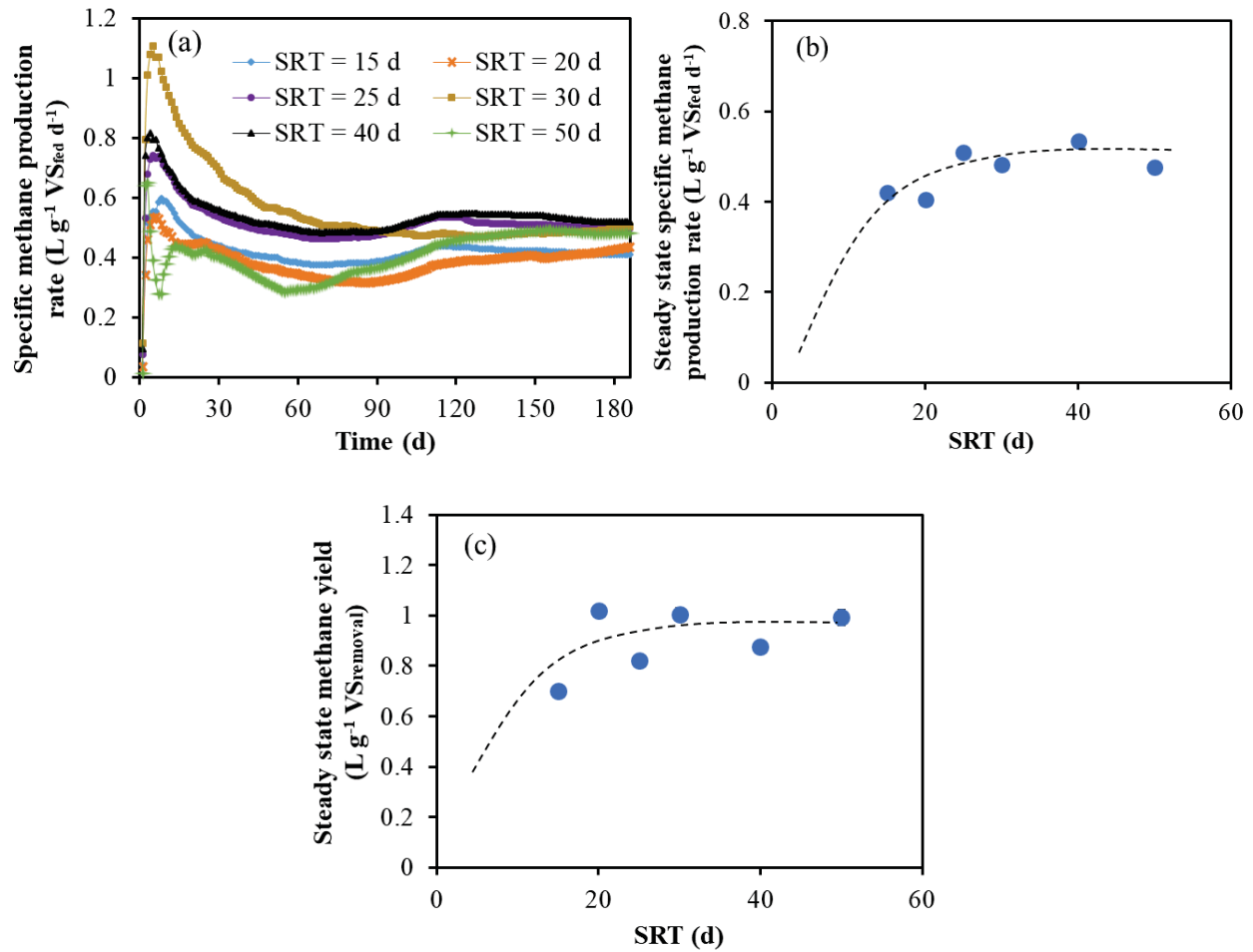


Figure 3.3 Effect of SRT on a) specific methane production rate in the course of 186-day operation; b) the steady state specific methane production rate and c) yield averaged over the day 120 to 186.

Figure 3.4a showed that the pH of all digesters fluctuated within a narrow range between 7.1 and 7.4 throughout the 186 days' experiment regardless of the SRT, indicating the excellent stability of digesters under all SRTs. This might be attributable to the high alkalinity measured in all digesters (Figure 3.4b), despite that VFA accumulation was observed in shorter SRT digesters (Figure 3.4c). It is known that alkalinity above 3500 mg L^{-1} and a VFA-to-alkalinity ratio below 0.4 is sufficient to maintain the stability of mesophilic anaerobic digesters (Appels et al. 2008, Sri Bala Kameswari et al. 2012, Switzenbaum et al. 1990). VFAs were found to be inversely related to the SRT in Figure 3.4c, which is expected because higher VFA concentration is required to support the faster growth rate of many anaerobes including the slow growing methanogens. The accumulation of VFA also indicates that it is the methanogenesis but not the hydrolysis accounting for the rate-limiting step of the anaerobic digestion, which is in line with the independence of TS

reduction on SRT as observed in Figure 3.5a. Figure 3.4b and d shows that even the digester operated at 15-day SRT can actually achieve sufficiently high alkalinity and low VFA-to-Alkalinity ratio to ensure the digester stability.

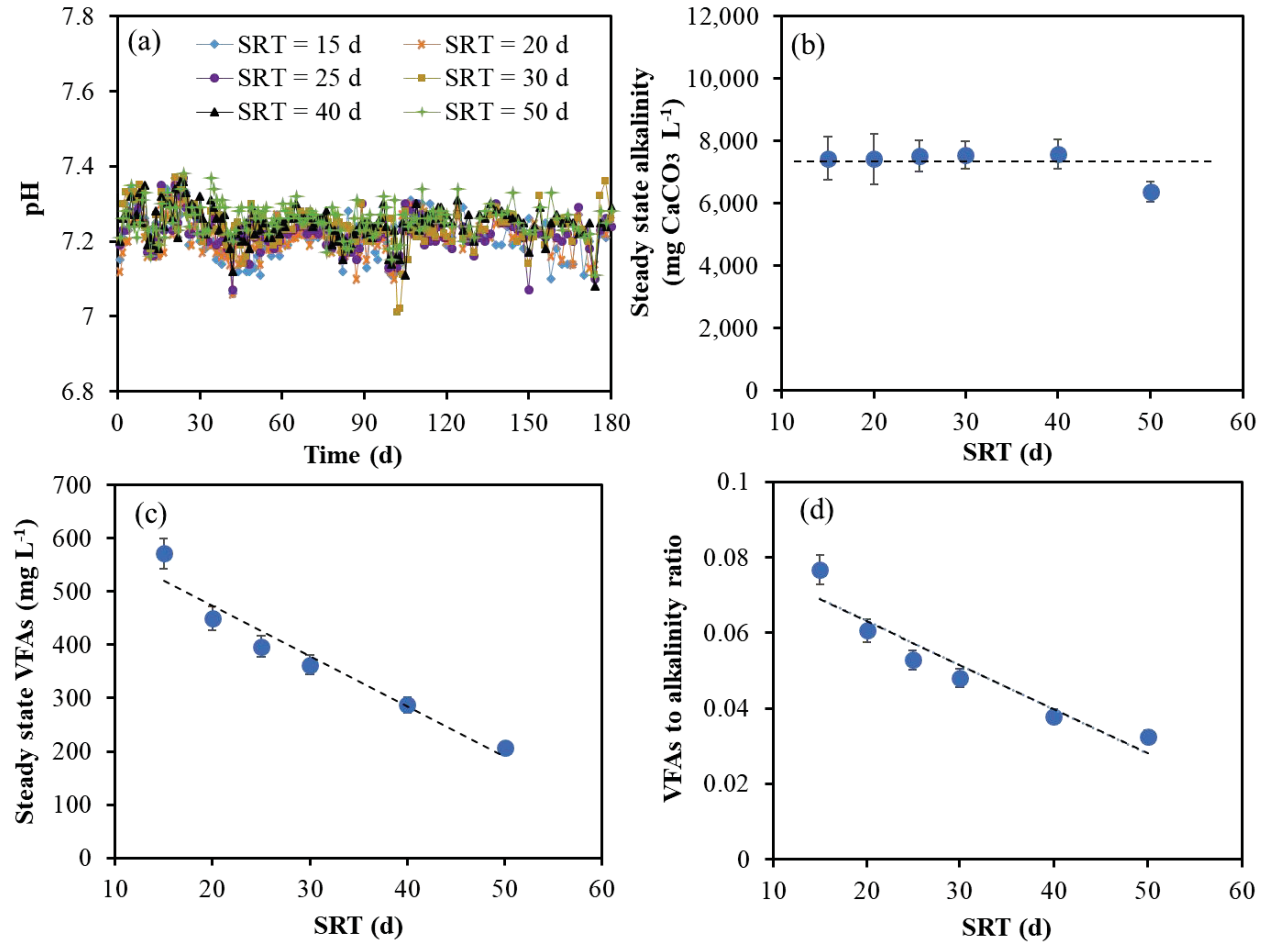


Figure 3.4 Effect of SRT on a) digester pH in the course of 186-day operation, and b) Alkalinity, c) VFAs, and d) VFA-to-Alkalinity ratio averaged from day 120 to 186.

3.6.2 Effect of SRT on solid reduction and effluent protein contents

Figure 3.5a shows average TS and VS reduction around 45% and 55-58%, respectively, which reveals that the SRT increase from 15 to 50 days had an insignificant effect on solids reduction indicated by p-values in a range of 0.17 - 0.48. The similar observation was also reported by others (Chen et al. 2012, Hindin and Dunstan 1960, Lee et al. 2011, Rittmann and McCarty 2012). Figure 3.5a implies that increasing SRTs from 15 to 50 days can only slightly improve the solids reduction, and almost the same level of VS should be expected in the anaerobic digestion effluent. As mentioned previously, the protein contents remaining in biosolids after anaerobic digestion are

highly correlated to VOSCs production. Thus, the protein contents in digester effluent were measured and shown in Figure 3.5b. It can be seen that the protein content decreased from 0.37 to 0.35 and then to 0.31 g g⁻¹ dry solids with the increase of SRT from 15 days to 30 days and then to 50 days, respectively. Consistent with the effect of SRT on VS reduction, the effluent protein level showed insignificant difference as SRT increased, which is indicated by p-values in a range of 0.13 - 0.46. This implies that effective anaerobic digestion in terms of organic and protein reduction can be achieved with a SRT as short as 15 days, and increasing SRT to 50 days cannot substantially attenuate the concentration of effluent protein, the odor precursors.

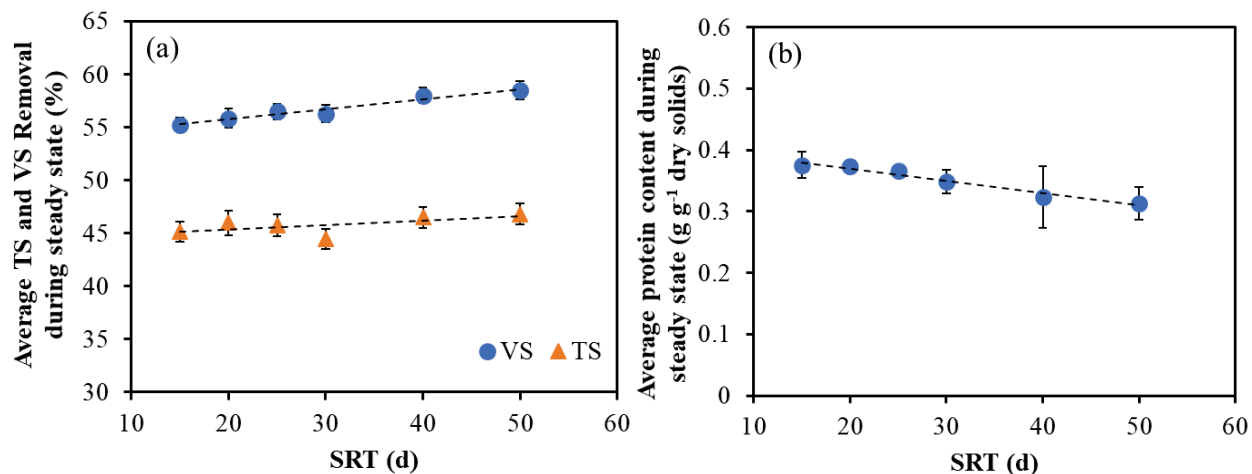


Figure 3.5 Effect of SRT on a) TS and VS reduction, and b) protein content averaged over the steady state operation from day 120 to 186.

3.6.3 Effect of SRT on biosolids dewaterability at various polymer doses and shear intensity

The cake dryness under each SRT used in this study was examined in Figure 3.6 in response to various dewatering shear intensity and polymer doses. To the best of our knowledge, the effect of SRT on the dewaterability of anaerobic digestion biosolids has not been reported in literature. It can be seen that SRTs actually had very limited effect on the cake dryness. For example, the dewaterability of the 15-day SRT biosolids is almost the same as that of the 50-day SRT biosolids cake prepared with the same polymer doses and shear intensity. Again, ANOVA results indicated that SRT played insignificant effect on cake TS ($p = 0.66$). Indeed, the shear intensity and polymer doses demonstrated greater impact than SRT on the cake dryness (Figure 3.6) with a p-value of 0.007 and 0.004, respectively. It can be observed in Figure 3.6 that higher shear intensity tends to consume more polymer to achieve the same cake dryness. Figure 3.6 demonstrates that 30% more

polymer dose was required to achieve the same cake dryness when the shear intensity ($G \cdot t$) was increased from 1×10^4 in belt press to 11×10^4 in centrifuge (Figure 3.6). In addition, Figure 3.6 also revealed that increasing polymer dose can greatly improve cake dryness e.g. for 10% as TS, however, the cake dryness can decline when polymer was overdosed beyond the optimal dose.

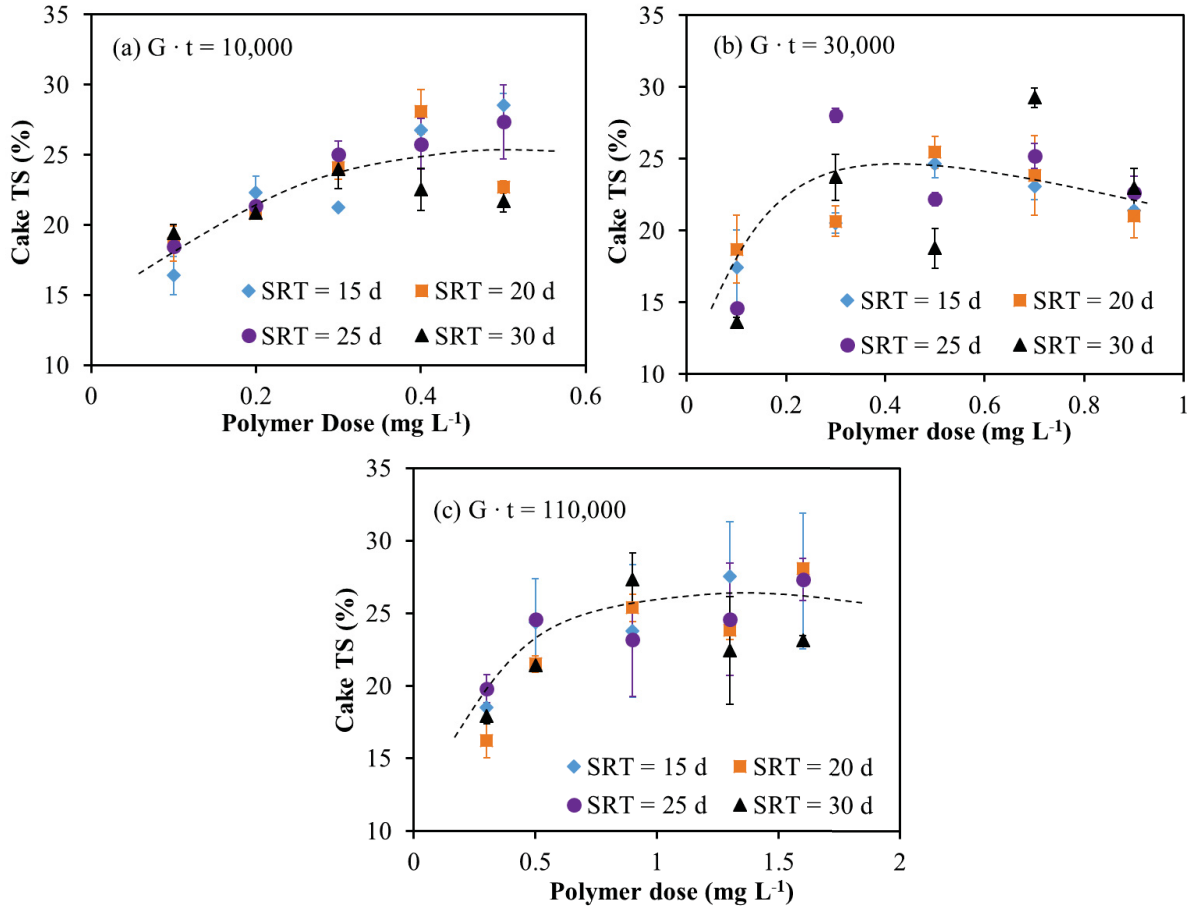


Figure 3.6 Effect of SRT on cake dryness as a function of polymer dose under the shear intensity of a) belt press at $G \cdot t = 1 \times 10^4$, b) plate and frame press at $G \cdot t = 3 \times 10^4$, and c) centrifuge dewatering process at $G \cdot t = 11 \times 10^4$.

3.6.4 Effect of SRT on odor emission during the cake storage

Figure 3.7 reveals the bell curves of MT and DMS concentration over the storage time. The MT and DMS concentrations increased during anaerobic storage, peaked at about day 2, and then declined to nearly undetectable levels between day 4 and 5. It has been recognized that MT and DMS are produced from the microbial utilization of sulfur-containing proteins, more specifically the amino acids of methionine and cysteine, and both MT and DMS can be utilized by

methanogens as substrates (Du and Parker 2012, Higgins et al. 2006, Zhang et al. 2018). Thus, the bell curves in Figure 3.7 can be explained by a fast regrowth of odor producing microbes and a delayed regrowth of methanogens following biosolids dewatering. Probably due to the little difference in protein contents of the biosolids obtained at different SRTs (Figure 3.5b), there was no clear pattern observed for the effect of SRT on peak biosolids odor emission in Figure 3.7. There was only a small decrease on peak biosolids odor emission at low shear condition when a longer digester SRT was used in Figure 3.7a and b. Prominently, three-order of increase in both MT and DMS emission were resulted when the shear intensity increased about 11 times and the polymer dose increased four times (Figure 3.7).

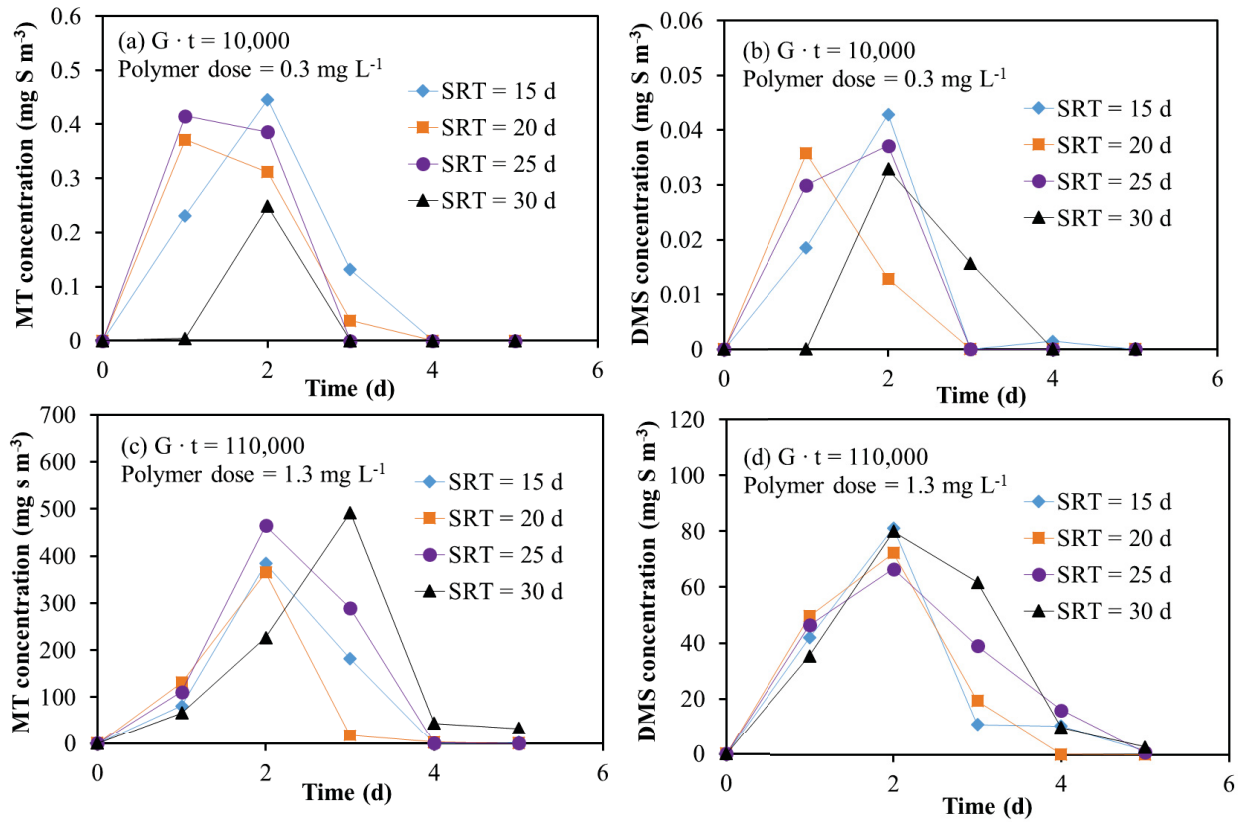


Figure 3.7 Effect of SRT on MT (a, c) and DMS (b, c) concentration emitted as a function of storage time from the cake subject to a combination of shear intensity of belt press at $G \cdot t = 1 \times 10^4$ and the polymer dose of 0.3 mg L^{-1} (a, b); and a combination of the shear intensity of centrifuge with $G \cdot t = 11 \times 10^4$ and a polymer dose of 1.3 mg L^{-1} (c, d).

3.6.5 Effect of shear intensity on peak odor emission during the cake storage

In view of the prominent impact of shear intensity, its effect on the peak MT and DMS emission was further investigated and plotted in Figure 3.8. In general, Figure 3.8 shows that the peak emissions exponentially increased with the shear intensity for sludges digested at different SRTs, indicating a more prominent effect of shear intensity within the range. For instance, the 30-day SRT biosolids dewatered by the centrifuge ($G \cdot t = 11 \times 10^4$) gave 70 and 36 times greater MT peak emission than those dewatered by belt press ($G \cdot t = 1 \times 10^4$) and plate and frame press ($G \cdot t = 3 \times 10^4$), respectively (Figure 3.8c). The significant influence of shear intensity on odor emission was shown with a p-value of 0.0002, while the SRT showed insignificant effect on odor emission with a p-value of 0.27.

Several mechanisms should be considered for the specific role of shear intensity in sulfur odor emission from dewatered cakes. First, high intensity shear forces have been shown to be capable of releasing sulfur-containing proteins wrapped in anaerobically digested sludge as odor precursors for microbial utilization (Chen et al. 2011a). Second, high shear intensity is known to lead to structural changes of proteins and enhance their bioavailability. Studies showed that shearing can cause conformational changes including molecule unfolds and peptide breakage in some proteins, and the structural changes were more substantial when high shear is applied at an air-liquid interface which is also the case in industrial dewatering centrifuge (Maa and Hsu 1996, Maa and Hsu 1997). Third, it has been recognized that methanogens need to work in proximity to acetogenic or hydrogen producing bacteria through a syntrophic relationship (McCarty and Smith 1986, Stroot et al. 2001). The dispersion of biosolids flocs by high shearing in centrifuge might have disturbed such syntrophic relationship by breaking up the microbial consortia, inhibiting the methanogenesis as well as the MT and DMS utilization by methanogens (Dolfing 1992, McCarty and Smith 1986, Stroot et al. 2001, Whitmore et al. 1987). This agrees with the observed bell-shaped MT and DMS profiles measured during cake storage in Figure 3.7, evidencing the delayed regrowth of methanogens.

Moreover, Figure 3.8 shows that the sludge treated under longer SRTs tends to give higher peak odor emissions in response to the higher shear intensity. A higher shear intensity must have released more sulfur-containing proteins contained in the digested sludge as odor precursors for microbial utilization (Chen et al. 2011a), which explains the exponential increase of odor emission

along with the shear intensity increase (Figure 3.8). Since insignificant difference in protein contents was measured in sludge stabilized at different SRTs (Figure 3.5b), the possible reason for the higher peak emission at longer SRTs may be attributed to the fact that the sludge treated under a longer SRT tends to retain greater abundances of microbes including those MT and DMS producers capable of driving faster odor production.

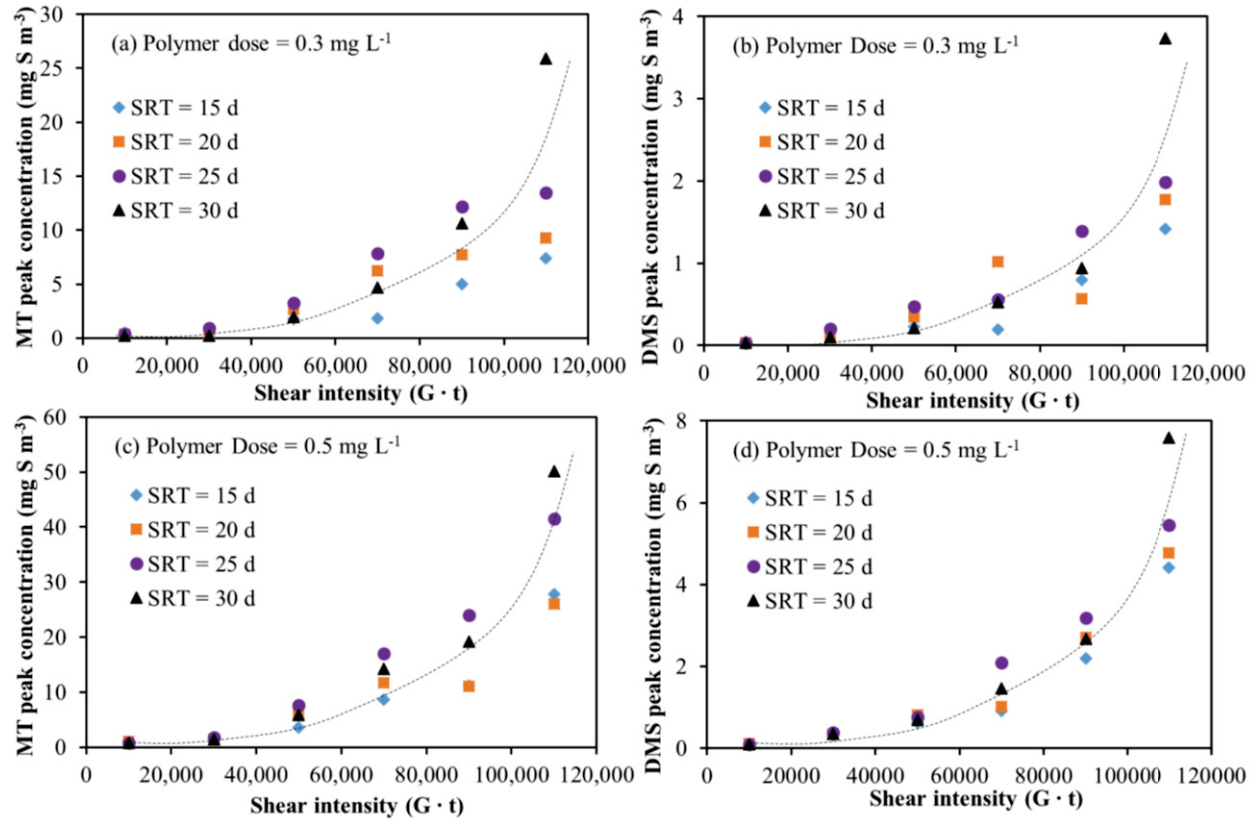


Figure 3.8 Effect of SRT on the peak MT (a, c) and DMS (b, d) concentrations emitted as a function of shear intensity at the polymer doses of 0.3 mg L⁻¹ (a, b) and 0.5 mg L⁻¹ (c, d) under various SRTs.

3.6.6 Effect of polymer doses on peak odor emission during the cake storage

The contribution of polymer doses to odor emission was further investigated in Figure 3.9, in which peak emissions of MT and DMS exponentially increased with the polymer doses. The influence of polymer dose on odor emission gave a p-value of 3×10^{-8} , while the SRT consistently showed insignificant effect on odor emission with a p-value of 0.23. It should be noted that dewatering under more intensive shearing typically result in a greater polymer demand to achieve the same cake dryness as shown in Figure 3.6, consistent with previous studies (Burton et al. 2013,

Werle et al. 1984). Figure 3.9 indicates that a high polymer dose (e.g. 1.6 mg L^{-1}) in combination with a high shear intensity ($G \cdot t = 11 \times 10^4$) produced the highest MT and DMS peak concentrations, for example 1400 and 210 mg S m^{-3} , respectively, from the 30-day SRT biosolids, which is three-order greater than the odor emission at the low shear intensity ($G \cdot t = 1 \times 10^4$) and polymer dose (e.g. 0.5 mg L^{-1}). These suggest that cationic polymer may also play an essential role in the aforementioned shear-protein interactions, i.e. a higher polymer dose might have coagulated more bioavailable proteins released from flocs dispersion in the cake and left less in the centrate, leading to more MT and DMS production during cake storage. Besides, possible conformational changes of proteins including molecule unfolds and peptide breakage under high shear may also have elevated protein coagulation by cationic polymer (Burton et al. 2013). Once again, Figure 3.9 shows that the sludge treated with longer SRT tends to give off more odor emission under a higher shear intensity ($G \cdot t = 11 \times 10^4$) likely due to the greater retention of the microbial population of MT and DMS producers and also the residual proteins (0.35 to 0.37 g g^{-1} dry solids) after anaerobic digestion (Figure 3.5b).

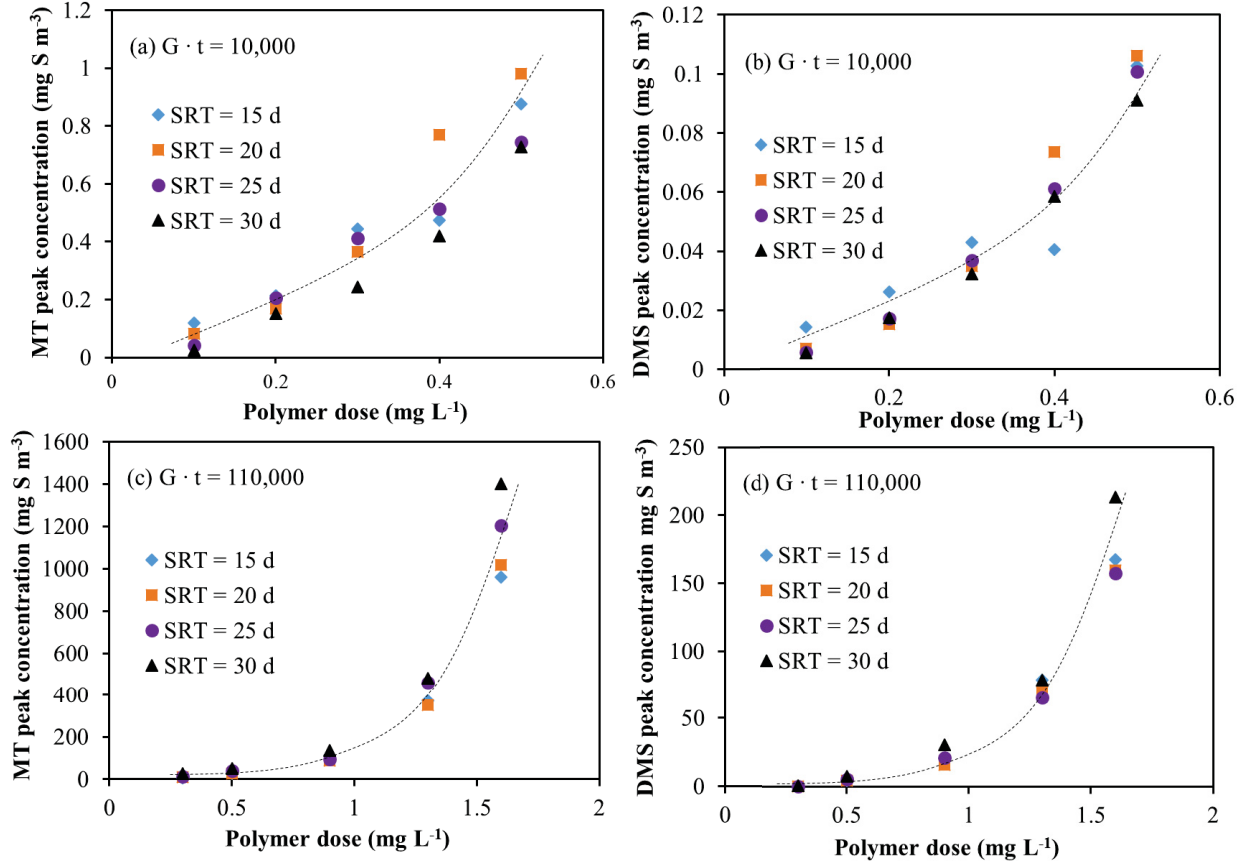


Figure 3.9 Effect of polymer dose on the peak MT (a, c) and DMS (b, d) concentrations emitted from dewatered cake under the shear intensity of belt press with $G \cdot t = 1 \times 10^4$ (a, b) and centrifuge with $G \cdot t = 11 \times 10^4$ (c, d) under various SRTs

3.7 Conclusions

During the anaerobic storage of biosolids, no significant variance on odor emission was observed for biosolids stabilized at different SRTs, which can be explained by the insignificant effect of SRT on protein contents in digested sludge. In addition, SRT also showed insignificant effect on biosolids dewaterability in terms of the cake dryness. In general, increasing SRT beyond 15 days provides little benefit on biosolids quality as well as the anaerobic digestion performance. In contrast, polymer dose and shear intensity typically involved in dewatering practices showed significant impact on biosolids dewaterability and odor emission. The peak odor emission was also found to exponentially increase with both the shear intensity and the polymer dose. The results imply that low shear dewatering methods such as belt press and plate & frame press are preferred

to high shear centrifuge for biosolids handling facilities with odor concerns, and incomplete anaerobic digestion at SRT as short as 15 days is not a major cause of odor nuisance.

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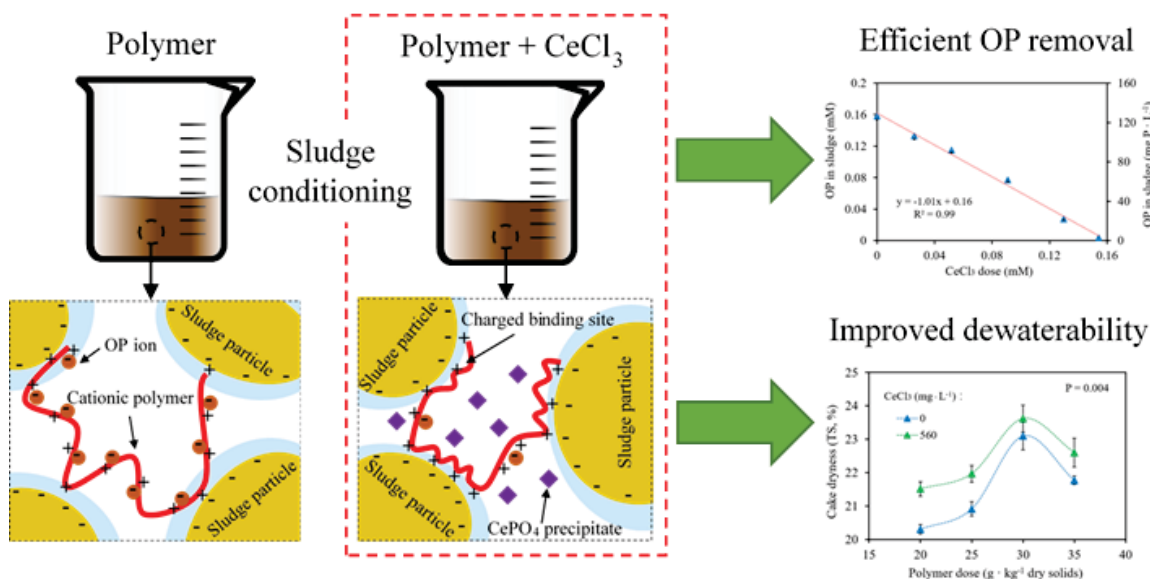
Chapter 4 : Using cerium chloride to control soluble orthophosphate concentration and improve the dewaterability of sludge: Part I. Mechanistic understanding

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4.1 Abstract

Cerium chloride (CeCl_3), being a superior OP precipitant, was found to be able to significantly improve sludge dewaterability in terms of sludge cake dryness and CST. In order to offer insights into the mechanism behind OP removal associated dewaterability improvement, the change in sludge SRF, compressibility (K), and bound water contents (U_b) in response to CeCl_3 and CePO_4 addition at the two cationic polymer doses was mathematically simulated. Results showed that $29.8 \text{ g} \cdot \text{kg}^{-1}$ dry solid CePO_4 addition was able to decrease the SRF by 52%, decrease the U_b by 42%, and reduce the K by 18%. Importantly, CeCl_3 addition of equal cerium molarity showed even higher SRF and U_b reductions by 67% and 54%, respectively, but the same K reduction. A new theory depicting how the OP has outcompeted negatively charged sludge particles for cationic polymers is put forward in this study to interpret the effect of OP removal on sludge dewaterability improvement.

4.2 Graphical abstract



4.3 Keywords

Biosolids; Dewaterability; Struvite; CST; Compressibility; Sludge dryness

4.4 Introduction

The release of OP from processes, e.g. anaerobic digestion, handling HRAS that contains high phosphorus content often leads to the formation of crystalline phosphate scale on the internal walls of pipes, pumps, and valves used in the post digestion processes, a problem that increases the maintenance cost and reduces the hydraulic capacity of pipes (Doyle & Parsons, 2002). This problem becomes even more prominent when centrifuges are used for sludge dewatering in that it results in carbon dioxide degassing and thus an increase in the pH to above 7. At this elevated pH, the crystallization of struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) can result when ammonia, magnesium, and OP ions are above 20, 27, and 106 $\text{mg} \cdot \text{L}^{-1}$, respectively, as has often been measured in the liquid phase of anaerobically digested sludges (Marti, Bouzas, Seco, & Ferrer, 2008; Ohlinger, Young, & Schroeder, 1998; Stratful, Scrimshaw, & Lester, 2001).

OP removal through precipitation by adding metal ions such as magnesium, aluminum, and iron (III) is a common approach employed for preventing phosphate deposition (Huang, Liu, & Ding, 2015; Marti et al., 2008; Stabnikov, Tay, Tay, & Ivanov, 2004). It is noteworthy that the OP removal efficiency is limited by the solubility of the metal precipitates as well as the pH. In order

to further improve the OP removal efficiency, overdosing and/or pH adjustment is often needed, leading to added chemical usage and sludge volume increases. For example, 72% aluminum overdosing was used to achieve OP reduction of 85%, and this resulted in a 50% sludge volume increase and 42% sludge mass increase as reported by Baillod, Cressey, and Beaupre (1977), Schmidtke (1985), and Wang, Vaccari, Li, and Shammas (2005). Clearly, a more efficient OP precipitation is desired to achieve high OP removal with low sludge volume increases at minimum chemical addition. We used CeCl_3 as a more efficient OP precipitant in this study due to the low solubility of CePO_4 ($\text{pK}_s = 25.3$) compared to other precipitates (MgNH_4PO_4 , $\text{pK}_s = 13.2$; AlPO_4 , $\text{pK}_s = 20.0$; and $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$, $\text{pK}_s = 15.0$) at neutral pH (CRC, 2007; Johannesson, Lyons, Stetzenbach, & Byrne, 1995; Musvoto, Wentzel, & Ekama, 2000).

In addition to the scale prevention, sludge with lower OP concentration has also been found to possess better sludge dewaterability. The potential mechanism behind was attributed to the higher equilibrium divalent cation content at lower OP concentration, which might have enhanced the sludge dewaterability through the particle bridging (Alm, Sealock, Nollet, & Sprouse, 2016; Benisch, Schauer, & Neethling, 2014; Bergmans, Veltman, van Loosdrecht, van Lier, & Rietveld, 2014; Higgins, Bott, Schauer, & Beightol, 2014; Wu, Bishop, & Keener, 2005; Zhou, Kelly, Mavinic, & Ramey, 2001). Given the potentially higher OP removal capacity of CeCl_3 , we anticipate that its effect on sludge dewaterability will be more substantial. However, the application of CeCl_3 as a more effective OP precipitant has rarely been reported in the field of wastewater treatment, and its potential effect on the dewaterability improvement has not been investigated either.

Various models have been proposed to define water distribution in sludge (Möller, 1983; Mowla, Tran, & Allen, 2013; Sanin, Clarkson, & Vesilind, 2011; Sfflollen, 1988). One frequently used approach generally classified the water distribution in sludge as free and bound water as illustrated in Figure 4.1 (Colin & Gazbar, 1995; Dick & Ball, 1980; Herwijn, 1996; Vaxelaire & Cézac, 2004; Vesilind & Hsu, 1997). The free water usually refers to: i) the interspace water that is distributed outside of the flocs and can be easily separated by sludge settling; and ii) the floc water that is trapped within the sludge floc structure under gravity but can be partially separated from the sludge by the mechanical strains provided by dewatering methods such as compression or centrifuge. The bound water usually denotes: iii) the surface and hydrated water that is physically and chemically

bound to the sludge particle surfaces; and iv) the intracellular water that is trapped within cells. It is our hypothesis that the OP removal along with the CeCl_3 addition may change the sludge water distribution shown in Figure 4.1. Thus, in this study, we not only evaluated the effect of CeCl_3 addition on the OP removal efficiency, the CST, and the sludge cake dryness, but also we estimated the changes of the SRF, compressibility, and bound water content of the sludge in response to the CeCl_3 addition. This study is designed to provide insight into the mechanism of the sludge dewatering improvement through OP removal by CeCl_3 addition.

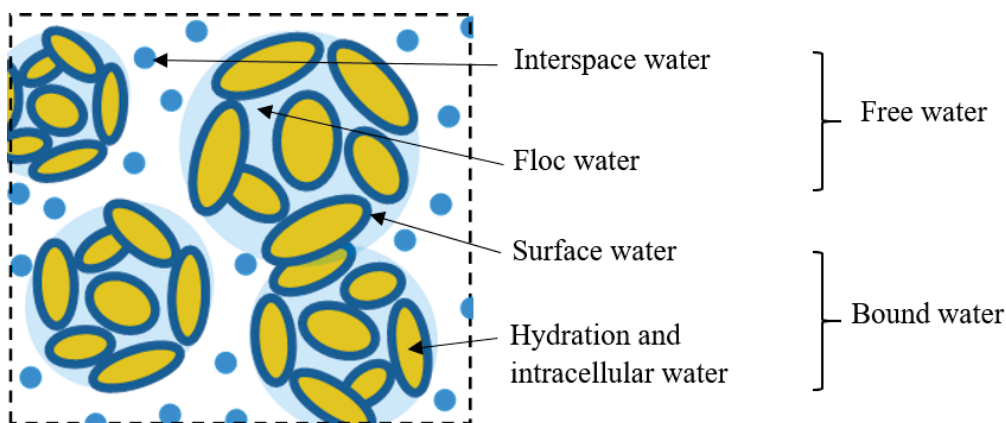


Figure 4.1 Illustration of water distribution in biological sludge

4.5 Materials and methods

4.5.1 Sample collection and preparation

Thickened waste activated sludge (TWAS) and digestate were collected from the Upper Occoquan Service Authority (UOSA) at Centreville, Virginia where the modified Ludzack-Ettinger process is used for biological nutrient removal, while lime and alum are used for mainstream phosphorus removal. TWAS stabilization is performed in mesophilic anaerobic digesters, and the excessive TWAS is blended with digestate for dewatering at a volumetric ratio between 10% and 20% in the centrifuge feed tank at the UOSA. Sludge samples collected and blended on different days were used for this study. To avoid significant OP and dewaterability changes due to long-term storage during the experiment period, the fresh sludge sample had to be collected multiple times when they were needed for the experiment. The characteristics of these multiple samples, denoted as the Sample A, B, and C, are listed in the Table 4.1. The 5.6 g L^{-1} cationic polymer (Clarifloc SE-587,

Riceboro, GA) was dosed in this study for sludge conditioning. The 1.62 M CeCl_3 solution (Neo RE300, Toronto, Canada) was added for the OP removal.

Table 4.1 Characteristics of the sludge samples

Sludge	TWAS blended (% v/v)	Blend TS (% m/m)	OP ($\text{mg P} \cdot \text{L}^{-1}$)	pH
Sample A	20%	3.12%	122.5	7.08
Sample B	10%	2.84%	161.0	7.18
Sample C	20%	3.25%	161.0	7.10

4.5.2 Analytical procedures

To quantify the sludge OP, the mixed sludge sample was first centrifuged at $1000 \times g$ for 20 minutes. Then, the centrate was filtered through 1.5 μm and 0.45 μm filters in sequence. The final filtrate was measured for OP using a molybdovanadate method test kit (TNT plus 846, Hach, Ontario, Canada). The particle size distribution in the sludge was quantified using a laser scattering particle size distribution analyzer (Horiba Partica LA-950V2, Kyoto, Japan). The CST was measured according to Standard Methods (APHA, 2012) using a capillary suction timer (OFITE 294-50, Houston, USA). Whatman # 17 filter paper (Maidstone, UK) was used in the capillary suction apparatus. TS was measured according to Standard Methods (APHA, 2012).

4.5.3 Laboratory centrifuge dewatering setup

A laboratory dewatering protocol was established to simulate the industrial centrifuge dewatering processes. Three key steps were included in the protocol as shown in Figure 4.2: (a) polymer conditioning (with or without CeCl_3 addition) under mechanical shearing; (b) centrifugal sedimentation; and (c) cake compression. Briefly, 500 ml blended sludge samples were first mixed with polymer and/or CeCl_3 , and then sheared in a high-power commercial blender (Waring MX1200XTXP, Stamford, CT) under the controlled root mean velocity gradient (G , s^{-1}) for the duration (t_s , s) as indicated in Figure 4.2a. $G \cdot t_s$ is commonly used as a non-dimensional expression to quantify shear input that can be calculated using the shear duration, and the root mean velocity gradient in Eq. (4.1) according to the studies by Letterman, Quon, and Gemmell (1973) and Werle, Novak, Knocke, and Sherrard (1984):

$$G = \sqrt{\frac{2\pi \cdot g \cdot N \cdot T}{60 \cdot V_s \cdot v \cdot \rho_w}} \quad (4.1)$$

in which g is gravitational acceleration constant ($\text{m} \cdot \text{s}^{-2}$); N is the paddle rotational speed (rpm); T is the net torque on paddle ($\text{kg} \cdot \text{m}$); V_s is the sample volume (m^3); v is the sludge kinematic viscosity ($\text{m}^2 \cdot \text{s}^{-1}$) measured for each sludge samples using a viscometer (Size 1C, Cannon, State college, PA); and ρ_w is the density of water ($\text{kg} \cdot \text{m}^{-3}$). The blender was modified according to the work by Camp (1968) in order to measure the torque and paddle rotational speed. The $G \cdot t_s$ was controlled at 10^5 which is a typical value of the industrial centrifuges including the one used in UOSA (Murthy, 2004; Novak, 2006).

For the second and third steps shown in Figure 4.2b and c, 105 ml sheared sludge was preliminarily dewatered with a lab centrifuge (Sorvall Legend X1R, Fisher Scientific, Waltham, MA) under constant centrifugal acceleration of $5000 \times g$ and a time of 20 min. Then, 15 ml centrifuged solids was further compressed to produce the dewatered cake using a piston press under controlled pressure of 448 kPa for 30 min. The dryness of the centrifuged solids and the dewatered cake in terms of TS were measured immediately after the step b and c, respectively.

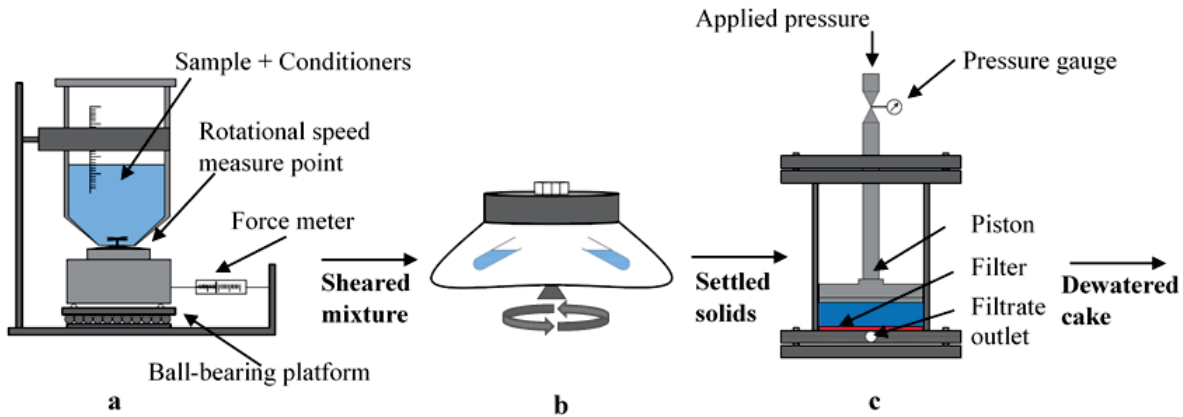


Figure 4.2 Laboratory setup mimicking centrifuge dewatering: a) conditioning under mechanical shearing; b) centrifugal sedimentation; and c) cake compression

4.5.4 Evaluations of the specific resistance to filtration, compressibility, and bound water content

In addition to experimentally measure cake dryness and CST, the other sludge dewatering properties such as SRF, compressibility, and bound water content were also mathematically

calculated as a cost-effective and time-saving measure to offer theoretical insights behind the experimental observation. CST was first developed by Baskerville and Gale (1968) as a more convenient method to substitute the complex and expensive SRF test. Thereafter, intensive mathematical modeling effort has been made to relate the two parameters (Christensen, Sorensen, Christensen, & Hansen, 1993; Ma, Zhao, & Kearney, 2007; Pan, Huang, Cherng, Li, & Lin, 2003; Sawalha & Scholz, 2010). In this study, the average SRF ($\text{m} \cdot \text{kg}^{-1}$) was estimated using CST data based on the Eq. (4.2) developed by Ma, Zhao, and Kearney (2007) from the conventional compressible cake filtration theory (Christensen, Sorensen, Christensen, & Hansen, 1993),

$$CST = t_2 - t_1 = \frac{\mu_f \cdot SRF \cdot w}{2 \cdot A^2 \cdot P} (V_2^2 - V_1^2) + \frac{\mu_f \cdot R_m}{A \cdot P} (V_2 - V_1) \quad (4.2)$$

in which CST is capillary suction time (s), namely the time difference for filtrate to travel in the filter paper to the first (t_1) and second (t_2) sensor of the capillary suction apparatus as a result of the capillary suction during the filtration process; V_1 and V_2 are the filtrate volume at t_1 and t_2 , respectively (m^3); μ_f is the filtrate viscosity ($\text{Pa} \cdot \text{s}$); w is the mass of dry solids deposited per unit volume of filtrate ($\text{kg} \cdot \text{m}^{-3}$); A is the CST filter paper area (m^2); P is the pressure of the sludge column on the filter paper (Pa); R_m is the filter paper resistance to the filtration (m^{-1}). The second term on the right-hand side of Eq. (4.2) can be determined by measuring the CST of pure water (CST_w),

$$CST_w = \frac{\mu_f \cdot R_m}{A \cdot P} (V_2 - V_1) \quad (4.3)$$

Substituting Eq. (4.3) into (4.2) gives,

$$CST - CST_w = \frac{\mu_f \cdot SRF \cdot w}{2 \cdot A^2 \cdot P} (V_2^2 - V_1^2) \quad (4.4)$$

Solving Eq. (4.4) for SRF gives,

$$SRF = \frac{2 \cdot A^2 \cdot P \cdot (CST - CST_w)}{\mu_f \cdot w \cdot (V_2^2 - V_1^2)} \quad (4.5)$$

In order to determine the SRF using Eq. (4.5), the 23 mm diameter of a filter media placed at the bottom of the CST device was used to calculate the A ; CST_w , V_1 , and V_2 were measured as 7 ± 0.2 s, $1.5 \pm 0.1 \times 10^{-7} \text{ m}^3$, and $7.0 \pm 0.1 \times 10^{-7} \text{ m}^3$, respectively, at the room temperature by using ultrapure water. w was calculated using the sludge TS of Sample C shown in the Table 4.1 and a sludge density of $1103.18 \text{ kg} \cdot \text{m}^{-3}$ monitored by UOSA. The sum of the sludge head and filter

paper suction pressure of 15 kPa and the water viscosity of $1.1 \times 10^{-3} \text{ Pa} \cdot \text{s}$ at room temperature were used for P and μ_f , respectively (Baskerville & Gale, 1968; Leu, 1981). Eq. (4.5) shows that SRF linearly correlates with the CST normalized by the sample solids content for given capillary suction apparatus. The same correlation has also been established by others (Christensen, Sorensen, Christensen, & Hansen, 1993; Ma, Zhao, & Kearney, 2007; Peng, Ye, & Li, 2011; Vesilind, 1988).

The bound water content was determined in the Eq. (4.6) based on the classical method of compression-permeability cell which has the same configuration as the lab piston press shown in Figure 4.2c (Herwijn, 1996; Ruth, 1946):

$$U_w = U_b + \frac{\varepsilon}{(1 - \varepsilon)} \cdot \left(\frac{\rho_w}{\rho_s} + U_b \right) \quad (4.6)$$

in which U_w is the sample moisture content ($\text{kg water} \cdot \text{kg}^{-1} \text{ dry solids}$); U_b is the sample bound water content ($\text{kg bound water} \cdot \text{kg}^{-1} \text{ dry solids}$); ε is the cake porosity; ρ_w is the density of the water ($\text{kg} \cdot \text{m}^{-3}$); and ρ_s is the density of the dry solids ($\text{kg} \cdot \text{m}^{-3}$). $1400 \text{ kg} \cdot \text{m}^{-3}$ was used for ρ_s (Qasim, 1998). As described in the introduction and illustrated in Figure 4.1, Eq. (4.6) is established based on the assumption that the sludge consists of dry solids as well as the free and bound waters. Applying Eq. (4.6) to the sludge at the initial and final stages of the cake compression test, assuming mechanical compression does not change the sludge bound water content, gives,

$$U_w^o = U_b + \frac{\varepsilon_o}{(1 - \varepsilon_o)} \cdot \left(\frac{\rho_w}{\rho_s} + U_b \right) \quad (4.7)$$

$$U'_w = U_b + \frac{\varepsilon'}{(1 - \varepsilon')} \cdot \left(\frac{\rho_w}{\rho_s} + U_b \right) \quad (4.8)$$

in which U_w^o and U'_w are the initial and final cake moisture contents ($\text{kg water} \cdot \text{kg}^{-1} \text{ dry solids}$) that can be calculated from the sludge TS measured after the Step b and c shown in Figure 4.2, respectively; very similar U_w^o ($10.7\% \pm 0.094$) was obtained under the polymer dose of 25 and 30 $\text{g} \cdot \text{kg}^{-1} \text{ dry solids}$, respectively for sample C; ε_o and ε' are the initial and final cake porosity that can be estimated from Eq. (4.15). First, solving ε_o and ε' from Eqs. (4.7) and (4.8), respectively. Then, solving U_b from the ratio of the two ε_o and ε' equations gives,

$$U_b = \frac{U_w^o \cdot (\rho_w + \rho_s \cdot U_w') - \frac{\varepsilon_o}{\varepsilon'} \cdot U_w' \cdot (\rho_w + \rho_s \cdot U_w^o)}{(\rho_w + \rho_s \cdot U_w') - \frac{\varepsilon_o}{\varepsilon'} \cdot (\rho_w + \rho_s \cdot U_w^o)} \quad (4.9)$$

It should be noted that the ratio $\frac{\varepsilon_o}{\varepsilon'}$ represents the cake porosity change as a result of the piston compression. Apparently, a smaller final cake porosity (ε') after dewatering will indicate a better cake compressibility, thus a larger $\frac{\varepsilon_o}{\varepsilon'}$. If we define the sludge compressibility as K, namely $\frac{\varepsilon_o}{\varepsilon'} = K$, Eq. (4.9) can be rewritten as,

$$U_b = \frac{U_w^o \cdot (\rho_w + \rho_s \cdot U_w') - K \cdot U_w' \cdot (\rho_w + \rho_s \cdot U_w^o)}{(\rho_w + \rho_s \cdot U_w') - K \cdot (\rho_w + \rho_s \cdot U_w^o)} \quad (4.10)$$

In order to determine the cake compressibility coefficient K, the following correlations can be applied given the filtrate was generated from the change of free water volume contained in the porous spaces, and its volume equals to the sludge volume change after the piston compression,

$$V_f = \varepsilon_o \cdot V_o - \varepsilon' \cdot V_c \quad (4.11)$$

$$V_f = V_o - V_c \quad (4.12)$$

in which V_f is the filtrate volume (m^3), V_o and V_c are the initial and final cake volume (m^3), respectively. Thus, K can be derived from Eqs. (4.11) and (4.12) as,

$$K = \frac{\varepsilon_o(V_o - V_f)}{\varepsilon_o \cdot V_o - V_f} \quad (4.13)$$

V_o is the known sample volume loaded into the piston press, namely 15 ml; V_f can be calculated using the mass balance, i.e. the initial mass of sludge equals the sum of the mass of dewatered cake and the filtrate,

$$V_f = \left(\frac{m_s}{S_o} - \frac{m_s}{S_c} \right) \cdot \frac{1}{\rho_w} \quad (4.14)$$

in which m_s is the mass of dry solids (kg); S_o and S_c are the TS (%) of the sludge and dewatered cake. The only unknown for the determination of U_b and K in Eqs. (4.10) and (4.13) is ε_o , namely the porosity of the settled solids obtained from lab centrifuge in the second step of Figure 4.2. Assuming the primary particle in the compressed cake is incompressible, the well-known Kozeny-Carman equation developed for incompressible particles as shown in Eq. (4.15) can be applied to estimate ε_o using the SRF obtained from Eq. (4.5),

$$SRF = \frac{180 \cdot (1 - \varepsilon_0)}{\rho_s \cdot d_p^2 \cdot \varepsilon_0^3} \quad (4.15)$$

in which d_p is the diameter of the primary particle that constitutes the aggregate (m). $0.4 \mu\text{m}$ which is 1/300 to 1/100 of the average aggregate sizes measured in this study was used for d_p according to the work by Guan, Amal, and Waite (2001).

4.5.5 Statistical analysis

All experiment was conducted in duplicate. One-sample t-test was used to test the null hypothesis that the mean difference between the control and the cerium addition groups is equal to zero in terms of the cake dryness and the average aggregate size data presented in Figures 4.4 and 4.5a. The changes of average aggregate size due to CeCl_3 addition was shown in a box plot (Figure 4.5b) marked with extremes, quartiles, median, and mean. For the cake dryness, CST, SRF, K, and U_b , as shown in Figures 4.6 and 4.7, unpaired two-sample t-test was used to compare the control, CePO_4 , and CeCl_3 groups with each other. The P-value < 0.05 was used to reject the null hypothesis.

4.6 Results and discussion

4.6.1 Efficiency of OP removal by CeCl_3 addition

OP removal was investigated by measuring the equilibrium OP concentrations in 40 mL Sample A at various CeCl_3 doses. Figure 4.3 presented the remaining OP concentrations in Sample A at the CeCl_3 doses of 0, 0.65, 1.30, 2.27, 3.25, and 3.85 mM, respectively. It can be seen that the remaining OP concentration decreased linearly from 122.5 to 3.05 mg P $\cdot$ L⁻¹ with an increase in the CeCl_3 dose, and 97.5% OP removal was achieved at a CeCl_3 dose of 3.85 mM. The regressed trend line in Figure 4.3 showed an approximately 1 mole OP removal with each mole of cerium addition which corresponds to the theoretical stoichiometric ratio required for OP precipitation by cerium: $\text{Ce}^{3+} + \text{PO}_4^{3-} = \text{CePO}_4$. For Sample A at a sludge pH of 7.08 as shown in Table 4.1, the OP removal efficiency observed indicates that CeCl_3 is more efficient than magnesium, calcium, aluminum, or iron (III) ions, which have been reported to give 0.36, 0.43, 0.8, and 0.37 mole phosphate removed for each mole of metal ion added at a similar pH (Huang et al., 2015; Mamais, Pitt, Cheng, Loiacono, & Jenkins, 1994). The higher OP removal efficiency for CeCl_3 can be attributed to the lower solubility of CePO_4 ($\text{pK}_s = 25.3$) than that of MgNH_4PO_4 ($\text{pK}_s = 13.2$),

AlPO_4 ($\text{pK}_s = 20.0$), or $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$ ($\text{pK}_s = 15.0$) at neutral pH (CRC, 2007; Musvoto et al., 2000; Johannesson et al., 1995). Additionally, the higher valence of Ce^{3+} than that of Ca^{2+} likely also plays an advantageous role through its higher stoichiometric ratio in reaction with OP, even though $\text{Ca}_3(\text{PO}_4)_2 \cdot x\text{H}_2\text{O}$ formed at neutral pH has a similar pK_s of 25.5 (CRC, 2007; Musvoto et al., 2000).

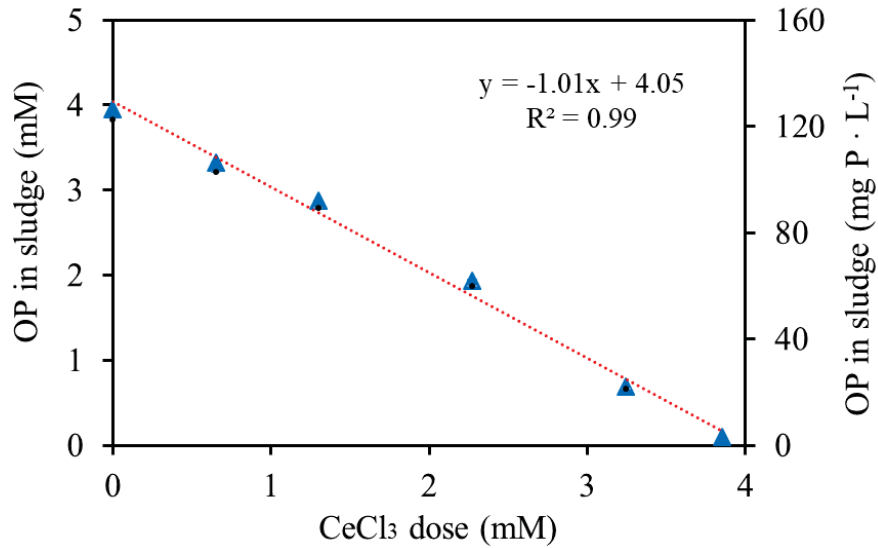


Figure 4.3 Equilibrium OP concentrations in Sample A at various CeCl_3 doses

4.6.2 Effect of CeCl_3 addition on dewatered cake dryness at various polymer doses

We evaluated the effect of CeCl_3 addition on the dewaterability of the sludge blended with TWAS and the digestate (Sample B in Table 4.1) at various polymer doses. A dose of 2.27 mM CeCl_3 was added to achieve an equilibrium OP concentration of $90.5 \text{ mg P} \cdot \text{L}^{-1}$ which is below the critical OP concentration of $106 \text{ mg} \cdot \text{L}^{-1}$ required for the struvite formation at a pH of 7 to 10 (Marti et al., 2008). The TS for the dewatered cake obtained from these experiments using the laboratory dewatering system are shown in Figure 4.4. The dewatered cake dryness increased as polymer dose increased until a peak was reached at an optimal polymer dose around $30 \text{ g} \cdot \text{kg}^{-1}$ dry solids. Above $30 \text{ g} \cdot \text{kg}^{-1}$ dry solids, the dewatered cake dryness decreased with increasing polymer dose. Interestingly, significant improvement ($P = 0.004$) to sludge dewaterability was observed with CeCl_3 addition compared to the control that did not have CeCl_3 added at all polymer doses used in Figure 4.4. With 2.27 mM CeCl_3 added to the sludge samples, improvements in sludge cake dryness of 5.9%, 5.0%, 2.3%, and 3.8% were achieved with polymer doses of 20, 25, 30, and 35

$\text{g} \cdot \text{kg}^{-1}$ dry solids, respectively, resulting in a parallel cake dryness profile with sludge cake dryness for CeCl_3 addition running above the control that had no CeCl_3 added. It is noteworthy that the cake dryness improvements with CeCl_3 addition appeared more prominent at the lower polymer doses than those at the higher doses (Figure 4.4).

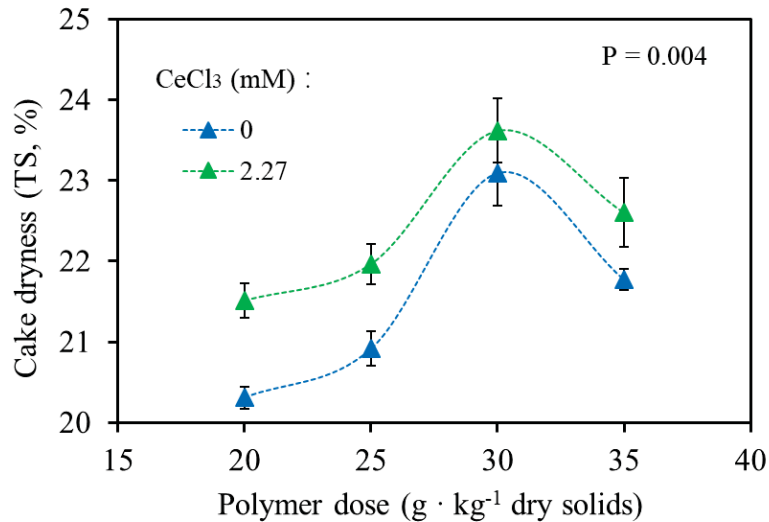


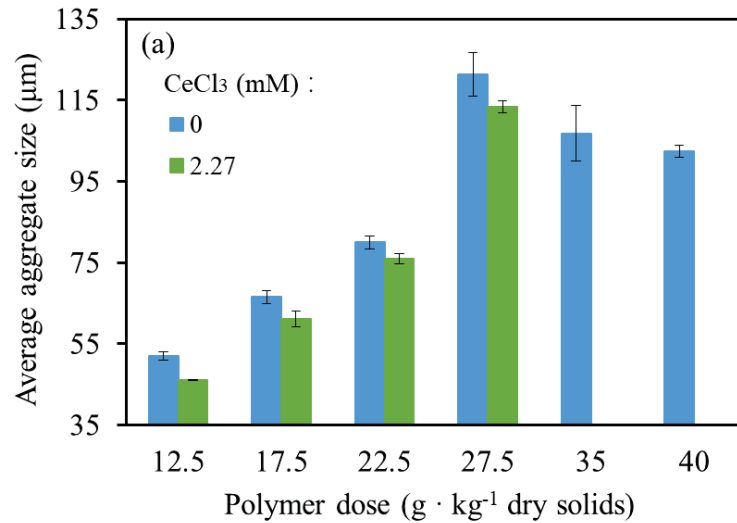
Figure 4.4 The dewatered cake dryness obtained at various polymer doses, with and without CeCl_3 , added into Sample B (characterized in the Table 4.1). The P value represents the significance of cake dryness changes as a result of the CeCl_3 addition.

4.6.3 Effects of CePO_4 precipitate and OP removal on the sludge dewatering

Sludge conditioning using cationic polymer simultaneously involves charge neutralization and polymer bridge formation, through which the negatively charged sludge particles can be flocculated (Jarvis, Jefferson, Gregory, & Parsons, 2005; Metcalf & Eddy, 2014). In order to verify whether CeCl_3 affected the sludge polymer conditioning, we evaluated the effect of CeCl_3 addition on the sludge aggregate sizes. Figure 4.5 presented the average aggregate size of the conditioned and sheared sludge blends under different polymer doses with and without CeCl_3 addition for Sample B in Table 4.1. The average aggregate size increased with polymer dose until a peak is reached around $27.5 \text{ g} \cdot \text{kg}^{-1}$ dry solids which is very close to the optimal polymer dose measured in the Figure 4.4 at $30 \text{ g} \cdot \text{kg}^{-1}$ dry solids. Beyond that, it started to decrease as more polymer was added. By comparing Figure 4.5a to Figure 4.4, it can be seen that the maximum cake dryness was achieved around the same optimal polymer dose ($30 \text{ g} \cdot \text{kg}^{-1}$ dry solids) which coincided with the

largest average aggregate size. Interestingly, the statistical analysis graphically presented in Figure 4.5b shows that the CeCl_3 additions in Figure 4.5a have significantly ($P = 0.003$) decreased the average aggregate sizes by about 4 to 8 μm under the same shear intensity.

In fact, several studies have reported the decrease of sludge aggregate size with the increase of polymer charge density, namely the number of charged binding sites per unit molecular weight or length of the polymer (Eriksson & Alm, 1992; Hjorth & Jørgensen, 2012; Nasser & James, 2007). A higher charge density will result in an increased possibility that multiple charged binding sites will attach to the same sludge particle through charge neutralization, and in turn lead to diminished polymer bridging due to the reduced binding sites and space available for further bridging, thus smaller aggregates tend to be formed (Hjorth & Jørgensen, 2012; Nasser & James, 2007). Consequently, the decrease in the average aggregate size as observed in Figure 4.5 indicated that the particle charge neutralization effect of the cationic polymer might have been enhanced by CeCl_3 addition. This is likely through the precipitation of negatively charged OP that competes with negatively charged sludge particles for the binding sites on the cationic polymers (Jiang & Graham, 1998; Özacar & Şengil, 2003; Tatsi, Zouboulis, Matis, & Samaras, 2003).



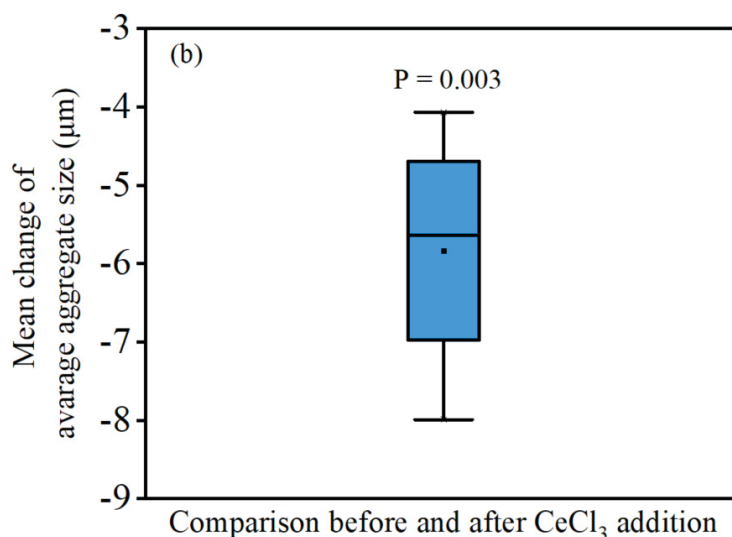


Figure 4.5 a) Average sludge aggregate sizes at various polymer doses for Sample B characterized in Table 4.1 with and without CeCl_3 addition; and b) Statistical analysis of the particle size change (particle size with CeCl_3 addition minus that without) as a result of the CeCl_3 addition

Since CeCl_3 addition significantly decreased the sludge OP level (Figure 4.3), we hypothesized that the dewaterability improvement caused by cerium addition has to do with either the CePO_4 precipitate formation or the removal of OP from the sludge, or both. In order to test this hypothesis, we investigated the respective effect of CePO_4 precipitate addition and OP removal on the dewaterability improvement of Sample C (characterized in Table 4.1) by using the CST and cake dryness as indicators. Basically, CePO_4 and CeCl_3 solutions of equal molarity (2.27 mM) were added into the sludge that had been dosed with polymer at 25 and 30 $\text{g} \cdot \text{kg}^{-1}$ dry solids, respectively. The resulting CST and cake dryness were compared to reflect the individual effect of CePO_4 precipitate and CeCl_3 addition. A control experiment without any cerium addition was also carried out to provide a baseline for the comparison. As can be seen in Figure 4.6, at the lower polymer dose of 25 $\text{g} \cdot \text{kg}^{-1}$ dry solids, both CePO_4 and CeCl_3 addition led to considerable dewaterability improvement. In comparison with the control, the sludge CST decreased 44% and 56%, and the cake dryness increased 5.2% and 9.2% with CePO_4 and CeCl_3 addition, respectively, indicating both the CePO_4 precipitate and CeCl_3 addition played significant roles ($P < 0.05$) in enhancing the sludge dewaterability (Figure 4.6). Moreover, the significant difference ($P < 0.05$) in the dewaterability improvement between CePO_4 and CeCl_3 addition further revealed that the removal of OP also contributed to the dewaterability improvement, since CeCl_3 addition not only formed

the same amount of CePO_4 precipitate ($29.79 \text{ g} \cdot \text{kg}^{-1}$ dry solid) as that was directly added in the CePO_4 addition group, it but also removed $12.04 \text{ g} \cdot \text{kg}^{-1}$ dry solid OP that the CePO_4 addition experiment didn't offer. It also can be seen from Figure 4.6 that, at the higher polymer dose of $30 \text{ g} \cdot \text{kg}^{-1}$ dry solids, the overall dewaterability was improved for all groups as compared to the lower polymer dose of $25 \text{ g} \cdot \text{kg}^{-1}$ dry solids, but the difference made by CePO_4 and CeCl_3 additions became less significant ($P > 0.05$). This observation indicates that the increase of polymer dose from 25 to $30 \text{ g} \cdot \text{kg}^{-1}$ dry solids might have overshadowed the effect of CeCl_3 addition, and thus the cationic polymer and the CeCl_3 addition might have interacted with each other in contributing to the sludge dewaterability improvement as reflected by the CST and cake dryness trends in Figure 4.6.

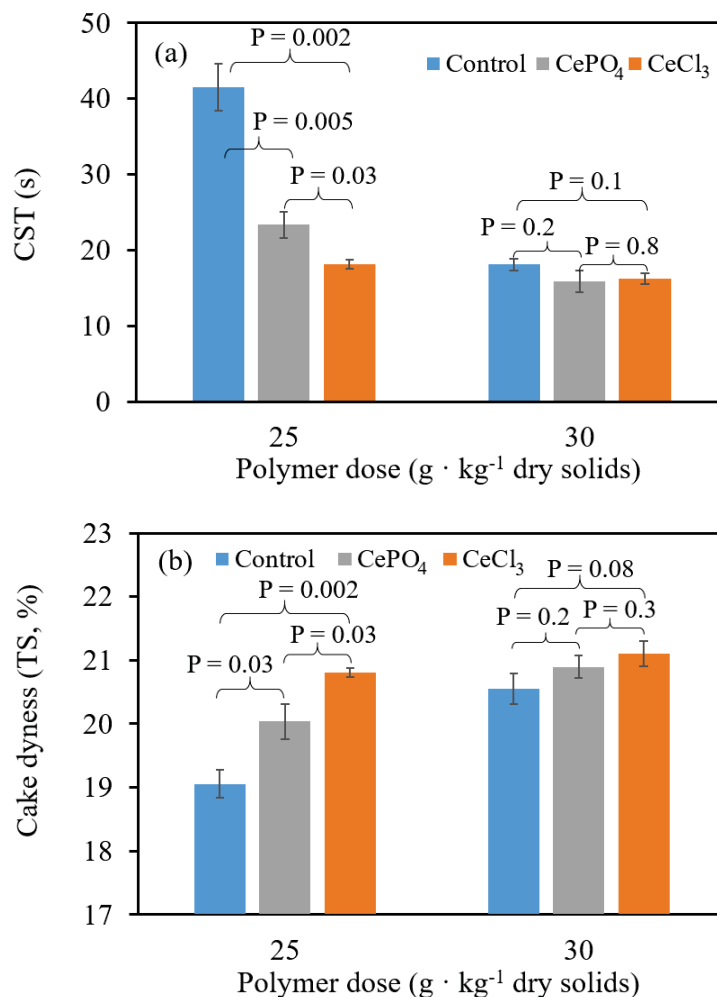


Figure 4.6 a) CST and b) cake dryness in terms of TS measured at the polymer doses of 25 and $30 \text{ g} \cdot \text{kg}^{-1}$ dry solids for Sample C in Table 4.1 with no cerium, 2.27 mM CePO_4 , or 2.27 mM CeCl_3 addition.

4.6.4 Improvement of the specific resistance to filtration, compressibility, and bound water content as a result of the CeCl₃ addition

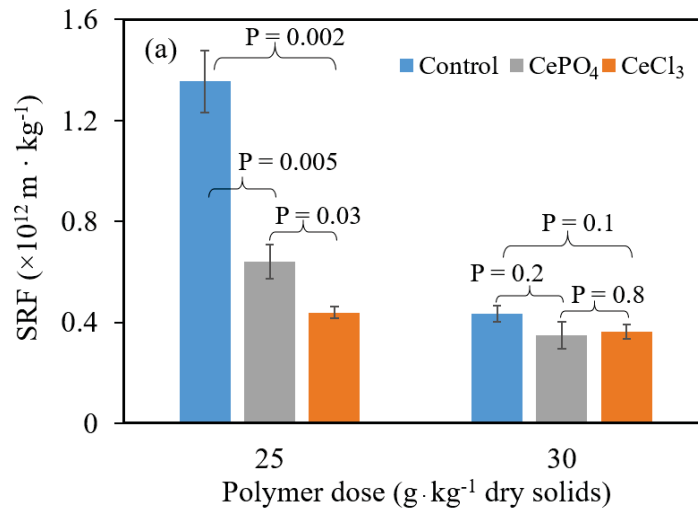
Comparisons of the dewaterability improvement between CePO₄ and CeCl₃ addition in Figure 4.6 indicated that both CePO₄ precipitate and OP removal had contributed to the sludge dewaterability. In order to offer additional insights into the mechanistic role that CePO₄ precipitate plays on dewatering improvements compared to the role of OP removal, the SRF, U_b , and K were calculated using Eqs. (2.5), (2.10), and (2.13) for the data from Figure 4.6.

SRF represents the resistance of the sludge to the drainage of its liquid component (Lue-Hing, 1998). As shown in Figure 4.7a, at a relatively lower polymer dose ($25 \text{ g} \cdot \text{kg}^{-1}$ dry solids), adding 2.27 mM CePO₄ precipitate and 2.27 mM CeCl₃ both significantly ($P < 0.05$) reduced the sludge SRF from $1.3 \pm 0.1 \times 10^{12}$ to $6.4 \pm 0.7 \times 10^{11}$ (52% reduction) and $4.4 \pm 0.2 \times 10^{11} \text{ m} \cdot \text{kg}^{-1}$ (67% reduction), respectively. Dosing CeCl₃ not only formed the same amount of CePO₄ precipitate as that added to the other samples, but it also removed soluble OP from the sludge. Thus, the 15% SRF reduction by CeCl₃ addition over that of CePO₄ precipitate addition can be attributed to the OP removal (Figure 4.7a). At the higher polymer dose ($30 \text{ g} \cdot \text{kg}^{-1}$ dry solids), the control SRF with no cerium addition was 68% lower than the control SRF at the polymer dose of $25 \text{ g} \cdot \text{kg}^{-1}$ dry solids. Again, adding CePO₄ precipitate and CeCl₃ reduced the sludge SRF from $4.3 \pm 0.3 \times 10^{11}$ to $3.5 \pm 0.5 \times 10^{11}$ (20% reduction) and $3.6 \pm 0.3 \times 10^{11} \text{ m} \cdot \text{kg}^{-1}$ (17% reduction), respectively. Interestingly, CeCl₃ addition brought no significant ($P > 0.05$) SRF reduction over CePO₄ precipitate addition at the higher polymer dose of $30 \text{ g} \cdot \text{kg}^{-1}$ dry solids.

Figure 4.7b presented the effects of CePO₄ and CeCl₃ addition on sludge compressibility. It revealed that the addition of CePO₄ precipitate or CeCl₃ decreased the sludge compressibility by 18% at the lower polymer dose of $25 \text{ g} \cdot \text{kg}^{-1}$ dry solids. Additionally, since less significant ($P > 0.05$) compressibility difference was observed between CePO₄ and CeCl₃ addition (Figure 4.7b), it can be concluded that the OP removal had very limited effect on the sludge compressibility improvement.

Sludge conditioning by cationic polymer as well as CePO₄ and CeCl₃ addition also led to decreased bound water according to Figure 4.7c. At the lower polymer dose of $25 \text{ g} \cdot \text{kg}^{-1}$ dry solids, CePO₄ and CeCl₃ significantly ($P < 0.05$) reduced the sludge bound water content from 2.2 ± 0.1 to 1.3 ± 0.1 (42% reduction) and $1.0 \pm 0.05 \text{ kg bound water} \cdot \text{kg}^{-1}$ dry solids (54% reduction), respectively.

Thus, about 12% better bound water reduction can be attributed to OP removal by CeCl_3 while the other 43% may be attributed to the CePO_4 precipitation. However, at the higher polymer dose of $30 \text{ g} \cdot \text{kg}^{-1}$ dry solids, CePO_4 addition insignificantly ($P > 0.05$) affected the sludge bound water content, which again can be attributed to the overwhelming effect of the cationic polymer addition. However, CeCl_3 addition was found to significantly ($P < 0.05$) reduce the sludge bound water content even at the higher polymer dose. Clearly, Figure 4.7c shows that the OP removal played a more significant role than CePO_4 precipitate in reducing sludge bound water at higher polymer dose. Meanwhile, polymer addition alone (without Cerium addition) reduced the sludge bound water content by 55% in comparison with that at the lower polymer dose, indicating the essential role of polymer on the sludge bound water content reduction as has been shown in previous studies (Chu & Lee, 1999; Hjorth & Jørgensen, 2012; Katsiris & Kouzeli-Katsiri, 1987). Therefore, it can be concluded from Figure 4.7 that polymer, CePO_4 , and CeCl_3 addition all contribute to bound water reduction.



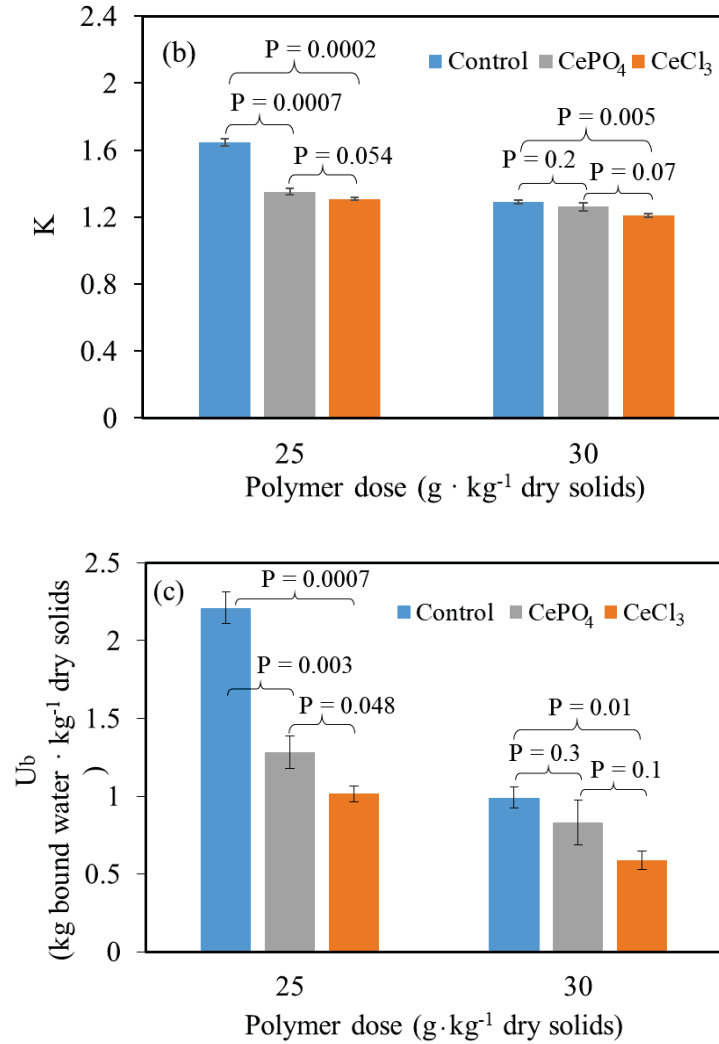


Figure 4.7 a) specific resistance to filtration (SRF), b) sludge compressibility (K), and c) bound water content (U_b) obtained from Eqs. (2.5), (2.13), and (2.10), respectively.

4.6.5 Mechanism of the sludge dewaterability improvement by cerium chloride addition

The results presented in Figure 4.7 revealed that the CePO₄ precipitate might have functionally acted as a physical conditioner, also known as a skeleton builder, that reduced the sludge SRF and the compressibility (Figure 4.7). It is known that the sludge mixed with such physical conditioners tends to form a rigid permeable lattice structure that maintains high sludge structural strength and thus impedes compression, such that the channel pore closure due to high pressure during the mechanical dewatering can be mitigated as illustrated in Figure 4.8 (Benitez, Rodriguez, & Suarez, 1994; Qi, Thapa, & Hoadley, 2011; Thapa, Qi, Clayton, & Hoadley, 2009). It is noteworthy that the phosphate precipitate was also found to be capable of reducing the bound water content in the

sludge (Figure 4.7c). As illustrated in Figure 4.1, biological sludge contains a high intracellular bound water content. Based on micrograph observations, Liu et al. (2013) concluded that under higher shear the physical conditioners such as phosphate precipitates with irregular shape inlayed or pierced microbial cells, and thus promoted the conversion of bound water (intracellular water) to free water. Thus can be seen, the addition of CePO_4 precipitate is mechanistically capable of reducing both the free and bound water contained in the sludge, which is consistent with the observations in Figures 4.6 and 4.7.

The sludge dewaterability improvement along with OP removal has been previously reported in the literature (Alm et al., 2016; Benisch et al., 2014; Higgins et al., 2014; Wu et al., 2005; Zhou et al., 2001). However, very limited mechanistic explanation was provided. The experimental results in this study showed that CeCl_3 addition offered even better sludge dewaterability improvement over CePO_4 precipitate addition (Figures 4.6 and 4.7), implying that the OP removal must have played some other roles that CePO_4 precipitate does not in contributing to the sludge dewaterability improvement. This study demonstrated that the OP removal is able to significantly reduce the sludge bound water content as well as the cake SRF but has very limited effect on the sludge compressibility improvement (Figure 4.7).

Previous interpretation of the dewaterability improvement by OP removal was made from a standpoint of the ratio of divalent to monovalent cations (Alm et al., 2016; Benisch et al., 2014; Higgins et al., 2014). Basically, it was believed that the increase of divalent to monovalent cation ratio will lead to better sludge dewaterability because the former can bridge negatively charged particles but the latter cannot (Agarwal, Abu-Orf, & Novak, 2005; Neyens, Baeyens, & Creemers, 2003; Novak, Sadler, & Murthy, 2003; Sobeck & Higgins, 2002). In this regard, Higgins et al. (2014) reported that digested sludge with lower OP concentration tended to have more divalent cation available in the sludge, and hypothesized this is due to the OP complexation and precipitation with divalent metal cations such as Ca^{2+} and Mg^{2+} . Following the same hypothesis, if existing OP complexation and precipitation was in significant amount, extra OP being released from the existing phosphate precipitates in the sludge should be expected in the course of CeCl_3 addition due to equilibrium shift, especially at the low end of remaining OP concentration. However, the linear stoichiometric correlation between equilibrium OP and the CeCl_3 addition as observed in Figure 4.3 indicates no significant OP release in our experiment.

It should be realized that the cationic polymers have been used in sludge conditioning for the previous studies that have reported the dewaterability improvement along with the OP removal, and polymer savings were observed when metal ions were added to remove OP (Alm et al., 2016; Benisch et al., 2014; Bergmans et al., 2014; Higgins et al., 2014; Kopp & Benisch, 2018; Wu et al., 2005; Zhou et al., 2001). Theoretically, the negatively charged OP is able to compete with the same negatively charged sludge particles for the positively charged binding sites on cationic polymers. As a matter of fact, several previous studies have confirmed the binding of OP with either the organic or inorganic cationic polymers (Jiang & Graham, 1998; Özacar & Şengil, 2003; Tatsi et al., 2003). It has been long recognized that the surface charge neutralization and particle bridging are the two mostly accepted principles dominating sludge conditioning by cationic polymers (Jarvis et al., 2005; Metcalf & Eddy, 2014). Reasonably, OP removal through metal phosphate precipitation must have left more binding sites available on the cationic polymers for neutralizing and flocculating sludge particles. This theory can explain many phenomena observed in this study. First of all, when the length of the polymers remains unchanged, the OP removal by CeCl_3 will increase the availability of the charged binding site on polymers. Consequently, more binding sites can be attached to a single sludge particle and thus leave less space for inter-particle bridging (Figure 4.8b). This will lead to smaller aggregates being formed as observed in Figure 4.5 and previous studies (Eriksson & Alm, 1992; Hjorth & Jørgensen, 2012; Nasser & James, 2007). Second, the excessive binding sites provided by the high polymer dosing in Figures 4.6 to 4.7 can explain the less prominent effect of the OP removal on the sludge dewaterability improvement because the number of new binding sites vacated by phosphate precipitation might have been overshadowed by the extra binding sites brought along with the extra polymer dosing (Figure 4.6). Third, the vacated cationic polymer binding sites can help flocculate the colloidal particles through bridging and charge neutralization, resulting in better solid-liquid separation and thus better sludge filterability as indicated by the reduction of the sludge filtration resistance in Figure 4.7a. Finally, the vacated cationic polymer binding sites also can release the bound water content in biological sludge through the replacement of water molecules by adsorbing polymer on the particle surfaces as a result of the charge neutralization (Katsiris & Kouzeli-Katsiri, 1987). With this being said, we propose that the competition between OP and negatively charged sludge particles for cationic polymers ought to be the mechanism explaining the dewaterability

improvement along with the OP removal that was observed in this and many other OP precipitation studies. A schematic illustration of the proposed mechanism is shown in Figure 4.8.

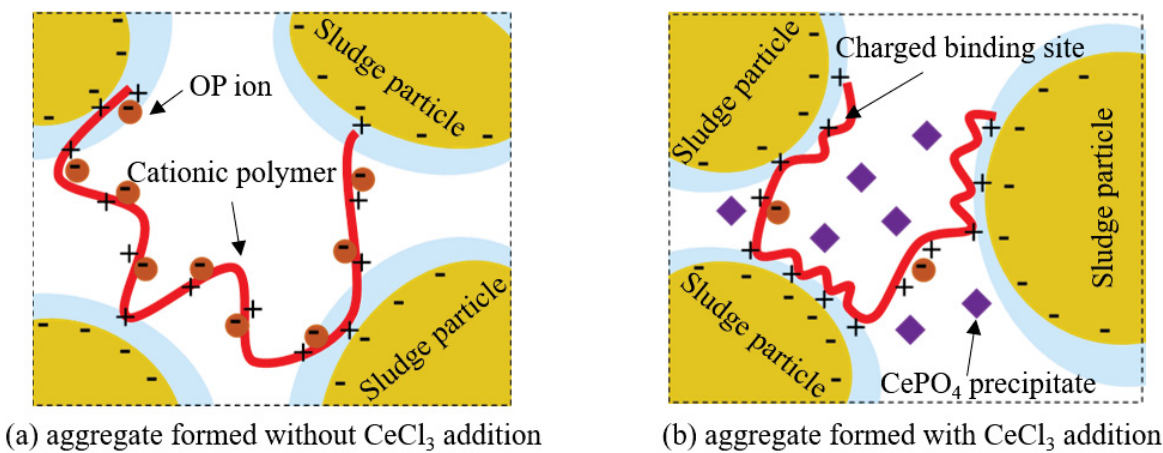


Figure 4.8 Schematic illustration of the interaction between cationic polymer and sludge particles (a) without and (b) with CeCl_3 addition during sludge conditioning

4.7 Conclusions

The effects of CeCl_3 addition on the OP removal and sludge dewaterability improvement have been investigated in this study. Following conclusive remarks can be drawn from this study:

1. CeCl_3 showed high sludge OP removal efficiency at neutral pH which was approximately equal to the theoretical stoichiometric ratio of 1 mole OP removal with each mole of cerium addition.
2. The dewaterability improvement in terms of cake dryness was also achieved with the CeCl_3 addition. The improvement was more significant at the polymer dose lower than the optimal level.
3. The improved sludge dewaterability by CeCl_3 addition can be possibly attributed to the formation of CePO_4 precipitate and the removal of OP ion from the sludge. CePO_4 precipitate might have created skeleton builders for enhancing sludge filterability, released intracellular water, and reduced sludge compressibility, while the OP ion removal has further improved the sludge filterability and bound water release but left the sludge compressibility unchanged.

4. The competition between OP and negatively charged sludge particles for the positively charged binding sites on cationic polymers is put forward as a new theory to shed light on the observed dewaterability improvement by OP removal.

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Chapter 5 : Using cerium chloride to control soluble orthophosphate concentration and improve the dewaterability of Sludge: Part II. Case study

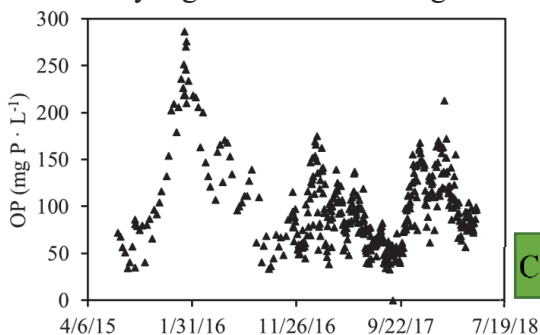
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5.1 Abstract

High concentration of OP in anaerobically digested sludge can lead to struvite crystallization, deterioration of sludge dewaterability, and elevated mainstream OP loading through centrate recirculation. The UOSA has observed seasonally high OP levels in its dewatering blend tank, which was found in this study to be a consequence of unwanted biological phosphorus accumulation in HRAS during the intensified winter denitrification operation and the subsequent OP release in the course of anaerobic digestion. In order to control the nuisance struvite scaling issues, a bench study was conducted and CeCl_3 was dosed as an effective OP precipitant. The results of this study demonstrated that CeCl_3 dosing showed higher OP removal efficiency than other commonly used OP precipitants. In addition, bench scale simulations indicated sludge dewaterability improvements which were used to predict lower polymer and dewatering energy demands at the full scale. The economic analysis conducted in this case study showed that the seasonal dosing of CeCl_3 at UOSA has the potential to provide a net annual saving of US \$47,000.

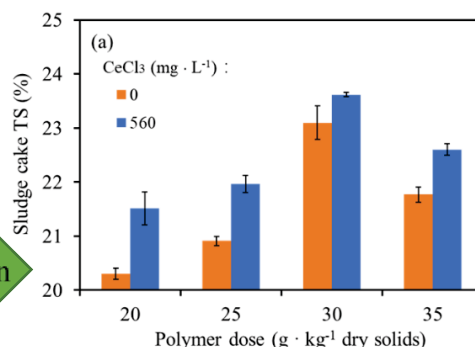
5.2 Graphical abstract

Seasonally high OP in dewatering blend tank



CeCl₃ addition

Dewaterability improvement



Struvite scaling issues during sludge handling



Cost effective struvite control



5.3 Keywords

Cerium chloride; Dewatering; Struvite; Economic analysis; Phosphate removal

5.4 Introduction

Biological phosphorus accumulation occurs in activated sludge processes that are subjected to alternating anaerobic and aerobic conditions in the presence of influent VFAs (Maurer, Gujer, Hany, & Bachmann, 1997). In the course of biological phosphorus accumulation, OP is released under anaerobic conditions and stored in greater amounts as intracellular polyphosphate under aerobic conditions (Yeoman, Stephenson, Lester, & Perry, 1988). Phosphorus-enriched sludge is produced at WWTPs that either intentionally adopt biological phosphorus accumulation as a phosphorus limit compliance approach or unintentionally achieve biological phosphorus accumulation as a result of intensified denitrification (Cokro et al., 2017; Østgaard, Christensson, Lie, Jönsson, & Welander, 1997). Recently, many WWTPs have observed the negative impacts of biological phosphorus accumulation on their downstream sludge handling process, especially when anaerobic digestion is applied to stabilize the phosphorus-enriched sludge (Alm, Sealock,

Nollet, & Sprouse, 2016; Higgins, Bott, Schauer, & Beightol, 2014; Zhou, Kelly, Mavinic, & Ramey, 2001).

Significant amounts of OP release during anaerobic digestion of phosphorus-enriched sludge are often observed with deteriorated sludge dewaterability, severe struvite scaling on dewatering equipment, and increased OP loading to the mainstream treatment processes from dewatering recycles (Benisch, Schauer, & Neethling, 2014; Doyle & Parsons, 2002; Wu, Bishop, & Keener, 2005). The UOSA, a 54 million gallons per day (MGD) WWTP, has observed seasonally higher OP levels in its dewatering blend tank that receives TWAS and anaerobically digested sludge (ADS) during periods of intensified denitrification in winter. As a consequence, nuisance struvite crystallization occurred at UOSA when elevated OP in sludge was sent to dewatering, resulting in scale formation in centrifuges, piping, and return pumps used to handle centrate, giving rise to increased maintenance and costly downtime of centrate handling facilities.

Chemical precipitation has long been applied as a stable, reliable, and efficient OP removal strategy for wastewater treatment, and is often applied to control struvite formation following anaerobic digestion (Huang, Liu, & Ding, 2015; Marti, Bouzas, Seco, & Ferrer, 2008; Stabnikov, Tay, Tay, & Ivanov, 2004). In this study, CeCl_3 was evaluated by UOSA as an OP precipitant because of constraints imposed by its sludge dewatering and pelletizing facilities that don't align well with the use of other more conventional chemicals such as magnesium, iron or aluminum salts. For example, the iron content in the dry biosolid pellets of UOSA already approaches the dryer manufacturer's recommended limit to avoid catalyzed hot spots and pellet fires in storage silos. Aluminum addition was tested and resulted in deteriorated sludge dewaterability. Moreover, CeCl_3 showed the potential to act as a more effective OP precipitant for the low solubility of CePO_4 ($\text{pK}_s = 25.3$) compared to other common OP precipitates (e.g., MgNH_4PO_4 , $\text{pK}_s = 13.2$; AlPO_4 , $\text{pK}_s = 20.0$; and FePO_4 , $\text{pK}_s = 15.0$) at neutral pH (CRC, 2007; Johannesson, Lyons, Stetzenbach, & Byrne, 1995; Musvoto, Wentzel, & Ekama, 2000). Literature reported deteriorated dewaterability with high OP levels in sludge. Therefore, this study was designed to test the hypothesis that the higher OP removal capacity of CeCl_3 will likely substantially improve sludge dewaterability (Alm et al., 2016; Benisch et al., 2014; Bergmans, Veltman, van Loosdrecht, van Lier, & Rietveld, 2014; Higgins et al., 2014; Wu et al., 2005; Zhou et al., 2001).

The authors were unable to find reported examples in literature where CeCl_3 was applied to control struvite production from unwanted phosphorus accumulation resulting from seasonally intensified denitrification. To fill this knowledge gap, an evaluation of the seasonal OP contents of TWAS, ADS, and blended sludge in the full scale plant was performed to verify that high OP levels in the centrate at UOSA coincided with its seasonally intensified denitrification practice. Then, the effect of CeCl_3 on sludge OP removal and dewaterability was evaluated on bench scale. Finally, an economic analysis was conducted by applying the bench scale results to the full-scale plant as a case study to determine if CeCl_3 application is a cost-effective OP precipitant for UOSA to consider for the future.

5.5 Materials and methods

5.5.1 WWTP description

The Regional Water Reclamation Plant operated by UOSA at Centreville, Virginia has an operating permit to produce 54 MGD of clean water for potable reuse. UOSA uses the modified Ludzack-Ettinger process for biological nutrient removal. Lime and alum are used for post-secondary advanced treatment for mainstream phosphorus removal. Mesophilic anaerobic digestion is used for primary sludge and TWAS stabilization. Any excess TWAS that cannot be processed in the anaerobic digesters is directly blended with ADS in the dewatering blending tank, then transferred to a centrifuge feed tank. Centrifuges and rotary drum dryers are used for sludge dewatering to produce biosolid pellets.

Previous research conducted on the Occoquan Reservoir since 2005 has described the nitrogen control strategy of UOSA for the downstream drinking water supply and the Chesapeake Bay. The nitrate-rich reclaimed water from UOSA provides a beneficial oxidizing environment at the bottom of the Occoquan Reservoir, thereby preventing water quality degradation when the reservoir is thermally stratified in warm weather with low dissolved oxygen in the hypolimnion (Cubas Suazo, 2012). Under these conditions the nitrate discharged from UOSA is denitrified in the hypolimnion and doesn't reach the Chesapeake Bay. Denitrification in the reservoir is limited once the reservoir turns over and the water column is mixed, which results in delivering dissolved oxygen throughout the water column. For this reason, UOSA must operate a more intensified denitrification process during non-stratified periods in order to meet its annual total nitrogen load limit imposed for the

Chesapeake Bay Program. As a compliance approach for nitrogen removal, UOSA uses a single anoxic zone in the Modified Ludzack-Ettinger process and operates other bioreactor zones at very low dissolved oxygen concentrations to stimulate simultaneous denitrification during cold weather periods when the reservoir is mixed. Seasonally high OP content was observed in the dewatering blended sludge at UOSA which was found to be coincident with periods of intensified denitrification (Figure 5.1).

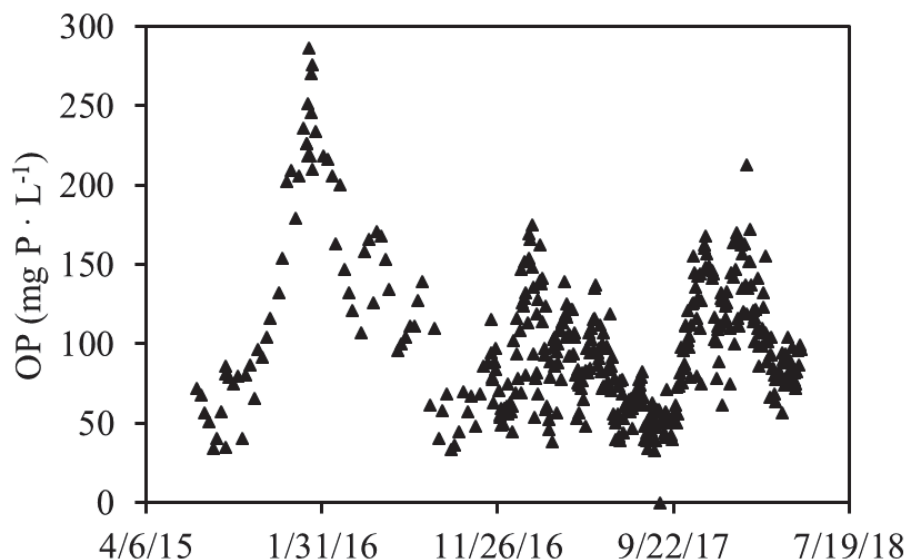


Figure 5.1 OP levels measured at the dewatering blend tank of UOSA since 2015

5.5.2 Sample collection and preparation

Fresh TWAS and ADS samples were collected from UOSA during the summer and winter of 2017 and analyzed for OP concentrations. These samples were then blended at a 20% TWAS and 80% ADS volumetric ratio similar to the sludge blends used in the full-scale dewatering blend tank at UOSA. These blended sludge samples were evaluated at the bench scale for cerium addition and dewaterability tests.

5.5.3 Analytical procedures

The sludge OP measurements were conducted using sludge sample that was centrifuged at $1000 \times g$ for 20 minutes. Then, the supernatant was filtered through $1.5 \mu\text{m}$ and $0.45 \mu\text{m}$ membrane filters in sequence to remove the suspended solids. The final filtrate was measured for OP using a molybdovanadate method test kit (TNT plus 846, Hach, Ontario, Canada). TS was measured

according to Standard Methods (APHA, 2012). Sludge was conditioned with cationic polymer (Clarifloc SE-587, Riceboro, GA) and OP removal was examined using a CeCl_3 solution (Neo RE300, Toronto, Canada).

5.5.4 Laboratory centrifuge dewatering setup

A laboratory dewatering protocol was established to simulate the full scale industrial centrifuge dewatering processes. Three key stages of the centrifuge process were simulated in the bench scale system. The initial step included chemical conditioning using cationic polymer (with or without CeCl_3) under controlled mechanical shearing at a $G \cdot t$ value of 10^5 using a modified high-power commercial blender (Waring MX1200XTXP, Stamford, CT). The selected $G \cdot t$ value is similar to the typical value of industrial centrifuges including the ones used at UOSA (Novak, 2006). The second stage simulated centrifugal sedimentation using a lab centrifuge (Sorvall Legend X1R, Fisher Scientific, Waltham, MA) under constant centrifugal acceleration of $5000 \times g$ and 20 minutes of spin time. Finally, cake compression that occurs as cake is scrolled out of the centrifuge was simulated using a piston under controlled pressure to obtain the final cake dryness. The blended sludge samples used for the dewaterability tests had an initial OP concentration of $161 \text{ mg P} \cdot \text{L}^{-1}$. A dose of $560 \text{ mg} \cdot \text{L}^{-1}$ CeCl_3 was added to achieve an equilibrium OP concentration of $90.5 \text{ mg P} \cdot \text{L}^{-1}$ which is below the target OP concentration of $100 \text{ mg P} \cdot \text{L}^{-1}$ determined in previous studies at UOSA to effectively minimize struvite scaling in the full scale.

5.6 Results and discussion

5.6.1 Effect of seasonal denitrification change at UOSA on sludge OP content

As revealed in Figure 5.2a, ADS and TWAS collected during the summer of 2017 produced very similar OP concentrations with an average OP concentration of 65 and $74 \text{ mg P} \cdot \text{L}^{-1}$, respectively, indicating that biological phosphorus accumulation and the subsequent OP release in anaerobic digestion was insignificant during the summer periods when less intensive denitrification was carried out because the OP concentration did not change much before and after anaerobic digestion (Figure 5.2a). However, for the ADS samples collected during the winter of 2017 when denitrification was being intensified, the OP concentration in ADS ($151 \text{ mg P} \cdot \text{L}^{-1}$) doubled that in the undigested TWAS ($71 \text{ mg P} \cdot \text{L}^{-1}$) as shown in Figure 5.2a. The significant intracellular polyphosphate storage and the subsequent OP release in the course of anaerobic digestion was

further confirmed by the observed OP increase after anaerobic storage of the winter TWAS. As shown in Figure 5.2b, the OP concentration in blended sludge of 20% TWAS and 80% ADS collected during winter of 2017 increased 75% from 102 to 179 mg P · L⁻¹, when it was exposed to more than seven hours of anaerobic storage. Figure 5.2 indicates significant polyphosphate accumulation in TWAS during winter periods of intensified denitrification likely due to the occurrence of unintentional biological phosphorus accumulation.

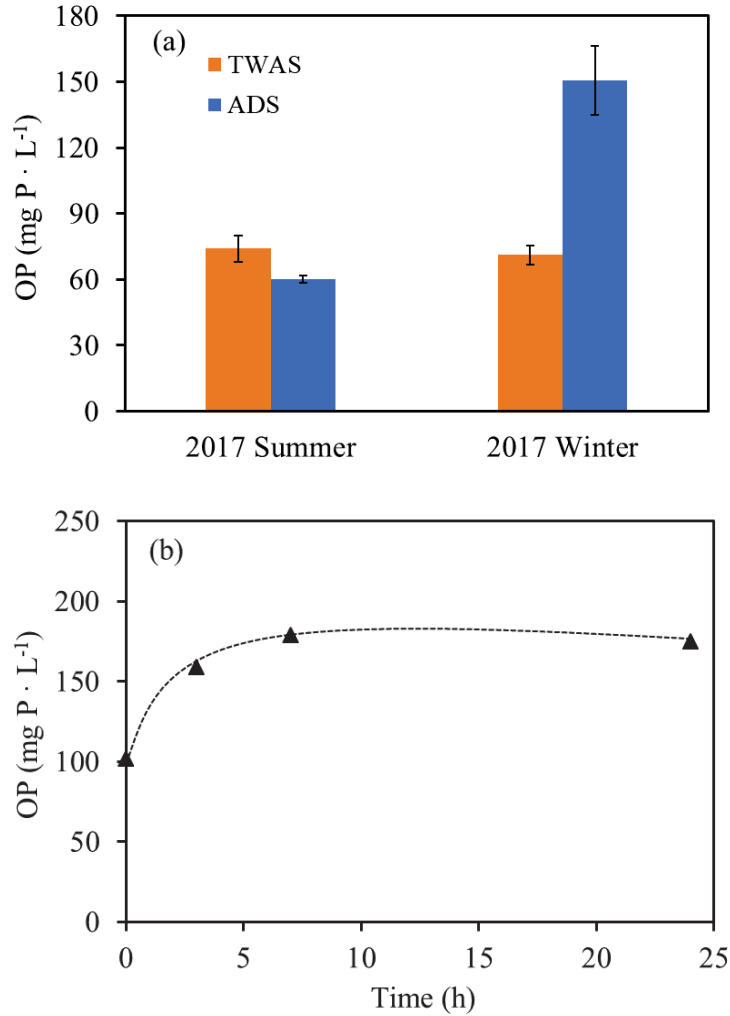


Figure 5.2 a) OP concentration in TWAS and ADS collected during 2017 winter and summer; b) OP release along with time under anaerobic storage from 20% TWAS and 80% ADS blended sludge collected during 2017 winter

5.6.2 Effect of CeCl_3 addition on OP removal

Blended sludge of 20% TWAS and 80% ADS collected during winter period with a OP concentration of $122.5 \text{ mg P} \cdot \text{L}^{-1}$ and a pH of 7.08 was used to investigate the OP removal efficiency of CeCl_3 . The equilibrium OP concentrations were measured at CeCl_3 doses of 0, 160, 320, 560, 800, and 950 $\text{mg} \cdot \text{L}^{-1}$. As shown in Figure 5.3, cerium addition achieved 97.5% OP removal at a $\Delta\text{OP} : \Delta\text{Ce}^{3+}$ molar ratio of 1 at neutral pH in blended sludge. This indicated that CeCl_3 is more effective in OP precipitation than other metal precipitants such as calcium, magnesium, aluminum, and iron salts, which have been reported to give 0.43, 0.38, 0.8, and 0.37 mole OP removed for each mole of metal ion added at neutral pH (Huang et al., 2015; Mamais, Pitt, Cheng, Loiacono, & Jenkins, 1994).

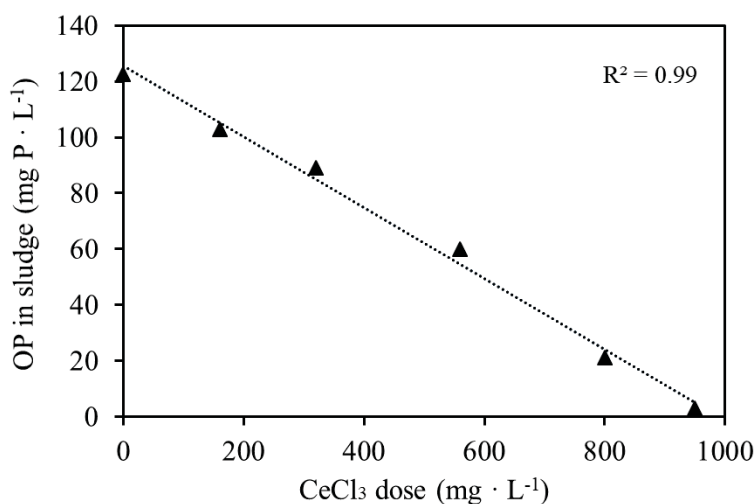


Figure 5.3 Equilibrium OP concentrations at various CeCl_3 doses

5.6.3 Effect of CeCl_3 addition on sludge cake dewaterability

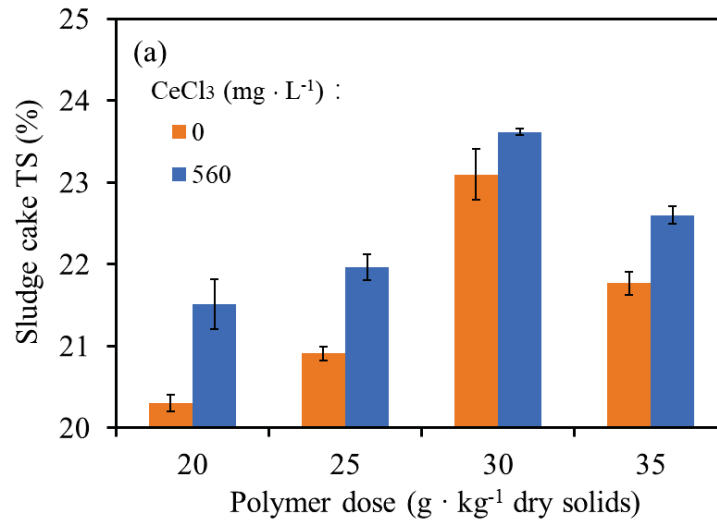
In addition to the high OP removal efficiency, significant sludge dewaterability improvement in terms of sludge cake dryness was also observed when CeCl_3 was used with different polymer doses, or under different dewatering pressures. With $560 \text{ mg} \cdot \text{L}^{-1}$ CeCl_3 added to the blended sludge samples of 20% TWAS and 80% ADS, improvements in sludge cake dryness of 5.9%, 5.0%, 2.3%, and 3.8% were achieved with polymer doses of 20, 25, 30, and $35 \text{ g} \cdot \text{kg}^{-1}$ dry solids, respectively as compared to the case when CeCl_3 was not added (Figure 5.4a). Figure 5.4b shows that 2.5% and 1.2% improvements in sludge cake dryness were achieved at the piston pressure of 241 and 310 kPa, respectively, with $560 \text{ mg} \cdot \text{L}^{-1}$ CeCl_3 added. However, at a higher pressure of 379 and

448 kPa, the improvement became insignificant. The different responses of cake dryness to piston pressure increase with and without CeCl_3 addition shown in Figure 5.4b implied that CeCl_3 addition may have affected sludge compressibility possibly due to the formation of CePO_4 precipitate which functionally acted as a physical conditioner, also known as a skeleton builder (Qi, Thapa, & Hoadley, 2011; Tiller & Yeh, 1987). The potential dewatering energy saving due to CeCl_3 addition is shown in Figure 5.4c, in which the piston energy applied to the sludge during dewatering was estimated using Eq. (5.1):

$$E_p = P \cdot \Delta V \cdot m^{-1} \quad (5.1)$$

in which E_p is piston energy input per dry mass cake (J g^{-1} dry solids); ΔV is sludge volume reduction (m^3); m is cake dry mass of 3.76 g; and P is piston pressure (Pa).

As shown in Figure 5.4c, to achieve the same cake dryness, sludge samples with CeCl_3 addition required lower piston energy input than those without cerium addition. For example, in order to achieve the target cake TS content of 21%, about 20% less energy was needed for the sludge sample with $560 \text{ mg} \cdot \text{L}^{-1}$ CeCl_3 addition compared to the sludge sample that received no CeCl_3 dose. It should be noted that the energy savings as a result of CeCl_3 addition decreased at higher pressures of 379 and 448 kPa.



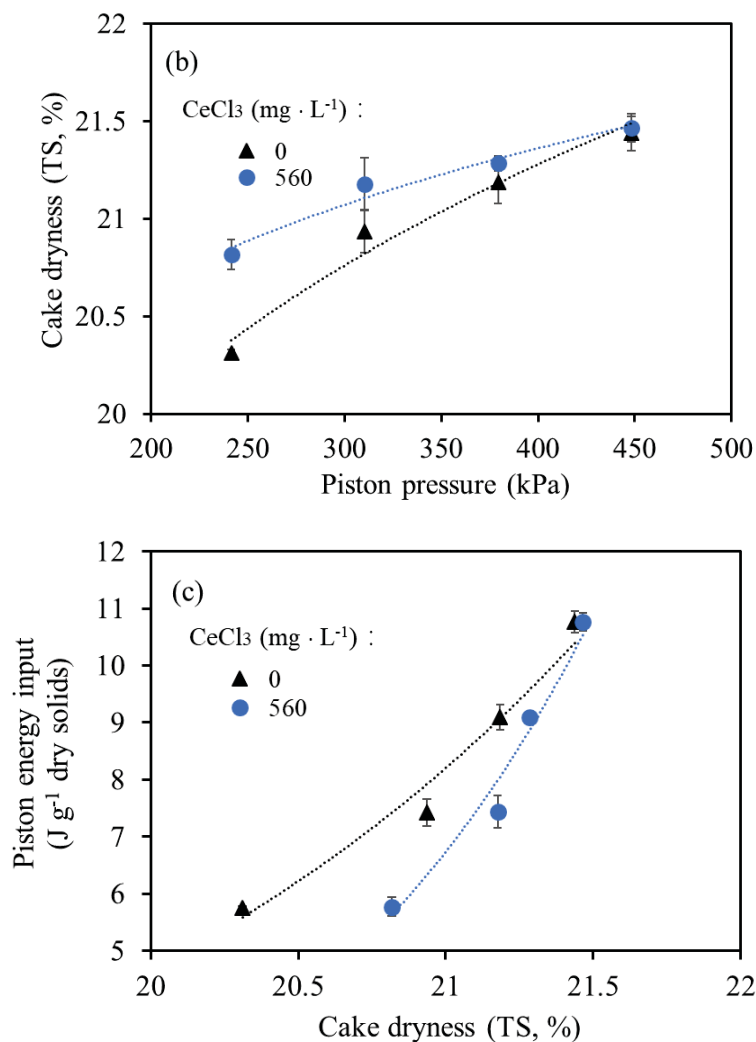


Figure 5.4 Effect of CeCl_3 addition on sludge cake dryness, a) dewatered cake dryness obtained at various polymer doses; b) dewatered cake dryness obtained at various piston pressures; c) piston energy input for obtaining various cake dryness

5.6.4 Economic impact of cerium chloride utilization at UOSA

Full scale field trials to control struvite by removing OP using CeCl_3 were conducted at UOSA. Figure 5.5 shows the severe struvite scaling issues observed at the centrifuges and the centrate pump station at UOSA due to the high winter OP in sludge. Figure 5.5a, b and c show the degree of struvite scaling without CeCl_3 addition. The centrate pump shown in Figure 5.5a was completely locked up by struvite after only 18 days of operation without CeCl_3 addition in winter. In contrast, Figure 5.5d shows almost no struvite formation on the centrate pump after a one-year operation with CeCl_3 addition at a dose targeted to keep the OP level below $100 \text{ mg P} \cdot \text{L}^{-1}$ in the

blended sludge. The CeCl_3 feed point was between the dewatering blend tank and the centrifuge feed tank.

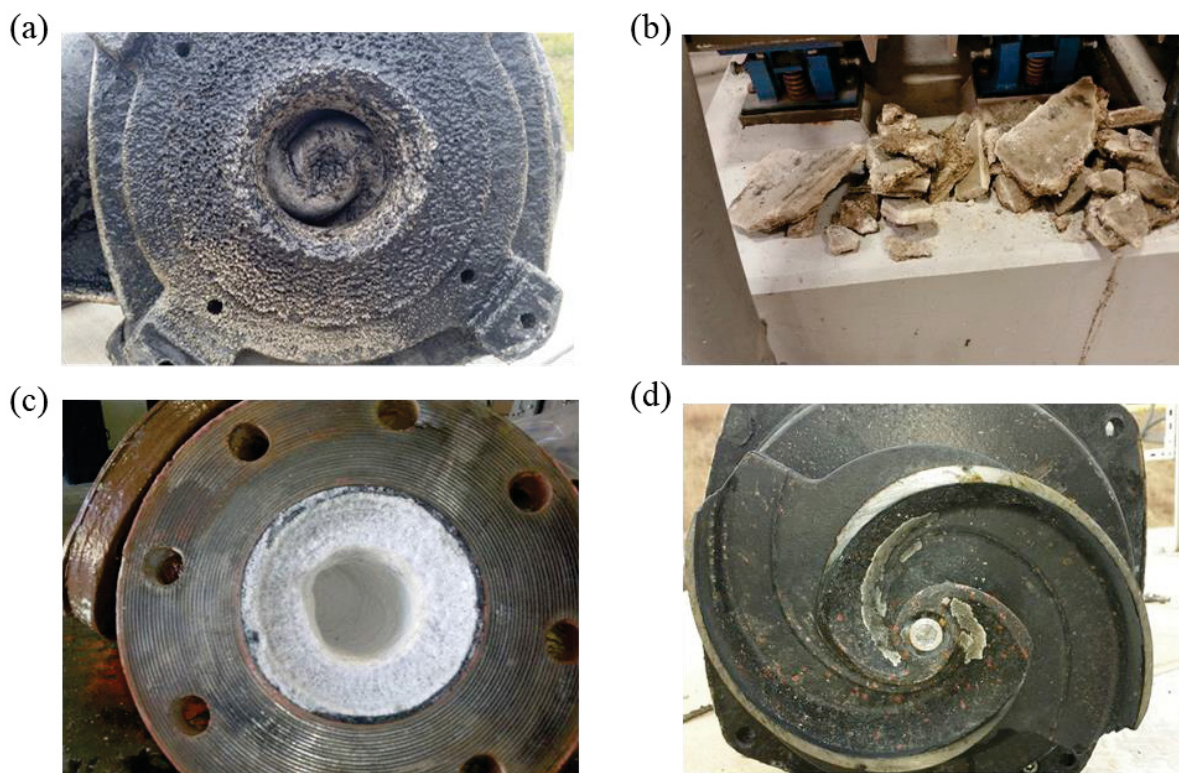


Figure 5.5 Photographs taken from the centrate pump station and the centrifuge of UOSA: a) centrate pump locked up by struvite; b) struvite scale removed from the centrifuge; c) struvite scale in centrate pump discharge pipe; d) centrate pump condition after CeCl_3 addition.

In addition to effective struvite control, Table 5.1 shows the benefits of CeCl_3 application on sludge dewatering based on estimated average operational conditions at UOSA. The application of CeCl_3 only led to a 0.8% increase in dry sludge production. The experimental results of this study (Figure 5.4 a) indicated that the current polymer consumption of $28 \text{ g} \cdot \text{kg}^{-1}$ dry solids can be reduced to $25 \text{ g} \cdot \text{kg}^{-1}$ dry solids (10%) with CeCl_3 addition, when the same cake dryness of 22% was achieved. Alternatively, 1% dryer cake could be achieved if the polymer consumption was kept the same, i.e. $28 \text{ g} \cdot \text{kg}^{-1}$ dry solids (Figure 5.4 a), which would lead to a 3% mass reduction for the dewatered cake as shown in Table 5.1 and likely additional energy saving in thermal dryer due to the decreased cake moisture content (Vaxelaire, Bongiovanni, Mousques, & Puiggali, 2000).

Although CeCl_3 showed a better OP removal efficiency in terms of OP removal per mole metal ion consumption, the chemical cost for CeCl_3 was much higher than other commonly used OP precipitants such as aluminum and iron due to the current high price of the commercially available cerium products for wastewater treatment. For example, the CeCl_3 product used by UOSA has a unit cost of $3.5 \text{ US } \$ \cdot \text{kg}^{-1}$, while the commonly used $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ costs 0.25 and $0.35 \text{ US } \$ \cdot \text{kg}^{-1}$, respectively (Sun et al., 2017). It should be noted that the total costs for sludge OP control may be highly dependent on-site specific characteristics and the flexibility of the current process design. CeCl_3 was selected by UOSA mainly because of constraints imposed by its sludge dewatering and pelletizing facilities, and the much lower capital investment for CeCl_3 addition. Previous studies conducted at UOSA demonstrated that the large quantity of aluminum salt (alum) required to reduce OP under the critical level for struvite control ($100 \text{ mg P} \cdot \text{L}^{-1}$) created significant dewaterability issues and negatively affected the quality of the dried biosolids pellets. As for ferric salts, iron content in the dry biosolid pellets of UOSA already approaches the dryer manufacturer's recommended limit to avoid catalyzed hot spots and pellet fires in its pellet storage silos without external ferric addition. Finally, both aluminum and ferric based precipitants may require special chemical storage and delivery facilities due to their corrosive nature and temperature sensitivity. None of these concerns exist when using the cerium precipitant. Taking these issues into account for the specific case of UOSA, although the chemical cost of aluminum and ferric products are more cost competitive, the capital costs to modify the existing facilities for dewatering, drying, pelletizing, and chemical storage may lead to significantly higher life cycle costs which make those alternatives less attractive as options for UOSA.

Another option considered by UOSA for OP removal (and recovery) is to promote struvite formation through magnesium salt addition in a separate designed and constructed process reactor and produce struvite crystals which can be separated or pelletized then marketed to fertilizer blenders (Shu, Schneider, Jegatheesan, & Johnson, 2006; Smith, Triner, & Knight, 2002; Yetilmezsoy, Ilhan, Kocak, & Akbin, 2017). However, this solution requires significant capital investment, operational labor, and chemical costs. According to Montag, Gethke, and Pinnekamp (2009), the investment costs for a 100,000 population equivalent WWTP amount to US \$1,607,221 corresponding to the recovery of phosphorus from sludge, which is equivalent to about 20 years cost of CeCl_3 addition. Moreover, a high influent OP concentration, e.g. between 150 to 450 mg L^{-1} , is usually required for revenue recovery from struvite fertilizer (Geerts, Marchi, & Weemaes,

2014; Li et al., 2019; Yetilmezsoy, Ilhan, Kocak, & Akbin, 2017). However, UOSA does not intentionally operate its biological reactors for biological phosphorus accumulation. In fact, UOSA operates its biological reactors in an aerobic mode for a good portion of each year to produce higher nitrate concentrations in its reclaimed water in the warmer seasons. A situation like this results in revenue recovery from a marketable struvite product to be an insignificant driver when examining economic options.

Table 5.1 Summary of the predicted operational benefits of using CeCl_3 for OP removal.

Components	Without CeCl_3	With CeCl_3
Sludge flow ($\text{m}^3 \cdot \text{day}^{-1}$)	500	500
CeCl_3 dose ($\text{kg} \cdot \text{day}^{-1}$)	0	119
OP concentration ($\text{mg P} \cdot \text{L}^{-1}$)	130	100
CePO_4 production ($\text{kg} \cdot \text{day}^{-1}$)	0	113
Dry sludge production ($\text{kg} \cdot \text{day}^{-1}$)	14,563	14,676
Dewater polymer dose ($\text{g} \cdot \text{kg}^{-1}$ dry solids)	28	25
Cake dryness as TS* (%)	22%	23%
Dewatered cake mass* ($\text{kg} \cdot \text{day}^{-1}$)	66,194	64,369
The calculation is based on a blended sludge TS of 2.64% and density of $1103 \text{ kg} \cdot \text{m}^{-3}$ as observed at UOSA during the period of this study.		
* At polymer dose of $28 \text{ g} \cdot \text{kg}^{-1}$ dry solids		

Table 5.2 shows the breakdown of the net annual savings to UOSA for CeCl_3 addition to control OP in its blended sludge. The savings add to approximately US \$47,000 per year based on the results of this study and the historical cost tracking information gathered from the facility cost records of UOSA. The chemical cost is based on seasonal CeCl_3 consumption during the periods of intensified denitrification operation (six months) when the centrate OP level would typically be above the target level of $100 \text{ mg P} \cdot \text{L}^{-1}$ in the dewatering blend tank. The full scale CeCl_3 solution dosing is accomplished using a small automated dosing system that uses totes and a metering pump that comes with negligible construction, operation and maintenance efforts. The capital cost needed to put the system together was very low. The cost savings on polymer was based on a 10% reduction of the current polymer consumption observed in the bench study (Table 5.1). Obviously,

the most significant savings came from lower cleaning and maintenance cost for the centrate pump station and the centrifuge system which together contributed to 75% of the total savings (Table 5.2).

The annual cost saving for UOSA assessed in Table 5.2 is based on their operational strategy of reducing polymer dose to maintain the same cake dryness, instead of keeping the same polymer dose but using the CeCl_3 to produce a dryer cake. As a matter of fact, other potential cost savings corresponding to CeCl_3 utilization can also be generated from the energy saving for mechanical and thermal dewatering equipment (Figure 5.4c), and sludge quantity reduction for transportation and disposal (predicted as US \$8,500 year⁻¹), which was not taken into the consideration of Table 5.2.

Table 5.2 Predicted annual savings based on the results from this study and the tracked facility costs from UOSA.

Components	US \$ year ⁻¹
Chemical cost of CeCl_3	75,000
Operation and maintenance cost of CeCl_3 addition	negligible
Chemical saving of lime for return OP reduction	3,000
Polymer saving due to CeCl_3 addition	18,000
Centrate pump station maintenance saving due to struvite control	41,000
Centrate pump station value reclaimed due to struvite control	10,000
Centrifuge maintenance saving due to struvite control	50,000
Total savings	47,000

5.7 Conclusions

Results from this case study demonstrated that:

- Biological phosphorus accumulation and the subsequent OP release in the course of anaerobic digestion were responsible for the high OP content in sludge during the periods of intensified denitrification operation at UOSA.
- CeCl_3 dosing showed higher OP removal efficiency than other commonly used OP precipitants.

7. CeCl_3 dosing also achieved apparent dewaterability improvements, which can potentially result in savings on polymer and energy during sludge dewatering.
8. The CeCl_3 is a preferred OP precipitant for UOSA where aluminum and ferric salts were determined to be unfavorable for the case-specific circumstances of UOSA.
9. Seasonal dosing of CeCl_3 at UOSA provided favorable economics with a predicted net annual savings of US \$47,000.

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Chapter 6 : Understanding the dewaterability of aerobic granular sludge formed in continuous flow bioreactors treating real domestic wastewater: is it really better than the dewaterability of activated sludge?

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6.1 Abstract

The continuous flow aerobic granulation technique has been successfully developed for domestic wastewater treatment. Yet, the impact of the technique on downstream dewatering process is still unknown. This study is intended to help fill this knowledge gap by providing a comprehensive examination of the dewaterability difference between aerobic granular sludge and activated sludge in response to thickening, polymer dose, and shear force effects. Four parameters, namely CST, SRF, compressibility, and dewatered cake dryness, were collectively employed to evaluate the sludge dewaterability. Without polymer, insignificant dewaterability difference was found between aerobic granular sludge and activated sludge thickened to 3% TS. With polymer, the thickened aerobic granular sludge demonstrated prominently better dewaterability at an optimal polymer dose only 25% that of the thickened activated sludge. However, it was discovered in this study that aerobic granular sludge quickly lost its dewaterability advantage upon being disintegrated under shear force equivalent to the level used in typical industrial dewatering centrifuges. Therefore, utilities pursuing continuous flow aerobic granulation should be aware of this discovery when considering transitions from activate sludge to aerobic granular sludge and how that transition could impact dewatering process performance and equipment selection.

6.2 Keywords

Continuous flow, Aerobic granular sludge, Dewaterability, Compressibility, SRF, CST

6.3 Introduction

Conventional biological wastewater treatment processes produce a large amount of excess sludge difficult to settle and dewater, which is a key bottleneck in sludge treatment and final disposal. A continuous flow aerobic granulation technique holds promise to intensify the capacity of biological treatment trains leading to greater biomass retention and faster sludge sedimentation with minor modification of the existing infrastructure in WWTPs (Kent et al. 2018, Sun et al. 2019). Recent study demonstrated that aerobic granular sludge with superior settleability can be formed from activated sludge inoculated into a plug-flow reactor (PFR) fed with real domestic wastewater (Sun et al. 2019). As a result, additional interests arose to evaluate the dewaterability enhancement of the aerobic granular sludge formed within the continuous flow system by using dewatering methods commonly applied in WWTPs. Therefore, the goal of this study was to provide a comprehensive examination of the dewaterability of continuous flow aerobic granular sludge by measuring the CST, cake dryness, SRF, and compressibility in response to the thickening, polymer dose, and shear force effects commonly encountered in WWTP dewatering processes. The outcome from this study will hopefully provide additional insights into granular sludge dewaterability and offer helpful guidance to dewatering process optimization and equipment selections in WWTPs considering the adoption of a continuous flow aerobic granulation technique.

6.4 Materials and methods

6.4.1 Cultivation of aerobic granular sludge in a continuous flow reactor fed with real domestic wastewater

A pilot-scale continuous flow aerobic granulation reactor illustrated in Figure 6.1a was operated at the UOSA, a local WWTP located in Centreville, VA, USA. All detailed reactor design, operation, performance, as well as the sludge characteristic information was reported in a recent study (Sun et al. 2019). Briefly, the PFR was made of ten completely stirred tank reactors (CSTRs) in series (Figure 6.1a). A column selector was used to selectively retain granular sludge that settled faster than 10 m h^{-1} . Figure 6.1b displays the morphology of the sludge stabilized in the pilot-scale reactors after 90 days of continuous operation.

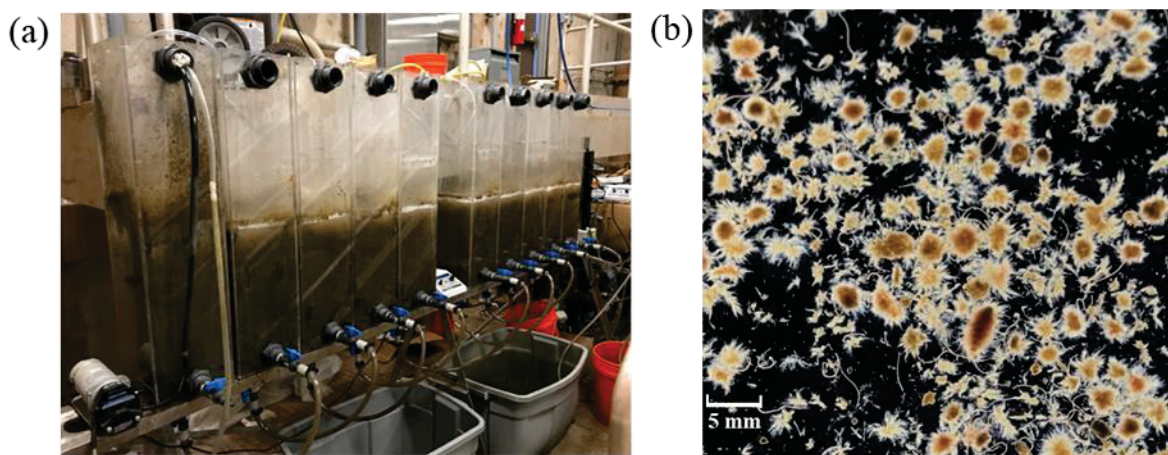


Figure 6.1 a) a pilot-scale continuous flow aerobic granulation PFR; and b) morphology of the granular sludge stabilized in it

6.4.2 Sludge characteristics analysis

The median diameters (d_{50}) was calculated from the particle size distribution using image processing software (ImageJ 2.0.0). Other sludge characteristics including sludge volume index for 5 min (SVI_5) and 30 min (SVI_{30}), volatile suspended solid (VSS), mixed liquor suspended solids (MLSS), and TS were analyzed according to the standard methods (APHA 2012). Chemical oxygen demand (COD) was analyzed using COD TNT plus 820 vials and a spectrophotometer (Hach, Loveland, CO, USA). Ammonia was measured using a QuikChem® 8500 series 2 flow injection analysis system (Lachat, Loveland, CO, USA). The method described by Liu and Fang (2002) was used for extracellular polymeric substances (EPS) extraction. Briefly, EPS were extracted by 2% EDTA, then EPS with a molecular weight greater than 3.5 kDa were separated using Dialysis Kits (Spectrum™ Labs Spectra/Por™ 3500 D MWCO Standard RC Pre-Treated, Waltham, MA USA). The polysaccharide content (PS) in EPS was determined by a phenol-sulfuric acid method (Nielsen 2010). Pierce modified Lowry protein assays were used for the EPS protein content (PN) analysis (Thermo Scientific, Waltham, MA, USA).

6.4.3 Dewaterability analysis

6.4.3.1 Normalized capillary suction time

CST is a commonly used method for quantifying sludge filterability by measuring the time required for drawing filtrate through capillary force (Scholz 2005). The CST was measured

according to standard methods (APHA 2012) using a capillary suction timer (OFITE 294-50, Houston, TX) equipped with Whatman no. 17 chromatography-grade paper (Maidstone, UK). Since CST is affected by the TS concentration, the CST values were normalized by the TS in the thickened sludge (Yu et al. 2008).

6.4.3.2. Specific resistance to filtration

Different from CST, SRF describes the sludge filterability by quantifying the resistance of the sludge to the drainage of its liquid component through a porous medium by vacuum or pressure (Graham 1999). The SRF tests were conducted using a piston press (FANN 207174, Houston, TX) at controlled pressures, i.e. 138 to 345 kPa (20 - 50 psi) similar to the pressure range of industrial dewatering equipment, e.g. belt press and plate & frame press (Burton et al. 2013). The filtrate volume change along with time was recorded. The SRF was calculated by Eq. (6.1) as defined elsewhere (Christensen and Dick 1985):

$$SRF = \frac{2 \cdot \Delta P \cdot A^2}{\mu \cdot w} \cdot b \quad (6.1)$$

in which ΔP is the piston pressure, A is the filtration area, μ is the filtrate viscosity measured by a viscometer (Size 1C, Cannon, State college, PA), w is the weight of dry cake solids per unit volume of filtrate, b is the slope of the profile of filtration time divided by filtrate volume as a function of filtrate volume as illustrated in Figure 6.2. It should be noted that the mechanical dewatering actually consists of two phases, i.e. filtration and expression. CST and SRF mainly measure the filtration phases (To et al. 2016). Figure 6.2 shows an example of recorded dewatering time divided by filtrate volume as a function of filtrate volume over 30 min. The SRF was calculated using the slope, namely b in Eq. (6.1), of the linear portion during the filtration phase (Figure 6.2). As shown in Figure 6.2, after the filtration phase, b in Eq. (6.1) increased remarkably during the expression phase (Novak et al. 1999).

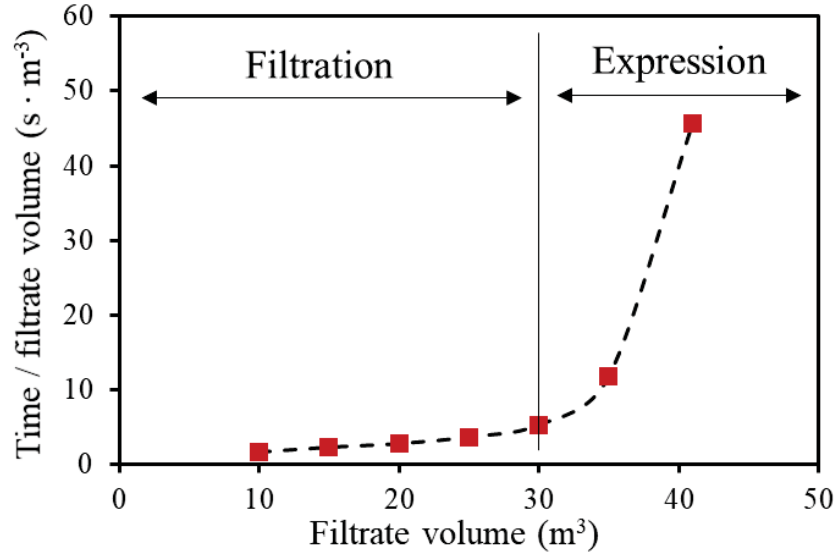


Figure 6.2 A plot of the filtration time divided by filtrate volume as a function of filtrate volume during a 30-min SRF test of this study.

6.4.3.3 Compressibility

In contrast to CST and SRF measuring sludge filterability at constant capillary or piston pressure, the parameter describing the filtration in response to increasing pressure is called sludge compressibility (Novak et al. 1999). The compressibility of sludge is defined as the degree of SRF increase with pressure increase. For more compressible sludge, e.g. biological sludge, the filtration rate, namely filtrate volume divided by filtration time, will increase at a slower pace as the pressure increase due to the concurrent SRF increase in Eq. (6.1) caused by the porosity loss during compression (Novak et al. 1999, Sørensen and Sørensen 1997, Sørensen and Hansen 1993). This phenomenon is commonly described by an empirical function in Eq. (6.2) (Pollice et al. 2007, Sørensen and Hansen 1993),

$$SRF = a \cdot \Delta P^k \quad (6.2)$$

in which a is a cake constant, k is the coefficient of compressibility which has a value close to 1 for biological sludge and a value around 0.6 for chemical sludge (Qi et al. 2011). A larger k indicates a more compressible sludge. The compressibility in this study was determined using the SRF values measured at the pressures of 138, 207, 276, and 345 kPa (20, 30, 40, and 50 psi).

6.4.3.4 Cake dryness

Since both filtration and expression in Figure 6.2 should be collectively evaluated in sludge dewatering (Novak et al. 1999), the cake dryness (i.e., TS content in dewatered sludge) was measured as an overall dewatering efficiency indicator using the final sludge cake obtained after 30 min of the SRF test (Figure 6.2).

6.4.4 Sludge thickening

The aerobic granular sludge collected from the pilot-scale PFR and the activated sludge collected from the aeration basin at UOSA were settled, then the sediment was gently thickened using a bench-top centrifuge at $968 \times g$ (Clinical-428, IEC, Boston, MA) for 1 min to achieve similar TS around $2.91 \pm 0.14 \%$ for both samples. The purpose was to simulate the thickening effect in a full-scale application. The thickened sludge was evaluated for dewaterability.

6.4.5 Polymer conditioning

The effect of polymer dose on sludge dewaterability was also investigated. The polymer used in the present study was the same high molecular weight cationic polymer (Clarifloc SE-587, Riceboro, GA) used in the full-scale centrifuge dewatering process at UOSA. The conditioning was conducted by gently hand shaking the sludge and polymer mixture for 10 seconds.

6.4.6 Shear intensity

As specified elsewhere (Zhang et al. 2019), a modified high-power commercial blender (Waring MX1200XTXP, Stamford, CT) was used to provide shear under the controlled root mean velocity gradient (G, s^{-1}) for a given duration (t, s). $G \cdot t$ is a commonly used non-dimensional expression to quantify shear intensity in which G was determined by measuring the torque and paddle rotational speed of the blender according to the study by Letterman et al. (1973). It is known that mechanical shearing exerted by dewatering equipment at a typical $G \cdot t$ in the range of 1 to 11×10^4 can substantially change the sludge dewatering performance (Murthy 2004, Novak 2006). Thus, the effect of shear was investigated in this study at the intensity ($G \cdot t$) levels of 0, 1, 5, and $11 (\times 10^4)$.

6.5 Results

6.5.1 Characteristics of aerobic granular sludge formed in a continuous flow bioreactor

Table 6.1 showed that the un-thickened granular sludge and un-thickened activated sludge had very different MLSS. The granular sludge possessed a d_{50} around 3.4 mm which was 10 times that of the activated sludge even though exactly the same real domestic wastewater and hydraulic retention time (HRT) were used for their cultivation. Besides, the SVI_5 and SVI_{30} values of granular sludge were 35% and 59% of those the activated sludge, respectively, indicating the superior settleability of the former to the latter (Table 6.1). The PS and PN are the major components of EPS (Sheng et al. 2010). The PS, PN, and EPS contents of aerobic granular sludge were 2.4, 1.8, and 1.9 times greater than those of activated sludge, respectively (Table 6.1). Regardless, the total COD and ammonia removals were similar between reactors using granular sludge and activated sludge.

Table 6.1 Characteristics of the granular sludge and activated sludge cultivated at steady state

Parameters	Unit	Granular sludge	Activated sludge
MLSS	$\text{mg} \cdot \text{L}^{-1}$	2341 ± 108	4131 ± 535
d_{50}	mm	3.4	0.3
SVI_5	$\text{mL} \cdot \text{g}^{-1}$	70.9 ± 2.6	201.6 ± 28.3
SVI_{30}	$\text{mL} \cdot \text{g}^{-1}$	63.9 ± 2.0	108.5 ± 12.8
PS	$\text{mg} \cdot \text{g VSS}^{-1}$	14.9 ± 0.1	6.3 ± 0.4
PN	$\text{mg} \cdot \text{g VSS}^{-1}$	58.9 ± 2.3	33.1 ± 1.1
EPS	$\text{mg} \cdot \text{g VSS}^{-1}$	73.8 ± 2.4	39.4 ± 1.5
COD removal	%	88.9 ± 1.5	94.1 ± 1.4
Ammonia removal	%	99.7 ± 0.2	99.5 ± 0.5

6.5.2 CST, SRF, and cake dryness comparison

The normalized CST, SRF, and cake dryness of the thickened aerobic granular sludge were compared to those of the thickened activated sludge without polymer addition and shear effect. As shown in Table 6.2, the average normalized CST and SRF of aerobic granular sludge were about 8% and 18% lower than that of activated sludge. The dewatered cake of aerobic granular sludge was about 4% dryer than that of activated sludge. Although these results indicate that granular sludge appeared to possess superior dewaterability to activated sludge, t-test shows that the p-values of 0.11, 0.24, and 0.32 were calculated for the normalized CST, SRF, and cake dryness between the two type of sludge, implying the dewaterability difference between thickened granular sludge and thickened activated sludge was insignificant (p-value > 0.05, Table 6.2).

Table 6.2 Comparison of normalized CST, SRF, and cake dryness of aerobic granular sludge and activated sludge without polymer addition and shear effect

	Normalized CST (s · g ⁻¹)	SRF (× 10 ¹³ m · kg ⁻¹)	Cake dryness (TS %)
Activated sludge	0.82 ± 0.04	6.58 ± 0.95	10.53 ± 0.63
Granular sludge	0.88 ± 0.01	5.55 ± 0.77	10.90 ± 0.26
p-value (α = 0.05)	0.11	0.24	0.32

6.5.3 Compressibility comparison

Figure 6.3 showed the SRF and cake dryness of the thickened aerobic granular sludge and thickened activated sludge determined at increasing dewatering pressures of 138, 207, 276, and 345 kPa (20, 30, 40, and 50 psi) without polymer addition and shear effect. The k in Eq. (6.2) determined for granular sludge and activated sludge from Figure 6.3a was 0.900 and 0.956, respectively, indicating thickened aerobic granular sludge was slightly (p-value = 0.45) less compressible. It should be noted that biological sludge was often reported to be highly compressible, i.e. with a k value in Eq. (6.2) close to (6.1), indicating there was almost no filtration rate increase in response to pressure increase according to Eq. (6.1) (Novak et al. 1999, Pollice et al. 2007, Sørensen and Hansen 1993). This was further demonstrated in Figure 6.3 b. As pressure

increased from 138 to 345 kPa, the dewatered cake dryness only improved less than 1% in terms of TS. In addition, the slope of the cake dryness, with increasing pressure, for the thickened aerobic granular sludge was only slightly greater (p-value = 0.32) than that of the thickened activated sludge (Figure 6.3b). In short, the difference in the compressibility between thickened aerobic granular sludge and thickened activated sludge was also insignificant.

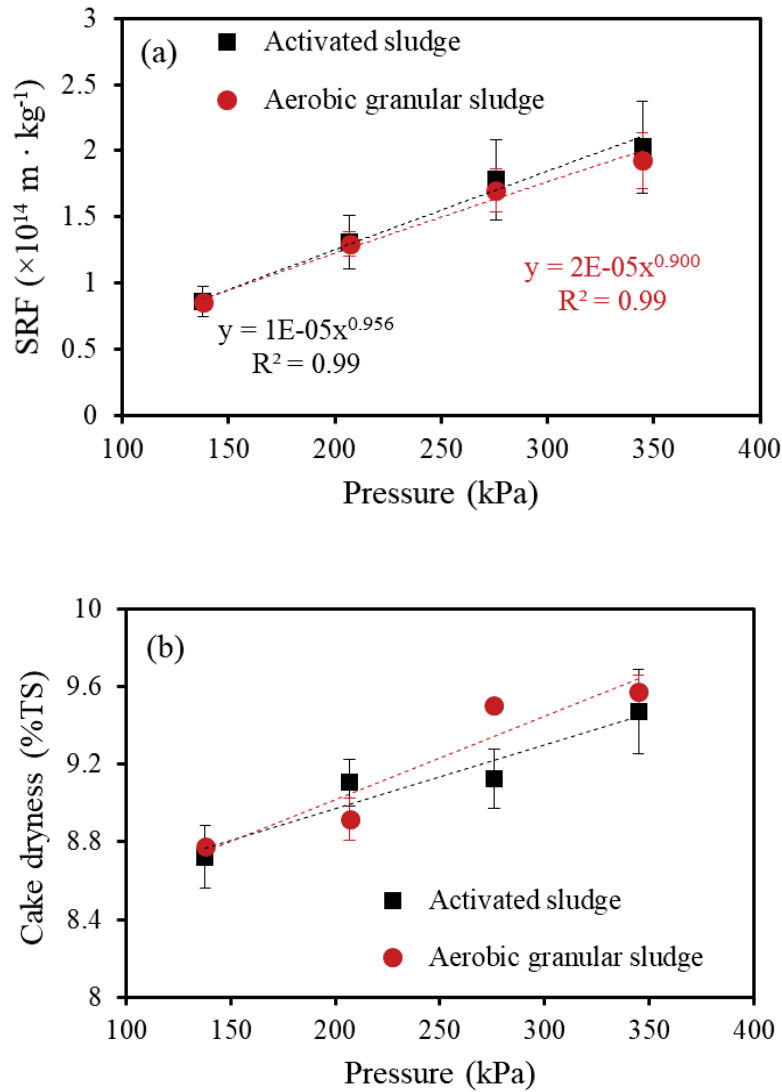


Figure 6.3 Effect of pressure on a) SRF and b) cake dryness for thickened aerobic granular sludge and thickened activated sludge

6.5.4 Effect of polymer addition

In practice, polymer is frequently added to thickened sludge in WWTPs to condition it for enhanced dewaterability (Burton et al. 2013). Therefore, polymer doses ranging from 0 to 16 g · kg⁻¹ dry solids were added to thickened granular sludge and thickened activated sludge to compare their dewaterability after sludge conditioning. It is known that there exists an optimal polymer dose for maximum sludge dewaterability. Both under- and over-dosing of polymer has been observed to worsen dewaterability (Lynch and Novak 1991, Werle et al. 1984). As shown in Figure 6.4a and b, the optimal polymer dose to achieve maximum filtration, as indicated by normalized CST and SRF, was only 3 g · kg⁻¹ dry solids for thickened aerobic granular sludge, which was only 25% of the dose needed to achieve optimum dewatering for thickened activated sludge (around 12 g · kg⁻¹ dry solids). This suggests there will be 75% saving on polymer dose and also 32% and 10% improvements in normalized CST and SRF, respectively. T-test shows that the p-values of 0.006 and 0.019 were calculated for the normalized CST and SRF between the two type of sludge, indicating the significant advantages in dewatering if aerobic granular sludge is used in place of the conventional activated sludge (Figure 6.4).

As explained in Figure 6.2, cake dryness is an overall dewaterability indicator, reflecting both filtration and expression characteristics of the sludge. Figure 6.4c demonstrates similar observations in that the optimal dose for achieving the maximum dewaterability of aerobic granular sludge in terms of cake dryness is around 7 g kg⁻¹ dry solids which was much lower than the 16 g kg⁻¹ dry solids dose observed for activated sludge. Perhaps more importantly, the maximum cake dryness achieved at the lower optimal dose was 13.8% TS for aerobic granular sludge while the maximum cake dryness was only 11.5% TS for activated sludge, a difference of 2.3% TS. This suggests aerobic granular sludge may save 56% polymer and achieve 17% greater cake dryness than using conventional activated sludge. A p-value of 0.047 between the two type of sludge indicated that aerobic granulation offered a significant improvement on cake dryness with polymer addition.

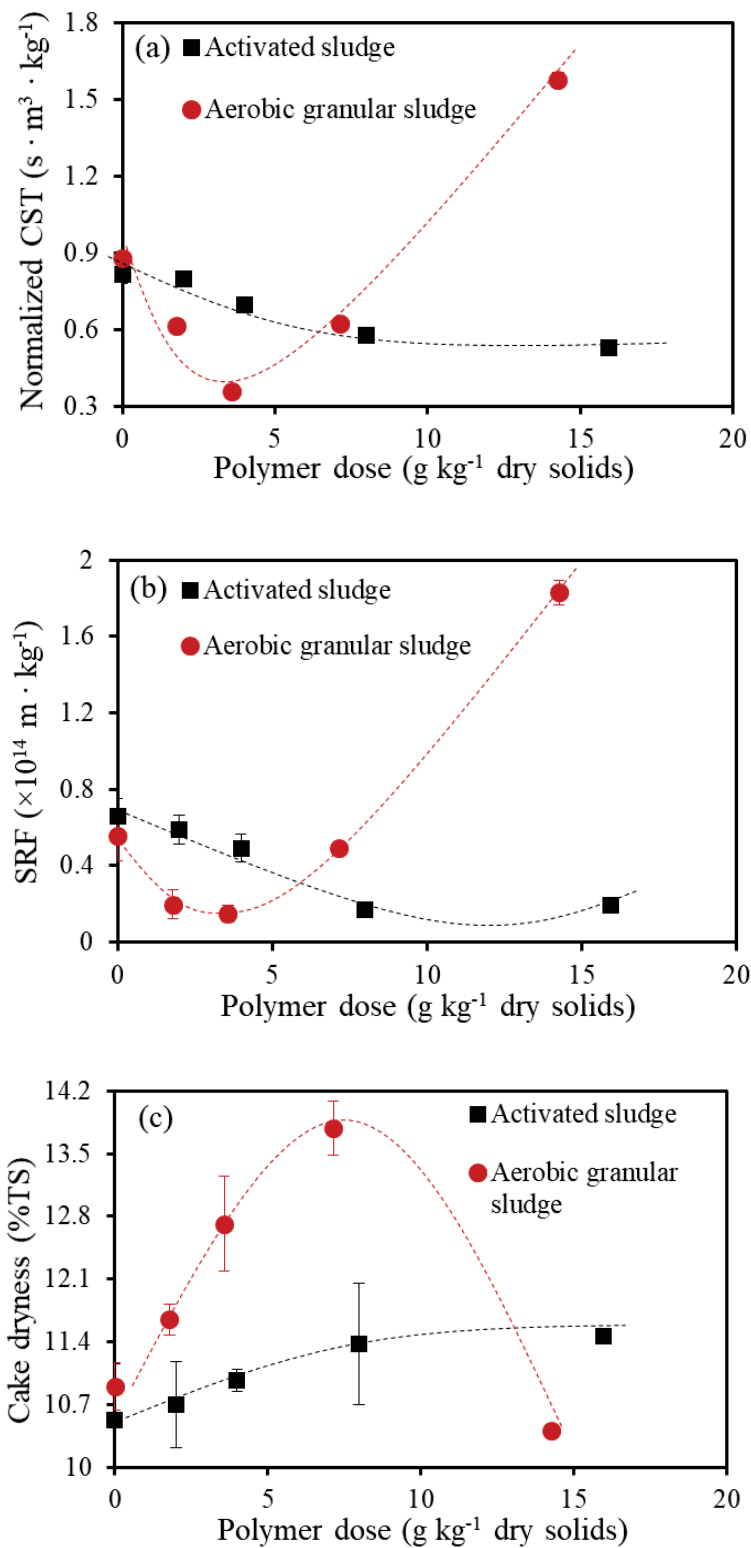
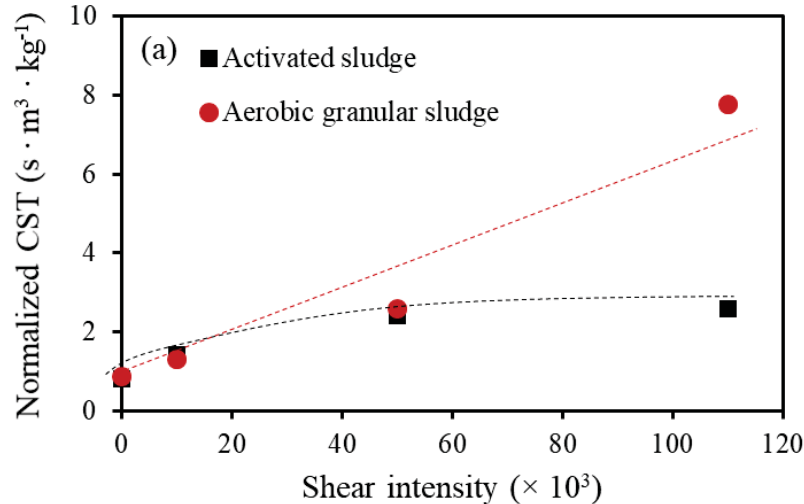


Figure 6.4 a) Normalized CST, b) SRF, and c) cake dryness obtained at various polymer doses

6.5.5 Effect of shear intensity

Shear intensity is a known factor impacting sludge dewaterability (Murthy 2004, Novak 2006). In the course of dewatering processes in WWTPs, thickened sludge endures shear intensity ($G \cdot t$) in magnitudes of 1, 3, and 11 ($\times 10^4$) in industrial belt press, plate & frame press, and centrifuge dewatering processes, respectively (Novak 2006). Therefore, the effect of shear intensity on normalized CST, SRF, and cake dryness of the thickened aerobic granular sludge and thickened activated sludge was measured in Figure 6.5. It can be seen that the strength of shear intensity was inversely correlated to the sludge dewaterability. In other words, sludge dewaterability in terms of normalized CST, SRF, and cake dryness worsened as shear intensity increased from 1 to 11 ($\times 10^4$). It should be pointed out that the difference in dewaterability between granular sludge and activated sludge was not evident with p-values ranged from 0.11 to 0.32 until the shear intensity increased to levels greater than 5×10^4 (Figure 6.5). At the high shear intensity, e.g. 11 ($\times 10^4$), thickened aerobic granular sludge consistently demonstrated significantly worse dewaterability than thickened activated sludge in terms of normalized CST, SRF, and cake dryness with a p-value of 0.0005, 0.013, and 0.031, respectively (Figure 6.5).



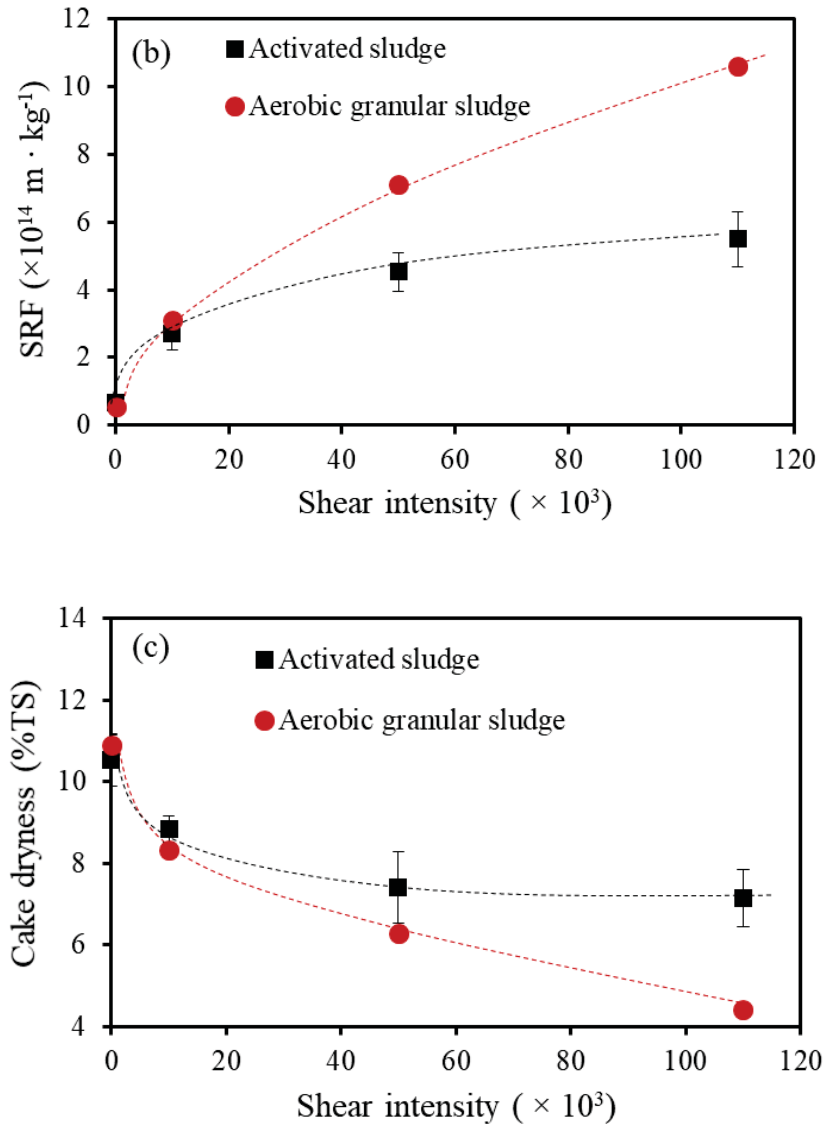


Figure 6.5 Effect of shear intensity ($G \cdot t$) on a) normalized CST, b) SRF, and c) cake dryness

6.6 Discussion

6.6.1 Without polymer addition, thickened aerobic granular sludge does not exhibit superior dewaterability to thickened activated sludge

Most previous aerobic granular sludge dewaterability studies were performed without consideration of sludge thickening and thus concluded that granular sludge possesses superior dewaterability over activated sludge (Csempesz et al. 1998, Zhou et al. 2011). Without thickening,

the TS of aerobic granular sludge was as low as 0.2% (Table 6.1). Under this low TS condition, bioparticles in aerobic granular sludge demonstrated discrete settling characteristics for their large particle size, compact structure, and more hydrophobic surfaces (Gao et al. 2011). Hence, superior dewaterability of aerobic granular sludge compared to activated sludge is expected (Cydzik-Kwiatkowska et al. 2019, Zhou et al. 2011). However, thickened sludge at least possesses TS concentrations greater than 1% as can be estimated from the SVI_{30} in Table 6.1. It was recognized that sludge particles tend to form a continuous particle network at a solids concentration above the gel-point which typically was reported to be around 1% as TS (Buscall and White 1987, D. Stickland et al. 2008, Krysiak-Baltyn et al. 2019, Legrand et al. 1998, Skinner et al. 2015, Thapa et al. 2009). Hence, thickened granular sludge may not possess superior dewaterability to thickened activated sludge as shown in Table 6.2 and Figure 6.3 when polymer is not added. A similar conclusion was drawn in the study by Krysiak-Baltyn et al. (2019), i.e., the filterability quantified by compressional rheology approach for granular and activated sludge from sequential batch reactors was similar at a solids concentration beyond the gel-point.

6.6.2 With polymer addition, thickened aerobic granular sludge exhibits superior dewaterability and lower optimal polymer dose to thickened activated sludge

The polymer conditioning process involves the destabilization of the gel particle network and the reformation of particle-particle interactions through surface charge neutralization (Csempesz et al. 1998, Legrand et al. 1998, Mikkelsen and Keiding 2001, Novak 2006, Zhang et al. 2014). It has been recognized that microbial cells in granular sludge or activated sludge are wrapped in EPS which is a major sludge component affecting sludge dewaterability (Mikkelsen and Keiding 2002, Yang and Li 2009, Yu et al. 2008, Zhang et al. 2016). The cationic polymer with a high positive charged density generally showed greater improvement on the dewaterability of sludge with negatively charged EPS (Chang et al. 2001, Christensen et al. 2015, Tiravanti et al. 1985, Zhang et al. 2014). Figure 6.4 showed that the thickened aerobic granular sludge consumed much less polymer and achieved better dewaterability than the thickened activated sludge, suggesting that the same cationic polymer appeared to be more effective in neutralizing the charge of EPS on the surface of thickened aerobic granular sludge than thickened activated sludge, despite the fact that the aerobic granular sludge actually possesses 1.9 times more EPS than the activated sludge as shown in Table 6.1. As a matter of fact, the similar observation was reported for aerobic granular sludge formed in sequential batch reactors, i.e., much less polymer was consumed for dewatering

aerobic granular sludge than for dewatering activated sludge even though the former contains 1.8 times more EPS than the latter as reported in the study by Cydzik-Kwiatkowska et al. (2019). The difference was attributed to the higher tightly bound EPS content within the granular sludge (Cydzik-Kwiatkowska et al. 2019). Accumulated evidences suggest that aerobic granular sludge possesses more hydrophobic and less negatively charged surfaces than activated sludge due to the variance in EPS contents and characteristics (Liao et al. 2001, Wilén et al. 2018, Zhang et al. 2007, Zhu et al. 2012). Hence, it is not difficult to understand that the lower surface charge on aerobic granular sludge in turn consumed less polymer for neutralization.

6.6.3 Shear force deteriorates the dewaterability of aerobic granular sludge more than activated sludge

Sludge particle disintegration under mechanical shear was demonstrated to have a negative impact on dewaterability as shown in Figure 6.5. When the smaller sludge particles were formed in sludge cake and filter medium during filtration, they resulted in greater resistance for filtrate to flow through the pores (Hogg 2000, Karr and Keinath 1978). In addition, particle disintegration led to greater particle surface area and possibly greater exposure of charged EPS which has been reported to deteriorate dewaterability through osmosis effect and increase the polymer consumption for charge neutralization (Mikkelsen and Keiding 2002, Sheng et al. 2010, Werle et al. 1984). This mechanism explains the observed dewaterability deterioration for both thickened aerobic granular sludge and thickened activated sludge observed in Figure 6.5. For aerobic granular sludge, the more dramatically dewaterability deterioration could be also related to the special EPS distribution. Aerobic granular sludge is known to possess a heterogeneous EPS distribution with an outer hydrophobic shell and an inner hydrophilic core (Wang et al. 2005). The hydrophobic shell has been found to be mainly distributed with non-readily biodegradable EPS, and most biodegradable EPS was concentrated in the core (Wang et al. 2007). Increasing evidences also suggested that the highly anionic PN content of EPS (Table 6.2) is also mainly located at the inner core of granular sludge (Caudan et al. 2012, Caudan et al. 2014, McSwain et al. 2005). As the structure of aerobic granular sludge was disintegrated under high shear intensity, the more hydrophilic EPS that originally wrapped inside the aerobic granules might have been released and deteriorated sludge dewaterability (Figure 6.6). Figure 6.5 suggests that the dewaterability deterioration due to increased shear intensity and particle disintegration aggravated more for granular sludge than for activated sludge as shear intensity increased. This may be attributed to a rapid disappearance of

the ten times particle size difference between aerobic granular sludge and activated sludge (Table 6.1), particularly under strong shear intensity $> 5 \times 10^4$ (Figure 6.5). This is coupled with the quick exposure of higher EPS content in aerobic granular sludge, which can be 1.9 times higher than that of conventional activated sludge (Table 6.1; Figure 6.6).

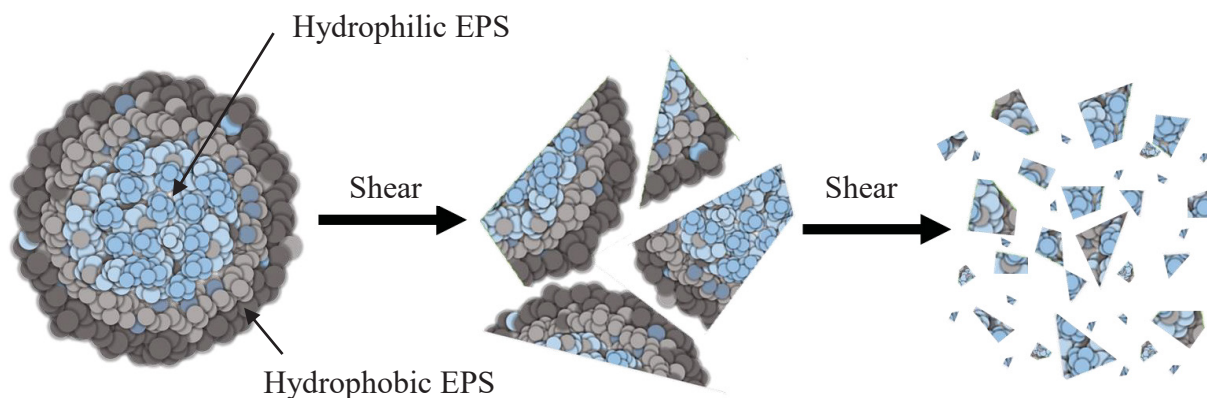


Figure 6.6 Schematic illustration of the EPS distribution in aerobic granular sludge and the effect of structural disintegration by shear force

6.6.4 Implication to practical application

The results shown in Figure 6.4 suggest that sludge conditioning is still essential for dewatering aerobic granular sludge. In this study, use of aerobic granular sludge could save 56 - 75% polymer dose, achieving 17% dryer sludge cake as compared to a conventional activated sludge system. However, the aerobic granular sludge exhibited even greater dewaterability deterioration than activated sludge when subjected to high shear intensity, e.g. $G \cdot t$ of $11 (\times 10^4)$ used in dewatering centrifuges (Novak 2006). For this reason, low shear dewatering methods such as belt presses (1×10^4) or plate & frame presses (3×10^4) may be more appropriate for dewatering aerobic granular sludge.

6.7 Conclusions

The following concluding remarks can be drawn from this study:

1. Without polymer addition, insignificant dewaterability differences were measured between thickened aerobic granular sludge and thickened activated sludge.
2. With polymer addition, thickened aerobic granular sludge exhibited superior dewaterability to thickened activated sludge. This was indicated by 32%, 10%, and 17% improvement in normalized CST, SRT, and cake dryness at the optimal polymer doses, respectively.
3. Using aerobic granular sludge may provide an opportunity to save dewatering polymer costs. A range in polymer dose savings of 56 - 75% was observed if dewatering conditioned aerobic granular sludge compared to conventional activated sludge.
4. The dewaterability of the aerobic granular sludge exhibited greater dewaterability deterioration than activated sludge in this study under high shear intensity, e.g. $G \cdot t$ of $11 (\times 10^4)$ used in dewatering centrifuges (Novak 2006). For this reason, low shear dewatering methods such as belt presses ($1 \times 10^4 G \cdot t$) and plate & frame presses ($3 \times 10^4 G \cdot t$) may be better dewatering technologies for use with aerobic granular sludge.

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Chapter 7 : Effect of temperature-phased anaerobic digestion and thermal hydrolysis pretreatment on the process intensification of anaerobic digestion

(This chapter is based on a project in collaboration with Arlington County and Alexandria Renew Enterprises. The content below contains unpublished results.)

7.1 Abstract

Rapid urbanization brings dramatic increase of sludge production, overloading many existing anaerobic digestion infrastructures built in the past decades. In this study, we explored the technical feasibility of four different types of thermal processes for enabling the existing anaerobic digesters to handle significantly greater loading rate through high-rate anaerobic digestion. The enhancement by using pre-pasteurization, thermophilic anaerobic digestion, temperature-phased anaerobic digestion (TPAD), and THP were comprehensively evaluated against the conventional mesophilic anaerobic digestion as a benchmark from the standpoints of digester startup, methane production, pathogen destruction, system stability, foaming potential, biosolids dewaterability, and odor emission. The results demonstrated that TPAD and THP were able to increase the methane productivity, namely the methane production rate from unit mass of feedstock, by an average of 20% and 24%, respectively, under even two times greater organic loading rate than that in conventional anaerobic digestion. Likewise, pre-pasteurization and thermophilic anaerobic digestion can respectively improve methane productivity by 30% at the same organic loading rate as in the conventional anaerobic digestion. Furthermore, the digesters using pre-pasteurization, thermophilic anaerobic digestion, TPAD, and THP can tolerate three times higher VFA-to-alkalinity ratios without notable digester upset. The involvement of any high-temperature processes (thermophilic anaerobic digestion, TPAD, and THP) produced Class A biosolids, improved dewaterability by 2 - 5%, and reduced biosolids odor production by 70-90% in terms of peak volatile sulfur emission. Notably, a 3% d⁻¹ gradual loading increase in synergy with bicarbonate addition was verified to be an effective means to overcome the challenge during startup of all high-rate processes.

7.2 Keywords

Anaerobic digestion; Temperature phased anaerobic digestion; Thermal hydrolysis pretreatment; Process intensification

7.3 Introduction

Rapid urbanization results in drastic increase of sewage sludge and the biosolids production, which leads to challenges such as the added cost for biosolids disposal, the land limitation for facility expansion, and the increasing odor nuisance complaints from the neighbor communities. One of the best choices for urban WWTPs to cope with these challenges is to seek process intensification technique to accommodate the increased biosolid treatment loading within their existing infrastructure without significant footprint expansion and additional capital investment.

Comparing to the mesophilic anaerobic digestion, thermophilic anaerobic digestion is able to improve pathogen destruction, accommodate higher organic loading, and enhance biomethanation by accelerating hydrolysis and methanogenesis, namely the two typical rate-limiting steps of anaerobic digestion (Converti et al. 1999). This is because that high temperature is able to lyse the microbial cells in biosolids and loosen up the structure of complex organics so that their accessibility to hydrolytic enzymes can be improved and in turn promotes the growth of methanogenic microbes for methane production (Lv et al. 2010). Despite these advantages, effluent from thermophilic digesters were reported to be odorous and of high VFA content (Bivins and Novak 2001). To alleviate this drawback, TPAD was developed in 1990s to utilize mesophilic reactor as a polishing reactor following the thermophilic reactor to further reduce VFAs, enabling greater process stability and higher effluent quality (Han and Dague 1997). The TPAD process includes a thermophilic digester operated typically at 55°C which is followed by a mesophilic digester operated at 35°C. In TPAD, both thermophilic and mesophilic reactors share the organic loading in sequence and collectively carry out the four steps of biomethanation, i.e. hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

Using high temperatures (130 – 170 °C) and pressures (2 – 10 bar), the complex organic contents of biosolids can be pretreated in the THP to achieve better biodegradability and pumpability for anaerobic digestion process intensification. anaerobic digestion with THP usually shows improved organic reduction and quicker biogas production, which will accommodate significantly higher

loading rate, and thus allow maximum utilization of the digester volume. Its reported advantages include: (i) reducing sludge viscosity to allow handling higher solids; (ii) improving sludge biodegradability for high rate AD; (iii) enhancing sludge dewaterability and killing pathogens for less disposal cost; and (iv) reducing odor production for enhanced protein degradation (Barber 2016).

Both TPAD and THP hold promise to accelerate the anaerobic digestion rate for intensified biosolid treatment. TPAD leverages a thermophilic anaerobic digester to pretreat the biosolids in preparation for the subsequent mesophilic anaerobic digester (Han and Dague 1997). Due to the higher temperature and accelerated microbial reaction in the thermophilic phase, TPAD may increase the biogas production rate, solids reduction, coliform removal, and odor mitigation. Likewise, the principle behind THP involves the hydrolysis and solubilization of the biosolids at elevated temperatures and pressures (Barber 2016). Although some studies have been conducted to evaluate the performance of TPAD and THP, knowledge gaps and research needs exist in understanding effect of TPAD and THP on process reliability, sludge dewaterability, and biosolids odor emission in comparison with conventional anaerobic digestion. Hence, this study was particularly focused on evaluating TPAD and THP performance including methane production, organic reduction, stability, dewaterability, pathogen destruction, and odor emission as potential process intensification technologies for urban WWTPs;

7.4 Material and methods

7.4.1 Digester operation

Six lab-scale stainless steel anaerobic digesters as shown in Figure 7.1a were operated under mesophilic (35 ± 0.3 °C) or thermophilic (55 ± 0.3 °C) conditions, and under completely mixed semi-continuous operation. The experiment was carried out in three phases. The experimental design is summarized in Figure 7.1b.

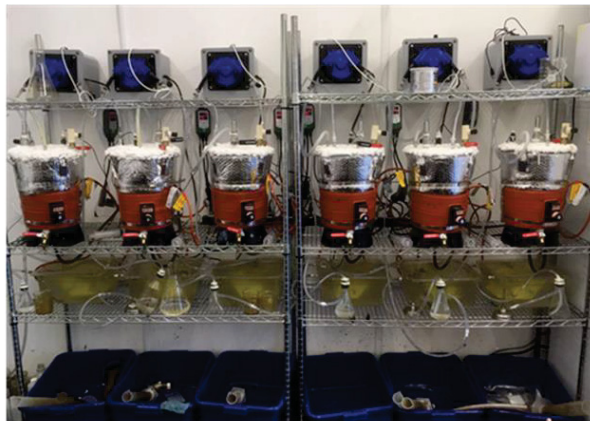
Phase I: in this phase of the study the pre-pasteurized sludge collected from the WWTP of AlexRenew (Alexandria, VA) was fed to a mesophilic digester and a thermophilic digester operated under a 25-day SRT in each as controls. Meanwhile, a TPAD system with a combination of a 11-day SRT of thermophilic phase and a 14-day SRT of mesophilic phase was operated to

digest the same pre-pasteurized sludge collected from AlexRenew. The THP effect on anaerobic digestion was studied by pretreating the sludge of 7.4% TS from the WWTP of Arlington County (Arlington, VA) with THP at 150 °C using a 2 L pressure vessels (Parr Instrument, Moline, IL). The THP pretreated sludge was diluted to 3.7% TS using dewatering centrate, then fed into a mesophilic digester operated under a 15-day SRT. Another mesophilic digester operated under the same 15-day SRT but without feedstock pretreatment with THP was used as a control.

Phase II: in this phase of the study the pasteurized sludge was replaced with non-pasteurized sludge to investigate the effect of pre-pasteurization on TPAD. Meanwhile, the TS was increased from 3.7% to 7.4% to study the TS effect on THP-pretreated anaerobic digestion. A TPAD system with a combination of a 7-day SRT of thermophilic phase and an 18-day SRT of mesophilic phase was operated in this phase.

Phase III: The temperature of THP was further varied from 150 °C to 130 °C and then 170 °C, respectively, to study the effect of THP temperature on anaerobic digestion.

(a)



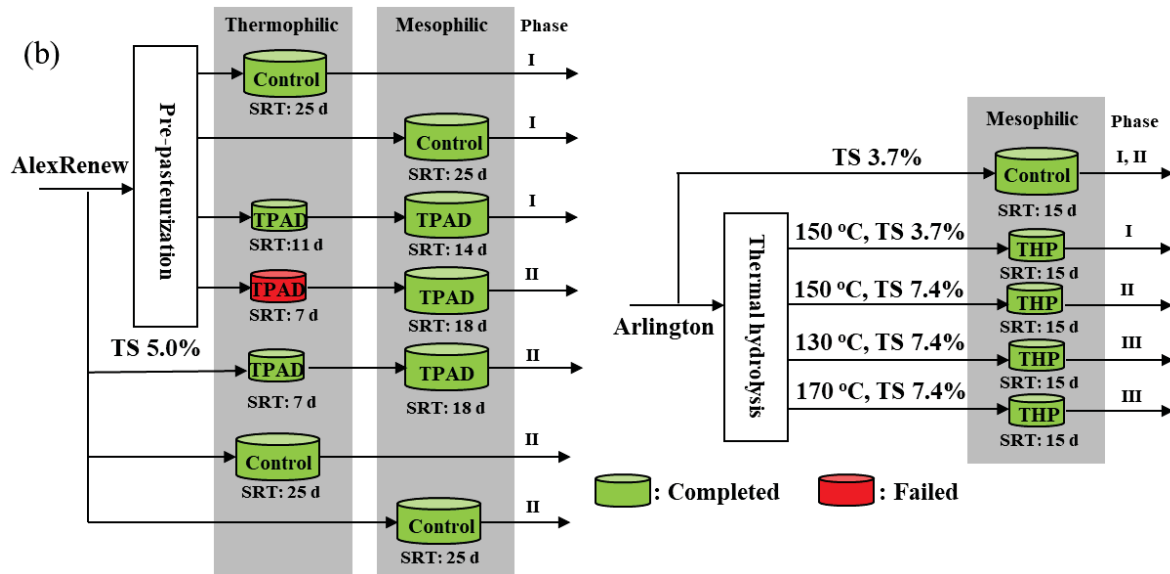


Figure 7.1 a) stainless steel anaerobic digesters used for this study; b) a summary of the experimental design

7.4.2 Performance evaluation

When all the digesters reached steady state, the performance of the TPAD and THP systems were compared with that of the controls of conventional process. The performance parameters are listed in Table 7.1.

Table 7.1 Summary of basic digestion performance parameters

Parameters	Measurements	Methods
Biogas production	Volumetric production rate	Tipping-bucket gas meter
Biogas composition	CH ₄ , CO ₂ , H ₂ , HS, MT, DMS	GC
Organic reduction	VS	APHA (2012)
pH, VFA, Alkalinity		APHA (2012)
Fecal destruction	<i>E. Coli</i> and total coli. MPN	EPA Method 1603
Biosolids dryness and odor	TS, H ₂ S, MT, DMS	APHA (2012) and GC

7.5 Results and discussion

7.5.1 Digester operation and gas production

Since late June 2018, all six digesters were operated for about 14 months as shown in Figure 7.2. The startup of the thermophilic digester of TPAD and the mesophilic THP digester in Figure 7.2 took a much longer startup time, e.g. five more months, than their experimental controls which took only less than one month. The thermophilic phase of the TPAD and the digesters with THP pretreatment were found liable to souring problem during the startup for the significant feedstock biodegradability improvement. Several countermeasures including different pace of SRT adjustment and NaHCO_3 addition were used to achieve the successful reactor startup. Digester failures of the thermophilic phase at a short SRT of 7 days was observed when fed with pasteurized sludge. As can be seen in Figure 7.2, TPAD and THP were finally capable of tolerating higher loading, leading to substantially higher biogas production rates in comparison with their controls.

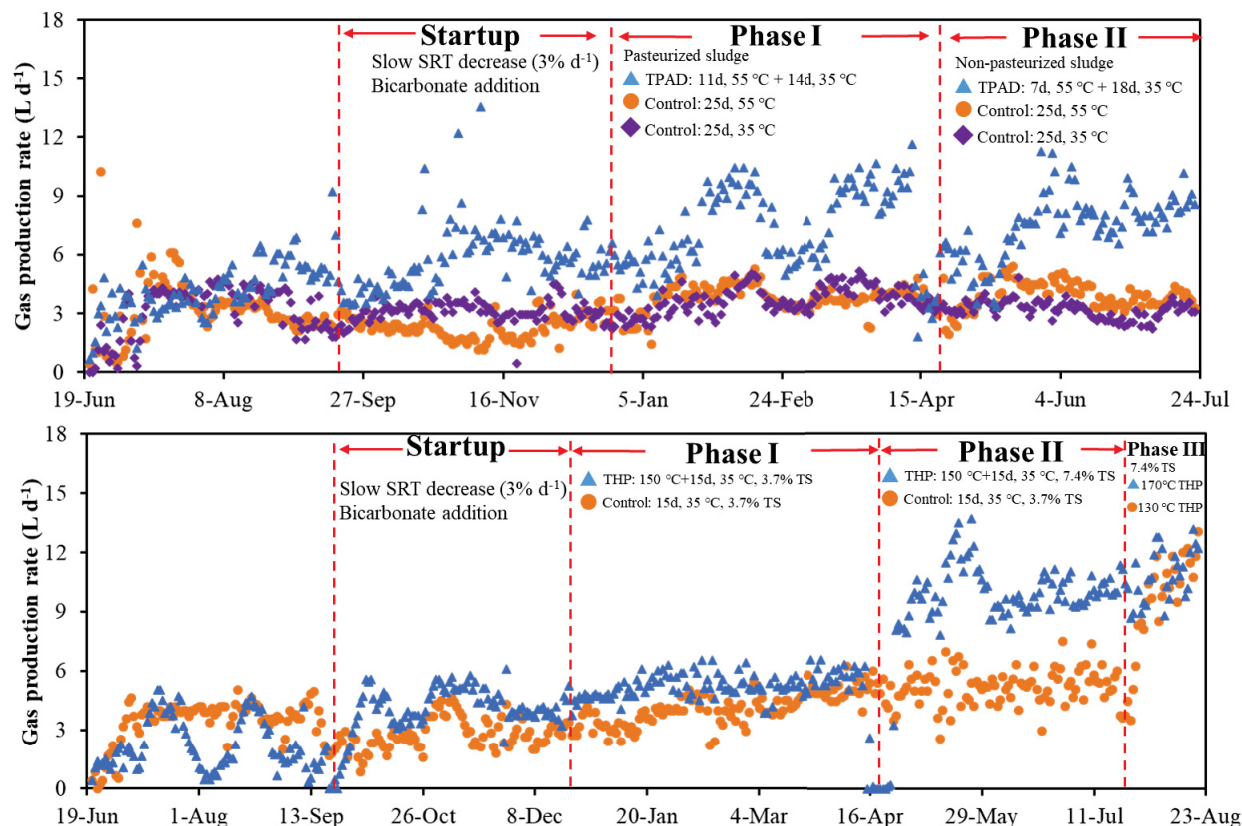
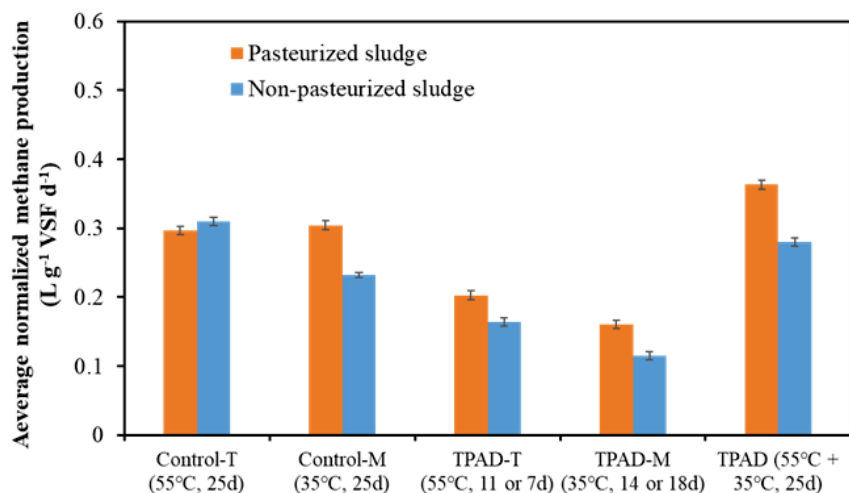


Figure 7.2 Effect of TPAD and THP on the biogas production during 14-month operation.

7.5.2 Normalized methane production rate

Due to the different SRTs and organic loading rates used for the TPAD and the THP experiment in this study, the methane production was normalized to the total VS loaded into each digester. As shown in Figure 7.3, TPAD fed with pre-pasteurized sludge operated with a combination of 11-day SRT in the thermophilic phase and a 14-day SRT in the mesophilic phase was able to increase the normalized methane production rate by an average of 20% in comparison with the mesophilic and thermophilic controls. However, the TPAD fed with non-pasteurized sludge and operated with a combination of 7-day SRT in the thermophilic phase and 18-day SRT in the mesophilic phase produced 22% more methane from unit VS fed than its mesophilic control, but 9% less than its thermophilic control. Figure 7.3 further showed that the digester receiving THP sludge of 3.7% TS produced about 36% more methane from the unit influent VS than its control. In addition, this improvement in methane production remained stable at different feed sludge TS (e.g. 3.7% and 7.4%) or THP temperatures (e.g. 130, 150, and 170 °C).



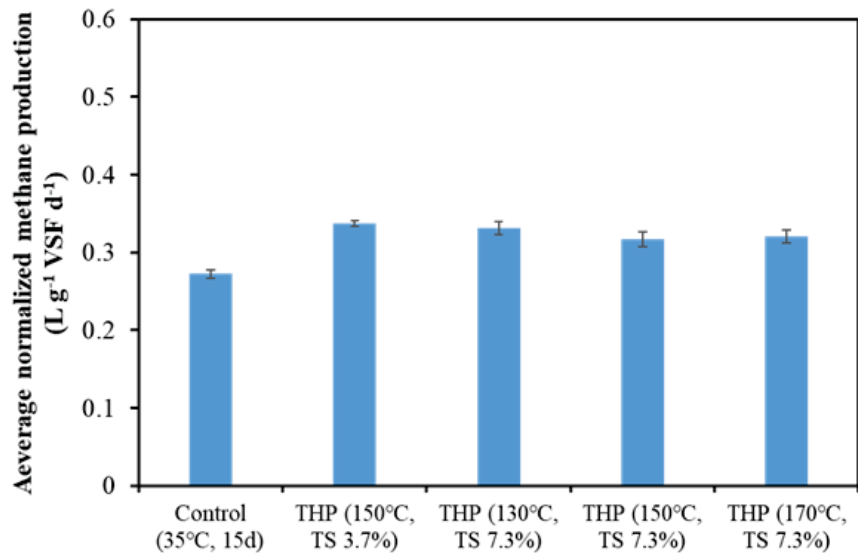


Figure 7.3 Effect of TPAD and THP on the normalized methane production rate averaged over the steady-state operations.

7.5.3 VS reduction

As shown in Figure 7.4, thermophilic AD and TPAD led to 6% and 8% greater VS reductions than the conventional mesophilic AD, respectively. THP-pretreated anaerobic digestion improved the VS reduction by 5% in comparison with the control without THP, when fed with the sludge of the same TS of 3.7%. However, as the THP-pretreated sludge TS increased to 7.3%, reduced VS reductions by 2 -7% were observed in Figure 7.4. The VS reduction increased with THP temperature increases.

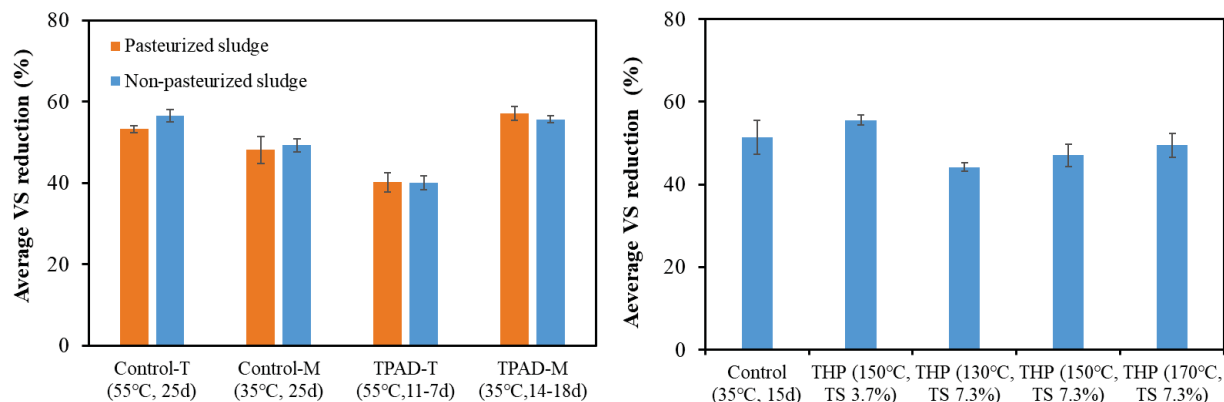


Figure 7.4 Effect of TPAD and THP on the VS reduction averaged over the steady-state operations.

7.5.4 VFA-to-alkalinity ratio

The VFA-to-alkalinity ratio is a commonly used indicator of digester stability with a typical range reported from 0.2 to 0.4 (Sri Bala Kameswari et al. 2012, Switzenbaum et al. 1990). It can be seen from Figure 7.5 that the thermophilic control receiving pre-pasteurized sludge and the thermophilic phases of TPAD have much higher VFA-to-alkalinity ratios ranging from 0.8 to 1.2, suggesting a lower stability due to potential VFA accumulation. Nevertheless, all digesters were maintained with a steady gas production and pH without NaHCO_3 addition. For the same reason, THP under the higher feed TS, e.g. 7.3%, also showed an about two times higher VFA-to-alkalinity ratio than its control.

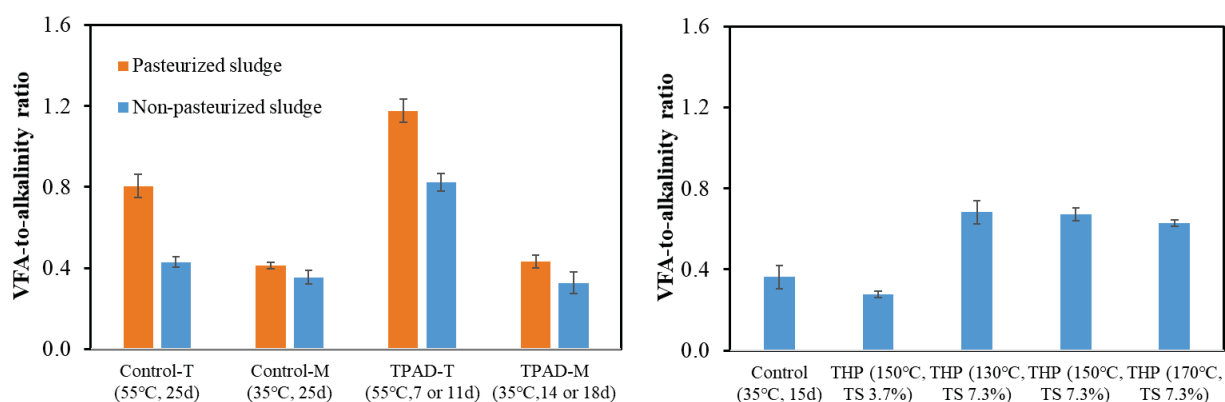


Figure 7.5 Effect of TPAD and THP on the VFA-to-Alkalinity ratio averaged over the steady-state operations.

7.5.5 Pathogen destruction

Figure 7.6 shows that the *E. Coli* and total coliform density in raw sludge from AlexRenew and Arlington was in a range of 10^6 to 10^8 MPN g^{-1} TS. The conventional mesophilic anaerobic digestion was able to achieve only one log reduction of *E. Coli* and total coliform under the SRT of either 15 or 25 days. The *E. Coli* and total coliform density in effluent from processes related to elevated temperature, i.e., the anaerobic digestion with pre-pasteurization, the anaerobic digestion with THP, the anaerobic digestion with SRT of 25 days in the thermophilic phase, and the TPAD, were all below the detection limit of 10^3 MPN g^{-1} TS.

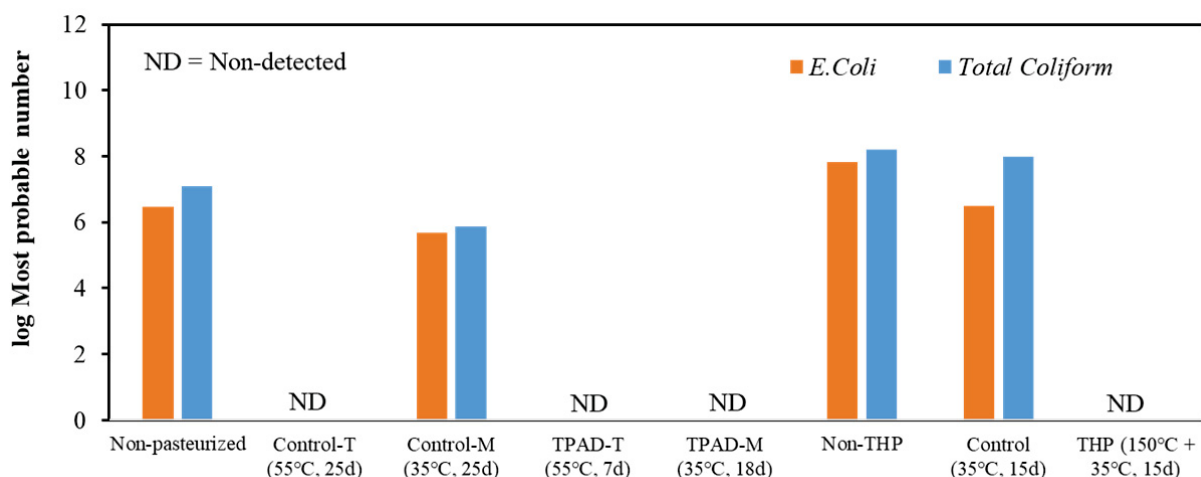


Figure 7.6 Effect of TPAD and THP on the pathogen destructions averaged over the steady-state operations.

7.5.6 Dewaterability

It can be seen from Figure 7.7 that pre-pasteurization pretreatment improved sludge dewaterability by 2% in terms of cake TS for both mesophilic and thermophilic digesters operated under the 25-day SRT, and the TPAD operated with SRTs of 11 days in the thermophilic phase and 14 days in the mesophilic phase further improved dewatered cake TS by 2%. However, the dewaterability improvement became negligible for the TPAD with non-pasteurized sludge operated with SRTs of 7 days in the thermophilic phase and 18 days in the mesophilic phase. The dewaterability improvement for THP was 1.5% in terms of cake TS with a feed sludge TS of 3.7%, and 4% with a feed sludge TS of 7.4%. In addition, increasing sludge THP temperature led to decreased biosolids dewaterability.

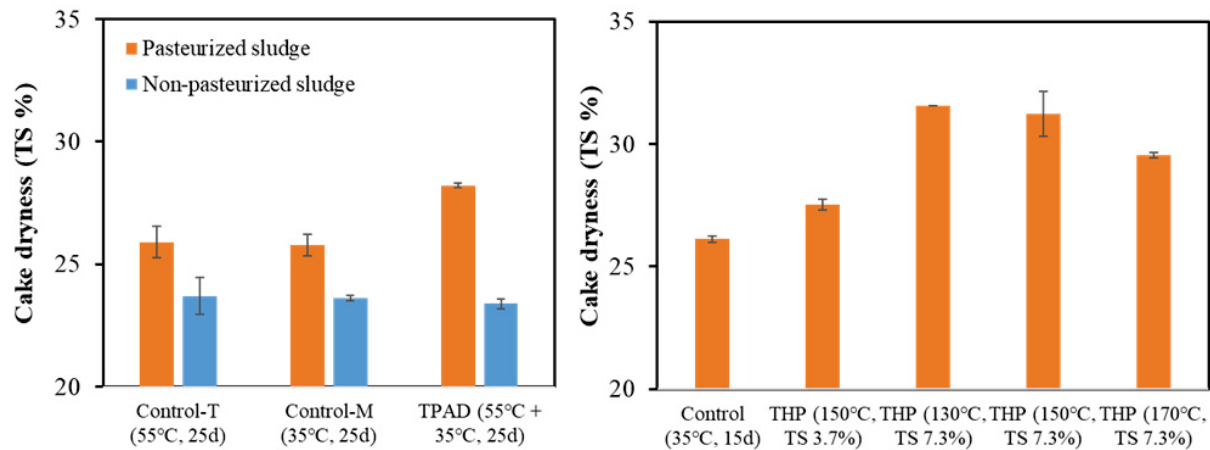


Figure 7.7 Effect of TPAD and THP on cake dryness dewatered at the shear intensity of $G \cdot t = 9 \times 10^4$ and polymer dose of 25 g kg^{-1} dry solids.

7.5.7 Volatile sulfur emission from biosolids

The digested and dewatered sludge in Figure 7.7 was stored in airtight containers to study the extent of VSCs emission during anaerobic storage. Figure 7.8 showed the peak concentration of total VSCs including HS, MT, and DMS. It can be seen that the mesophilic digesters fed with non-pasteurized sludge showed substantially higher VSCs emission. While fed with pasteurized sludge, TPAD in operated with SRTs of 11 days in the thermophilic phase and 14 days in the mesophilic phase increased the peak sulfur odor emitted from the dewatered biosolids by 78% in comparison with its controls, however, it then achieved the lowest sulfur odor emission when fed with non-pasteurized sludge and operated with a longer mesophilic phase of 18 days. THP was able to reduce VSCs production by 68% and 90%, with a feed sludge TS of 3.7% and 7.4%, respectively. In addition, the VSCs emission declined with THP temperature increases.

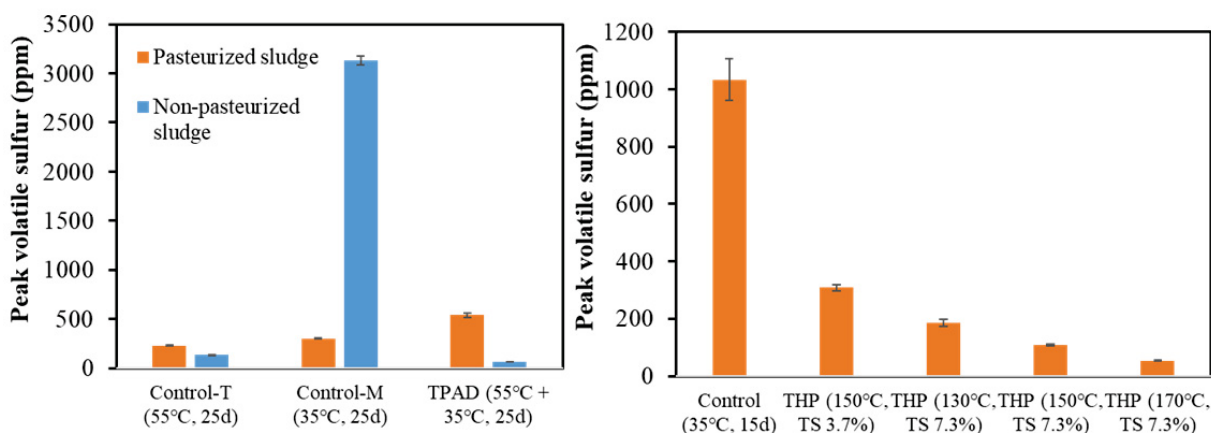


Figure 7.8 Effect of TPAD and THP on VSCs emission from biosolids dewatered at the shear intensity of $G \cdot t = 9 \times 10^4$ and polymer dose of 25 g kg^{-1} dry solids.

7.6 Conclusions

The following concluding remarks can be drawn from this study:

1. TPAD with pasteurized sludge and THP-pretreated AD required longer startup, e.g. five months in this study. Buffer addition and slow SRT adjustment are recommended for a successful startup of TPAD and THP.
2. The integration of pre-pasteurization and thermophilic AD is subjected to a high risk of failure.
3. Pasteurization played similar role in sludge hydrolysis as THP indicated by pH, alkalinity, and VFA increases.
4. TPAD and THP produced 20% and 24% more methane per unit VS fed than the control without thermal treatment.
5. The TPAD and high solids THP digestion can be stabilized at a much higher solids loading rate and VFA-to-alkalinity ratio.
6. All thermal processes including pasteurization, thermophilic digestion, TPAD, and THP produced Class A biosolids.
7. Pasteurization, TPAD, and THP improved biosolids dewaterability and reduced cake odor production.

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Chapter 8 : Recalcitrant dissolved organic nitrogen formation in thermal hydrolysis pretreatment of municipal sludge

(This chapter has been accepted for publishing as “Zhang D., Feng Y.M., Huang H.B., Khunjarc W, Wang Z.W. (2020) Recalcitrant dissolved organic nitrogen formation in thermal hydrolysis pretreatment of municipal sludge, Environment International”)

8.1 Abstract

THP has been considered as an advanced approach to enhance the performance of anaerobic digestion treating municipal sludge. However, several drawbacks were also identified with THP including the formation of brown and ultraviolet-quenching compounds that contain rDON. Melanoidins produced from the Maillard reaction between reducing sugar and amino group have been regarded as a representative of such compounds. This review presented the state-of-the-art understanding of the mechanism of melanoidin formation derived from the research of sludge THP, food processing, and model Maillard reaction systems. Special attentions were paid to factors affecting melanoidin formation and their implications to the control of rDON in the sludge THP process. These factors include reactant availability, heating temperature and time, pH, and the presence of metallic ions. It was concluded that efforts need to be focused on elucidating the extent of the Maillard reaction in sludge THP. This paper aims to provide a mechanistic recommendation on the research and control of THP-resulted rDON in municipal wastewater treatment plants.

8.2 Keywords

Maillard reaction; Melanoidins; Sludge; Thermal hydrolysis pretreatment; Recalcitrant dissolved nitrogen

8.3 Introduction

Thermal hydrolysis pretreatment is known as an effective municipal sludge pre-treatment process for enhancing anaerobic digestion (Barber 2016). In recent years, increasing applications of sludge THP have been reported in full-scale WWTPs. 75 THP facilities are currently either in operation

or in planning across the world treating 1.65 million metric dry tons of sludge per year (Barber 2016). The principle behind THP involves the hydrolysis and solubilization of the organic component of municipal sludge at elevated temperatures and pressures, commonly reported in the ranges of 160-190 °C and 480 to 1260 kPa (4.8 to 12.6 bar) (Wilson et al. 2011). The advantages of THP on sludge treatment have been well documented including: (i) reduction of sludge viscosity so that the sludge with higher solids content, e.g. 10 % TS, can still be pumped and mixed in anaerobic digesters (Bougrier et al. 2006, Oosterhuis et al. 2014); (ii) improvement of sludge biodegradability and thus biogas production (Liu et al. 2012, Stuckey and McCarty 1984); (iii) enhancement of sludge dewaterability (Higgins et al. 2017, Phothilangka et al. 2008); (iv) pathogen sterilization and odor reduction (Murthy et al. 2009, Neyens and Baeyens 2003). Along with the advantages it brings, there also comes the disadvantages that have not been fully resolved, e.g., the production of substances with high color and ultraviolet (UV)-quenching ability, as well as the generation of recalcitrant carbon, nitrogen, and phosphorus. Among these disadvantages, the production of rDON from THP is selected as a major focus of this review because rDON is known to not only deteriorate the efficiency of UV-disinfection through UV-quenching but also substantially increase the total nitrogen (TN) in the discharge of WWTPs. Dwyer et al. (2008b) has reported a 18% decrease of UV-transmission and a 50% increase of effluent rDON concentration from 1.8 to 2.8 mg L⁻¹ based on the historical data of a full-scale WWTP after commissioning sludge THP. It should be realized that the effluent TN limits of 3 mg L⁻¹ or less are common in regions such as the Chesapeake Bay watershed, coastal areas of North Carolina, mid-Colorado, and Okanagan Lake area of British Columbia, Canada, and increasingly stringent effluent discharge limits will be imposed in the near future (Moore 2010). For example, the 2000 Chesapeake Bay agreement mandated 48% reduction of TN loads from WWTPs based on the 1990 levels, which has led to more stringent effluent TN limits as low as 3 mg L⁻¹ by 2011 (Mulholland et al. 2007). By 2017, only 40% of the nitrogen reduction goal was achieved, which has failed to meet the midpoint-goal of 60% of the necessary TN reduction (EPA 2019). Clearly, as more WWTPs apply THP, the substantial rDON increases observed after THP implementation, e.g. 50%, can become a real threat for WWTPs to meet the increasingly stringent TN limits (Dwyer et al. 2008b). Therefore, serious considerations need to be taken towards the possible rDON formation as a result of THP.

The study by Stuckey and McCarty (1984) was probably the first report of rDON production from THP of activated sludge. Considerable reduction of nitrogen compounds biodegradability was observed in THP operated at 150 to 200 °C, even though the biodegradability of the treated activated sludge was significantly improved. For example, the biodegradability of mixed amino acids decreased 18 to 22% after THP at 200 °C in comparison with untreated samples (Stuckey and McCarty 1984). Penaud et al. (2000) further characterized the recalcitrant soluble compounds produced from the THP of microbial biomass, and then related the recalcitrant soluble compounds to the products from Maillard reaction based on the similarities in terms of supernatant color, product molecular weight, biodegradability, as well as the influence of acid precipitation. More recently, increased color, decreased UV-transmission, and increased DON concentration in plant effluent after the implementation of sludge THP have been reported in a full-scale biological nutrient removal system located at Queensland, AU (Dwyer et al. 2008b). The study conducted within the full-scale WWTPs further inferred that the Maillard reaction products, e.g. melanoidins, may be responsible for the rDON production during the sludge THP based on the comparisons of color, UV-quenching, dissolved organic carbon (DOC), DON, and molecular weight between THP effluent, synthetic melanoidin solution, and the plant effluents from WWTPs with and without THP treated sludge centrate return. As a newly identified issue, in-depth understanding of rDON formation mechanism during THP is essential to ensure the sustainable application of THP. Therefore, in this review, the mechanism of Maillard reaction in the context of THP is discussed to provide a technical guidance for rDON control and also for future research need identification.

8.4 What is the Maillard reaction and why it is relevant to rDON formation in sludge THP?

It has been recognized that the characteristics such as molecular weight, DON and DOC contents, color, UV-quenching, fluorescence, and aromaticity between the synthetic melanoidins formed from the Maillard reaction, e.g., glucose-glycine, and the nitrogen-containing, macromolecular, dissolved substances separated from THP effluent are very similar (Ahuja et al. 2015, Dwyer et al. 2008b, Gupta et al. 2015, Higgins et al. 2017, Penaud et al. 2000). Hence, it is important to understand the principle of the Maillard reaction that dictates the production of melanoidins, a primary type of rDON.

The Maillard reaction is named after French chemist Louis-Camille Maillard who first described the reaction in 1912 (Maillard 1912). The Maillard reaction is a non-enzymatic browning reaction that occurs between reducing sugar and amino group at an elevated temperature, forming dark-colored, UV-quenching, and hardly biodegradable polymers (Hodge 1953, Maillard 1912, Reynolds 1965). Another similar non-enzymatic browning reaction also contributing to the color development is the caramelization which usually occurs at a low water activity (Buera et al. 1987). Since the caramelization reaction does not involve nitrogen and thus is not covered in in this review.

Although the Maillard reaction was discovered one hundred years ago, it was not until recently that its implication in THP of municipal sludge was partially understood. In fact, most research related to the Maillard reaction was performed in the field of food and flavor industry where the reaction is intentionally facilitated to provide brown color and generate flavors and aromas of cooked foods (Martins et al. 2000). Since the typical THP conditions, e.g. 165 °C for 30 min, overlap with that of the Maillard reaction, and municipal sludge is full of polysaccharides (20% - 40%) and proteins (30% - 50%) as potential reactants for Maillard reaction, the knowledge derived from the food industry provides a mechanistic basis for understanding and controlling the rDON production during the THP of municipal sludge (Jimenez et al. 2013).

Nitrogen-containing recalcitrant organics can be produced through the Maillard reaction via the chemical reaction between the carbonyl groups of reducing sugar and the amino groups of amino acids, peptides, or proteins (Hodge 1953). The chemistry underlying the Maillard reaction is of complexity due to the large variety of reaction pathways including condensation, cyclisations, dehydrations, retroaldolisations, rearrangements, and isomerisations, and their strong dependence on reaction conditions such as temperature, time, pH, and the composition of reactants (Labuza et al. 1998, Martins et al. 2000). For this reason, Maillard reaction products are believed to be a mixture of heterogeneous compounds including those with relatively low molecular weights (LMWs, < 3.5 kDa) such as aldehydes, ketones, dicarbonyls, acrylamides, heterocyclic amines, and those polymeric compounds with large molecular weights (HMWs, > 10 kDa) such as advanced glycation end-products (Wang et al. 2011).

Among all these complex Maillard reaction products, melanoidins are the most intensively studied heterogeneous and nitrogen-containing brown pigments which have been suspected to be the major rDON produced during THP (Ahuja et al. 2015, Dwyer et al. 2008b, Gupta et al. 2015, Higgins et

al. 2017, Penaud et al. 2000, Wang et al. 2011, Wilson and Novak 2009). Melanoidins were reported to be negatively charged molecules (Bekedam et al. 2008b, Ćosović et al. 2010, Morales 2002). The molecular weight of melanoidins obtained from model Maillard systems, i.e. simplifications of complex natural reactants with pure reducing sugars and amino acids, was believed to be dependent on heating temperature and time. A lower heating temperature and time, for example 95 – 100 °C for 2 – 5 hours, produced primarily LMW (< 3.5 kDa) melanoidins (Hofmann 1998b, Kim and Lee 2008a, Ramonaitytė et al. 2009), while HMW (>10 kDa) melanoidins were predominately obtained from real foods or model Maillard systems heated under higher temperature or longer time, e.g. 121 °C for 30 min or 100 °C for more than 48 hours (Brudzynski and Miotto 2011, Ćosović et al. 2010, Ibarz et al. 2009, Morales 2002). Under the typical sludge THP condition, e.g. 140 to 165 °C for 30 min, the increased color, UV-quenching, DON, and organic recalcitrance have been largely attributed to the melanoidins with HMW (> 10 kDa) (Dwyer et al. 2008b, Penaud et al. 2000).

The color and UV-quenching are distinct characteristics of melanoidins. Many attempts have been made to quantify the color development and UV-quenching of melanoidins in model Maillard systems by using spectrophotometric approach and the Lambert-Beer equation in Eq. (8.1) (Brands et al. 2002, Kim and Lee 2008b, Rufián-Henares and Morales 2007):

$$A = \varepsilon \cdot l \cdot c \quad (8.1)$$

in which A is the light/UV absorbance, ε is the extinction coefficient at a given wave length, l is the length of light/UV passing through in solution, and c is the concentration of absorbent. For example, Rufián-Henares and Morales (2007) determined the ε in Eq. (8.1) at 420 nm for glucose-tryptophan and glucose-lysine melanoidins to be 0.225 and 4.315 ml mg⁻¹ cm⁻¹, respectively. However, for complicated reactants such as real food and sludge, Eq. (8.1) and ε become less useful due to the complicated chemical composition and thus the unknown concentrations of each component, e.g. c in Eq. (8.1), of melanoidins formed with those reactants. Instead, the increase in light or UV absorbance (A) before and after THP was often directly used as indicators for melanoidin production (Ahuja et al. 2015, Dwyer et al. 2008b, Higgins et al. 2017, Penaud et al. 2000).

With regard to the nitrogen content in Maillard reaction products, Cämmerer and Kroh (1995) reported that the C:N ratio varies from 7.4 to 26.3 when different reducing sugars reacted with

glycine following a molar ratio of 1 to 1. Ahuja et al. (2015), Dwyer et al. (2008b), and Higgins et al. (2017) reported substantial DON increase after sludge THP likely due to the production of melanoidins, and the DON production has been observed to increase with THP operating temperature. As recalcitrant organic compounds, melanoidins are also known for poor microbial degradability and even adverse biological effects including genotoxicity, cytotoxicity, and antimicrobial activity (Chandra et al. 2008, Wang et al. 2011). Ivarson and Benzing-Purdie (1987) evaluated the biodegradation of U-¹⁴C labeled synthetic melanoidins with soil microorganisms and reported only 1 mg out of 1.42 g reduction of melanoidins after aerobic incubation for 25 days. For sludge THP, Stuckey and McCarty (1984) reported that the anaerobic biodegradability of mixed amino acids decreased 18-22% after THP at 200 °C in comparison with untreated samples, despite the overall biodegradability improvement of the activated sludge. Penaud et al. (2000) reported that the removal of HMW melanoidins by resin decolorization or acid precipitation led to the improvement of sludge anaerobic biodegradability of 26% in terms of biogas conversion. In addition, Gupta et al. (2015) reported that the aerobic biological treatment had little effect on the organic nitrogen in the returned liquor collected from a full-scale plant sequentially processed with THP, anaerobic digestion, and dewatering. These observations are closely related to the characteristics of melanoidins as summarized in Table 8.1.

Table 8.1 Observations related to the production of melanoidins in THP.

Feedstock	Temperature & Time ^a	Color (ADMI ^b)	UV-Absorbance	DON (mg L ⁻¹)	MW (kDa)	Biodegradability	Reference
Secondary sludge	140-165 °C 30 min	THP: 4000-12500 THP-AD ^c : 4000-8750	THP: 60-120 THP-AD: 20-40	THP: 2000-3000	> 10	Anaerobic biodegradability was not affected by THP temperature	Dwyer et al. (2008b)
Industrial microbial biomass	140 °C 30 min	-	-	-	9-28	HWM melanoidins reduced anaerobic biodegradability	Penaud et al. (2000)
Primary & secondary sludge	130-170 °C 30 min	-	THP-AD: 22 - 32	THP: 1300-2300 THP-AD: 400	-	Anaerobic biodegradability was not affected by THP temperature	Higgins et al. (2017)
Primary & secondary sludge	130-170 °C 30 min	-	THP-AD, permeate: 6.1 - 13.5	THP-AD, permeate: 112 - 328	-	-	Ahuja et al. (2015)
Synthetic nitrogenous organics	200 °C 60 min	-	-	-	-	Anaerobic biodegradability decreased 18 to 22% after THP	Stuckey and McCarty (1984)
Primary & secondary sludge	165 °C 30 min	THP-AD, permeate: 14904	THP-AD, permeate: 38	THP-AD, permeate: 280	> 300	DON was recalcitrant to aerobic biodegradation	Gupta et al. (2015)

^a Temperature increase corresponded to the increases of color, UV-absorbance, and DON within the provided ranges.

^b ADMI: color index developed by the American Dye Manufacturers Institute

^c THP-AD: anaerobic digestion receiving THP-pretreated feedstock.

8.5 What factors affect the Maillard reaction and rDON production?

In this section, we present internal and external factors that affect both the rate and extent of Maillard reaction, with the hope of providing a fundamental understanding for developing strategies to control Maillard reactions and formation of rDON in THP conditions. Five predominating reaction factors, including reactants, heating temperature and time, pH, and the presence of metallic ions are discussed to reveal their impact on Maillard reaction and the properties of derived rDON products.

8.5.1 Effect of reactants

Municipal sludge is rich of polysaccharides (20% - 40%) and proteins (30% - 50%), providing abundant reactants for Maillard reaction at THP conditions (Jimenez et al. 2013). For both pure reactants such as reducing sugars and amino acids used in model systems and the real biomass such as food products, the properties of melanoidins formed was found to be reactant specific (Bekedam et al. 2008a, Bekedam et al. 2008b, Cämmerer and Kroh 1995, Ortega-Heras and González-Sanjosé 2009, Rufián-Henares and Morales 2007, Van Chuyen et al. 1973a, Van Chuyen et al. 1973b). As mentioned previously, the Maillard reactions between glycine and six different D-carbohydrates yielded melanoidins with distinct relative content of DON, i.e. C:N ratios varying from 7.4 to 26.3 under the same reaction condition (Cämmerer and Kroh 1995). However, the results from the same study also indicated that the molar ratio of the reactants had little effect on C:N ratio of the melanoidins produced (Cämmerer and Kroh 1995). The browning effect, e.g. light absorbance of melanoidins purified from different reactants, also showed significant differences. For example, ϵ in Eq. (8.1) determined for glucose-tryptophan and glucose-lysine melanoidins has been reported to be of 19-fold different with values of 0.225 and 4.315 ml mg⁻¹ cm⁻¹, respectively (Rufián-Henares and Morales 2007).

The reactivity of reactants was also reported to affect the kinetics of the Maillard reaction and in turn the characteristics of melanoidins formed. For example, Van Chuyen et al. (1973a, 1973b) quantified the rate of amines degradation and color development at 80 °C when glyoxal reacted with amines with reducing peptides amount, i.e., in the order of three (tetraglycine), two (triglycine), one (diglycine), and zero (glycine) peptides. It showed that the reactivity of the peptides, namely amino acids with short chain, was much greater than that of the single amino acid in terms of color development and amine utilization rates.

The composition and characteristics of melanoidins in real biomass is more complex than in model systems due to its diverse pool of reactants. Many studies in food science have demonstrated that specific food-originated compounds may play important roles in formulating melanoidin compositions. For example, the presence of phenolics in coffee melanoidins has been largely attributed to the presence of chlorogenic acids in coffee beans (Bekedam et al. 2008a, Bekedam et al. 2008b). Polyphenols, the natural micronutrients found in grape, have been shown to contribute to the melanoidins of grape syrup and sweet wine (Ortega-Heras and González-Sanjosé 2009). For municipal sludge THP, the effect of reactants on melanoidin production is largely unknown. Further investigations, for example on the extent of recuing sugar production and the reactivity of various amino groups in sludge, are important to assess the melanoidin production during THP.

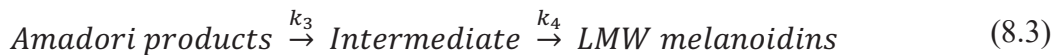
8.5.2 Effect of heating temperature and time

Since the discovery of Maillard reaction, heating temperature and time have been major factors investigated. The kinetics of Maillard reaction can be considered as three steps as described in Eqs. (8.2) to (8.4), and elevated temperature ($> 70\text{ }^{\circ}\text{C}$) is generally favorable for each step: (i) the early-stage to form Schiff base complex and Amadori compound from amino group and sugar is shown in Eq. (8.2) (Ge and Lee 1997); (ii) an intermediate stage for color formation through a range of reactions to form LMW ($< 3.5\text{ kDa}$) melanoidins is shown in Eq. (8.3) (Wedzicha and Leong 2005), and (iii) an advanced stage to form HMW ($> 10\text{ kDa}$) melanoidins through polymerization (Hayase et al. 2006, Tressl et al. 1998) and/or cross-linking of LMW melanoidins is shown in Eq. (8.4) (Hofmann 1998a, b).

(i) Early-stage:



(ii) Intermediate stage:



(iii) Advanced stage:



Temperature plays an important role in determining the Maillard reaction rate constant of each step and in turn govern the dominating reaction pathways and the degree of polymerization and/or cross-linking. Based on the study by Ge and Lee (1997), using a phenylalanine-glucose model

system, the formation of Schiff base complex rather than the formation of Amadori products was determined to be the rate-limiting step during the early stage of the Maillard reaction (Eq. (8.2)) indicated by a three magnitude higher of k_1 than k_2 , namely 3.97 and $3.54 \times 10^{-3} \text{ h}^{-1}$ at the temperature of 97°C , respectively.

It is known that the effect of temperature on reaction rate constant can be described by using the well-known Arrhenius equation, which shows that reaction steps associated with higher activation energy (E_a) are more sensitive to temperature changes (Martins et al. 2000). Ge and Lee (1997) observed that the formation rate of Amadori products from phenylalanine and glucose in Eq. (8.2) with an E_a of 33.5 kJ mol^{-1} started to slowly increase at a temperature around 70°C , and then sharply increased beyond 90°C , while the formation rate of Schiff base complex in Eq. (8.2) with an E_a of 27.3 kJ mol^{-1} increased much slower with temperature. During the intermediate stage in Eq. (8.3), k_3 (first order) and k_4 (second order) in Eq. (8.4) for intermediate and LMW melanoidins formation were in the range of 0.6 to $4 \times 10^{-2} \text{ h}^{-1}$ and 0.7 to $21.6 \text{ mol}^{-1} \text{ L h}^{-1}$, respectively, which are much greater than the kinetic constants of early stage in Eq. (8.2), e.g., 0.7 to $3 \times 10^{-4} \text{ mol}^{-1} \text{ L h}^{-1}$ as determined for glucose reacting with lysine, arginine, glycine, serine, valine, glutamic acid, aspartic acid, or alanine (Wedzicha and Leong 2005). Despite the large variety of reactants and pathways in the advanced stage of the Maillard reaction (Eq. (8.4)), the formation kinetics of HMW melanoidins was often reported as zero order (Brands and van Boekel 2002, Morales and Van Boekel 1996, Morales et al. 1995, Van Boekel 2001). Brands and van Boekel (2002) reported that the kinetic constant in the advanced stage (Eq. (8.4)) was the greatest among all reaction steps with a k_5 of 1.14 h^{-1} and E_a of 128 kJ mol^{-1} for glucose-lysine and a k_5 of 1.86 h^{-1} and E_a of 75 kJ mol^{-1} for fructose- lysine systems. It should be noted that most of the Maillard reaction kinetic constants in the aforementioned studies were obtained at relatively low temperatures e.g. $70 - 100^\circ\text{C}$, and substantially higher reaction kinetic constants can be expected under the typical THP temperature, e.g. 165°C , according to the Arrhenius equation.

With those being said, the first step of the Maillard reaction, i.e., the formation of Schiff base complex from amine and sugar (k_1 in Eq. (8.2)), is usually believed to be the rate-limiting step for melanoidin production. Consequently, further regulation of this rate-limiting step may serve as an efficient measure for rDON control. This can be potentially achieved by reducing the availability of the essential reactants, e.g. amines, through pH adjustment or amine wrapping by coagulants. It

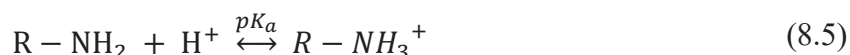
has been reported that the Amadori product formation rate in Eq. (8.2) exponentially decreased with pH decrease, which is in line with the effect of pH on the concentration of reactive unprotonated amino groups (Ge and Lee 1997).

In addition to kinetic constants, temperature also affects the molecular weight, biodegradability, color, and element composition of the melanoidins formed. Wang et al. (2011) summarized that a heating temperature lower than 50 °C and a duration of more than 30 days produced mainly LMW (< 12 -14 kDa) melanoidins during the fermentation and storage of beer, sweet wine, and grape syrup. In contrast, the heating temperature higher than 150 °C and a duration less than 2 hours produced HMW (>12 -14 kDa) melanoidins during the production of bread, coffee, roasted malt, cocoa, and biscuits. Notably, temperature increase was also reported to decrease the biodegradability of synthesized melanoidins by both aerobic and anaerobic microorganisms (Ivarson and Benzing-Purdie 1987), which is undesired for WWTP with high effluent TN standard. The color formation is more associated with the formation of LMW and HMW melanoidins in Eqs. (8.3) and (8.4) (Wedzicha and Leong 2005). The color of HMW melanoidins has been reported to increase with increasing heating temperature and time (Brands et al. 2002, Echavarría et al. 2013, Fogliano et al. 1999). Moreover, Motai (1974) investigated the molecular weights of color components in glycine-xylose melanoidins, and concluded that the darkness of melanoidins was increased by the polymerization of LMW melanoidins to form HMW melanoidins in the advanced stage (Eq. (8.4)). Finally, it was also revealed that temperature may alter the element composition of melanoidins. For example, Cämmerer and Kroh (1995) reported that temperature increase from 60 to 170 °C increased the C:N ratio from 6.2 to 12.5 for glucose/glycine melanoidins. For sludge THP, operating temperature increase, e.g. from 140 to 165 °C, have been demonstrated to lead to more melanoidin production, as indicated by the increased darkness, UV-quenching, DON, and organic recalcitrance (Ahuja et al. 2015, Dwyer et al. 2008b, Higgins et al. 2017). Unfortunately, systematic investigations of the effects of temperature on the characteristics of melanoidins formed during sludge THP remain absent.

8.5.3 Effects of pH

pH has been demonstrated to be an important factor that influences the reactant reactivity and the structure of the melanoidins. For example, pH determined the extent to which the reactions proceed in Eq. (8.3), e.g. the formation of reductones (pH > 7), Fission products (pH = 7), or Schiff's base

of furfural ($\text{pH} < 7$) (Martins et al. 2000). The fundamentals of the effect of pH on the Maillard reaction have to do with the changes in the availability of reactive forms of reducing sugar and amino group in response to pH changes. The un-protonated form of amino group and the open-chain form of reducing sugar at higher pH are considered to be the reactive forms (Martins et al. 2000). Depending on the pK_a of a specific amino group-containing compound, as pH decreases, amino groups will be protonated and gradually lose their reactivity with reducing sugar as shown in Eq. (8.5),



To better illustrate the availability of the reactive forms of amino groups at different pH, Figure 8.1a illustrates an example of the effect of pH on the availability of un-protonated glycine which has a pK_a of 9.6. When the pH is below 7, the content of reactive amino group is less than 0.3%, and the reactive amino group content increases exponentially with pH (Martins et al. 2000). Figure 8.1b shows the effect of pH on the formation rate of the phenylalanine Amadori compound, namely a product of early-stage Maillard reaction in Eq. (8.2). Again, as pH increased towards alkaline condition, the proton releasing from the amine ion increased the reactive amino acid content, leading to an exponential increase of the Amadori formation rate (Ge and Lee 1997).

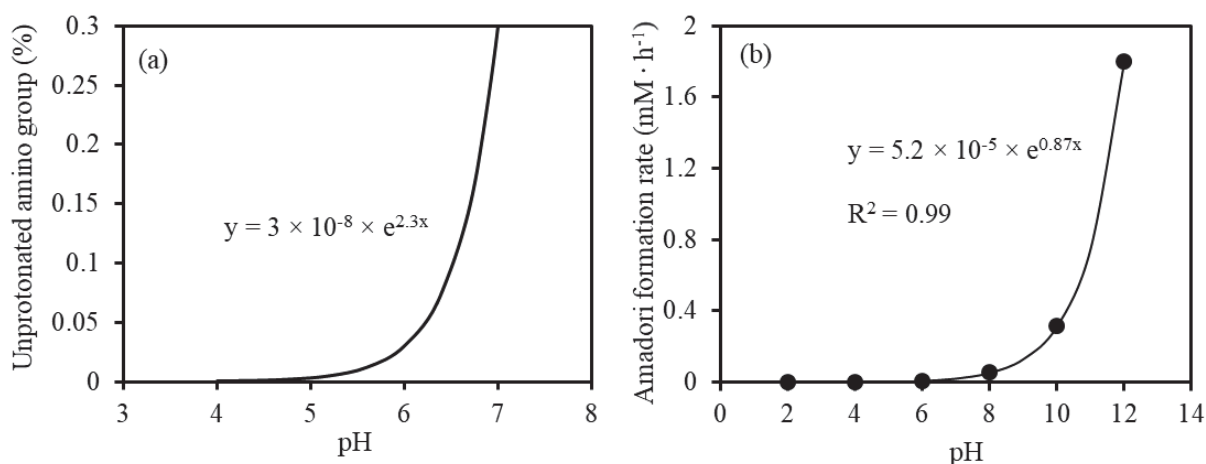


Figure 8.1 a) Effect of pH on the reactive form of the amino group of glycine (Martins et al. 2000); b) Effect of pH on the formation rate of phenylalanine Amadori compound (Ge and Lee 1997).

The reaction pathways and products of Maillard reaction also depend on the types of reducing sugars involved and pH. In aqueous solution, reducing sugars present in several chemical

configurations, including open chain form, cyclic form, ionized form, enediol anion (enolization), and isomers in equilibrium (Martins et al. 2000). It has been reported that the degree of sugar ionization, enolization, and the isomerisations increases as pH increases, which further result in different color patterns of melanoidins formed after Maillard reaction (Kim and Lee 2008a, Laroque et al. 2008, Martins et al. 2000). More thorough studies are still needed to further demonstrate the dependence of melanoidin properties on sugar structure and explain the mechanisms.

It should be noted that the pH usually drops as a result of THP, probably because of the formation of reductone along with the development of the Maillard reaction. The drop of pH has recently been confirmed in glucose-lysine system without buffer (Han et al. 2017, Kwak et al. 2005, Wang et al. 2009). Consistent with the aforementioned pH effect, less dark and polymerized melanoidins were formed from the same reactants when pH dropped without buffer addition (Kwak et al. 2005). Thus, for sludge THP, not only the initial sludge pH, but also the pH dynamics in the course of THP is important for controlling the rDON production.

8.5.4 Effect of metallic ions

Metallic ions such as calcium, aluminum, and iron are commonly used in municipal sludge handling processes to assist sludge thickening, phosphorus precipitation, and odor mitigation (Dassey and Theegala 2012, Morse et al. 1998, Park and Novak 2013, Zhang et al. 2019). As anionic compounds, melanoidins and some Maillard reaction intermediates have been reported to bind with metallic ions. However, the effect of metallic ion binding on the color development of melanoidins is still under debate. O'brien and Morrissey (1997) have reported that the Amadori compounds formed at the early-stage (Eq. (8.2)) of Maillard reaction were able to bind metallic ions with varying binding strength in the order of $Mg^{2+} > Cu^{2+} = Ca^{2+} > Zn^{2+}$. Gomyo and Horikoshi (1976) showed that melanoidins have a remarkable coagulation capacity with various metallic ions including Fe^{3+} , Al^{3+} , Cu^{2+} , Zn^{2+} , Co^{2+} , and Mn^{2+} , and their browning was suppressed by metallic ion additions. However, Morales et al. (2005) examined the iron (II)–binding ability of several food-originated melanoidins and found no relationship between browning and iron binding ability of melanoidins.

On the contrary, Morita and Kashimura (1991) proposed that transition metallic ions such as Cu^{2+} , Fe^{2+} , Fe^{3+} , and Mn^{2+} were able to catalyze the Maillard reaction and promote the formation of chromophores through oxidative pathway indicated by a 48% to 107% increase in 330 nm

absorbance with metal additions. The study by Kwak and Lim (2004) found that the presence of metallic ions can either accelerate or inhibit browning depending on what kinds of amino acids involved, and transition metals Cu^{2+} and Fe^{2+} accelerated the browning of melanoidins the most in general.

Apparently, various mechanisms including chelation, catalyzation, and coagulation may be involved in the interaction of melanoidins and metallic ions. Additionally, the metal binding of melanoidins has also been related to the antioxidant and antimicrobial properties of melanoidions (Rufián-Henares and de la Cueva 2009, Wang et al. 2011).

8.6 Current understanding of the Maillard reaction in THP of municipal sludge

The organic component of municipal sludge (about 80% as VS) typically contains polysaccharides (20% -40%), proteins (30% -50%), and lipids (< 10%), which provides a pool of reactants to fuel Maillard reaction (Jimenez et al. 2013). Moreover, the heating condition of THP, for example 165 °C for 30 min in commercialized THP processes (e.g. CAMB), overlaps with the conditions ideal for the formation of melanoidins through the Maillard reaction (Barber 2016). Despite the high possibility for the Maillard reaction to take place in the THP of municipal sludge, the study of the Maillard reaction in the field of wastewater treatment is still in its early stage.

Many studies have related the increase of DON, browning, and enhanced UV-quenching in the effluent of sludge THP or downstream anaerobic digestion to the formation of melanoidins as summarized in Table 8.1 (Ahuja et al. 2015, Dwyer et al. 2008b, Gupta et al. 2015, Higgins et al. 2017, Penaud et al. 2000, Wilson and Novak 2009). Wilson and Novak (2009) further reported that the THP of polysaccharides alone in a model system at a temperature below 190 °C resulted in little UV-quenching, suggesting sugar browning through caramelization unlikely occurred at the typical temperature of THP. Despite these evidence, little information has been provided to describe to what extent the organics in sludge have participated in the Maillard reaction and formed melanoidins. This is particularly important because THP is aimed to increase the biodegradability of sludge by hydrolysis of the macromolecular organics, while the Maillard reaction and the formation of highly recalcitrant melanoidins play an opposite role. In fact, controversial study results were reported related to the significance of Maillard reaction with respect to sludge biodegradability. Most studies regarding the Maillard reaction reviewed in this paper were at

temperatures below 200 °C. As mentioned previously, recalcitrant melanoidins tend to form with a much faster kinetics at a temperature above 90 °C, and the reaction rate increases with temperature. However, studies showed that sludge biodegradability, as indicated by the change of methane yield before and after THP, increases with THP temperature starting from 100 °C until a maximum level was reached in the temperature range of 175 to 190 °C (Higgins et al. 2017). Clearly, this indicated when the Maillard reaction happened in parallel with thermal hydrolysis, the impact of Maillard reaction on the production of non-biodegradable rDON including melanoidins is minor as compared to the overall biodegradability improvement. At a temperature above 190 °C, the sludge biodegradability has been reported to decrease, and this has been also related to the Maillard reaction in previous studies (Bougrier et al. 2008, Pinnekamp 1988, Stuckey and McCarty 1984). However, at a temperature above 190 °C, the effect of sugar caramerlization must be taken into consideration and compared with the effect of Maillard reaction to better explain the contribution of Maillard reaction to the observed sludge biodegradability decrease. Furthermore, very few studies directly investigated the effect of Maillard reaction on rDON during sludge THP. The study by Higgins et al. (2017) showed insignificant increase of DON concentration in the anaerobic digester effluent as a results of the increase of THP temperature from 130 to 170 °C, which does not support the well-accepted knowledge of enhanced melanoidins formation at high temperature (Brands et al. 2002, Echavarria et al. 2013, Fogliano et al. 1999, Ge and Lee 1997). In addition, previous studies on the effect of THP on rDON production by Higgins et al. (2017) and Dwyer et al. (2008b) only examined the DON concentration in THP or anaerobic digester effluent but didn't test the aerobic biodegradability of DON, namely rDON. It should be realized that DON from anaerobic digester effluent following THP may not be a good measurement of rDON. This is because the recalcitrance of DON should be evaluated in aerobic condition similar to the mainstream treatment.

Furthermore, only a few works were performed to characterize melanoidins formed from sludge THP, and no study has been conducted to address the effect of sludge type and composition, e.g. primary, secondary, or chemical sludge. Dwyer et al. (2008b) compared the molecular weight, DON and DOC contents, color, UV-quenching, fluorescence, and aromaticity between the synthetic melanoidins (glucose-glycine) and the macromolecular dissolved substances separated from THP effluent and also the effluents of two full-scale WWTPs with and without THP. Similarities were identified for the compounds with molecular weight above 10 kDa. However,

biodegradability test was not carried out to verify the recalcitrance of THP effluent or to confirm that melanoidin can represent the increased rDON in plant effluent. Penaud et al. (2000) reported that the browning effect was mostly provided by compounds with molecular weight of 9 to 28 kDa, while compounds with molecular weight greater than 100 kDa contribute the most to the low biodegradability of THP sludge. Studies from other fields demonstrated that the property of melanoidins such as color, molecular weight, and nitrogen content can be highly reactant specific as discussed in section 3.1 (Cämmerer and Kroh 1995, Rufián-Henares and Morales 2007). In order to better evaluate the significance of the Maillard reaction in sludge THP and have a better understanding of sludge-originated melanoidins, the effect of various chemical components of primary treatment, secondary treatment, etc., on melanoidin production in THP should be studied. For example, comparing primary to secondary sludge, the former typically has more VS (75% vs 70% of TS) and polysaccharide (44% vs 26% of TS), less protein (25% vs 36% of TS) and nitrogen (2.5% vs 3.8% TS), and more acidic pH (6 vs 7.1) (Burton et al. 2013). This implies that the THP of secondary sludge may be liable to a higher rDON production potential than the THP of primary sludge due to the higher protein contents and pH. In addition, melanoidins have been demonstrated to have multiple biological effects such as genotoxicity, cytotoxicity, antioxidative effect, and antimicrobial activity (Chandra et al. 2008, Wang et al. 2011). These topics are currently absent in the study of sludge THP, and whether these properties impact the microbial activity in the downstream anaerobic digestion and biological nitrogen removal is also unknown.

Finally, the effects of THP operational conditions other than temperature and time, such as moisture, pressure, pH, buffer capacity, and metal addition on rDON generation have not been investigated. pH is an important factor determining the reactivity of reducing sugar and amino group. It has been reported that THP can reduce sludge pH and increase the sludge alkalinity, likely due to the formation of reductone and the release of weak acids such as ammonia (Han et al. 2017, Wang et al. 2009). Thus, Maillard reaction is not only affected by the initial pH of the sludge but also by the dynamics of pH during THP (Han et al. 2017, Wang et al. 2009). Besides, some sludge THP has been hybridized with acid and alkaline treatment, which potentially has a more substantial effect on melanoidin formation due to the more extreme pH conditions (Neyens et al. 2003a, Neyens et al. 2003b, Shehu et al. 2012, Vlyssides and Karlis 2004). Although the effect of metallic ions on the Maillard reaction is still unclear, significant changes of melanoidin production with and without metallic ion additions have been observed (Gomyo and Horikoshi 1976, Kwak and

Lim 2004, Morita and Kashimura 1991, O'brien and Morrissey 1997). Both transition (Fe) and non-transition (Al, Ca, Mg) metallic ions are commonly used in WWTPs (Dassey and Theegala 2012, Morse et al. 1998, Park and Novak 2013, Zhang et al. 2019). Thus, their impacts on rDON formation during sludge THP are worth investigation.

8.7 Potential strategies for rDON control during and after sludge THP

Melanoidins may appear following THP and create operational problem on UV-disinfection and biological nutrient removal due to their recalcitrant and UV-quenching nature. Thus, strategies for melanoidin reduction are desired in the application of THP in WWTPs.

8.7.1 Lower THP temperature

Theoretically, a lower temperature will result in lower reaction coefficients (Eqs. (8.2) to (8.4)), especially for the rate limiting step (k_1) in Eq. (8.1), hence provides a possible strategy to reduce melanoidin and rDON production. Many researches have reported that a low THP temperature (e.g. 130 to 150 °C) can reduce UV-quenching in the anaerobic digestion effluents, however doing so was also reported to reduce the sludge digestibility (Higgins et al. 2017, Wilson and Novak 2009). Differently, the study by Dwyer et al. (2008b) suggested that low THP temperature (140 to 165 °C) reduced the production of melanoidins but left the sludge digestibility unaffected. These reports imply a potential trade-off between sludge digestibility improvement and the inhibition of melanoidins by decreasing the THP temperature.

8.7.2 pH Adjustment

Homma et al. (1982) measured the isoelectric points of various Maillard reaction products, at which these compounds can be precipitated. The results showed that all Maillard reaction products evaluated showed isoelectric points around pH = 3. Later, Penaud et al. (2000) studied the performance of acid precipitation at the pH of 3 in precipitating melanoidins from sludge THP effluent and observed considerable improvement (over 20%) in sludge digestibility after the acid treatment. Although adjusting pH to this extreme level (pH = 3) is not feasible in practice to control melanoidins, the reactivity of amino group remarkably declines at pH below 7 (Figure 8.1), which indicated that pH adjustment and control within a narrow range around 5 - 7 may also lead to effective melanoidin reduction. Further investigation is warranted to verify this hypothesis.

8.7.3 Upstream metallic ion addition

Metallic ions such as calcium, aluminum, and iron are commonly used in wastewater treatment to assist sludge thickening, odor prevention, and/or phosphorus precipitation (Burton et al. 2013, Dassey and Theegala 2012, Morse et al. 1998, Park and Novak 2013, Zhang et al. 2019). As mentioned previously, some of the metallic ions, e.g. Al^{3+} and Ca^{2+} , bind melanoidins, leading to rDON reduction after THP, yet some of the transition metallic ions, e.g. Fe^{3+} and Mn^{2+} , catalyze the Maillard reaction and lead to increased rDON production (Gomyo and Horikoshi 1976, Morita and Kashimura 1991). Therefore, selection of right metallic coagulants in the processes prior to sludge THP may also serve as a potential strategy for melanoidin control. For example, substitution of Fe^{3+} with Al^{3+} coagulant prior to sludge THP offers possibility to substantially reduce melanoidin production.

8.7.4 Sludge conditioning and dewatering

The anaerobically digested sludge following THP is often subjected to conditioning and dewatering, in which cationic coagulants such as metallic ions and organic polymer are added to improve dewaterability, and the centrate is returned to the mainstream (Burton et al. 2013). Due to the anionic nature of melanoidins, coagulation with various metallic ions have been demonstrated as an effective way for melanoidin precipitation (Gomyo and Horikoshi 1976). Dwyer et al. (2009) reported simultaneous color and DON removal with alum addition into melanoidin-containing sewage treatment plant effluent. The results showed that an alum dose of 30 mg Al L^{-1} was able to achieve color, DON, and DOC removal of 75%, 42%, and 30%, respectively. In another study, adding cationic organic polymer was reported to remove 55% of DON and 45% of UV-quenching in sludge THP return liquor after dewatering, and doing so was particularly effective for removal of DON with molecular weight above 3 kDa which has a higher potential to bind with cationic polymer (Ahuja et al. 2016). Effective DON and UV-quenching mitigation were also reported by using dual conditioning with cationic polymer and ferric chloride during dewatering (Wilson et al. 2011).

8.7.5 Other post-THP treatment technologies

Melanoidins removal by adsorption has been investigated and showed good removal performance. Penaud et al. (2000) studied the color removal effectiveness of adsorbent resin (Amberlite XAD 7HP from Rhom and Haas) in sludge THP effluent and achieved a maximum color removal of

58%. The molecular weight analysis further revealed that melanoidins with a molecular weight higher than 100 kDa were eliminated, but the ones with a relatively low molecular weight of 9 – 82 kDa were only partially removed. Activated carbon adsorption of melanoidins was also investigated using isotherm approach and the best melanoidin adsorption capacity was 450 ± 10 mg COD g⁻¹ (Figaro et al. 2006).

Advanced oxidation processes have also been tested for decolorization of melanoidins prepared from model system. Hayase et al. (1984) reported about 64% and 97% color reduction of melanoidins using hydrogen peroxide in pH of 7 and 10, respectively. Kim et al. (1985) reported 84% and 97% removal of melanoidins by ozone treatment for 10 min and 90 min, respectively. Dwyer et al. (2008a) reported that UV irradiation of hydrogen peroxide was capable of removing 99% color, 50% DOC, and 25% DON from the synthetic melanoidin solution at the hydrogen peroxide dose of 3.3 g L⁻¹.

Several microorganisms have been identified with the ability to utilize melanoidins even though they are well-known to be recalcitrant and even toxic to living organisms. Tiwari et al. (2012) reported that yeast *Candida tropicalis* RG-9 was able to degrade melanoidins and showed a maximum decolonization rate of 75% within 24 hours when incubated at 45 °C with additional carbon (glucose) and nitrogen (peptone) added. In addition, several white-rot fungus species also showed decolonization capacity of melanoidins (Dahiya et al. 2001, Raghukumar et al. 2004, Raghukumar and Rivonkar 2001). A comprehensive review on microbial and enzymatic degradation of melanoidins was published by Chandra et al. (2008)

8.8 Conclusions

The following concluding remarks can be drawn from this review:

1. Maillard reactions are a group of browning reactions between sugar and amino group at elevated temperature and responsible for the formation of heterogeneous pigments known as melanoidins. The Maillard reaction occurs during sludge THP and leads to increased rDON in WWTP effluent. This is because THP conditions overlap with that of the Maillard reaction, and municipal sludge is rich of potential reactants for the Maillard reaction.

2. Melanoidins are recalcitrant, nitrogen-containing, negatively charged, with color and UV-quenching ability, and can be either of high (> 10 kDa) or low (< 3.5 kDa) molecular weight. Very often, melanoidins were used as the representative compounds of rDON in THP pretreated sludge.
3. Different reactant compositions are known to affect the rDON content, color development, reaction kinetics, and reaction pathways.
4. High temperature accelerates the rate-limiting step of the Maillard reaction, namely the formation of Schiff's base complex in early-stage, hence affects the physiochemical properties of melanoidins including molecular weight, color, and the rDON content.
5. pH affects the reactivity of amino group and the structure of sugar via the equilibrium shifts of different forms of the reactants. In general, low pH mitigates rDON production.
6. Metallic ions affect the formation and solubility of rDON through the chelation, the transition metal catalyzation, and the coagulation.
7. The significance of the Maillard reaction in contribution to rDON production during sludge THP has not been fully verified, especially in terms of the recalcitrance to aerobic treatment.
8. Few studies focused on the characteristics of rDON produced from sludge THP. The impacts of many operational conditions of THP such as moisture, pressure, pH, buffer capacity, and metal addition on the production of rDON are still unknown.
9. Lowering THP temperature and pH, proper selection of coagulants for upstream treatment, and sludge conditioning prior to dewatering were proposed in this review as potential strategies to control rDON production. Approaches for post-THP rDON removal are also evaluated.

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Chapter 9 : Impact of thermal hydrolysis pretreatment and anaerobic digestion on the formation of dissolved organic nitrogen recalcitrant to aerobic treatment

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9.1 Abstract

This study for the first time tracked the turnover of DON in the THP operated at different temperatures and followed by anaerobic digestion (AD) and/or aerobic incubation (AI). The results showed that the increasing THP temperature substantially increased the levels of rDON as defined by the DON that can survive aerobic biodegradation. The mesophilic AD operated with 15 days of hydraulic retention time cannot reduce the total rDON level but did increase the high molecular weight fractions of rDON, rCOD, color, and UV-quenching. Because of this anaerobic transformation, the strong correlation of rDON to the THP temperature, rCOD, color, and UV-quenching as detected in the THP effluent disappeared when AD was involved. Attention should be paid to the role of AD in transforming the characteristics of rDON returned to the mainstream treatment trains.

9.2 Keywords

THP, rDON, UV-quenching, color, Maillard reaction

9.3 Introduction

Municipal sludge THP at elevated temperature has been increasingly implemented for enhancing anaerobic digestion (AD) by improving the sludge viscosity, digestibility, dewaterability, and pathogen sterilization (Bougrier et al., 2006; Higgins et al., 2017; Liu et al., 2012; Murthy et al., 2009; Neyens and Baeyens, 2003; Oosterhuis et al., 2014; Phothilangka et al., 2008). However, a hidden risk of using THP lies in its production of rDON via Maillard reaction as elaborated in a recent review (Zhang et al., 2020). This is because the rDON returning from the sidestream to the mainstream through the filtrate or centrate recirculation may contribute to the TN level in the WWTP discharge along with the increase of recalcitrant chemical oxygen demand (rCOD), color,

and UV-quenching substances. For instance, rDON increasing from 1.8 to 2.8 mg N L⁻¹ and UV-transmission decreasing from 59 to 41% have been observed in plant effluent based on the historical data of a full-scale WWTP after commissioning sludge THP (Dwyer et al., 2008). It should be realized that the effluent TN limits of 3 mg L⁻¹ or less are common in regions such as the Chesapeake Bay watershed, coastal areas of North Carolina, mid-Colorado, and Okanagan Lake area of British Columbia, Canada, and increasingly stringent effluent discharge limits will surely be imposed in the near future (Moore, 2010). Clearly, as more WWTPs apply THP, the rDON formation after THP implementation may become a real threat for WWTPs to meet the increasingly stringent TN limits (Dwyer et al., 2008). Therefore, serious considerations need to be taken towards the possible rDON formation as a result of THP. To this end, this study investigated the effects of THP temperature, AD, and aerobic incubation (AI) on the formation and turnover of rDON that is bound to end up in the mainstream treatment trains. It is anticipated that the knowledge obtained from this study will offer insights into the potential impact of the sidestream THP and AD on the mainstream wastewater treatment.

9.4 Materials and methods

9.4.1 Experimental design

The pre-dewatered sludge with a total solids content of 7.4% was collected from a local WWTP. As illustrated in Figure 9.1, sludge THP was conducted at different temperatures of 110, 130, 150, 170, and 190 °C using a 2 L pressure vessel (4600, Parr Instrument, Moline, IL) heated in a muffle furnace for 2.5 hours. The sludge processed in THP at 130, 150, and 170 °C was fed to mesophilic anaerobic digesters stabilized for 60 days at a SRT of 15 days. The digesters were operated with a working volume of 5 L at a constant temperature of 35.5 ± 0.3 °C, and were well-mixed via biogas recirculation at the flow rate of 0.5 L min⁻¹ from the headspace to the conical bottom.

Referring to Figure 9.1, filtrates were harvested through 0.45 µm filters (Millex-HV, Merck Millipore Darmstadt, Germany) from the pre-dewatered sludge, THP effluent, THP-AD effluent, THP-AI effluent, and THP-AD-AI effluent. These filtrates were then dialyzed using 10 kDa and 100 kDa membranes (Por 7, Spectrum, Waltham, MA) to separate the low molecular weight (LMW, <10 kDa), medium molecular weight (MMW, 10 – 100 kDa), and high molecular weight (> 100 kDa) fractions. The dialyzed samples were subsequently quantified for DON, COD, UV-

254 nm absorbance, and 450 nm absorbance. In the context of this study, rDON and rCOD are defined as the DON and COD recalcitrant to AI (Figure 9.1). This is because the real concern actually comes from the DON and COD that can survive the aerobic mainstream treatment, ending up in the WWPT discharge. Therefore, an aerobic respirometer (ARE-800, Challenge, Springdale, AR) was utilized to provide AI at room temperature by real-time monitoring the organics oxidation by fresh activated sludge collected from the aeration basin of a local WWTP until the oxygen utilization rates (OURs) dropped to negligible levels, e.g. $< 0.002 \text{ ml L}^{-1} \text{ h}^{-1}$, for more than 3 days. Prior to the respirometer test, all filtrates were diluted with deionized water to ammonia concentrations of 45 mg N L^{-1} in similarity to that in the mainstream conditions.

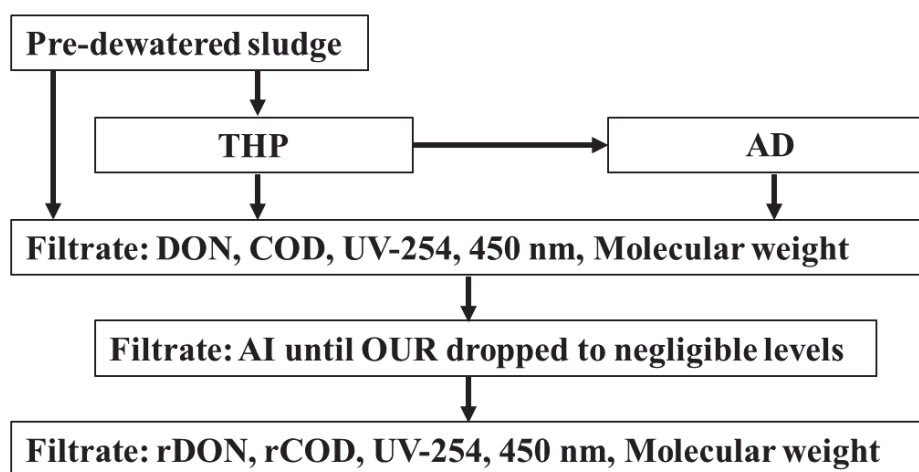


Figure 9.1 A schematic illustration of the experimental design.

9.4.2 Chemical analysis

DON was determined by the concentration difference between TN and inorganic nitrogen including ammonia, nitrate, and nitrite. The ammonia, nitrate, nitrite, and, TN concentrations were measured using the Hach test kits of TNT 832, NitriVer 3, TNT835, and Method 10072, respectively. COD was measured with the Hach TNTplus Vial test kit. UV-254 nm, and 450 nm absorbance were determined using a plate reader (Synergy H1, BioTeck, Winooski, VT).

9.4.3 Statistical analysis

All the sludge pretreatment, sample preparation, incubation, and chemical analysis were conducted in duplicate. Pearson correlation was used to analyze the multivariate relationships among rDON,

rCOD, UV-254 nm absorbance, 450 nm absorbance, and THP temperature. T-test was conducted for the correlation analysis, and results with a p-value < 0.05 were considered significant.

9.5 Results

9.5.1 Effect of THP, AD, and AI on DON and rDON formation and turnover

As can be seen in Figure 9.2a, the DON concentration gradually increased from 502 to 874 mg N L⁻¹ with the THP temperature increase from 110 to 170 °C and then slightly drop to 811 mg N L⁻¹ at 190 °C. The major fraction of the DON was made of LMW which followed the same trend as the total DON (Figure 9.2a). In contrast, the THP temperature did not show much influence on the HMW DON fraction which remained at almost the same concentration around 50 mg N L⁻¹ as in the raw sludge (Figure 9.2a). In addition, the DON with MMW that originally did not exist in the raw sludge started to show up in THP, and its concentration monotonously increased from 24 to 126 mg N L⁻¹ with the THP temperature from 110 to 190 °C (Figure 9.2a).

The AI experiment in Figure 9.2b revealed that majority of the DON (84 to 88%) was actually biodegradable in comparison with the results in Figure 9.2a. Yet, the concentration of the DON remaining after AI, namely rDON, still followed the same trends as that of the DON before AI (Figures 9.2 a and b). For example, the LMW fraction increased from 66 to 114 mg N L⁻¹ with THP temperature increase from 110 to 170 °C and then dropped to 73 mg N L⁻¹ at THP temperature of 190 °C (Figure 9.2b). The HMW fraction remained almost unchanged at 15 mg N L⁻¹ which was about the same as the fraction in raw sludge that survived AI (Figure 9.2b). Again, the MMW fraction monotonously increased from 3 mg N L⁻¹ to 8 mg N L⁻¹ with the THP temperature increase from 110 °C to 190 °C. Differently, this MMW fraction started to show up in the raw sludge after AI (Figure 9.2b).

The anaerobic degradability of DON shown in Figure 9.2c was not as good as that under the aerobic condition in Figure 9.2b. For example, the concentrations of DON survived AD were still 28, 48, and 39 mg N L⁻¹ higher than those survived AI at all THP temperatures tested. Interestingly, the major fraction of DON became HMW DON as a result of AD, which averagely increased 2.6 times for all three THP conditions (Figure 9.2c). Such a substantial increase of HMW DON might be attributable to the hydrolysis of particulate organics during AD in that the THP effluent was fed

to AD in Figure 9.2c but only filtrate was fed to the AI in Figure 9.2b (Mata-Alvarez et al., 2000). In comparison, only minor amount (4%) of LMW DON originally contained in THP effluent remained after AD. The MMW DON was only detected in the filtrate from the AD operated with THP at 170 °C (Figure 9.2c). It is noteworthy that the total rDON concentrations with AD in Figure 9.2d were not much different from that without AD in Figure 9.2b at the same THP temperature. The only difference is that the fraction of HMW rDON increased from 12% in the filtrate without AD (Figure 9.2b) to 25% in the filtrate with AD (Figure 9.2d), suggesting that the AD has potentially increased the molecular weight of rDON.

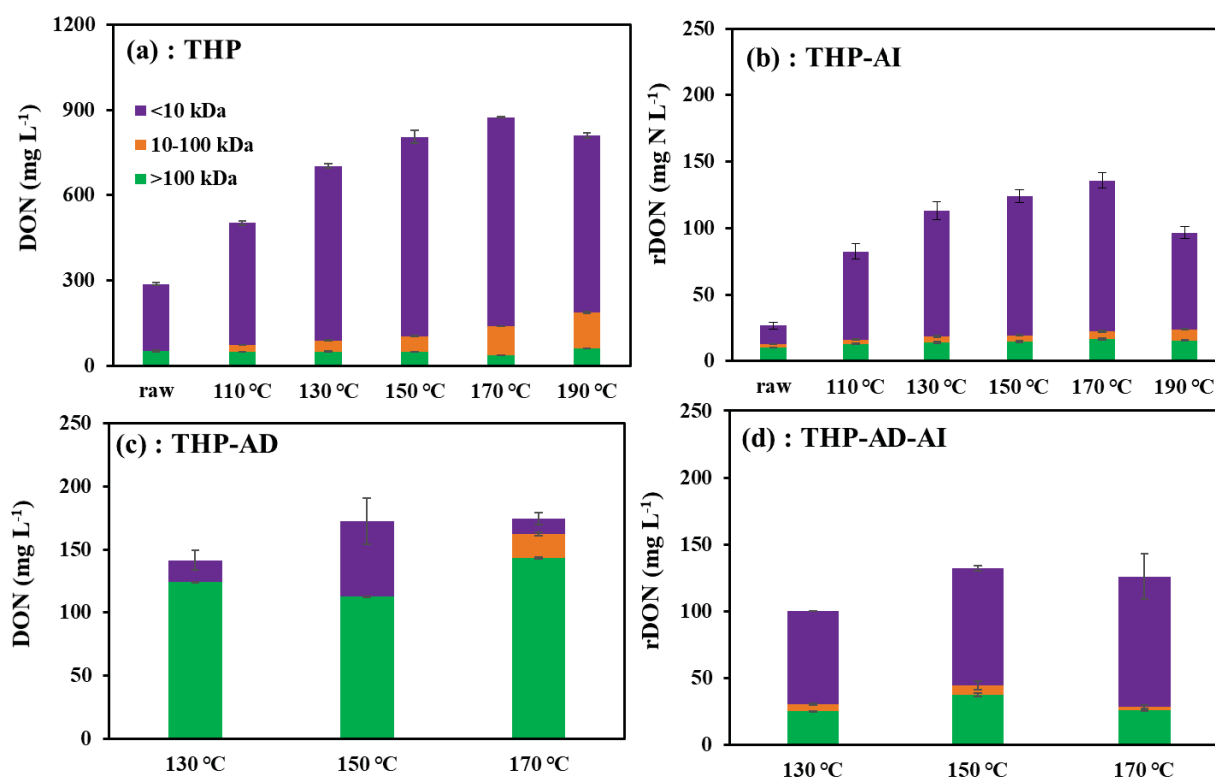


Figure 9.2 Effect of THP temperature on filtrate DON and rDON contents within different molecular weight ranges as a result of: a) THP; b) THP-AI; c) THP-AD; and d) THP-AD-AI.

9.5.2 Effect of THP, AD, and AI on COD and rCOD formation and turnover

The soluble COD demonstrated a similar trend as that of the DON in response to the THP temperature increase (Figures 9.2a and 9.3a), i.e., as the THP temperature increased from 110 to 190 °C, both the LMW and MMW COD increased while the HMW COD remained almost unchanged (Figure 9.3a). Likewise to the observation in Figure 9.2b, most of these three fractions

of COD were biodegradable under aerobic condition with LMW COD as the major remaining fraction (Figure 9.3b). There was about 0.3 g L^{-1} HMW COD remaining regardless of the THP temperature, which was about 3 times that in the aerobically treated raw sludge (Figure 9.3b). Similar to the observation in Figure 9.2c, the soluble COD reduction in AD was not as much as that in AI (Figure 9.3c), i.e., there was still about 6.5 g L^{-1} soluble COD remaining after AD regardless of the THP temperature (Figure 9.3c). Very similar to Figure 9.2c, the concentration of HMW COD actually increased after AD, and the MMW COD only showed up in the AD THP at 170°C (Figure 9.3c). Very different from Figure 9.2d, Figure 9.3d shows that the AI following AD can substantially removal rCOD with only 1.7 to 2.2 g L^{-1} remaining, indicating AD has possibly improved the biodegradability of rCOD generated in THP. The majority of rCOD remaining after THP-AD-AI was with HMW (Figure 9.3d).

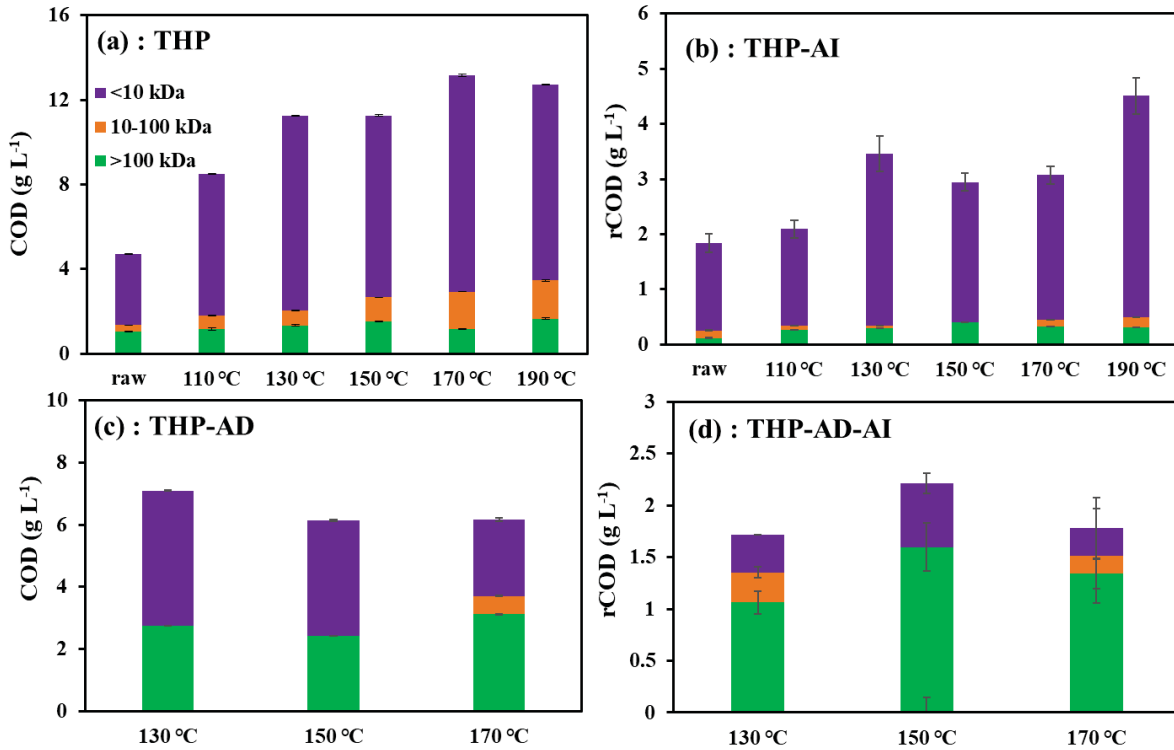


Figure 9.3 Effect of THP temperature on COD and rCOD contents within different molecular weight ranges as a result of: a) THP; b) THP-AI, c) THP-AD; and d) THP-AD-AI.

9.5.3 Effect of THP, AD, and AI on UV-quenching substance formation and turnover

The THP temperature effect on UV-quenching molecule production in Figure 9.4a followed the same trends as those of the rDON and rCOD (Figures 9.2a and 9.3a). Basically, majority of the

quenching was contributed by LMW substances (Figure 9.4a). The contribution from HMW substances remained the same as in the raw sludge regardless of the THP temperature. The contribution from MMW substance monotonously increased with THP temperature (Figure 9.4a). AI can remove greater than 3 orders of magnitude of UV-quenching substances (Figure 9.4b). In the minor remaining UV-quenching substances, MMW became a major contributor (Figure 9.4b). Only minor contribution was made by the LMW and HMW compounds to the UV-quenching regardless of the THP temperature (Figure 9.4b).

The AD also removed majority of the UV-quenching substances generated in THP even though not as thorough as the AI did (Figure 9.4c). Interestingly, almost all UV-quenching substances remained after AD were with HMW (Figure 9.4c). In contrast, the LMW UV-quenching substances were almost completely removed. Only minor contribution from MMW substances to UV-quenching was observed in the 170 °C THP sample after AD, which was consistent with the DON and COD patterns measured in Figures 9.2c and 9.3c, respectively. It is noteworthy that UV-quenching in the AI effluent with AD in Figure 9.4d was actually higher than that without AD (Figure 9.4b). This indicated that, besides THP, sludge AD also contributed to UV-quenching increase. LMW and MMW substances contributed almost equally to the UV quenching while HMW substances contributed much less (Figure 9.4d).

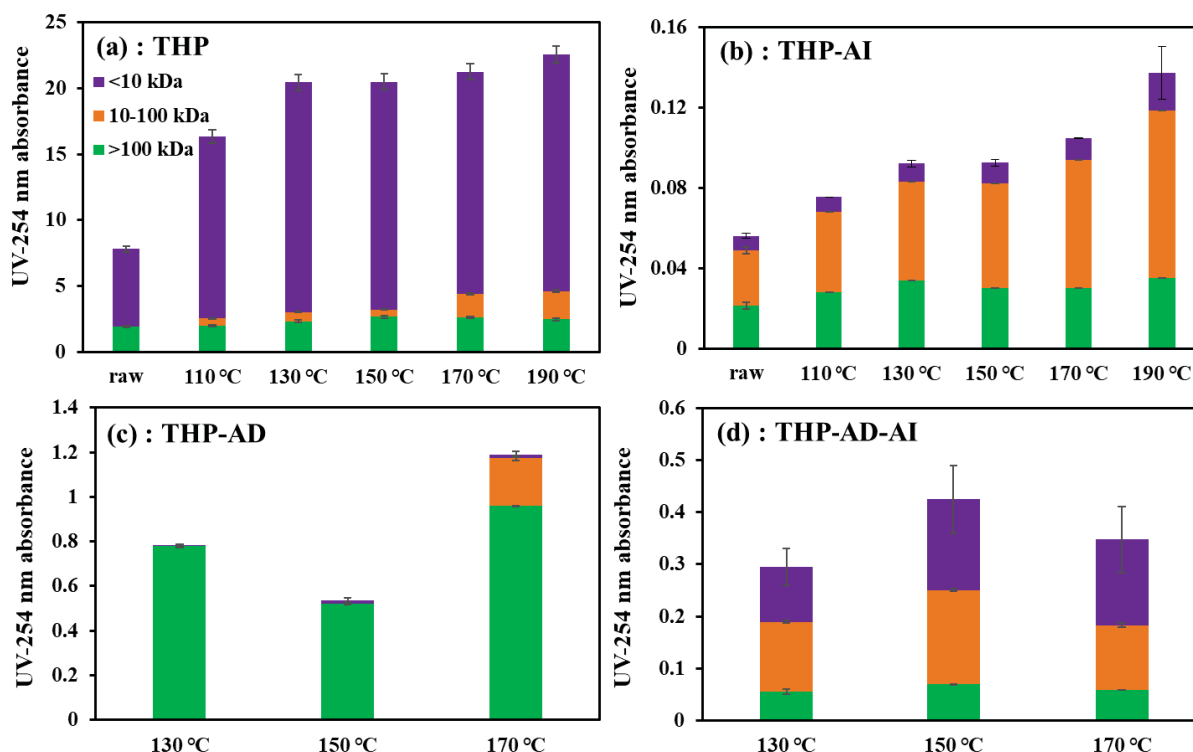


Figure 9.4 Effect of THP temperature on UV-quenching within different molecular weight ranges as a result of: a) THP; b) THP-AI, c) THP-AD; and d) THP-AD-AI.

9.5.4 Effect of THP, AD, and AI on color formation and turnover

Figure 9.5a shows that the THP temperature between 110 and 170 °C substantially increased the color absorbance from 0.42 to 0.77 even though this trend did not sustain in the effluent from 190 °C THP. The major contributor to the color increase were HMW substances (Figure 9.5a). In contrast, the color contribution from LMW and MMW substances were minor. The AI removed greater than 97% of the color created by THP (Figure 9.5b). The majority of the color remaining after the AI was contributed by LMW substances. The AD only removed 54% to 74% of the color created by THP with majority of the remaining color contributed from HMW substances (Figure 9.5c). Again, the color in the AI effluent with AD in Figure 9.5d was still higher than that without AD (Figure 9.5b) even though 95% color was removed in the THP-AD-AI effluent (Figure 9.5d).

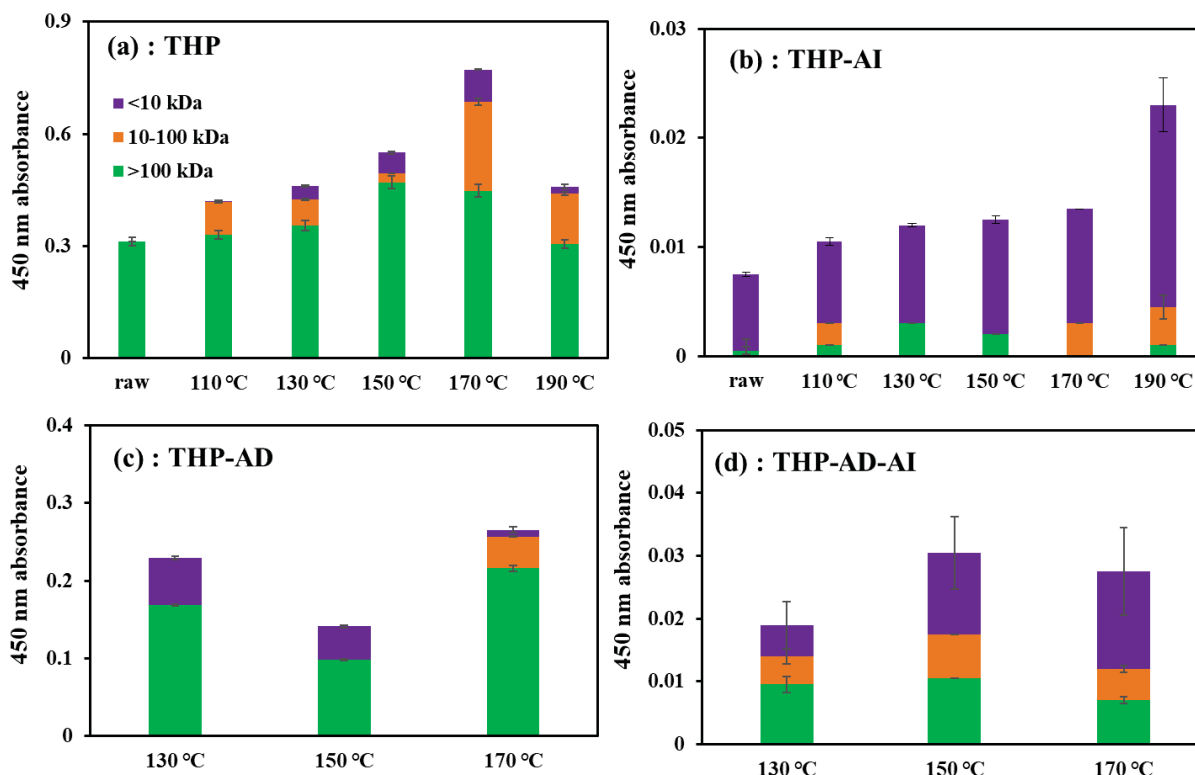


Figure 9.5 Effect of THP temperature on the color within different molecular weight ranges after: a) THP; b) THP-AI; c) THP-AD; and d) THP-AD-AI.

9.5.5 Aerobic degradation kinetics of THP or THP-AD effluent filtrate

The OUR profiles presented in Figure 9.6 display the aerobic degradation kinetics of THP and THP-AD filtrates. Overall, it can be seen that the OUR profile without AD was always above that with AD, indicating AD has removed a substantial amount of dissolved organic matters and thus decreased the aerobic biodegradability of the filtrate (Figure 9.6). However, the considerable aerobic oxidation of the organic matters remaining in AD effluent also indicates that anaerobic degradation alone is actually insufficient for evaluating the recalcitrance of the organics returned to the mainstream treatment trains as done in previous studies (Dwyer et al., 2008; Higgins et al., 2017).

In general, three phases of aerobic degradation can be identified in all profiles in Figure 9.6: Phase I) a period of high OUR peaks likely due to oxidizing easily biodegradable organics; Phase II) a period of medium OUR for oxidizing organics with medium biodegradability; and Phase III) a period of slow OUR for oxidizing organics with low biodegradability. Figure 9.6 showed that the

OUR trends were almost identical for treating the THP effluent without AD even though the THP temperature increased from 130 to 170 °C, suggesting that the THP temperature ranging from 130 to 170 °C didn't change the overall aerobic biodegradability of filtrates probably because the non-biodegradable component was actually minor in THP effluent regardless of the THP temperature (Figures 9.2 and 9.3).

The effect of THP temperature on recalcitrant organics was more notable during the AI of THP-AD effluent in that most of the readily biodegradable fraction has been removed by AD. As the THP temperature increased from 130 °C in Figure 9.6a to 150 °C in Figure 9.6b, the peak for the utilizations of easily biodegradable organics in AD effluent disappeared, and a faster utilization of the medium biodegradability was observed (Figure 9.6b). This indicated that the THP temperature increase from 130 to 150 °C provided more readily biodegradable organics for AD, hence less available for the AI. However, at the temperature of 170 °C, the OUR profile in Figure 9.6c became very similar to that of 130 °C in Figure 9.6a, suggesting that the organics bioavailability for AD was the best at 150 °C but not at the 170 °C of THP. This is in line with the COD reductions in Figures 9.3c and d in which the aerobic COD reduction of THP-AD effluent first decreased from 76% at 130 °C to 64% at 150 °C, and then increased to 71% at 170 °C. Figure 9.6 implies a potential trade-off between the sludge digestibility improvement and the recalcitrant organics formation at elevated THP temperatures. Previous studies reported that the increase in THP temperature not only improved organic digestibility but also increased the formation of dissolved recalcitrant organics (Higgins et al., 2017; Wilson and Novak, 2009).

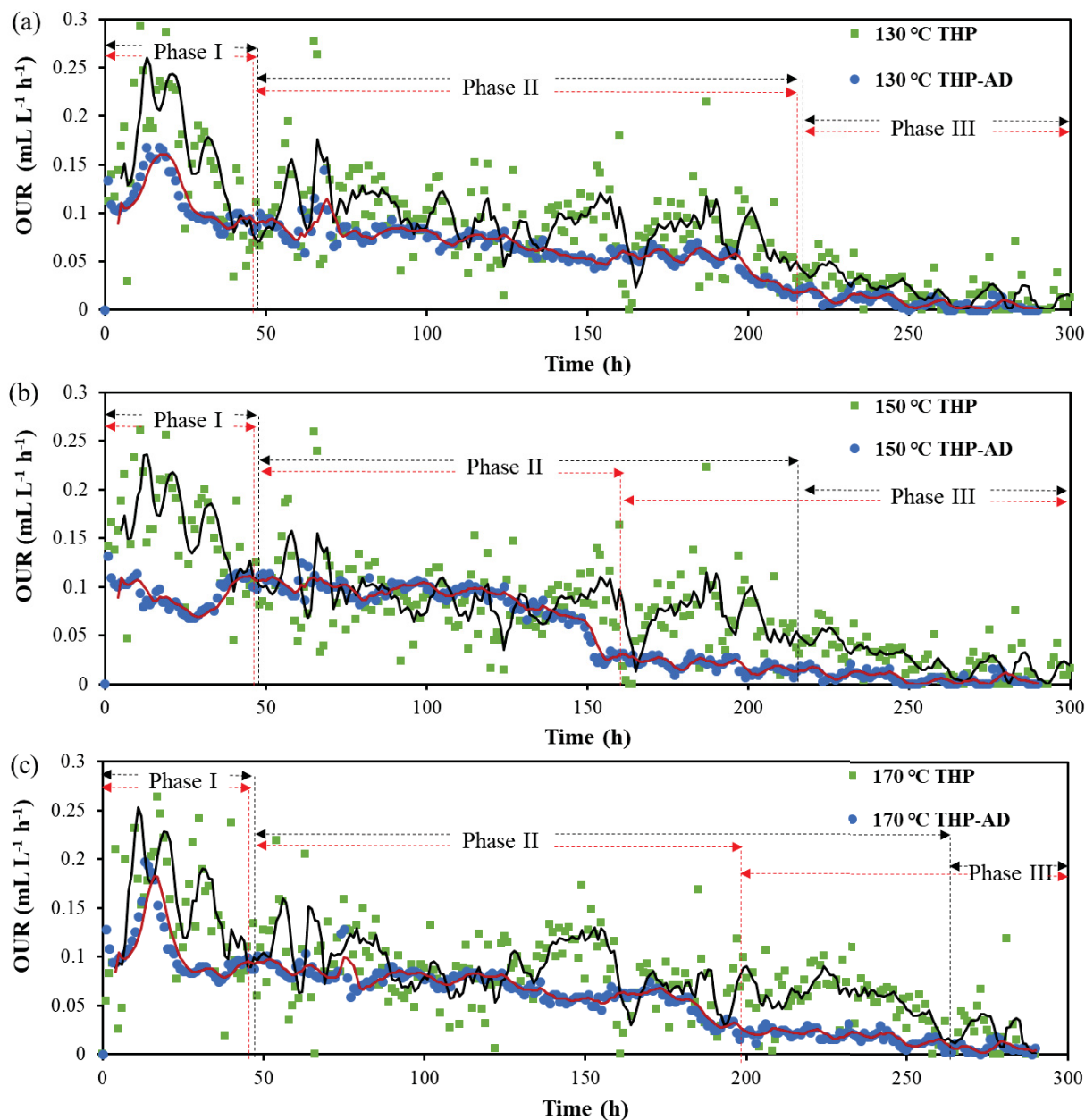


Figure 9.6 Effect of THP temperature and AD on OUR during aerobic incubation.

9.6 Discussion

9.6.1 Impact of THP and AD on rDON

The Maillard reaction between protein and sugar at temperature ranging from 110 to 190 °C has been suspected as a possible mechanism responsible for the rDON production during THP (Zhang

et al., 2020). It was recognized that the melanoidins formed from the Maillard reaction and the dissolved substances separated from the sludge THP share very similar characteristics in terms of molecular weight, DON and COD contents, color, UV-quenching, fluorescence, and aromaticity (Ahuja et al., 2015; Dwyer et al., 2008; Gupta et al., 2015; Higgins et al., 2017; Penaud et al., 2000). The results from Figures 9.2b to 5b exhibited that the rDON, rCOD, color, and UV-quenching monotonously increased with THP temperature ranging from 110 to 170 °C when AD was not applied. This is in agreement with the Maillard reaction kinetics, i.e., high temperature leads to high rate of Maillard reaction and in turn the formation of more rDON (Ge and Lee, 1997; Martins et al., 2000; Minowa et al., 2004; Nursten, 2005; Wang et al., 2011; Wang, 2011).

There was some inconsistency observed at the THP temperature of 190 °C, i.e. although rCOD (Figure 9.3b), UV-quenching (Figure 9.4b), and color (Figure 9.5b) have dramatically increased at this temperature, the rDON level actually has dropped (Figure 9.2b). One possible explanation of this inconsistency at 190°C is related to sugar caramelization which is another non-enzymatic browning reaction contributing to the color, UV-quenching, and rCOD production but not to the rDON production (Buera et al., 1987). Wilson and Novak (2009) reported that the sugar caramelization occurred at the temperature of 190 °C and above in the THP of polysaccharides, which overlapped with the highest THP temperature used in this study. However, sugar caramelization does not involve nitrogen and thus only leads to the increase of color, UV-quenching, and rCOD but not the rDON. The reduced rDON production was possibly due to the competition between the caramelization reaction and the Maillard reaction for sugar at 190 °C.

AD effectively removed about 82% DON and 46% soluble COD generated from THP (Figures 9.2 a and c). This observation is consistent with the finding reported by Higgins et al. (2017), i.e., AD destroyed 71-79% of the DON generated from THP. Because of this excellent anaerobic degradation of DON, rDON eventually accounted for 71-77% of the DON coming out of the THP-AD (Figure 9.2 c and d) even though rDON was only 14-16% of the DON originally generated from THP (Figure 9.2 a and b). This indicates that majority of the DON coming out of AD was actually rDON which is detrimental to the TN compliance in the mainstream discharge. Contrary to the outstanding performance of AD in removing DON and COD, it made little improvement on the total rDON reduction (Figure 9.2 c and d), exhibited adverse effect on color and UV-quenching reduction (Figures 9.4d and 9.5d), and even shifted the molecular weight distribution towards the

HMW (Figures 9.2d to 9.5d). The molecular weight distribution analysis found that quite some HMW DON compounds formed during the AD of THP effluent, which is likely due to the anaerobic hydrolysis of particulate organic nitrogen compounds in sludge (Figure 9.2c), and these HMW DON compounds were only partially biodegradable in the subsequent AI (Figure 9.2d). Similar observation was also reported previously, i.e., more larger dissolved organic matters remained after AD than after aerobic digestion (Du and Li, 2017). In contrast, much LMW rDON that formed in THP but cannot be degraded under the aerobic condition (Figure 9.2b) were degraded under the anaerobic condition (Figure 9.2c). As a matter of fact, the superior ability of anaerobic condition over aerobic condition for the biodegradation of LMW dissolved organics has been recognized before (Amaral et al., 2009; Bilgili et al., 2008). A recent review pointed out that the LMW compounds actually form during the early and intermediate stages of the Maillard reaction (Zhang et al., 2020).

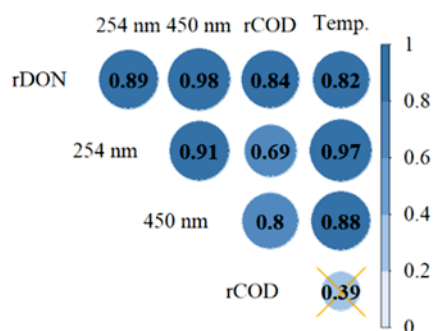
9.6.2 Correlations of rDON to THP temperature, rCOD, UV-quenching, and color in THP or THP-AD effluent

In order to better reveal the correlation between parameters related to rDON at the THP temperature ranging from 110 to 170 °C, Pearson's correlation coefficient was calculated with its significant level shown in Figure 9.7. As can be seen from Figure 9.7a and b, the overall and LMW rDON showed strong positive correlation with temperature, UV-quenching, color, and rCOD in the THP effluent without AD. Moreover, rDON increased with temperature and UV-quenching regardless the molecular weight distribution (Figures 9.7a to d). This is in line with the feature of the Maillard reaction in which the concentrations of the Maillard reaction products and UV-absorbance are always correlated with each other and increase with the temperature (Brands and van Boekel, 2002; Stamp and Labuza, 1983). Strong positive correlation between rDON and rCOD was also identified for both LMW and HMW compounds (Figure 9.7b and d) but not for the MMW compounds (Figures 9.7c). Theoretically, the correlation between rDON and rCOD depends on the molecular C:N ratio in the Maillard reaction products, which may be of greater variance for MMW compounds. For example, Cämmerer and Kroh (1995) reported that the C:N ratio varied from 7.4 to 26.3 when different reducing sugars reacted with glycine to produce melanoidins, a representative rDON, with a molecular weight above 14 kDa within the MMW range. In addition, color development showed strong positive correlation with rDON and temperature only for LMW compounds (Figure 9.7b). It should be noted that the LMW compounds

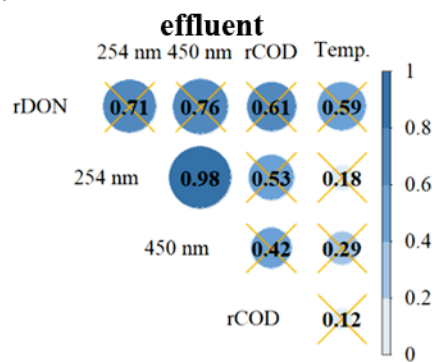
formation and color development actually occur during the early and intermediate stages of the Maillard reaction (Ge and Lee, 1997; Wedzicha and Leong, 2005). In contrast, MMW and HMW compounds form in the advanced stage of the Maillard reaction through polymerization and/or cross-linking of LMW compounds, and the color development for these compounds can vary greatly with the reactants of different nitrogen contents (Rufián-Henares and Morales, 2007). As can be seen in Figures 9.2b and 9.5b, the LMW compounds was the major contributors to both rDON and color, suggesting the early and intermediate stages of the Maillard reaction may be more predominant during the sludge THP.

When THP effluent was firstly processed in AD and then in AI, most of the correlations previously identified without AD involvement in Figure 9.7 disappeared. The overall rDON contents after AD showed insignificant correlation with THP temperature, rCOD, UV-quenching, and color (Figure 9.7e). The LMW rDON was only correlated to color (Figure 9.7f). As mentioned previously, the LMW rDON dominated in THP-AI effluent was likely degraded in AD (Figures 9.2b and c). For this reason, the strong correlations relating rDON to the Maillard reaction observed without AD involvement in Figure 9.7b disappeared in Figure 9.7f. Likewise, the MMW rDON was almost completely removed during AD (Figure 9.2), and thus showed no correlation with any other parameters in Figure 9.7g at all. Finally, as AD substantially increased the fraction of HMW compounds likely through anaerobic hydrolysis (Figures 9.2 to 9.5), the correlation identified between rDON, THP temperature, and rCOD in THP effluent for HMW in Figure 9.7d no longer exists in Figure 9.7h.

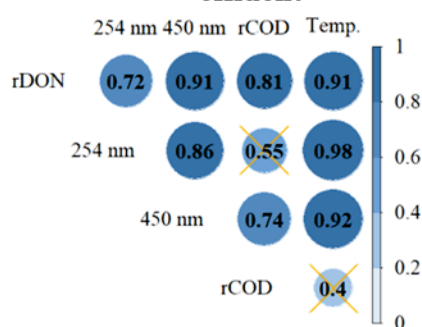
(a) Overall in THP-AI effluent



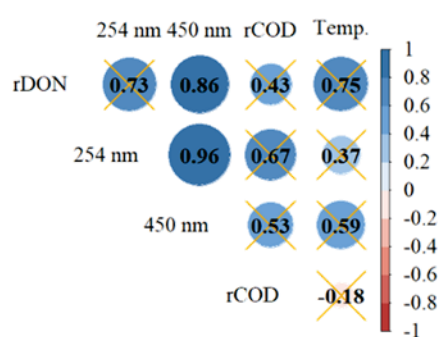
(e) Overall in THP-AD-AI effluent



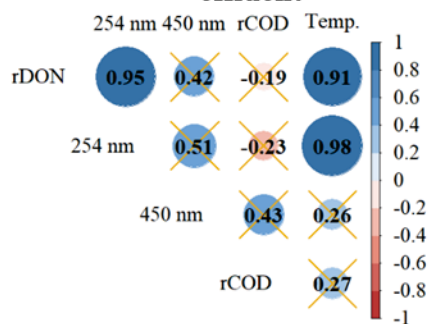
(b) LMW Compounds in THP-AI effluent



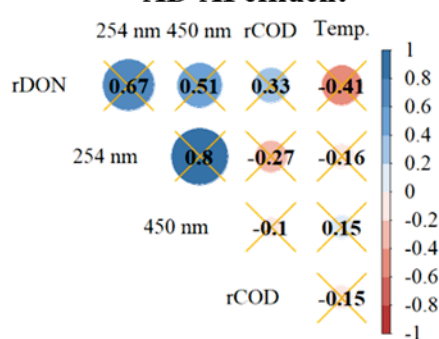
(f) LMW Compounds in THP-AD-AI effluent



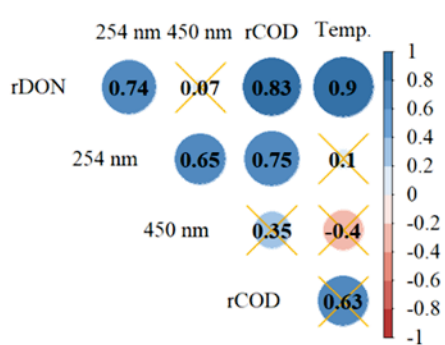
(c) MMW Compounds in THP-AI effluent



(g) MMW Compounds in THP-AD-AI effluent



(d) HMW Compounds in THP-AI effluent



(h) HMW Compounds in THP-AD-AI effluent

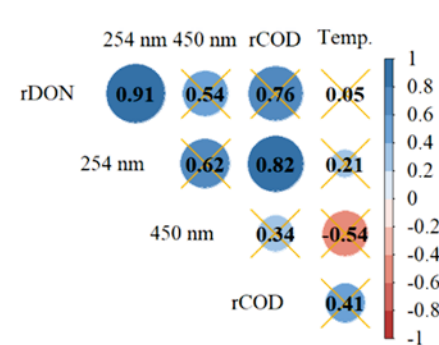


Figure 9.7 Correlations among THP temperature, rDON, rCOD, color, and UV-quenching within different molecular weight ranges in THP-AI or THP-AD-AI effluent (blue circle – positive Pearson correlation coefficient; red circle – negative Pearson correlation coefficient; yellow cross – insignificant correlation with a P value > 0.05).

9.7 Conclusions

The following concluding remarks can be drawn from this study:

1. The rDON contents increased 110 to 310% as the THP temperature increased from 110 to 170 °C as compared to that in the raw sludge without THP.
2. AD did not reduce the total rDON but reduced 6-11% rCOD generated in THP.
3. More high molecular weight rDON, rCOD, color, and UV-quenching showed up as a result of the AD.
4. Although rDON accounted for 14-16% of the DON generated from THP, it made up 71-77% of the DON coming out of the THP-AD, indicating majority of the DON in AD effluent was actually rDON that is detrimental to the TN compliance in the mainstream discharge.
5. In THP-AI effluent, rDON showed strong correlations with the THP temperature, rCOD, color, and UV-quenching, which is in line with the mechanism of the Maillard reaction. However, these correlations disappeared when AD was involved. Therefore, the color and UV-quenching can be used as a good indicator of rDON only for the THP or THP-AI effluent but not for the THP-AD-AI effluent.
6. Two to three orders of magnitude of UV-quenching reduction and more than 95% color reduction can be achieved whenever AI is involved, e.g. through THP-AI or THP-AD-AI.

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