A HYBRID PRE-TREATMENT WITH MICROWAVE AND HYDROGEN PEROXIDE FOR PERFORMANCE ENHANCEMENT IN TWO-STAGE ANAEROBIC DIGESTION

A THESIS

Submitted by

HERALD WILSON AMBROSE

for the award of the degree

of

DOCTOR OF PHILOSOPHY

JOINTLY WITH

DEPARTMENT OF BIOTECHNOLOGY

BHUPAT AND JYOTI MEHTA SCHOOL OF BIOSCIENCES

INDIAN INSTITUTE OF TECHNOLOGY MADRAS

JANUARY 2021

THESIS CERTIFICATE

This is to certify that the thesis titled **"A HYBRID PRE-TREATMENT WITH MICROWAVE AND HYDROGEN PEROXIDE FOR PERFORMANCE ENHANCEMENT IN TWO-STAGE ANAEROBIC DIGESTION",** submitted by **Mr. HERALD WILSON AMBROSE**, to the Indian Institute of Technology Madras, Chennai and Curtin University, Australia for the award of the degree of Doctor of Philosophy, is a bonafide record of research work carried out by him under our supervision. The contents of this thesis, in full or in parts, have not been submitted to any other institutes or University for the award of any degree or diploma.

Prof. G. K Suraishkumar
Professor
Department of Biotechnology
Bhupat and Jyoti Mehta School of Biosciences
Indian Institute of Technology Madras
Chennai-600036

Dr. Tushar Sen
Adjunct Associate Professor
WASM: MECE
Faculty of Science and Engineering
Curtin University
Western Australia-6102

Dr. Hussein Znad
Associate Professor
WASM: MECE
Faculty of Science and Engineering
Curtin University
Western Australia-6102

Date: 18.01.2021 **Place:** Chennai

ACKNOWLEDGEMENT

First and foremost, I thank almighty God for his immense blessings and guidance during the entire Ph.D. course. I would like to sincerely thank and deeply acknowledge Prof. G. K. Suraishkumar, Dr. Tushar Sen and Dr. Hussein Znad for their relentless support and guidance throughout my Ph.D. journey. Prof. G. K. has been my inspiration in various aspects, particularly in his teaching methods and approach towards students. Also, he has provided enormous moral support and lifted my spirits during the tough phases of my course. On the other hand, Dr. Tushar has provided excellent guidance and support during my research at Curtin University. He has been greatly helpful in the procurement of research equipment and establishing lab facilities at Curtin University. I also thank Dr. Hussein for his excellent support and guidance at Curtin and IIT Madras. I am highly indebted to my supervisors for the wonderful support they have provided throughout the course. I also specially thank Dr. Ligy Philip for providing lab facilities to conduct my experiments at IIT Madras.

I deeply appreciate and thank Prof. Abhijit Mukherjee, Dean South Asia and Director of IITM- Curtin Dual Doctoral Degree Program, Prof. Gary Allison, Associate Deputy Vice-Chancellor (Curtin University), Prof. A. K. Mishra, Dean Academic Research (IIT Madras) and Ms. Kavitha G. R. for all their support in smoothly conducting this collaborative Ph.D. program. I specially thank my dear friend Dr. Nilesh Kumar Jha for his invaluable contribution in completing all formalities related to this collaborative program.

I am extremely grateful to the Department of Biotechnology and WASM at IIT Madras and Curtin University, respectively for the academic support. I thank Dr. Smita Srivastava, Prof. K. Subramaniam, Dr. K. Chandraraj and Dr. Raghunathan Rengasamy for their thoughtful comments during all Doctoral Committee meetings at IITM. I thank Prof. Ming Ang for his insightful suggestions during the meetings held at Curtin. I especially thank Dr. Beng Chua, Life Sciences facility manager at Curtin, for his extraordinary help in establishing the lab facility at B300 and continuous support throughout my work. I also extend my sincere thanks to Dr. Roshanak Doroushi, Ms. Melina and Mr. Andrew Chan for their unwavering support in providing the necessary chemicals and equipment facility at Curtin. I also would like to thank Dr. Eugene and Dr. Anteneh for their ingenious ideas towards my experimental setup and expert guidance during my studies at Curtin.

My heartfelt thanks to all my lab mates at both IIT Madras and Curtin University. I take this moment to especially thank Dr. Archana Raghavan, Mr. Raja Murugan and Ms. Krishnapriya for being as close as a family in providing moral support throughout my Ph.D. course. I also thank Ms. Sandhya, Mr. Ravichandran, Mr. Puneet, Ms. Lakshmi, Mr. Balaji Manickavasagam, Dr. Abilarasu, Dr. Krithika and Mr. Arvind for helping conduct my experiments at IIT Madras. I specially thank my lab mates Dr. Subrata Biswas and Mr. Jinesh Machale at Curtin, for their unparalleled support.

I thank all my friends at both IIT Madras and Curtin, who have made this a wonderful journey. I am deeply indebted to my dear friends Mr. Ambuj and Mr. Santhosh at IIT Madras. They have been with me both in times of desperation and joy; whom I can rely on at any time in my life. And, I'm extremely grateful to all my Callan Close family and the Curtin- Indian community for the wonderful times spent together in Perth. I also thank my dear friends, Dr. Yogesh Kumar and Dr. Anbarasu for their support over the years. And, many thanks to my dear students Mr. Sivasankar and Mr. Suthersun for their assistance in sending consignments for my research. Finally, I would like to extend my deepest gratitude to my dear friend Mr. Calvin Liang, without whom my research at Curtin would have been difficult. I thank him for all the help in research, travel and exploration.

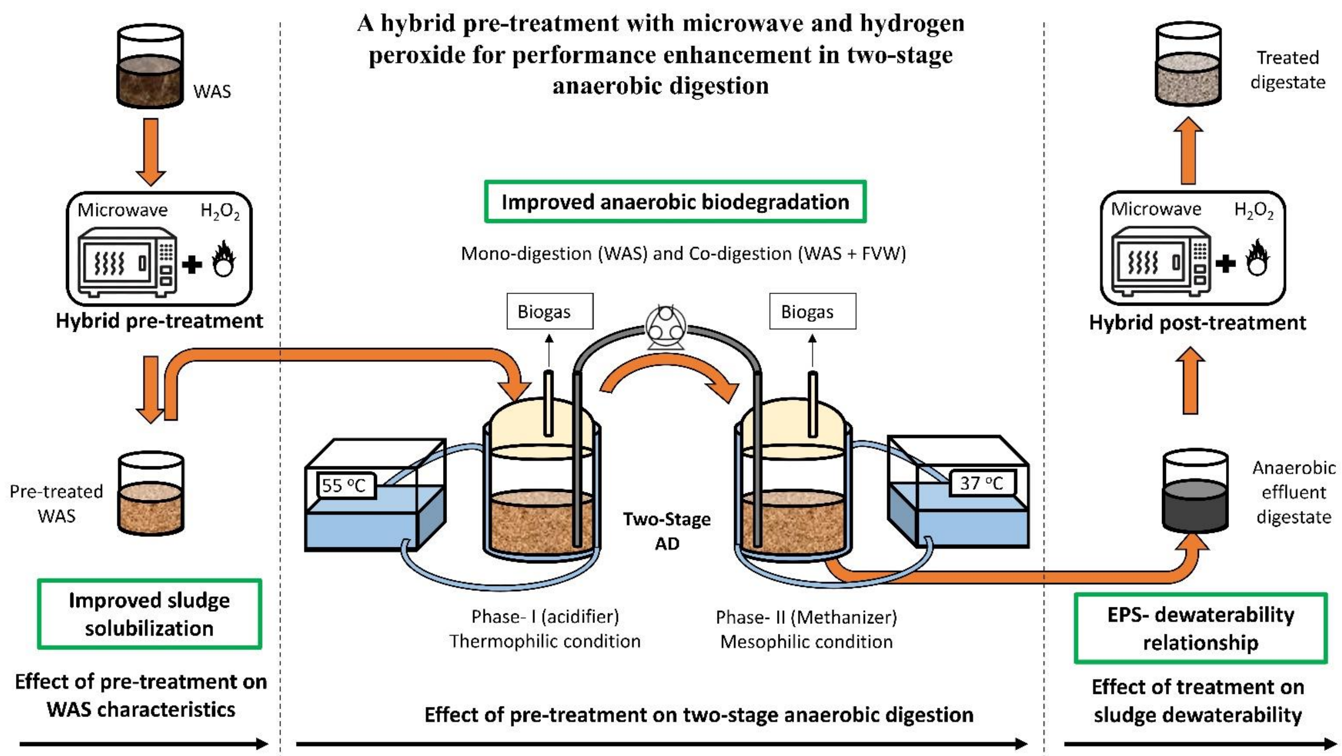
I would like to thank my family for being very supportive and patient throughout my studies. I thank my parents Mr. Ambrose and Ms. Amalorpavam, my brother Jerald Milton, my in-laws Ms. Jayashree, Dr. Anand, Dr. Soudaminy, Mr. Kiran and baby Izzy. Finally, I thank my beloved wife Ms. Monisha for being my pillar of strength throughout the Ph.D. tenure.

I dedicate this research work to my wife and family.

Date: 18-01-2021

Indian Institute of Technology Madras, India Herald Wilson

GRAPHICAL ABSTRACT



WAS- Waste activated sludge; FVW- Fruit and vegetable wastes; H_2O_2- Hydrogen peroxide; AD- Anaerobic digestion

ABSTRACT

The rapid rise in global population has led to increased production of wastewater in urban settlements. The treatment of such municipal wastewater at wastewater treatment plants (WWTPs) results in the production of waste activated sludge (WAS), a semi-solid by-product produced from the secondary treatment unit. The production of excess sludge, sludge bulking, and odor are disadvantageous in WWTPs, therefore various sludge conditioning methods are adopted. Anaerobic digestion (AD) is one among the sludge stabilization techniques, wherein total solids, pathogens, and odor are minimized through the metabolic action of anaerobic microorganisms. In addition, AD produces biogas, which can be utilized for energy recovery in WWTPs. However, the process efficiency of AD is limited due to the slow hydrolysis of WAS flocs resulting in poor anaerobic biodegradability. Therefore, in the current thesis work, a hybrid MW-H_2O_2 (microwave and hydrogen peroxide) pre-treatment technique was studied in a novel semi-continuous two-stage anaerobic digestion to enhance the anaerobic biodegradability of WAS.

The effect of hybrid treatment on sludge at three different stages of anaerobic digestion has been dealt sequentially in the thesis as shown in the graphical abstract. At first, the effect of hybrid pre-treatment on influent WAS characteristics was studied. Secondly, the pre-treated sludge was studied in two-stage anaerobic digestion, which includes mono-digestion of WAS and co-digestion of WAS and fruit and vegetable wastes (FVW). Finally, the pre-treatment effects on effluent digestate dewaterability were examined. To begin with, the hybrid pre-treatment effect on sludge solubilization was compared between two WAS from a different origin, namely Nesapakkam waste activated sludge (NWAS) and Beenyup mixed waste activated sludge (BMWAS). The optimum pre-treatment condition for both the sludges varied, as the composition of both the sludges was different. The maximum COD, biopolymer solubilization was achieved at 450 W + 1 % H_2O_2/TS and 660 W + 1 % H_2O_2/TS for NWAS and BMWAS for 2 min contact time, respectively. The hybrid (MW-H_2O_2) pre-treatment results in the breakdown of microbial cells and extracellular polymeric substances (EPS) matrix through the synergetic action of thermal (microwave) and oxidative stress (hydrogen peroxide) effect. This effect was witnessed through the increased solubilization of biopolymers with increasing microwave intensity in the hybrid pre-treatment. Besides, the hybrid pre-treatment produced 8- and 4.1- fold higher hydroxyl and

superoxide radicals respectively, compared to the untreated WAS, suggesting the elevated oxidative stress achieved through the pre-treatment.

Furthermore, a novel semi-continuous two-stage anaerobic digestion was developed for the current study. The two-stage digestion consists of two reactors namely, phase-I and phase-II, which serve as acidifier and methanizer, respectively. Both the reactors were maintained at different temperature and retention times to promote the microbial activity of respective anaerobic digestion. The phase-I was maintained at a constant thermophilic condition with a shorter retention time, while the phase-II was maintained at a constant mesophilic condition with a longer retention time. The performance of two-stage anaerobic digestion was compared with conventional single-stage digestion, in terms of biogas production and effluent digestate quality. In addition, a 660 W + 1 % H_2O_2/TS hybrid pre-treatment was applied in two-stage anaerobic digestion and the subsequent performance was evaluated. The two-stage operation achieved 76.4 ml/g tCOD methane yield compared to 40.4 ml/g tCOD achieved in single-stage anaerobic digestion, with an increase in average methane percentage. The application of pre-treatment increased the methane yield to 143.4 ml/g tCOD while sustaining 71 % methane in the peak methanogenesis days. This enhancement was attributed to the 22.12 % initial COD solubilization achieved through pre-treatment and elevated methanogenic activity in the two-phase operation. Moreover, the hybrid pre-treated two-stage operation achieved a 90 % reduction of faecal coliform, thereby improved the effluent digestate quality. Hence, the two-stage operation with pre-treatment has shown improved anaerobic biodegradability of WAS compared to conventional single-stage operation.

The research on novel two-stage anaerobic digestion with pre-treatment was further extended by the anaerobic co-digestion study. As the anaerobic digestion of fruit and vegetable wastes (FVW) face severe acidification issues in conventional single-stage digesters, co-digestion of FVW with BMWAS was investigated in the two-stage digester. The study revealed the importance of mixing ratio and the effects of hybrid pre-treatment on two-stage digester stability. A mixing ratio of 75 % BMWAS and 25 % FVW was compared to 50 % of each substrate. The former achieved a 1.6-fold increase in overall methane yield compared to the latter due to the difference in alkaline buffer capacity in both the digestions. Similar to the mono- digestion of BMWAS, the application of pre-treatment increased the anaerobic biodegradability in the former mixing ratio, achieving a cumulative methane yield of 169.1 ml/g tCOD, with an improved buffer capacity. This could be ascribed to the increased release of biopolymers and the resultant digester stability achieved through pre-treatment. Moreover, the pre-treatment induced prolonged oxidative stress through the generation of intracellular superoxide radicals and the levels were downregulated by sludge bioactivity during active methanogenesis. Therefore, two-stage anaerobic digestion with pre-treatment was found to be effective in digesting organic-rich substrates under optimum mixing condition.

Furthermore, the relationship between biopolymer distribution in the EPS matrix and sludge dewaterability was studied through microwave and hybrid treatments. The study was conducted in anaerobic effluent digestate and WAS. The study revealed that the release of biopolymers was observed to increase with increasing microwave intensities, accompanied by increasing dewaterability in both the sludges. And beyond a threshold, the dewaterability was worsened which was concurrent with a decrease in biopolymer release. Besides, the application of hybrid treatment accelerated the dewaterability at lower microwave intensities (220 W to 660 W).

PUBLICATIONS

Journal articles

1. Ambrose, H.W., Philip, L., Suraishkumar, G.K., Karthikaichamy, A., Sen, T.K., 2020. Anaerobic co-digestion of activated sludge and fruit and vegetable waste: Evaluation of mixing ratio and impact of hybrid (microwave and hydrogen peroxide) sludge pre- treatment on two-stage digester stability and biogas yield. J. Water Process Eng. 37. https://doi.org/10.1016/j.jwpe.2020.101498
2. Ambrose, H.W., Chin, C.T.L., Hong, E., Philip, L., Suraishkumar, G.K., Sen, T.K., Khiadani, M., 2020. Effect of hybrid (microwave-H2O2) feed sludge pretreatment on single and two-stage anaerobic digestion efficiency of real mixed sewage sludge. Process Saf. Environ. Prot. 136, 194–202. https://doi.org/10.1016/j.psep.2020.01.032

Book chapter

1. Ambrose, H.W., Philip, L., Sen, T.K., Suraishkumar, G.K., 2019. The Effect of Combined Microwave and Hydrogen Peroxide Pretreatment on Sludge Characteristics and Oxidation Status of Waste Activated Sludge, in: Lefèbvre, B. (Ed.), The Activated Sludge Process: Methods and Recent Developments. Nova Science Publishers, pp. 105–141.

TABLE OF CONTENTS

ABBREVIATIONS

AD	Anaerobic digestion
ADS	Anaerobically digested sludge
AOP	Advanced oxidation process
APF	Amino phenyl fluorescein
APHA	American public health association
ARGs	Antibiotic resistance genes
ASP	Activated sludge process
BCA	Bicinchoninic acid
BMWAS	Beenyup mixed waste activated sludge
BOD	Biological oxygen demand
BWWTP	Beenyup wastewater treatment plant
CFU	Colony-forming units
CMWSSB	Chennai Metropolitan Water Supply and Sewerage Board
COD	Chemical oxygen demand
CST	Capillary suction time
DHE	Dihydroethidium
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DS	Dry solids
EPS	Extracellular polymeric substances
EU	European union
EWRE	Environmental and water resources engineering
FAS	Ferrous ammonium sulfate
FOS/ TAC	The ratio of volatile organic acids to alkaline buffer capacity
FTIR	Fourier transform infrared spectroscopy
FVW	Fruit and vegetable waste
GHGs	Greenhouse gases
HRT	Hydraulic retention time
IIT	Indian Institute of Technology

IITMAS	Indian Institute of Technology activated sludge
IITMSTP	Indian Institute of Technology Madras sewage treatment plant
LB-EPS	Loosely bound extracellular polymeric substances
MLD	Million liters per day
MLSS	Mixed liquor suspended solids
MPN	Most probable number
MW	Microwave
MWAS	Mixed waste activated sludge
MW-H_2O_2	Microwave and hydrogen peroxide
NSTP	Nesapakkam sewage treatment plant
NWAS	Nesapakkam waste activated sludge
OFMSW	Organic fraction of municipal solid waste
OLR	Organic loading rate
PCB	Polychlorinated biphenyls
PLOOM	Perth long term ocean outlet monitoring
POME	Palm oil mill effluent
ROS	Reactive oxygen species
RPS	Raw primary sludge
SBM	Swachh bharat mission
SBR	Sequencing batch reactor
sCOD	Soluble chemical oxygen demand
SOFC	Solid oxide fuel cell
SRF	Specific resistance to filtration
SRT	Solids retention time
SS	Suspended solids
TB-EPS	Tightly bound extracellular polymeric substances
tCOD	Total chemical oxygen demand
TDS	Total dissolved solids
TEAS	Thickened excess activated sludge
TF	Triphenyl formazan
TOC	Total organic carbon

TPAD	Temperature phased anaerobic digestion
TS	Total solids
TSS	Total suspended solids
TTC-DHA	Triphenyl tetrazolium chloride-dehydrogenase
TWAS	Thickened waste activated sludge
UASB	Upflow anaerobic sludge blanket
UASFF	Upflow anaerobic sludge-fixed film
UFF	Upflow fixed film
UN	United Nations
UNICEF	United Nations Children's Fund
US EPA	United States Environmental Protection Agency
VFA	Volatile fatty acid
VS	Volatile solids
VSS	Volatile suspended solids
WAS	Waste activated sludge
WASM	WA School of Mines: Minerals, Energy and Chemical engineering
WHO	World Health Organization
WWTP	Wastewater treatment plant
ZP	Zeta potential

NOTATIONS, SYMBOLS AND ELEMENTS

$OH^{\bullet}$	Hydroxyl radicals
s[$OH^{\bullet}$]	Specific intracellular hydroxyl radicals
$O_2^{\bullet-}$	Superoxide radicals
s[$O_2^{\bullet-}$]	Specific intracellular superoxide radicals
$SO_4^{\cdot-}$	Sulfate radicals
O_3	Ozone
O_2	Oxygen
CO_2	Carbon dioxide
CH_4	Methane
H_2	Hydrogen
H_2O	Water
H_2O_2	Hydrogen peroxide
Fe^{2+}	Ferrous ion
Fe^{3+}	Ferric ion
HO^-	Hydroxide anion
$HO_2{}^{\bullet}$	Perhydroxyl radical
NaOH	Sodium hydroxide
$Ca(OH)_2$	Calcium hydroxide
HCl	Hydrochloric acid
H_2SO_4	Sulfuric acid
H_3PO_4	Phosphoric acid
HNO_2	Nitrous acid
$FeCl_2$	Ferrous chloride
$K_2Cr_2O_7$	Potassium dichromate
H_2S	Hydrogen sulfide
CH_3COOH	Acetic acid
C/ N	Carbon: Nitrogen
°C	Degree Celsius
s	Second

min	Minute
h	hour
µ	Microbial growth rate
k_h	Hydrolysis rate constant
GHz	Gigahertz
MHz	Megahertz
kHz	Kilohertz
%	Percentage
ε'	Dielectric constant
ε''	Dielectric loss factor
J	Joule
KJ	Kilojoule
HP	Horsepower
nm	nanometer
g	Gram
mg	Milligram
kg	Kilogram
M	Molar concentration
Es	Specific energy
W	Watt
kW	Kilowatt
l	liter
ml	Milliliter
µl	Microliter
m^3	Cubic meter
t	time

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Background

The global population is increasing at a rapid rate and it leads to a rise in population density at urban centers. The rise in population is so intense in developing countries like India, where an estimated 404 million urban dwellers will be added within 2050 (UN, 2014). These heavily populated cities will be producing billions of tons of wastewater and sewage sludge every year. Collection, treatment, transportation and disposal of such wastewater and sludge is subjected to strict environmental concerns due to the presence of organic pollutants and pathogenic organisms (Ariunbaatar et al., 2014; Katsoyiannis and Samara, 2005). As the urban population increase in size globally, the need to find efficient ways of wastewater treatment with low cost and eco-friendly effluent becomes imminent.

Wastewater treatment involves the collection of used water from various sources such as houses, industries, agriculture and cities through sewers/pipes and its subsequent treatment at established facilities called wastewater treatment plants (WWTPs) to remove contaminants and enrich for safe disposal (Mateo-sagasta et al., 2015). Within WTTPs, the wastewater undergoes sequential treatments such as primary, secondary, and tertiary levels to remove suspended solids, degrade soluble organics and achieve water reclamation. However, each treatment stage leads to the generation of residual solids/ sludge which incurs high costs for stabilization, transportation, and disposal. In particular, the activated sludge process (ASP) of secondary treatment produces biological flocs for the consumption of organic substrates in wastewater, which results in sludge bulking, excess sludge production and odor (Chen and Lo, 2003; Wu et al., 2009). The ASP leaves a huge volume of waste activated sludge (WAS) with biological flocs that are difficult to dewater and threatens the environment with the presence of harmful pathogens and secondary organic pollutants (Yu et al., 2013). Therefore, anaerobic digestion (AD) is employed to manage the huge volume of waste generated from the WAS system. Anaerobic digestion (AD) process is an established sludge stabilization technique that takes care of solid reduction, pathogen removal and also produces biogas for energy recovery (Cieślik et al., 2015). Biogas production from AD has gained global attention as it produces green energy with a reduction in the emission of greenhouse gases (Mao et al., 2015). AD also reduces odor, exhibits a high rate of killing pathogenic organisms and the resultant solid cakes can be used as soil conditioners (Gurjar and Tyagi, 2017).

The process of AD involves four major biological processes viz, hydrolysis, acidogenesis, acetogenesis and methanogenesis, taking place in the sequential order and are governed by distinct microbial communities (Anukam et al., 2019). Hydrolysis, being the first phase of anaerobic digestion involves the breakdown of complex organic matter into soluble organics (Turkdogan-Aydinol et al., 2011). During this process, hydrolytic fermentative bacteria breaks down complex high molecular weight carbohydrates, proteins and fats into their simple monomeric units through enzymatic action and facilitates subsequent acidogenesis in AD (Angelidaki et al., 2009). This process is the rate-limiting step of AD, as the presence of EPS (Extracellular polymeric substances) matrix in biological flocs, fiber content and a higher fraction of suspended solids make biological degradation difficult (Carrère et al., 2010; Chong et al., 2012; Izumi et al., 2010). Specific components in the EPS of activated sludge are resistant to aerobic and anaerobic biodegradability (Park et al., 2008). Due to this, the conversion of organics to biogas is reduced to half in conventional mesophilic anaerobic digesters (Parkin and Owen, 1986). Therefore, increasing the hydrolysis rate or the inherent biodegradability of the activated sludge becomes important to achieve maximum efficiency in AD.

Various pre-treatment methods and process modifications have been studied in the field of wastewater treatment, to facilitate faster hydrolysis and subsequent enhancement in AD (Carrère et al., 2010; Ji et al., 2017; Rajeshwari et al., 2001). Physical, chemical and biological pre-treatments methods have been reported to improve the sludge solubilization thereby, make the organics better accessible for the anaerobic bacteria (Pérez-Elvira et al., 2006). Physical treatments include thermal hydrolysis (Dessì et al., 2016; Ennouri et al., 2016; Gianico et al., 2016), high-pressure homogenizers (Wahidunnabi and Eskicioglu, 2014; S. Zhang et al., 2012), stirred ball mills (Kopp et al., 1997; Muller et al., 1998), ultrasonication (Gong et al., 2015; Jibao Liu et al., 2016b; Yeneneh et al., 2017a; Zhang and Jin, 2015) and microwave (Byun et al., 2018; Eskicioglu et al., 2007b; W. J. Park and Ahn, 2011; J. Zhang et al., 2016). Chemical treatments include acid or alkaline hydrolysis (Huang et al., 2016; E. Neyens et al., 2003a), oxidation by ozone (Chacana et al., 2017; Cheng et al., 2012) and hydrogen peroxide (Andreozzi, 1999; Jia et al., 2015; Liu et al., 2017) and surfactants (Hong et al., 2015; Poornima Devi et al., 2014; A. Zhou et al., 2015). And, biological pre-treatments include enzymatic hydrolysis (Nie et al., 2012; Yu et al., 2013) and microbial activity (Tang et al., 2012). All sludge pre-treatment technologies mentioned above work on the principle of the disintegration of cell walls, breakdown of particulate organic matter and dissolution of EPS matrix. Researches are constantly undertaken in the field of wastewater treatment to find the best individual pre-treatment or combinations to achieve maximum anaerobic digestion efficiency.

The application of microwave pre-treatment on sludge solubilization, biogas production and sludge dewaterability has been widely studied (Eskicioglu et al., 2007b; W. J. Park and Ahn, 2011; J. Zhang et al., 2016). Microwave irradiation found its importance as an alternative to thermal hydrolysis due to its reduced reaction time and energy requirements (Eskicioglu et al., 2007b). Therefore, microwave treatment was more cost-effective compared to conventional thermal treatment (Woon Ji Park and Ahn, 2011). This is due to the unique heating mechanism of microwave treatment, wherein the dielectric polarization caused by microwaves is responsible for heating water solutions (Zhang et al., 2006). As WAS is composed of numerous organic substances, microbial fractions and over 95 % water, it becomes a suitable candidate for the application of microwaves. Moreover, microwave irradiation also causes hotspots or overheated spots in the surface of microwave absorbent, causing rapid degradation of organic pollutants (Wang and Wang, 2016). Studies on microwave treatment in organic wastewater treatment are continuously undertaken and in combination with other physical/ chemical pre-treatments are also recently explored (Eskicioglu et al., 2006; Yeneneh et al., 2015a; J. Zhang et al., 2016).

Furthermore, the advanced oxidation process (AOP) is a chemical pre-treatment technology widely used for the treatment of wastewater characterized by non-easily removable organic compounds (Pera-Titus et al., 2004). Ozonation and hydrogen peroxide (H_2O_2) pre-treatments are the most widely studied AOP methods in recent times. Almost all chemical oxidation processes exploit the high reactivity of hydroxyl ($OH^{\bullet}$) radicals in driving oxidation reactions that abate organic pollutants (Andreozzi, 1999). $OH^{\bullet}$ radicals are classified as reactive oxygen species (ROS), that exhibit high reactivity against organic molecules with an order of 10^9 $M^{-1}s^{-1}$ (Slupphaug et al., 2003). The ROS generated during AOP are responsible for microbial cell lysis through cell membrane permeabilization, DNA damage and lipid peroxidation (Alvarez et al., 1978; Mishra et al., 2005). Apart from $OH^{\bullet}$ radical, there is another highly reactive oxygen intermediate viz, superoxide ($O_2^{\bullet -}$) that plays an important role in sludge solubilization during pre-treatment (Chacana et al., 2017). However, the amount of studies on ROS generation during pre-treatment and anaerobic digestion is scarce in the field of wastewater treatment. Besides, various studies on the chemical oxidation process have shown increased sludge solubilization and anaerobic biodegradability (Chacana et al., 2017; Collivignarelli et al., 2017; Neyens and Baeyens, 2003a).

Apart from the sludge pre-treatments, various process modifications have also been studied to improve the efficacy of anaerobic digestion (Pérez-Elvira et al., 2006). Phase separation of anaerobic digestion promotes the growth of distinct microbial consortium, thereby enhances the efficiency of individual metabolic processes involved within (Held et al., 2002). Two-stage anaerobic digestion involves the growth optimization of the hydrolytic-acidogenic and methanogenic microbial population in two separate digesters to enhance the respective metabolism (Held et al., 2002; Kumanowska et al., 2017). Furthermore, the two-stage process reduces reaction time, increases methane percentage and also maintains stable alkalinity (Ghosh et al., 1995; M. Kim et al., 2003). As the methanogenic process is isolated from hydrolytic-acidogenic reactions, a maximum of 80 % methane content can be achieved through the two-stage process (Kumanowska et al., 2017). Although, very few researches have been reported on temperature phased two-stage processes, wherein hydrolytic-acidogenic and methanogenic reactions are maintained at two different temperatures to enhance the overall anaerobic digestion (Borowski, 2015; Ge et al., 2010, 2011a). Besides, the effect of combined pre-treatment strategies in such two-stage operations is less reported in the treatment of WAS.

Therefore, this research investigates the effect of hybrid MW-H_2O_2 pre-treatment in the anaerobic digestion of real activated sludge. And the oxidative stress exerted by the pre-treatment on the sludge was evaluated by the measurement of hydroxyl and superoxide radicals. Besides, a novel two-stage anaerobic digestion was developed with hydrolytic-acidogenic and methanogenic processes maintained at thermophilic and mesophilic conditions, respectively. The effect of pre-treatment on anaerobic digestion efficiency of the novel two-stage process was evaluated with a focus on sludge solubilization, EPS disintegration, biogas production, pathogen removal and dewaterability. Moreover, the stability of the novel two-stage anaerobic digester was evaluated by the anaerobic co-digestion of activated sludge along with fruit and vegetable wastes (FVW), as the latter faces severe acidification issues in conventional mesophilic anaerobic digestion.

1.2 Problem statement

Anaerobic digestion (AD) and sludge dewatering are the most widely used sludge treatment methods at wastewater treatment plants (WWTPs). The recent passage of the Global Warming Solutions Act (AB32, 2006) makes AD an attractive option for controlling greenhouse gas emissions from organic waste treatment while producing renewable biogas energy (Chong et al., 2012; Rapport et al., 2011; Sen, 2015). Currently, conventional anaerobic digestion in WWTPs faces several challenges such as slow initial hydrolysis, poor biomethanation, low solid reduction, odor and bad dewaterability. Moreover, the costs associated with sludge handling, transportation and pathogen reduction have also gained attention from an economic and environmental-standard perspective. Various pre-treatments and process modifications have been reported to alleviate the problems associated with the poor performance of anaerobic digesters. Although individual treatments yield effective sludge disintegration, the similarity in the mechanism of different treatment methods has lead to combined/ hybrid pre-treatment technologies. The synergism between two pre-treatments not only enhances the effect of individual techniques but also helps to overcome some of their drawbacks. Various researchers have combined two pre-treatments for improving the digestion performance, treatment time, cost-effectiveness and eco-friendliness of individual treatments (Eswari et al., 2016; Penaud et al., 1999; Weavers and Hoffmann, 2000; Yeneneh et al., 2015b). In the case of AOP, the efficacy of pre-treatment is strongly dependent on the rate of generation of free radicals and the contact time of free radicals with the organic molecules (Eskicioglu et al., 2008; Parag R Gogate and Pandit, 2004). Since the decomposition rate of H_2O_2 to $OH^{\bullet}$ radicals is dependant on temperature, hybrid treatments such as microwave-H_2O_2 (MW-H_2O_2) and thermal- H_2O_2 were studied (Eskicioglu et al., 2008). However, the initial studies on the combined MW-H_2O_2 yielded contradicting results on biogas production, although an increase in sludge solubilization was achieved (Eskicioglu et al., 2008, 2007a). Further studies in hybrid MW-H_2O_2 pre-treatment suggested that the optimum dosage strategy of H_2O_2 has a significant effect on biogas production and reduction in generation of refractory compounds (Liu et al., 2017; Wang et al., 2009). These findings corroborate the effectiveness of hybrid MW-H_2O_2 pre-treatment in sludge solubilization and also biomethanation. Despite various individual studies on hybrid pre-treatment (MW-H_2O_2) and two-stage anaerobic digestion, the combination of both strategies have been scarcely reported. Moreover, the mechanism of sludge disintegration through the generation of ROS by hybrid pre-treatment (MW-H_2O_2) and the subsequent levels during anaerobic digestion has not been established yet.

Therefore, the overall objective of this research project is to undertake a fundamental study on hybrid pre-treatment for performance enhancement of anaerobic digestion to produce high yield and better dewaterability while achieving several other objectives of sludge processing at WWTPs. The current research focuses on exploring different combined technologies to improve the efficiency of anaerobic digestion along with expanding the current knowledge on the evolution of ROS in anaerobic digestion.

1.3 Research objectives

The general objective of the current research is to enhance the efficiency of anaerobic digestion through the combination of "hybrid pre-treatment" and "two-stage" technologies. The more specific objectives are as follow.

1. Characterization of municipal sewage sludge from the wastewater treatment plant.
2. Investigation of the effects of hybrid MW-H_2O_2 pre-treatment method on sludge solubilization and selection of the best pre-treatment condition for enhanced anaerobic digestion.
3. Investigation of the ROS levels from hybrid MW-H_2O_2 pre-treatment to better understand the mechanism for improvement.
4. To study the impact of hybrid MW-H_2O_2 pre-treatment in two-stage anaerobic digestion with real sewage sludge.
5. Evaluation of two-stage anaerobic digestion stability with co-digestion of sewage sludge and FVW.
6. Investigation of different treatment strategies on the dewaterability of the activated sludge and anaerobic digestate.

1.4 Scope of the current research

The current research encompasses the investigation of hybrid MW-H_2O_2 pre-treatment technology in two-stage anaerobic digestion of real sewage sludge and FVW. The efficiency of the combined technologies was evaluated based on biogas production, sludge solubilization, solid reduction, process stability, pathogen levels and dewaterability. The research was carried over at

IIT Madras and Curtin University with samples collected from sources such as the Beenyup wastewater treatment plant (Western Australia), Nesapakkam wastewater treatment plant (Chennai, India) and IIT water treatment unit (IITM, India). The current study expands the available understanding of combined technologies to improve the anaerobic biodegradability of waste activated sludge. Besides, it also adds valuable insights into the physicochemical changes imparted by pre-treatments on sludge.

1.5 Significance of the current research

The current research has the following significances.

1. The performance efficiency of a novel semi-continuous two-stage anaerobic digester was evaluated.
2. The feasibility of a combined technology involving hybrid pre-treatment followed by two-stage anaerobic digestion was examined.
3. The efficiency of the combined technology to digest highly acidifying FVW under co-digestion was evaluated.
4. The relationship between sludge dewaterability and EPS distribution was established.
5. The current knowledge on ROS generation during pre-treatment and anaerobic digestion was expanded.

1.6 Thesis organization

There is a total of 8 chapters in the current thesis work. The chapters are organized in the following chronological order.

Chapter one

This section gives an introduction to the current thesis work with a brief overview of the anaerobic digestion process and the associated challenges. The objectives, scope and significance of the current research have been described in detail. The thesis organization has also been provided for a better understanding of the reader.

Chapter two

This section provides a detailed literature review on anaerobic digestion, various pre-treatment strategies and process modifications adopted in the WWTP for sludge stabilization. An in-depth analysis on microwave, hydrogen peroxide and the scope of combined pre-treatment strategies based on available literature has been provided.

Chapter three

The details on experimental methodology, sample collection and analytical techniques have been presented in this chapter. The current research includes sludge samples collected from three different origins. A brief overview of the respective water treatment plants has been given in the chapter. Besides, all analytical techniques and the equipment used have been discussed in detail.

Chapter four

In this section, the effects of microwave, hydrogen peroxide and hybrid (MW-H_2O_2) pre-treatment on various WAS characteristics have been studied. The study involves a comparative analysis between two WASs from a different origin. The approach to achieve maximum solubilization without any negative impacts on subsequent anaerobic digestion have been discussed in detail in this section.

Chapter five

This chapter involves the anaerobic digestion of WAS under conventional single-stage and novel two-stage operations. The performance efficiency of such anaerobic digestions has been evaluated based on parameters such as biogas production, biogas composition, solids reduction and effluent digestate quality. Furthermore, an optimum hybrid pre-treatment was applied to two-stage anaerobic digestion and its impact on performance efficiency have been assessed. This chapter gives insights on two-stage anaerobic digestion and hybrid pre-treatment to the reader.

Chapter six

This chapter is a continuation of research work conducted in two-stage operation with a different substrate composition. It involves anaerobic co-digestion of WAS with fruit and vegetable wastes (FVW) at different mixing ratios and the subsequent performance efficiency has been evaluated. Similar to the previous chapter, the impact of hybrid pre-treatment on anaerobic co-digestion has been evaluated. This chapter enhances the understanding of the two-stage operation and, implications of pre-treatment on the oxidative status of anaerobic digestion.

Chapter seven

This penultimate chapter deals with the impact of microwave and hybrid (MW-H_2O_2) treatments on extracellular polymeric substances (EPS) distribution and the dewaterability of anaerobic digestate and activated sludge. The relationship between dewaterability and EPS distribution has been presented. This chapter enhances the understanding of hybrid (MW-H_2O_2) treatment on sludge characteristics, thereby adds more insights on sludge pre-treatment for the reader.

Chapter eight

A generalized conclusion with the research findings has been presented in this chapter. Some of the recommendations for future research in this field have also been given. Figure 1.1 provides the overall structure of the research work and the experimentations conducted at each stage to meet the objectives listed in section 1.3.

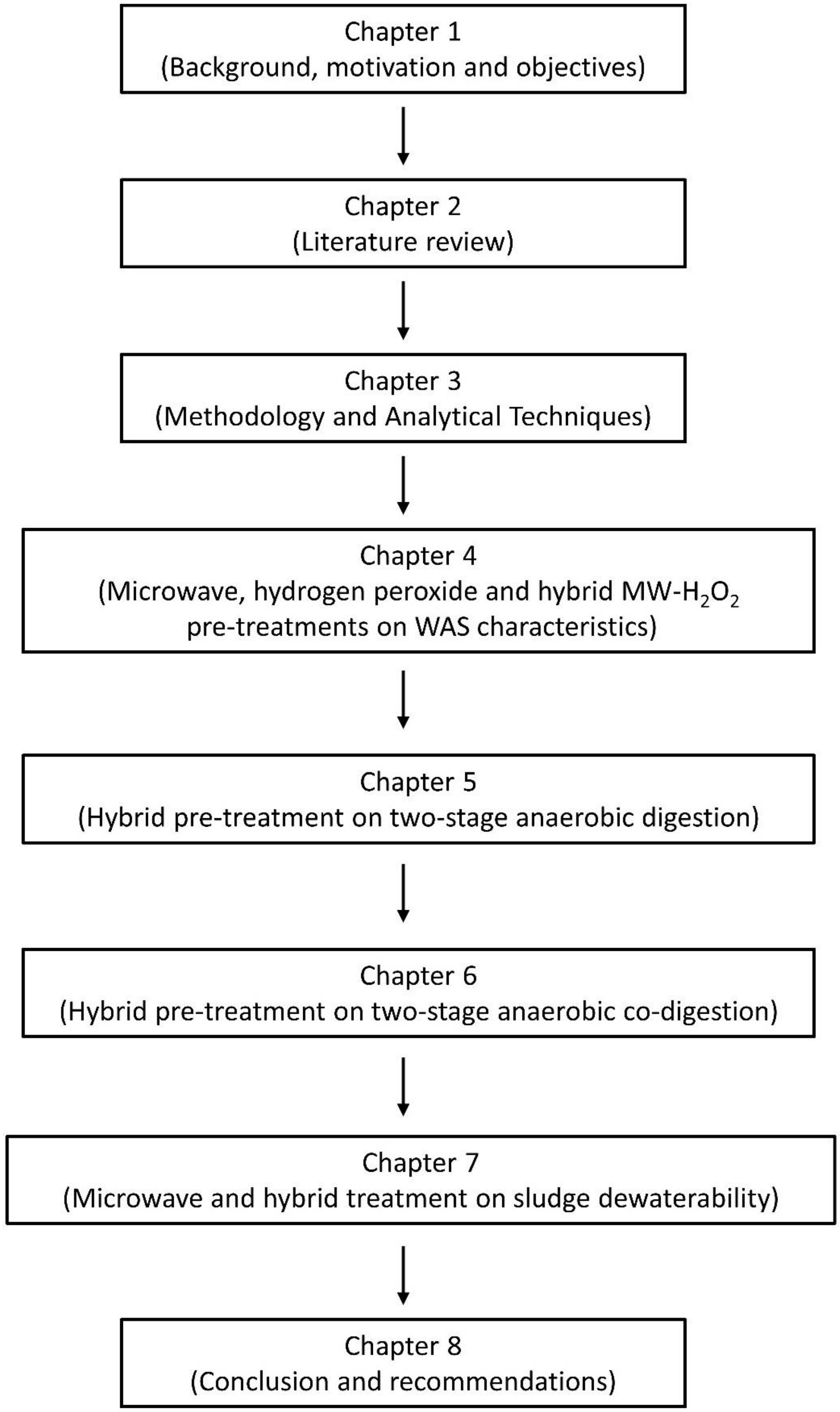


Figure 1.1: A schematic representation of the thesis work.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Wastewater management

With the rapid growth in global population, water management has created huge awareness across the nations (Mateo-sagasta et al., 2015). The need to save water resources, treat contaminated wastewater and replenish groundwater has been increasing over the years with the increasing industries and urbanization. Wastewater management has been a significant part of civilizations throughout the millennia, over various geographical settlements (Lofrano and Brown, 2010; Sharma and Sanghi, 2013). Besides, the lack of sanitation has affected human health and the surrounding environment throughout history, thereby steering both the people and the governments towards better wastewater management (Lofrano and Brown, 2010).

In Europe, the modern wastewater management was organized and reached an established status around the 19th century (Gallego-Schmid and Tarpani, 2019). Furthermore, sewers/pipes were designed to carry the domestic wastewater, through a network to wastewater treatment plants (WWTPs) for decontamination purposes. However, as per WHO estimation, by 2017 only 63 % of the global population in urban areas were able to use such established sanitation facilities around the globe (WHO/UNICEF, 2017). Most developing countries including India are still in progress towards sustainable wastewater management through modern technological advancements (Gallego-Schmid and Tarpani, 2019; R Kaur, S P Wani, 2014). Besides, the increasing pollutants in sewage due to industrial evolution have raised stringent environmental standards for the effluent water in developing countries (Walter and Lugelow, 1973). The advent of modern technologies in wastewater treatment plants aims to reduce residual sludge, pathogen levels, odor and increase the quality of effluent water before releasing it into the environment.

2.1.1 Wastewater Treatment Plants

The water used for bathing, laundry, dishwashing and industrial purposes, as well as human defecation, are collected as wastewater from cities/ suburbs and treated at off-site facilities called wastewater treatment plants (WWTPs) (Qadir et al., 2010). In some countries, the collection of wastewaters also includes rainwater that gets infiltrated into sewers during rainy seasons. These waters unless treated, carry accumulated pollutants, toxins, and pathogens, which are detrimental to the environment. Table 2.1 shows the proportion of wastewater treated by at least secondary treatment among countries with high population density (WHO and UNICEF, 2017). The need for improved wastewater management in developing countries like India is imminent due to rapid population growth and industrial development. Besides, complete termination of open defecation is still a challenge in rural areas of India. The recent inception of the Swachh Bharat Mission (SBM), a national sanitation program by the Government of India aims to eliminate open defecation and improve sanitation facilities nationwide (*Swachh Suvekshan Grameen*, 2019).

Table 2.1: Proportion of wastewater treated in various countries (WHO and UNICEF, 2017).

Sl. No	Countries	The proportion of wastewater treated by volume (%)
1	China	85
2	India	30
3	United states	99
4	Nigeria	32
5	Brazil	56
6	Russian federation	67
7	Mexico	54
8	Japan	100
9	Philippines	42
10	Egypt	72

2.1.1.1 Influent sewage characteristics

The term sewerage is used to describe the physical sewer infrastructure for the conveyance and treatment of sewage. Many developed countries have well-established sewerage system that separates rainwater from wastewater through separate pipelines. Whereas, in other countries, a combined sewerage system is adopted (Sperling, 2008). These sewer lines lead to the collection of wastewaters at WWTPs for further treatment and disposal. The major sources of influent wastewaters for WWTPs originate from houses and industries (WHO/UNICEF, 2017). The influent sewage is a heterogeneous mixture of micro-organisms, biomolecules (like proteins, carbohydrates, lipids and nucleic acids), plant/ animal remains, in-organic substances and grit (Bartram and Pedley, 1996; Henze and Comeau, 2008). The influent wastewater to municipal WWTPs can be categorized into the following (Sperling, 2008).

a) Domestic sewage

The sewage that originates from houses, commercial activities and institutions that are components of a locality are termed domestic sewage. Domestic sewage carries human faeces, other organic substances, soaps, and detergents as contaminants. The water consumption and wastewater generation are functions of corresponding population density in the concerned locality. Furthermore, community size, water availability and type of commercial establishments are some of the factors that determine the rate of wastewater generation in such localities (Sperling, 2008).

b) Infiltration

Infiltration is the collection of freely running water into the sewerage system through manholes, defective pipes, and broken connections/ joints. The quantity of infiltrated water in sewage is dependent on drainage area, topography, average rainfall, and population density. Besides, poor infiltration system in cities can lead to floods during heavy rainfall (Narasimhan et al., 2016; Sperling, 2008).

c) Industrial effluents

The industrial effluents may vary according to the type of manufacturing process, chemicals utilized, levels of recycling, water requirement, etc. The industrial effluents are characterized by higher suspended solids and chemical oxygen demand (COD) compared to the domestic sewage, thereby poses a higher threat to the environment (Gallego-Schmid and Tarpani, 2019; Henze and Comeau, 2008). Some of the contaminants in industrial effluents include toxic pollutants, pesticides, polychlorinated biphenyls (PCBs) and heavy metals (Katsoyiannis and Samara, 2005; Sponza, 2002).

2.1.1.2 Process hierarchy in WWTPs

A schematic of conventional WWTP is given in Figure 2.1. The influent sewage is treated sequentially through primary, secondary, and tertiary treatment stages. The residual matter separated from each treatment stage is referred to as sludge. The quantity of sludge generated from each stage is dependent on the degree of treatment. Higher sludge volumes lead to higher operating costs in WWTPs as the sludge needs to be stabilized, transported, and disposed of. Hence an efficient WWTP requires minimum sludge production with an eco-friendly sludge disposal mechanism. The stages involved and the corresponding sludge generated during the operation of a conventional WWTP are given below.

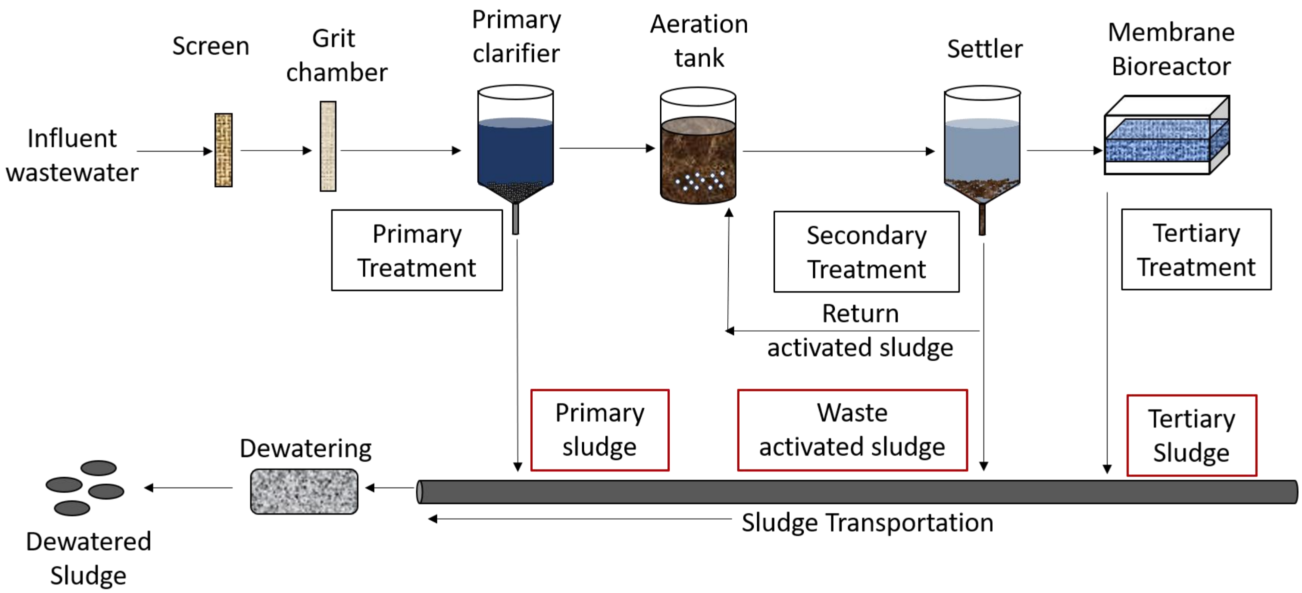


Figure 2.1: Overall schematic of a conventional wastewater treatment plant.

a) Primary treatment

Primary treatment removes the suspended solids and floating organic material (scum) by gravity sedimentation. An efficient primary sedimentation tank removes 50-70 % of suspended solids, 25-45 % of the biological oxygen demand (BOD) (Tchobanoglous et al., 2003) and 65 % of oil and grease (Sonune and Ghate, 2004). It also removes some heavy metals, organic nitrogen and phosphorous, however, colloidal, and dissolved organic constituents are not affected (Sonune and Ghate, 2004). The removal of colloidal and putrefiable substances can be accomplished by the secondary treatment. The sludge generated from the primary clarifier is raw primary sludge (RPS). RPS is usually grey in colour and slimy in texture. It is easily digestible by aerobic/ anaerobic bacteria, as it consists of highly degradable carbohydrates and fats (Hanjie, 2010; Yeneneh et al., 2017b). Therefore, primary treatment is mainly used as a precursor to secondary biological treatment (Doble and Geetha, 2011). Besides, biogas production is enhanced in the anaerobic digestion of RPS, due to higher VS content and methane potential (Hanjie, 2010). Table 2.2 illustrates the difference in biogas production from anaerobic digestion of primary sludge and activated sludge. The most widely used primary treatment methods are primary settlers, septic and Imhoff tanks (Mateo-sagasta et al., 2015).

Table 2.2: Biogas production from primary sludge and activated sludge.

Sl. No	Reference	Biogas production from sludge (ml/ gVS)			
		Primary sludge (RPS)	Activated sludge (WAS)	Mixed sludge (MS)	
				Biogas	(RPS: WAS) ratio
1	(Sato et al., 2001)	624	391	520	NA
2	(Nazari et al., 2017)	489.5	293	NA	-
3	(Dokulilová et al., 2018)	181	95	NA	-
4	(Pinto et al., 2016)	372	NA	218	40: 60
				316	60: 40
				302	80:20
5	(Yeneneh et al., 2017b)	NA	NA	36.5*	65: 35
				26.2*	50: 50
				25.9*	35: 65

Note: ‘NA’ means ‘not available’; ‘-‘ means ‘not applicable’; * data has been given in ml/ gTCOD

b) Secondary treatment

In secondary treatment, soluble biodegradable organic substances are metabolized and degraded by aerobic/ anaerobic micro-organisms. Aerated lagoons, activated sludge and trickling filters are commonly used secondary treatment processes. Microorganisms can be cultured in attached growth (biofilms) and suspended growth in the above processes (Lofrano and Brown, 2010). The activated sludge process (ASP) is the most widely used secondary treatment in WWTPs to reduce BOD, nutrients and some micro-pollutants (Du et al., 2018). In this method, the sludge is aerated in an aeration tank to increase the dissolved oxygen (DO) concentration, thereby facilitating the aerobic microbial population (mainly bacteria, protozoan, and fungi) to metabolize the organic matter (Figure 2.2).

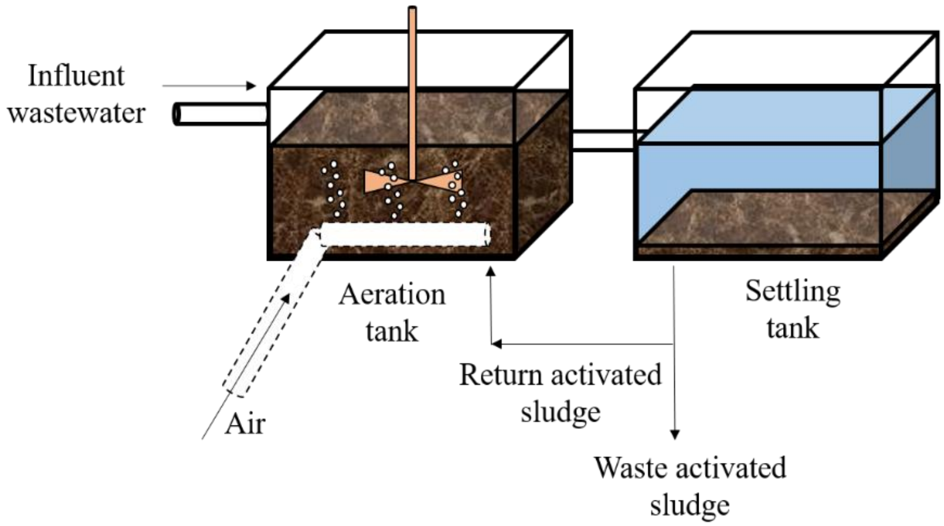


Figure 2.2: A simplified representation of the activated sludge process (ASP)

This process produces activated sludge as an unpleasant by-product for its large volume and odor (Guo et al., 2013). The highly distinguishing nature of activated sludge is the presence of microbial flocs. Besides, the sludge is also characterized by brownish colour and earthy odor. The presence of microbial flocs is the result of the overproduction of microorganisms in this process (Hanjie, 2010). This makes it more difficult to anaerobically digest compared to the RPS. Typically, a total of 0.94 kg DS/ 1000 gal of wastewater is produced as sludge from primary and secondary wastewater treatment (National Research Council, 1996). And the biological treatment results in the reduction of sludge volume through the oxidation of volatile organic solids present in it. The anaerobic digestion yields an average of 2.5 % dry solids from 5 % dry solids of WAS generated from centrifuge thickeners (Tchobanoglous et al., 2003).

Hence, both RPS and WAS can be subjected to anaerobic digestion, which results in the production of digested sludge. The digested sludge is reduced in volatile solid content, less odorous and decreased pathogen levels, which makes it safer for disposal (Yeneneh et al., 2015a). The difference in the chemical composition of digested and undigested sludge has been given in Table 2.3.

Table 2.3: Chemical composition of digested and undigested primary sludge (Tchobanoglous et al., 2003)

Parameter	Untreated sludge	Digested sludge
Total solids (TS) %	5.0-9.0	2.0-5.0
Volatile solids VS (% TS)	60-80	30-60
Soluble ether (% TS)	6-30	5-20
pH	5.0-8.0	6.5-7.5
Alkalinity (mg/ l $CaCO_3$)	500-1500	2500-3500
Energy content, kJ TS/ kg	23000-29000	9000-14000

c) Tertiary treatment

The tertiary or advanced treatment process is a specialized treatment strategy for the removal of specific pollutants or nutrients (nitrogen or phosphorous) to prevent eutrophication by the final effluent (Rattier et al., 2014; Sperling, 2008). Membrane bioreactors are incorporated in WWTPs to produce high-quality effluents, in many developed countries (Lofrano and Brown, 2010). The nature of tertiary sludge is dependent on the treatment strategy involved. The biological nitrification and denitrification yield a tertiary sludge similar to the activated sludge (Díaz et al., 2016).

2.1.1.3 Challenges faced in sludge management

WWTPs majorly produce two types of sludges viz, RPS from the primary settlers and WAS from the activated sludge process (Sperandio et al., 2012). Among these, the excess production of WAS is highly disadvantageous as it is composed of microbial flocs and it's biological degradation is difficult. Currently, the mitigation of excess sludge production is one of the major challenges in WWTPs (Kelessidis and Stasinakis, 2012). Sludge handling, treatment and disposal of sludge itself costs around 60 % of the total cost of wastewater treatment in the EU (Spellman, 1997; Wei et al., 2003). Besides, the main alternative methods for sludge disposal in the EU are landfilling and incineration (Wei et al., 2003). Both the methods possess potential health risks to human beings and livestock as the sludge is composed of toxic elements, pathogens and other pollutants. Moreover, the usage of available land space also requires approval through stringent environmental regulations and legal constraints. Therefore, the reduction of sludge production at its source or by efficient sludge treatment technologies are highly studied in the field of biological wastewater treatment.

2.1.1.4 Sludge stabilization

Raw sludge is rich in microorganisms, contains a high biodegradable organic fraction, releases offensive odor on degradation and threatens the environment with high pathogen levels. The sludge stabilization processes were developed to reduce the levels of biodegradable fraction and pathogens, thereby restricting the odor from the putrefaction process (Sperling, 2008). The stabilization processes can be classified as (a) Biological stabilization- use of aerobic/ anaerobic biological treatment for the stabilization of organic matter in sludge (b) Chemical stabilization- use of chemicals (hydrogen peroxide or lime) to treat the organic matter in sludge and (c) Physical stabilization- reduction of volatile substances in the organic matter through heating and vaporization.

Table 2.4: Different sludge stabilization techniques based on the type of process

Type of process	Stabilization technique	Advantages	Challenges
Biological	Anaerobic digestion	• Energy (biogas) generation • Sludge volume reduction	• Slow initial hydrolysis • Low methane production
	Aerobic digestion	• Low capital cost • Ideal for Class B biosolid requirements	• Low organic matter removal • Energy cost for blowers
Chemical	Alkaline treatment	• Improved sludge dewaterability • Pathogen inactivation	• Corrosion of equipment • Adverse effect on subsequent biological treatment
	Ozonation	• No odor formation • Pathogen inactivation	• Worsened sludge dewaterability
Physical	Thermal (Incineration)	• Reduction of disposable sludge volume • Suitable in areas with low land availability	• Environmental pollution by incineration products such as polycyclic aromatic hydrocarbons
	Co-incineration with coal or other fuels	• Energy generation	• Negative environmental impact by exhaust gases and fly ash

Based on the method of stabilization, the final output solids are qualified for soil conditioning/ fertilizers or sanitary landfill. Some of the sludge stabilization techniques that are followed in sludge treatment line at WWTPs have been classified in Table 2.4 (Anjum et al., 2016; Cieślik et al., 2015; Wang et al., 2017). Among various sludge stabilization techniques, anaerobic digestion gains attention due its economic value, eco-friendliness, and significant reduction of excess sludge.

2.1.2 Anaerobic digestion

Reduction of excess sludge production by anaerobic fermentation of effluent WAS is a standard sludge stabilization process in many WWTPs (Pérez-Elvira et al., 2006; Wang et al., 2017). As the WAS is composed of biological flocs, organic biodegradation by anaerobic digestion reduces the sludge volume significantly. Besides, it also recovers renewable energy in the form of biogas (Mata-Alvarez et al., 2000). In this process, the biodegradable organic substances of the substrate (WAS/ RPS) are converted into methane and carbon dioxide by anaerobic microorganisms through a series of biochemical reactions. The overall conversion of substrate to biogas is given in equation 2.1. The other products include nitrogen, ammonia, hydrogen and other by-products that are produced as a result of microbial metabolism. Moreover, the nitrogen which is not utilized for bacterial growth will be released as ammonia (Yeneneh, 2014).

$$Organic\ matter \xrightarrow[bacteria]{Anaerobic} CO_2 + CH_4 + New\ cells + energy\ for\ cells + other\ products$$

(equation 2.1)

The anaerobic digestion is carried out in an anoxic environment, which is the complete absence of oxygen. During anaerobic digestion, microbial activity leads to (1) solubilization of solids (2) metabolization of soluble solids and (3) gas production. Anaerobic digestion is a preferred biological sludge stabilization technique as it achieves sludge mass reduction, biogas production and improved dewaterability (Zhang et al., 2018; Q. Zhang et al., 2016). Moreover, with the rapid population growth and exhausting fossil fuels, biogas as a renewable energy source has gained global attention with its multiple environmental benefits (Cuéllar and Webber, 2008; Qi et al., 2005; Rehl and Müller, 2011). The biogas generation from anaerobic digestion is eco-friendly with the reduction of greenhouse gas emission (Anukam et al., 2019). Apart from biogas production, the effluent anaerobic digestate can also be used as crop fertilizers (Tambone et al., 2010). Therefore, anaerobic digestion is considered an efficient technology for the stabilization of excess sludge produced in WWTPs (Pérez-Elvira et al., 2006). A short schematic of WWTP along with the anaerobic digestion is given in Figure 2.3.

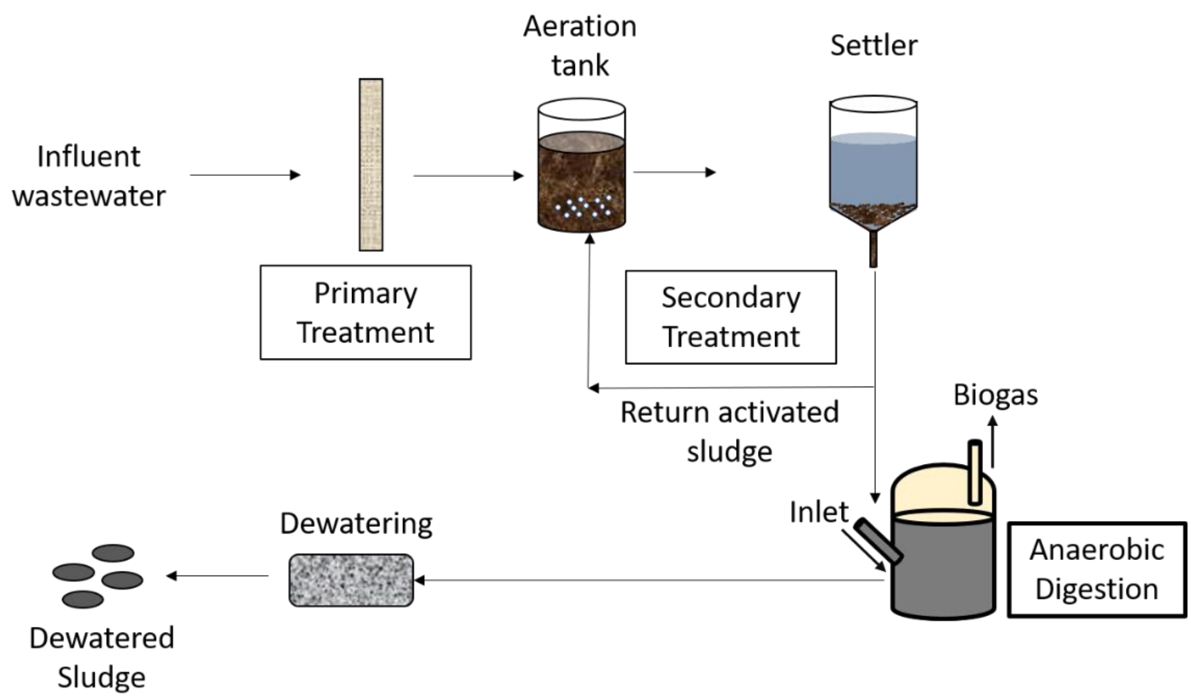


Figure 2.3: A short illustration of WWTP with the anaerobic digestion process

2.1.2.1 Biochemical processes involved in anaerobic digestion

Anaerobic digestion involves four major biochemical reactions, viz, hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 2.4) (Achinas and Euverink, 2016; Anukam et al., 2019). Each of these reactions is governed by distinct microbial communities, which ultimately converts the organic substrate to biogas (equation 2.1). A brief discussion about each of the reactions is given below.

a) Hydrolysis

Hydrolysis is the first step in the anaerobic digestion process. During hydrolysis, the complex organic matter is converted into soluble organic molecules by hydrolyzing bacteria. The bacteria secrete extracellular enzymes that break down carbohydrates, lipids and proteins into simple sugars, fatty acids and amino acids, respectively (Li et al., 2011). The process of hydrolysis initiates the drive for anaerobic digestion, which is subsequently taken over by acidogenic bacterium. However, hydrolysis is often considered the rate-limiting step of anaerobic digestion, due to the complexity of particulate organic matter in a typical activated sludge (Carrere et al., 2016; Tyagi and Lo, 2013; Yeneneh et al., 2015b).

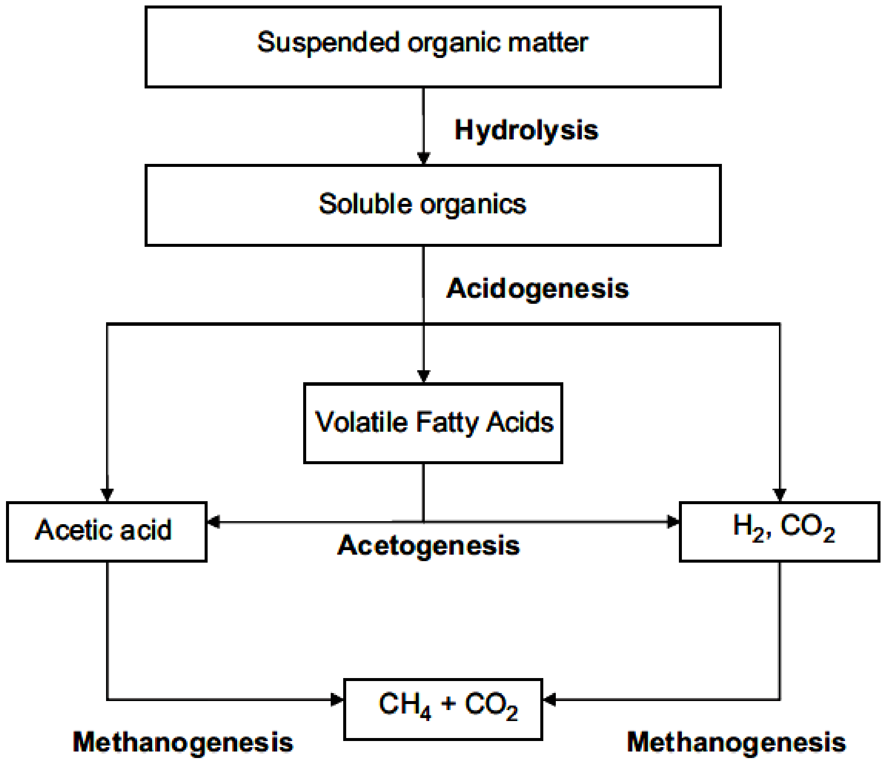


Figure 2.4: Biochemical processes involved in anaerobic digestion. (Dewil et al., 2008)

b) Acidogenesis

Acidogenesis succeeds hydrolysis in the anaerobic digestion process. During this stage, the soluble compounds formed during hydrolysis are converted into simple end products primarily volatile fatty acids (VFAs) and alcohols by acidogenic bacteria (Anukam et al., 2019; Wainaina et al., 2019). Moreover, CO_2 and H_2 gases are produced as the final metabolic products in this stage (Anukam et al., 2019). VFAs constitute a class of organic acids such as acetates, propionate and butyrate (Meegoda et al., 2018). The specific concentration of VFAs may depend on substrate characteristics and reactor type (Bouallagui et al., 2005; Huang et al., 2015). Both acidogenic and acetogenic reactions are catalyzed by a group of facultative and obligate anaerobes (Anukam et al., 2019).

c) Acetogenesis

Acetogenesis is the process where higher VFAs and other intermediates are converted into acetate with the production of H_2 gas (Meegoda et al., 2018). The H_2 gas produced during acetogenesis might inhibit the growth of acetogens, however, H_2 gas can be consumed to produce CH_4 by hydrogen scavenging bacteria (Anukam et al., 2019). This symbiosis between hydrogenotrophic methanogens (use H_2 to produce CH_4) and syntrophic acetate oxidation (acetate is oxidized to CO_2 and H_2) makes the reaction energetically favorable and enhances methanogenesis (Hattori, 2008).

d) Methanogenesis

This is the final stage of anaerobic digestion. During methanogenesis, the anaerobic methanogens convert CH_3COOH and H_2 into CH_4 and CO_2. The anaerobic methanogens involved in this stage are mostly obligate anaerobic archaea (e.g. *Methanococcus*, *Methanogenium*, *Methanobrevibacter*) (Anukam et al., 2019; Meegoda et al., 2018).

2.1.2.2 Process kinetics of anaerobic digestion

Anaerobic digestion is an elaborate scheme that involves sequential biochemical reactions that are driven by various factors such as substrate concentration, microbial biomass, temperature, pH and other associated factors (Fountoulakis et al., 2010; Grim et al., 2015; Yang et al., 2015). Understanding its process kinetics is helpful in the design and control of anaerobic digestion set-ups. Besides, the optimization of various parameters for the stable operation of anaerobic digestion requires an analysis of the process kinetics (Hassam et al., 2015; Zonta et al., 2013). The key reaction kinetics involved in anaerobic digestion are listed below.

a) Sludge solubilization kinetics

As hydrolysis is the major rate-limiting step of anaerobic digestion, the kinetics of initial sludge solubilization is a key factor in analyzing the process efficiency. The sludge solubilization can be analyzed by the rate of consumption of chemical oxygen demand, production of biogas and other intermediate components.

- Chemical oxygen demand (COD) is a measure of the amount of oxygen required to oxidize the organic content present in the given sample (Henze and Comeau, 2008). In other words, COD measures the amount of biologically active substances (microorganisms) and inactive organic matter present in the sample. The reduction of COD reflects the consumption of organics through anaerobic digestion and indirectly accounts for the solubilization of sludge (Meegoda et al., 2018). The hydrolysis rate constant (K_h) can be determined by the first-order hydrolysis model (L. Zhang et al., 2016) according to equation 2.2.

$$\frac{dCOD_t}{dt} = (-k_h).COD_t \qquad \text{(equation 2.2)}$$

Where,

t: Time (days)

COD_t: Chemical oxygen demand at time t (mg COD/ l)

- The methane yield is a function of the consumption of organic material for anaerobic digestion. The generation of methane signifies the reduction of the substrate (COD) and thereby reflects the sludge hydrolysis. The modified Gompertz equation represents the cumulative methane production as a function of methane production potential as given by the following equation 2.3.

$$M = P \times \exp\left(-\exp\left[\frac{Rm.e}{P}\,(\lambda - t) + 1\right]\right) \qquad \text{(equation 2.3)}$$

Where,

M= cumulative methane production (ml)

Rm= maximum methane production rate (ml/day)

P= methane production potential (ml), λ= lag phase (days)

t= time (days)

The hydrolysis rate constant (K_h) can be determined by assuming the first-order kinetics of methane production as per Veeken and Hamelers model (Veeken and Hamelers, 1999), according to equation 2.4.

$$M = P(1 - e^{-k_h t}) \qquad \text{(equation 2.4)}$$

- The progression of anaerobic digestion can also be quantified by the appearance and disappearance of products and substrates, respectively. During the initial phase of anaerobic digestion, the particulate organic matter is hydrolyzed by various extracellular enzymes of the hydrolytic bacterium, to produce soluble biomolecules such as monosaccharides, amino acids and simple fatty acids. During, the subsequent acidogenesis, these substrates (biomolecules) are converted to other products (VFAs) by the acidogenic microorganisms. The quantification of substrate disappearance or product appearance during the course of anaerobic digestion represents the hydrolysis of soluble substrates (Vavilin et al., 1996).

First-order reaction kinetics of product formation and substrate consumption can be used to quantify the rate of hydrolysis as given by the following equations (2.5 and 2.6).

$$\frac{d[S]}{dt} = -k.S \qquad \text{(equation 2.5)}$$

$$\frac{d[P]}{dt} = \alpha k.S \qquad \text{(equation 2.6)}$$

Where,

P is the product concentration, S is the substrate concentration, k is the first-order rate coefficient and α is the conversion coefficient of the substrate to product. The substrate mentioned here can be volatile solids (Vavilin et al., 2008). The rate of change of volatile solids concentration along with the anaerobic digestion gives a measure of the sludge solubilization or hydrolysis.

However, the mechanism of hydrolysis is carried over by extracellular enzymes secreted by the hydrolytic bacterium. Therefore, the hydrolysis rate (v) can also be analyzed from the quantification of substrates such as carbohydrates, proteins or lipids, as they are enzymatically hydrolyzed, using the Michaelis-Menten kinetics (Doran, 1995; Vavilin et al., 2008) according to the equation 2.7.

$$v = \frac{d[P]}{dt} = \frac{V_{max}.S}{K_m+S} = K_{cat}.E_0.\frac{S}{K_m+S} \qquad \text{(equation 2.7)}$$

Where,

v = d[P]/ dt = rate of product formation (or) the rate of hydrolysis reaction

S = substrate concentration

V_{max}= maximum rate of the reaction at an infinite substrate concentration

K_m= Michaelis-Menten constant for the substrate (S). K_m is the substrate concentration when the rate of the reaction, $v = \frac{V_{max}}{2}$

E_0= initial enzyme concentration

b) Microbial growth kinetics

Kinetics of the overall anaerobic microbial growth can be estimated through a mass balance equation based on COD with an assumption of 100 % consumption of substrate (C P Leslie Grady et al., 2011) (equation 2.8).

$$C_nH_aO_b \xrightarrow{\substack{Anaerobic \\ digestion}} f_eCH_4 + f_sC_5H_7O_2N \qquad \text{(equation 2.8)}$$

Where,

f_s= Fraction of electron for biomass growth

f_e= fraction of electron for biogas production

The specific growth rate (μ) of the anaerobic microbial biomass can be determined using the following equation 2.9 (Yang et al., 2015).

$$\mu = \frac{f_s}{f_e}\frac{d(V_{CH_4})}{dt}\frac{1}{X} \qquad \text{(equation 2.9)}$$

Where,

V_{CH_4}= Volume of methane produced in COD units

X= Cell biomass concentration (mg COD/ l)

The anaerobic microbial growth can also be estimated by applying the classic Monod equation (equation 2.10) (Owhondah et al., 2016; Pavlostathis and Giraldo-Gomez, 1991)

$$\mu = \frac{\mu_{max}.S}{K_m+S} \qquad \text{(equation 2.10)}$$

where S is the substrate concentration and K_m represents the substrate concentration, when $\mu = \frac{\mu_{max}}{2}$

2.1.2.3 Operational difficulties in anaerobic digestion

Although anaerobic digestion is considered an efficient sludge stabilization technique, it also suffers certain drawbacks pertaining to the optimum conditions for microbial growth, substrate or product inhibition and other relevant factors. Some of the parameters that affect the stable operation of anaerobic digestion are listed below.

a) Slow hydrolysis rate

Hydrolysis, the first stage of anaerobic digestion is considered the rate-limiting step of the overall process (Algapani et al., 2016; Carrère et al., 2010; Seng et al., 2010). Anaerobic digesters encounter a heterogenous mixture of biopolymers, organic substances and micro-organisms, that are inaccessible to the major methanogenic population unless broken down by hydrolysis. Thus, hydrolysis is an essential biochemical reaction that renders soluble substrates for subsequent anaerobic digestion. However, the complex nature of WAS makes it difficult to aerobic/ anaerobic biodegradation (Carrere et al., 2016). This is due to the presence of microbial flocs and the associated extracellular polymeric substances (EPS), a complex 3-dimensional matrix of biopolymers (Sheng et al., 2010). EPS is a mixture of macromolecules, such as carbohydrates, proteins, nucleic acids, lipids and other polymeric substances that are secreted by microorganisms or a product of cellular lysis during the activated sludge process (Carrère et al., 2010). Depending on sludge age and the nature of activated sludge, EPS remains recalcitrant to aerobic or anaerobic degradation (Wang et al., 1999, 2007; Zhang and Bishop, 2003). Besides, EPS has a significant effect on the physicochemical properties of the sludge, such as mass transfer, surface charge, flocculation ability and dewaterability, since it occupies the interior and exterior of the microbial aggregates (Ni et al., 2009). To overcome the ‘slow hydrolysis’ barrier, WWTPs adopt various pre-treatment techniques that accelerate anaerobic digestion (Carrère et al., 2010).

b) pH and volatile acids/ alkalinity stability

Anaerobic digestion involves various groups of microorganisms forming a microbial consortium that ultimately metabolize the organic substrates to biogas. And, each group in the consortium has a different optimum pH range. The pH optimum for the methanogenic bacteria is 6.5-7.8 (Izrail S. Turovskiy and Mathai, 2005; Jiunn-Jyi et al., 1997). The inhibition of methanogenesis is observed under weakly acidic pH conditions (Hwang et al., 2004). The acidogenic bacteria can function in the optimum pH range of 4.0-6.0 and they produce H_2 gas as the metabolic product (Hwang et al., 2004). During this phase, the VFAs produced by the acidogenic population tend to reduce the pH, which is unless countered by the methanogenic bacteria, causes pH instability in the anaerobic digestion (Izrail S. Turovskiy and Mathai, 2005).

The methanogenic population consumes the VFAs and maintains the pH stability by producing alkalinity in the form of CO_2, ammonia and bicarbonates. Therefore, a stable anaerobic digestion requires control over the optimum pH range for the respective microbial groups. In addition, the buffer stability of anaerobic digestion can be maintained by the addition of bicarbonate or phosphate buffer (Lin et al., 2013).

c) Temperature

The temperature of anaerobic digestion is an important parameter for the control and design of digesters, as it influences the microbial growth and the physicochemical properties of the components in the substrate. Thermophilic and mesophilic anaerobic digestions have been widely studied for biogas production, microbial growth and reactor stability (Gebreeyessus and Jenicek, 2016; Gou et al., 2014; L.-J. Wu et al., 2016; Zhou et al., 2002). Conventional mesophilic anaerobic digestion is the most widely practiced process due to its low energy demands and process stability. However, thermophilic anaerobic digestion provides faster reaction rates and low retention times compared to the former (Gebreeyessus and Jenicek, 2016). Besides, thermophilic digestion also produces "Class-A" biosolids, that are low on pathogen levels and preferred under the environmental regulation United States Environmental Protection Agency (US EPA) (Gebreeyessus and Jenicek, 2016). Nonetheless, thermophilic digestion can also inhibit the growth of certain methanogens because of the release of free ammonia and VFAs (Dewil et al., 2008). Therefore, it is important to maintain the optimum temperature conditions for microbial growth for the overall stability of anaerobic digestion.

d) Retention time

The average time spent by the substrates in contact with the anaerobic microbial inoculum in the reactor is called the retention time. The solids retention time (SRT) and hydraulic retention time (HRT) are the average time spent by the solids and the liquid sludge in the reactor, respectively (Dewil et al., 2008). The retention time is an important factor in the anaerobic digestion process, as it regulates various factors such as reaction time, microbial load and substrate/ product inhibition. A short SRT may decrease the reaction time and the removal of microorganisms from the reactor (Dewil et al., 2008). Besides, VFAs concentration increases as the methanogens are washed out in such short SRT and affects the efficiency of anaerobic digestion. Hence, stable anaerobic digestion requires a longer retention time (SRT>10) for the microorganisms to get acclimatized and perform methanogenesis (Dewil et al., 2008).

The above-mentioned factors are crucial to the successful implementation of anaerobic digestion as they play an important role in biogas production and optimum condition for anaerobic microbial activity. Despite maintaining optimum operational conditions such as pH, temperature and retention time, anaerobic digestion is highly inflicted by the recalcitrant nature of sludge to initial microbial hydrolysis. Therefore, the disintegration of complex WAS becomes essential before the application of anaerobic digestion, thus leading to the development of different pre-treatment methods.

2.2 Pre-treatment methods

Various sludge pre-treatment methods are studied over the years to overcome the slow hydrolysis rate and to breakdown the refractory molecules into simpler molecules for the anaerobic digestion process (Carrere et al., 2016; Carrère et al., 2010; Parag R Gogate and Pandit, 2004; Parag R. Gogate and Pandit, 2004). Apart from the sludge disintegration, pre-treatments also lead to sludge sanitation and improved dewaterability (Apul et al., 2010; Neyens and Baeyens, 2003a; Yeneneh et al., 2015a, 2017a). The selection of appropriate pre-treatment for anaerobic digestion may depend on factors such as substrate characteristics, cost-effectiveness, environmental impacts and regulations. For a better understanding of the current thesis work, the pre-treatment strategies are classified based on the pre-treatment count, as (1) mono pre-treatment and (2) hybrid pre-treatment.

2.2.1 Mono pre-treatment

The application of a single pre-treatment before anaerobic digestion was the primary initiative on improving the digestibility of primary and activated sludges (Haug et al., 1978). Since then, the amount of research on sludge pre-treatment techniques has been increasing in the past two decades (Tyagi and Lo, 2011). These techniques are followed to release the intracellular components from the microbial cells of activated sludge and solubilize the complex macromolecules in EPS fractions before the subsequent anaerobic digestion. The pre-treatment techniques can be generally classified into (a) physical (b) chemical and (c) biological pre-treatment methods based on the nature of pre-treatment (Pérez-Elvira et al., 2006).

2.2.1.1 Physical pre-treatment

The disintegration of sludge components by the application of kinetic energy can be classified under physical pre-treatment. Physical pre-treatments include thermal, mechanical, ultrasound, electric pulse, microwave and gamma irradiation (Neumann et al., 2016). The kinetic energy generated during the physical pre-treatment breaks down microbial cell walls, EPS fractions and particulate organic matter and thereby enhances the solubilization (Tyagi and Lo, 2011). For instance, the kinetic energy of microwave irradiation, causes water to reach the boiling point and causes microbial cell rupture (J. Zhang et al., 2016).

a) Thermal pre-treatment

The early report on thermal pre-treatment was studied to improve methane production and dewaterability (Haug et al., 1978). Before that, thermal energy was used as a treatment procedure for conditioning purpose and to enhance dewatering. The application of thermal energy breaks down the complex floc structure of sludge and releases the bound water (Carrère et al., 2010), which causes improved dewaterability (Anderson et al., 2002). Besides, thermal pre-treatment causes sludge solubilization which subsequently improved the efficiency of anaerobic digestion, as reported by many studies (Bougrier et al., 2008; Haug et al., 1978; Mottet et al., 2009; Wang et al., 1997). The operational parameters in thermal treatment are treatment temperature and treatment time.

The thermal treatment for sludge disintegration has been achieved through various equipment (or) modes. The most widely used strategies are hot water/ steam/ oil baths (Alfaro et al., 2014; Eskicioglu et al., 2006; Ferreira et al., 2014; Hong et al., 2004; Lee et al., 2019; Liu et al., 2012b; Lu et al., 2018; Taboada-Santos et al., 2019; Zhou et al., 2013), hot plates (Woon Ji Park and Ahn, 2011; Pino-Jelcic et al., 2006; Vergine et al., 2014), autoclaves (Braguglia et al., 2014; Chiappero et al., 2019; Gianico et al., 2016; Law et al., 2020; Lin and Lee, 2002; Mottet et al., 2009; Tanaka and Kamiyama, 2002) and autoclave reactors (Bjerg-Nielsen et al., 2018; Bougrier et al., 2008; Sarwar et al., 2018; Valo et al., 2004). The thermal treatment achieved through the conductive/ convective heat transfer from the source to sludge is considered as “conventional heating” by Eskicioglu et al. (Hosseini Koupaie and Eskicioglu, 2015; Koupaie and Eskicioglu, 2016; Mehdizadeh et al., 2013). Moreover, comparative studies of conventional heating with microwave heating were conducted for the evaluation of sludge disintegration and biogas production (Koupaie and Eskicioglu, 2016; Vergine et al., 2014). However, barely any research has gone through comparison of conventional heating among different equipment or modes.

The thermal treatment has been done widely in the range of 55 °C to 275 °C, with varying treatment times (Gonzalez et al., 2018). Conventionally, larger treatment times are adopted for lower temperatures and vice versa. Various studies have proposed a longer retention time from 9h to 7 days at a temperature range of 60-70 °C (Climent et al., 2007; Ferrer et al., 2008; Gavala et al., 2003; Skiadas et al., 2005; Toutian et al., 2020; Uma Rani et al., 2012; Vergine et al., 2014; Xu et al., 2014) for increasing the biogas production in anaerobic digestion of activated sludge. However, the temperature is considered a hyper-thermophilic or a mild-thermal condition and the corresponding hydrolysis is assumed to be the action of hydrolytic enzymes (Carrère et al., 2010; Gonzalez et al., 2018). Nevertheless, thermal treatment above 70 °C has caused increased hydrolysis, reduction in particle size (Laurent et al., 2009; Vavilin et al., 2008) and also lysis of bacterial cells (Prorot et al., 2011; Salton and Horne, 1951). The solubilization of proteins and carbohydrates are observed at temperatures above 75 °C (Appels et al., 2010; Dong et al., 2016). Various studies on thermal pre-treatment under 100 °C have shown increased methane production with enhanced sludge hydrolysis, and the observations are prominent in the range 60- 75 °C (Gonzalez et al., 2018).

Furthermore, thermal pre-treatments above 100 °C, are termed thermal hydrolysis or high-temperature thermal pre-treatment (Gonzalez et al., 2018). The thermal hydrolysis causes reduction of particle size till 120 °C (Gao et al., 2013) and temperature range 170- 190 °C was found to increase the particle size (Bougrier et al., 2006). Besides, extensive cell destruction and solubilization of intracellular content are salient features of thermal hydrolysis (Gonzalez et al., 2018). Also, thermal pre-treatment at a temperature range from 115 °C to 140 °C for about 20-60 min holding time, have shown improved methane production and anaerobic biodegradability (Braguglia et al., 2014; Chiappero et al., 2019; Gianico et al., 2016; Law et al., 2020; Park et al., 2014; Val Del Río et al., 2014).

In addition, thermal pre-treatment with an optimum temperature range of 160-190 °C at a shorter retention time (30- 60 min) achieved maximum methane production and process efficiency, in anaerobic digestion of activated sludge (Alfaro et al., 2014; Bjerg-Nielsen et al., 2018; Bougrier et al., 2006; Fdz-Polanco et al., 2008; Haug et al., 1978; Kobayashi et al., 2009; Oosterhuis et al., 2014; Pinnekamp, 1989; Stuckey and McCarty, 1984; Tanaka and Kamiyama, 2002). Besides, Dohanyas et al. achieved a 49 % increase in methane production with thermal treatment of 170 °C with a holding time of just 60s (Dohányos et al., 2004). Tanaka et al. achieved a 90 % increase in methane production with a treatment of 180 °C heat treatment for 1 min, in industrial activated sludge (Tanaka and Kamiyama, 2002).

However, thermal hydrolysis can sometimes generate refractory compounds at temperatures about 50-70 °C with a long treatment time (1-7 days), due to the Maillard reaction (Appels et al., 2010; Liao et al., 2016). Maillard reaction causes the formation of melanoidins, as a result of polymerization of reducing sugars and amino acids (Eskicioglu et al., 2007c). The refractory compounds generated as a result of Maillard reactions are indigestible and are detrimental to the microbial community (Szwergold, 2013). In addition, temperatures higher than 180 °C can also lead to Maillard reactions (Bougrier et al., 2008; Dwyer et al., 2008; Wilson and Novak, 2009). Besides, high temperatures (above 170 °C) can also lead to caramelization of carbohydrates (Gonzalez et al., 2018). Although thermal hydrolysis achieves improvement in biogas production and overall anaerobic digestion efficiency, it has certain drawbacks like extended treatment period and generation of refractory compounds.

b) Microwave

Microwaves are electromagnetic waves with a wide frequency (300 MHz to 300 GHz) range, and the most effective range for dielectric heating lies between 0.915 GHz and 2.45 GHz (Leonelli and Mason, 2010). The heating phenomenon created by microwaves is due to the electric-field induced polarization and the realignment of dipoles towards the oscillating electric field as indicated in Figure 2.5 (Leonelli and Mason, 2010). WAS, if applied microwave irradiation can be heated up either by the acceleration of ions colliding with each other or through the rapid realignment of water dipoles (Aylin Alagöz et al., 2016; Banik et al., 2003). Since more than 90 % of sludge (WAS or RPS) is composed of free water, microwave irradiation causes a faster heating effect (Pino-Jelcic et al., 2006). Besides, the bound water can also be heated up through microwave irradiation, due to its dielectric properties (Hasted and Roderick, 1958). This would cause the heating up of hydrophilic joints that are structural components of protein, DNA and lipid bilayer, thereby achieves targeted heating of biological solutions (Pino-Jelcic et al., 2006).

Therefore, microwave treatment achieves a targeted heating effect with a dramatic reduction in reaction time and stands as a potential alternative to thermal hydrolysis.

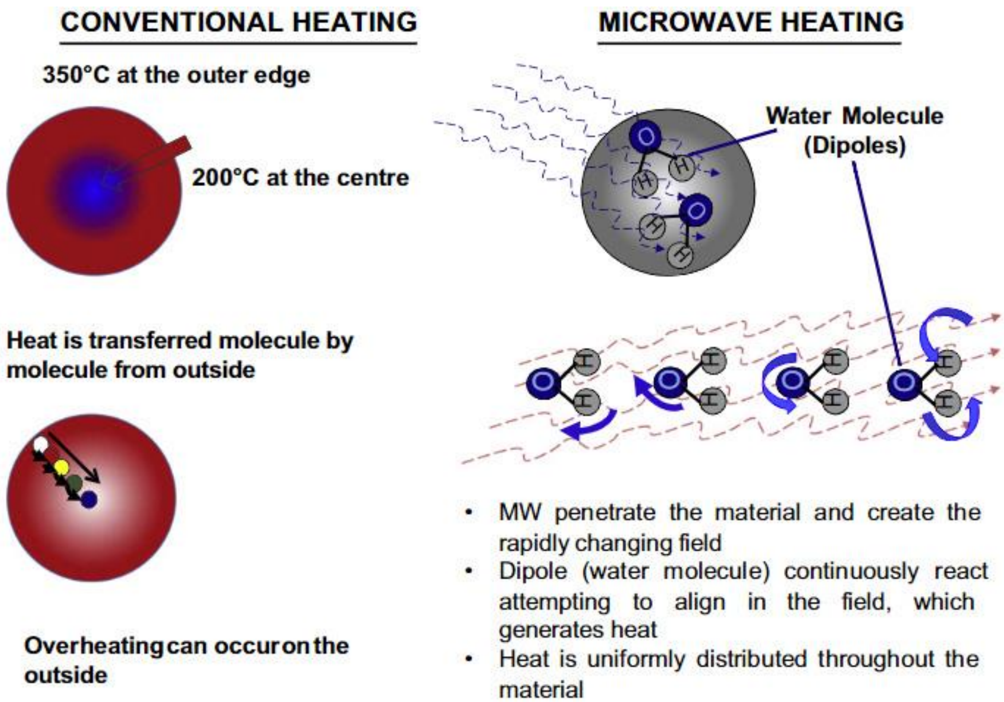


Figure 2.5: Difference between conventional thermal hydrolysis and microwave irradiation (Tyagi and Lo, 2013)

The efficiency of microwave treatment in heating biological solutions can be explained in terms of permittivity (ε*), which is given by the following equation 2.11 (Leonelli and Mason, 2010; Pino-Jelcic et al., 2006).

$$\varepsilon^* = \varepsilon' + i\varepsilon'' \qquad \text{(equation 2.11)}$$

Where,

ε' = dielectric constant (the ability of a component to be polarized by external electromagnetic field)

ε'' = dielectric loss factor (efficiency of conversion of electromagnetic energy into heat)

The ratio of dielectric loss factor to dielectric constant can be given as tan δ (equation 2.12), which tells us about the capacity of the materials to be heated. The higher the value of tan δ, the better is the heating efficiency.

$$\tan \delta = \frac{\varepsilon''}{\varepsilon'} \qquad \text{(equation 2.12)}$$

As the sludge is treated with microwave irradiation, the water dipoles and other induced dipoles (E.g. some EPS molecules, DNA, proteins) in the sludge align towards the applied electromagnetic field, thereby results in the heating effect. Besides, non-thermal effects of microwave irradiation in the treatment of sludge were also hypothesized by Eskicioglu et al and demonstrated insignificant compared to the thermal effects under identical treatment conditions (Eskicioglu et al., 2006; Koupaie and Eskicioglu, 2016; Mehdizadeh et al., 2013; Vergine et al., 2014).

The microwave treatment of activated sludge was initially studied for the pathogen removal (Hong, 2002; Hong et al., 2004; Pino-Jelcic et al., 2006), as a replacement to the time- consuming thermal hydrolysis. Besides, the heat loss through conduction and convection in the conventional thermal hydrolysis can be minimized (Tyagi and Lo, 2013). Similar to thermal hydrolysis, microwave pre-treatment causes sludge solubilization and biodegradation, while the latter is proportionally higher at temperatures above 110 °C (Toreci et al., 2011, 2010). However, microwave pre-treatment differs from thermal heating in the aspects of temperature increase rate and ramp rate (Koupaie and Eskicioglu, 2016). In addition, the sludge solids concentration is an important parameter to consider in microwave heating, as the amount of microwave absorption is dependent on the permittivity (ε) (Eskicioglu et al., 2007b). The sludge can be classified as an "absorber" of microwaves, based on the classification by Coelho (Coelho, 2012).

Microwave pre-treatment is usually done using microwave ovens (Figure 2.6) (Pino-Jelcic et al., 2006).

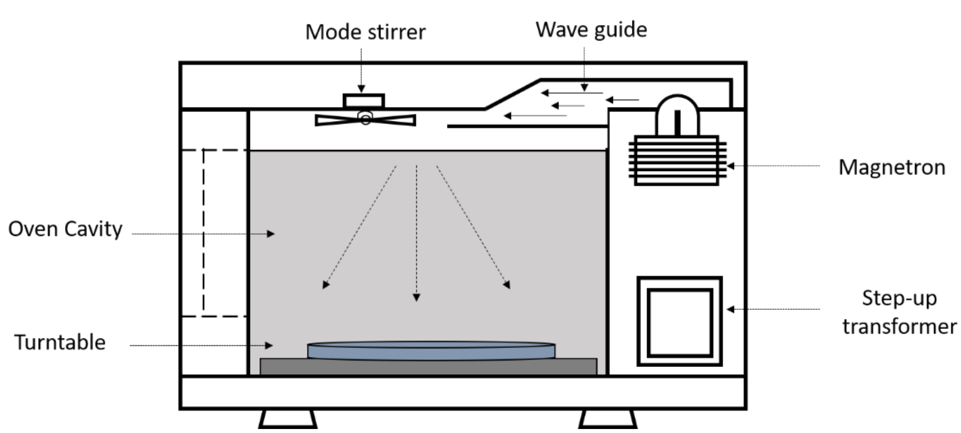


Figure 2.6: A simplified illustration of a conventional microwave oven

Microwave ovens are provided with six essential parts, namely, oven cavity, turntable, magnetron (the microwave generator), waveguide, mode stirrer (distributes microwave inside the microwave cavity) and a step-up transformer. The step-up transformer magnifies the power available to the oven, as the microwave oven requires more power than a normal voltage. Pre-treatment with microwave causes a reduction in particle size, similar to thermal pre-treatment under 100 °C (Kennedy et al., 2007; Yi et al., 2014). Regarding cell disruption, Cella et al. observed fourfold to fivefold higher microbial destruction with microwave irradiation at an intensity of 2.62 KJ/ g TS, the corresponding temperature of 80 °C with 9 min application time compared to the untreated thickened WAS (Cella et al., 2016). Besides, the authors also reported around 70 % reduction in faecal coliform levels in the anaerobic digestate of the microwave pre-treated sludge. The mechanism of cell destruction in a microwave pre-treatment could be both thermal and non-thermal effects caused by microwave irradiation. The non-thermal effects could be due to the absorption of microwaves by lipid bilayers, which lead to cell- disruption (Eskicioglu et al., 2007a). In addition, an increase in cell disruption was observed by microwave treatment as compared to the conventional thermal heating under similar conditions, supporting the non-thermal effects (Eskicioglu et al., 2007c; Hong et al., 2006, 2004; Kakita et al., 1995). In another study, non-thermal effects by microwave treatment in the inactivation of T4 Bacteriophage was studied and reported that the non-thermal effects resulted in minor killing and the thermal effects showed significant killing (Bryant et al., 2007). However, non-thermal effects on microbial disintegration are still inconclusive, as the control over temperature rate, quantification of molecular level absorption of microwaves are still challenging tasks (Bozkurt and Apul, 2020; Leonelli and Mason, 2010). Apart from thermal, non-thermal effects of the microwave, catalytic oxidation due to hydroxyl radical formation may also be responsible for the disintegration of cell walls and floc assemblies (Özön and Erdinçler, 2019).

Furthermore, the effect of microwave pre-treatment on the disintegration of biomolecules and sludge solubilization have been studied in the anaerobic digestion of WAS (Appels et al., 2013; Eskicioglu et al., 2007c; Kennedy et al., 2007; Uma Rani et al., 2013). A proportional increase in the soluble COD concentration (sCOD), was observed at temperatures below 100 °C and above 120 °C (Eskicioglu et al., 2007c; Kennedy et al., 2007). The increase in sCOD is considered an important parameter to evaluate the efficiency of pre-treatment. Pino Jelcic et al.

observed solubilization of 46 % non-soluble COD of WAS compared to 12 % solubilization of primary sludge, treated at temperatures around 60 °C (Pino-Jelcic et al., 2006). Similarly, a proportional increase in the concentration of soluble biomolecules (sugars, proteins and humic acids) was also observed at temperatures 50-160 °C (Eskicioglu et al., 2007a; Mehdizadeh et al., 2013). However, Eskicioglu et al. observed a decrease in reducing sugars concentration at temperature 96 °C, which could be the result of the Maillard reaction (Eskicioglu et al., 2007a). The solubilization of COD (% sCOD increase/ g VS) in relation to the specific energy input of microwaves is given in Figure 2.7. The given figure was compiled by (Bozkurt and Apul, 2020), with data drawn from various experiments across multiple journal articles. The solubilization increases with the increasing energy input, which is almost linear till 100 KJ/ g VS. However, the solubilization almost attains a saturation at energy input above 265 KJ/ g VS as observed in the figure. This could be due to Maillard reactions, as temperature level is increased beyond the threshold at higher energy input (Kuglarz et al., 2013; Yeneneh, 2014).

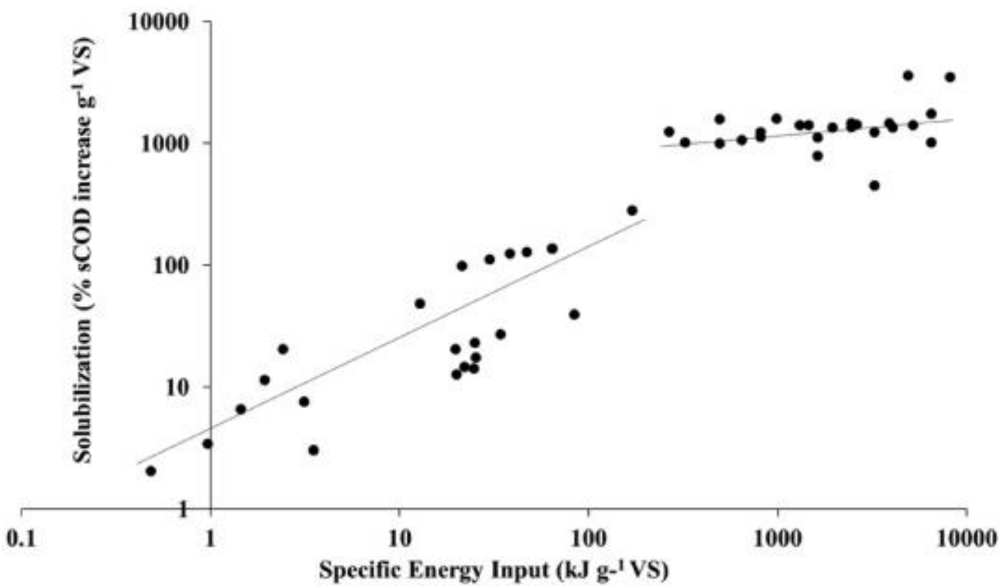


Figure 2.7: COD solubilization with increasing microwave specific energy input (Bozkurt and Apul, 2020).

Moreover, the effects of microwave irradiation on sludge biodegradation and biogas production have gone through some contradicting observations (Gonzalez et al., 2018). Most of the studies reported enhanced anaerobic biodegradation and biogas production (Alqaralleh et al., 2020; Elagroudy et al., 2020; Gonzalez et al., 2018; Jiang et al., 2018). Park et al. observed a 1.34-

fold increase in biogas production in the microwave pre-treated sludge compared to control, in mesophilic anaerobic digestion with 10 days hydraulic retention time (HRT) (Park et al., 2004). Similarly, microwave pre-treatment at 60-120 °C have shown increased biogas production compared to control in batch anaerobic digestion of WAS (Eskicioglu et al., 2007b; Hosseini Koupaie et al., 2017). Also, microwave pre-treatment at 175 °C has shown a 137 % increase in methane production in anaerobic co-digestion of thickened waste activated sludge (TWAS) with 60 % fat, oil and grease (Alqaralleh et al., 2019). However, an acute methanogenic inhibition was observed in a microwave (110-175 °C) pre-treated mesophilic anaerobic digestion of TWAS (Toreci et al., 2011). This could be due to the production of toxic by-products from microwave treatment or the lack of optimum acclimatization of inoculum (Toreci et al., 2011). Nevertheless, the high-temperature (above 175 °C) treatment of sludge has the disadvantage of the generation of refractory compounds from the Maillard reaction (Pinnekamp, 1989).

In addition, microwave pre-treatment at temperatures below 96 °C also improves the dewaterability of final anaerobic digestate (Coelho et al., 2011; Eskicioglu et al., 2007b; Jibao Liu et al., 2016a). Therefore, microwave treatment can be quite beneficial in terms of sludge solubilization, biogas production and dewaterability, however, the associated energy expenses are still a disadvantage in this method (Gonzalez et al., 2018).

c) Ultrasonication

Ultrasonication technology involves the formation of microscopic bubbles due to the cycles of ultrasound pressure waves and their subsequent collapse by a process known as “cavitation” (Figure 2.8). Such bubbles/ cavities result when a negative pressure exceeds the tensile strength of the liquid (Chen et al., 2012). The violent collapse of bubbles causes powerful waves of vibration in the cavitation field, which disrupts molecular interactions in the solution. Cavitation is a nucleated process, wherein the bubbles are formed in the weak points of the liquid, such as gas-filled crevices in the particulate organic matter (Chen et al., 2012). The efficiency of ultrasound pre-treatment in the pre-treatment of WAS depends on various parameters such as sludge characteristics, reactor geometry, ultrasound frequency, intensity and temperature (Delmas et al., 2015). The sludge disintegration caused by ultrasound is the result of shear forces generated by the cavitation process and/ or by the formation of reactive oxygen species such as hydroxyl radicals (Chen et al., 2012).

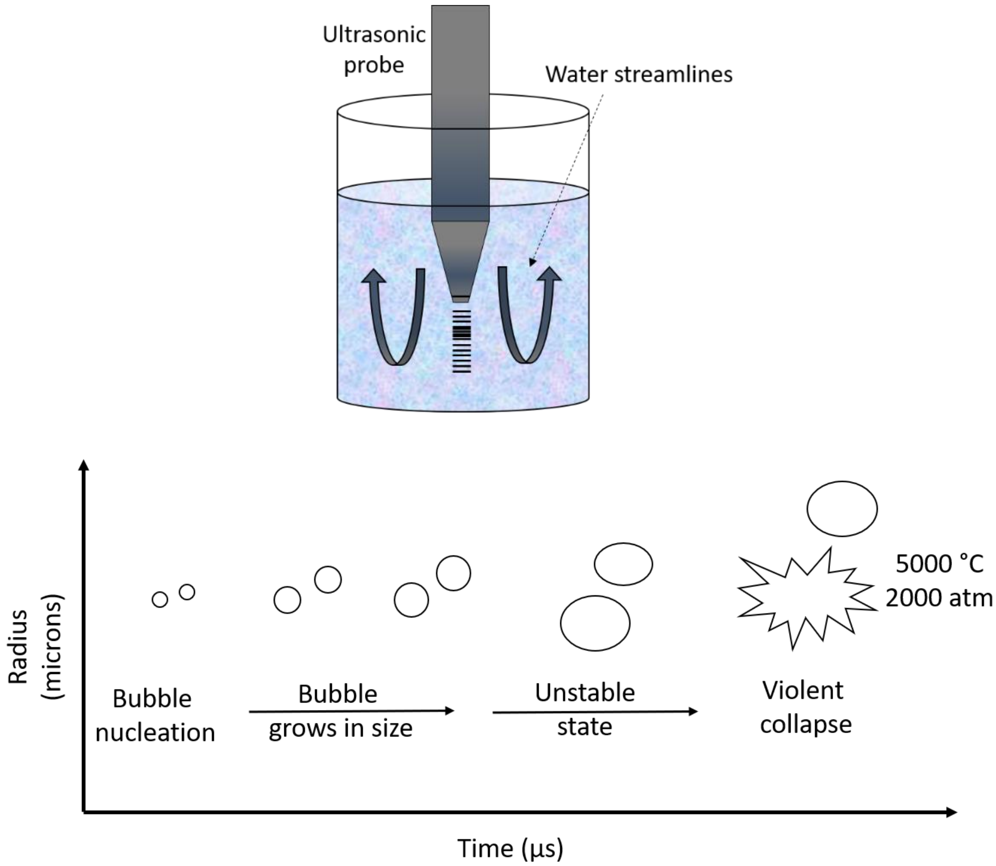


Figure 2.8: Mechanism of ultrasonication and cavitation phenomenon

One of the widely studied operational parameters of ultrasound pre-treatment is the specific energy (E_s). The specific energy of ultrasound encompasses the power, volume of the sample, application time and the solids concentration (equation 2.13).

$$E_s\left(\frac{KJ}{gTS}\right) = \frac{D\left(\frac{kW}{L}\right)*time(s)}{Solids\ concentration\ (\frac{gTS}{l})} \qquad \text{(equation 2.13)}$$

Where, D is the ultrasound density (power/ volume). Low-frequency ultrasound treatment (20-80 kHz) leads to shear stress as the dominant effect (Pilli et al., 2011), whereas high frequency (150-2000 kHz) induces the production of hydroxyl radicals (Tiehm et al., 2001). Besides, power density, application time, temperature and solids concentration of the sample have a direct influence on the efficiency of ultrasound pre-treatment (Gonzalez et al., 2018).

Ultrasound treatment has been used as a sludge homogenization technique for the disruption of bacterial cells and sludge floc structures (Cella et al., 2016; Foladori et al., 2007; Guo et al., 2014; Jorand et al., 1995). The rate of hydrolysis increases with ultrasound pre-treatment as reported by many studies (Braguglia et al., 2012; Kianmehr et al., 2013; Zorba and Sanin, 2013), which can be attributed to the sludge floc disintegration achieved by the pre-treatment (Bougrier et al., 2006; D. H. Kim et al., 2013; Rombaut et al., 2014; Sotodate et al., 2009). Besides, the particle size decreased with an increase in specific energy for a temperature-controlled ultrasonication (Feng et al., 2009). Also, ultrasonication caused higher solubilization of proteins compared to carbohydrates, at both temperature-controlled and uncontrolled conditions(Jaziri et al., 2012; Tian et al., 2015), which is similar to the effect of thermal hydrolysis (below 100).

Ultrasound pre-treatment is associated with temperature increase, dependent on the specific energy of the treatment (Gonzalez et al., 2018). Therefore, the sludge biodegradation or the biopolymer solubilization need to be assessed in terms of temperature controlled or uncontrolled experimentation, to distinguish the effects of sonication and thermal force. When the temperature was controlled below 45 °C, the increase in biodegradation was in the range of 1-5.5 % whereas, under un-controlled temperature, the increase was 7-18 % (Gonzalez et al., 2018). The increase in biogas production in the latter is most likely the results of thermal biodegradation. Besides, Le et al. suggested that an increase in biodegradation at uncontrolled temperature could be the result of combined effects of sonication and the temperature, similar to the results of thermal hydrolysis at temperatures around 100 °C (Le et al., 2016).

However, ultrasonication has energy requirement as a limiting factor as two energy conversions are involved, (1) electrical to mechanical and (2) mechanical to cavitation (Pérez-Elvira et al., 2010). Besides, it also requires the solids concentration to be less than 4 %, as higher solid concentrations have been reported to decrease the efficiency of ultrasonication (Pilli et al., 2016; Sahinkaya, 2015). Therefore, ultrasonication is often used in combination with other pre-treatments to achieve enhanced biodegradation (Yeneneh, 2014).

d) Freezing and thawing

Freezing biological substances at -20 °C for several hours and subsequent thawing at room temperature causes disintegration of microbial cells through the formation of ice crystals (Vaclavik and Christian, 2008). The process of crystallization depends on the rate of freezing. A slow freeze leads to faster extracellular crystallization than intracellular crystallization, thereby tears the microbial cell walls due to osmotic pressure (Wang et al., 2001). Further, prolonged exposure to freezing temperatures leads to intracellular crystallization, which causes ice formation internally that pierces out the cell membrane from the inside (Thomashow, 1998). As the WAS is composed of microbial cells and more than 90 % water, freezing and thawing have been employed as a pre-treatment technique to break down the microbial fraction (Hu et al., 2011; Vesilind and Martel, 1991; Wang et al., 2001).

Most of the studies in this pre-treatment have been focused on sludge dewaterability (Hu et al., 2011; Vesilind and Martel, 1991; Wang et al., 2001). Parameters such as rate of freezing, final temperature and pre-treatment time influence the efficiency of this pre-treatment technique. Besides, multiple freeze-thaw cycles and slower freezing rate have improved the efficiency of this pre-treatment (Vaclavik and Christian, 2008; Vesilind and Martel, 1991).

Furthermore, freezing and thawing tend to aggregate the particulate matter during the treatment and newly created aggregates remain together after thawing (Gao, 2011; Wang et al., 2001). Other studies suggest a decrease in particle size and floc size after this pre-treatment and thereby improved settleability (Gonzalez et al., 2018). However, the effects on floc architecture may vary, most of the studies suggest an improved dewaterability as the final result (Gonzalez et al., 2018; Hu et al., 2011). In addition, a decrease in viable cell count is expected in this pre-treatment, with a prominent decline through slow freezing (Diak and Örmeci, 2016). Nevertheless, the survival of bacterial cells at freezing temperatures has also been observed, possibly due to the presence of cryoprotectant components such as proteins, fats and glycerol in the solution (Montusiewicz et al., 2010). Additionally, increased biogas production has also been observed in the few studies reported in this pre-treatment (Jan et al., 2008; Montusiewicz et al., 2010; Pabón Pereira et al., 2012).

e) Hydrodynamic cavitation, milling and homogenization

In addition to various physical pre-treatment methods dealt with above, hydrodynamic cavitation, milling and homogenization are additional methods wherein application of direct mechanical force is involved. Hydrodynamic cavitation is similar to ultrasonic cavitation, except the cavitation bubbles are created by the flow of liquid under controlled conditions such as venturi tubes or orifice plates (Kumar and Pandit, 1999; Lee and Han, 2013). The inclination angle and the number of venturi constrictions are important parameters that affect the efficiency of this pre-treatment (Kim et al., 2008). Sludge milling/ ball milling involves a cylindrical grinding chamber filled with beads. The shear- and pressure- forces generated amidst the microbial fractions due to the agitation of these beads lead to sludge disintegration (Pérez-Elvira et al., 2006). The collision frequency between the moving beads is an important parameter in this method (Jung et al., 2001). Furthermore, homogenization can be achieved by the external application of pressure on sludge flocs and microbial cells (Gonzalez et al., 2018). During homogenization, the sludge is pressurized through an air pump, which forces the sludge through a narrow orifice (Wahidunnabi and Eskicioglu, 2014). The amount of pressure and the homogenization cycles are important parameters that determine the solubilization efficiency of this method (Y. Zhang et al., 2012).

The disintegration of floc structures and microbial cell lysis has been observed in ball milling and homogenization methods (Fang et al., 2015; Rai et al., 2008). The energy input of 5 KJ/ g TS achieved microbial inactivation of 30 %, and it increased to 80 % as the energy input is raised to 35 KJ/ g TS, using the ball milling method (Rai et al., 2008). In addition, a limited increase in hydrolysis rate and biodegradation have been observed in hydrodynamic cavitation and homogenization (Dhar et al., 2011b; Elliott and Mahmood, 2012; Lee and Han, 2013). Besides, the application of hydrodynamic cavitation in anaerobic co-digestion of WAS and oily wastewater was observed to increase biogas production (Habashi et al., 2016).

Although the above mechanical disintegration methods have shown sludge biodegradation and energy requirements that are comparable to other physical methods, they require installations such as pressurized vessels to carry out these pre-treatments.

2.2.1.2 Chemical pre-treatment

The disintegration of sludge can also be achieved by the addition of strong oxidants, acids, alkali or other chemicals (Pérez-Elvira et al., 2006). Ozonation and the addition of hydrogen peroxide are widely used chemical methods that work on the principle of chemical oxidation (Carrère et al., 2010). Chemical treatments have low energy requirements and they also enhance the final digestate dewaterability (Pérez-Elvira et al., 2006).

a) Advanced oxidation process

The advanced oxidation process (AOP) relies on the formation of reactive oxygen species such as hydroxyl radicals ($OH^{\bullet}$) or sulfate radicals ($SO_4^{\cdot-}$) in oxidizing the organic substances, refractory organic pollutants or inorganic contaminants present in the wastewater (Deng and Zhao, 2015). These radicals are extremely short-living and highly reactive, especially ($OH^{\bullet}$) radicals exhibit high reactivity in the order of 10^9 $M^{-1}s^{-1}$ (Slupphaug et al., 2003). The extreme reactivity of ($OH^{\bullet}$) radicals is due to its oxidation potential 2.8 V (pH 0) and 1.95 V (pH 14) vs standard electrode (Deng and Zhao, 2015). As a consequence of its high reactivity, ($OH^{\bullet}$) radicals oxidize almost all organic substances present in the activated sludge, without any selective mechanism (Gonzalez et al., 2018). The AOP of WAS have been widely studied with two oxidants, viz, ozone (O_3) and hydrogen peroxide (H_2O_2).

The most widely used AOP is ozonation. Ozone (O_3), is a strong oxidant with an oxidation potential of 2.07 V vs standard electrode (Deng and Zhao, 2015). Direct oxidation by O_3 is selective to the ionized and dissociated form of organic compounds rather than the natural form. However, once introduced into the solution, it decomposes itself to ($OH^{\bullet}$) radicals (equation 2.14) and perform the oxidation mechanism (Bougrier et al., 2006). O_3 breaks down heavier organic compounds into simpler molecules such as carboxylic acids, carbohydrates and amino acids (Bougrier et al., 2007, 2006; Salsabil et al., 2010). As a consequence of acid formation, the pH of the solution may drop 1 or 2 units after ozonation (Chu et al., 2008). Moreover, too high a dosage of O_3 could oxidize solubilized compounds and thereby reduce the apparent solubilization (Yeom et al., 2002).

$$3O_3 + H_2O \rightarrow 2OH^{\bullet} + 4O_2 \qquad \text{(equation 2.14)}$$

Therefore, one of the important parameters to be optimized in the case of chemical treatment is the dosage of the chemical. Several studies have shown optimum dosage for the biodegradation of WAS as 100 mg O_3/ g COD, 200 mg O_3/ g TSS and 150 mg O_3/ g TS (Carrère et al., 2010). Besides, 50 % of microorganisms were observed to be killed at an O_3 dose of 20 mg O_3/ g TSS (Chu et al., 2008). In addition, the reduction of particle size was observed as a result of floc disruption at an optimum dose of 50 mg O_3/ g TS (Demir and Filibeli, 2012). Floc disruption and reduction in mean particle size increases the hydrolysis rate. Yet, a higher O_3 dose (80 to 100 mg O_3/ g TSS) was observed to decrease the hydrolysis rate (Silvestre et al., 2015). Moreover, the optimum dosage of O_3 for enhanced anaerobic digestion efficiency may vary across different studies, as the WAS composition differs (Gonzalez et al., 2018).

Another chemical oxidant widely used in AOP is hydrogen peroxide (H_2O_2). The ($OH^{\bullet}$) radicals generated from H_2O_2 leads to the destruction of sludge floc structure and cell membranes. Besides, refractory organic pollutants can be effectively oxidized by ($OH^{\bullet}$) radicals, and converted into soluble molecules (He and Wei, 2010). In addition, the destruction of the floc structure can release the heavy metals into the aqueous phase from the bound state (Bouda et al., 2009). H_2O_2 based AOP has applications in sludge dewatering, minimization, pre-treatment for anaerobic digestion and removal of persistent pollutants (Guan et al., 2018).

Furthermore, H_2O_2 can be activated to produce the highly reactive oxidative species ($OH^\bullet$) radicals, by transition metals, microwave, ultrasound, electrolysis and light irradiation (Dong and Rosario-Ortiz, 2012; Eswari et al., 2016; Jiang et al., 2016; Xu and Li, 2010). Among various transition metals that can activate H_2O_2, iron is the most frequently used. The activation of H_2O_2 by Fe^{2+} -metal salt is referred to as "Fenton's reaction" (Dewil et al., 2005). The classical Fenton chemistry encompasses the following reactions.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^\bullet + HO^- \qquad \text{(equation 2.15)}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2{}^\bullet + H^+ \qquad \text{(equation 2.16)}$$

$$OH^\bullet + H_2O_2 \rightarrow HO_2{}^\bullet + H_2O \qquad \text{(equation 2.17)}$$

$$HO_2{}^\bullet + OH^\bullet \rightarrow H_2O + O_2{}^{\bullet -} \qquad \text{(equation 2.18)}$$

On the other hand, superoxide ($O_2^{\bullet -}$) radicals can be formed by a one-electron reduction mechanism from molecular oxygen (Prousek, 2007) as shown by equation 2.19, in biological systems.

$$O_2 \rightarrow O_2^{\cdot -} \rightarrow H_2O_2 \rightarrow HO^{\cdot} \rightarrow H_2O \qquad \text{(equation 2.19)}$$

($OH^\bullet$) radicals are produced through equation 2.15 by electron transfer. However, the produced ($OH^\bullet$) radicals can be scavenged by Fe^{2+} or H_2O_2 through equations 2.16 and 2.17. Therefore, the optimal ratio of iron salts to H_2O_2 needs to be determined, to avoid unwanted scavenging (Deng and Zhao, 2015). Nevertheless, H_2O_2 can be also be activated by the indigenous Fe^{2+} ion present in the sludge (X. Zhou et al., 2015). Besides the concentrations of iron and H_2O_2, additional parameters such as pH, temperature and reaction time also influence the Fenton reactions (Erden and Filibeli, 2011; Pilli et al., 2015; Sahinkaya, 2015). For instance, ferric ion precipitates and loses its catalytic activity at pH 4 and above (W. Zhang et al., 2015c). Similarly, high pH leads to the formation of hydroperoxyl radical ($HO_2^{\bullet}$), which acts as a scavenger of ($OH^{\bullet}$) radicals (equation 2.18) (Guan et al., 2018). Mostly, pH 2 or 3 have been applied for the pre-treatment of WAS in this technique (Andreozzi, 1999; Bao et al., 2015; Gong et al., 2015; Li and Zhang, 2014).

The oxidative mechanism of ($OH^{\bullet}$) radicals degrade the EPS matrix and destroy most of the microorganisms (Figure 2.9). Besides, it also leads to the mineralization of recalcitrant organics into CO_2 and H_2O (Rahmani et al., 2015).

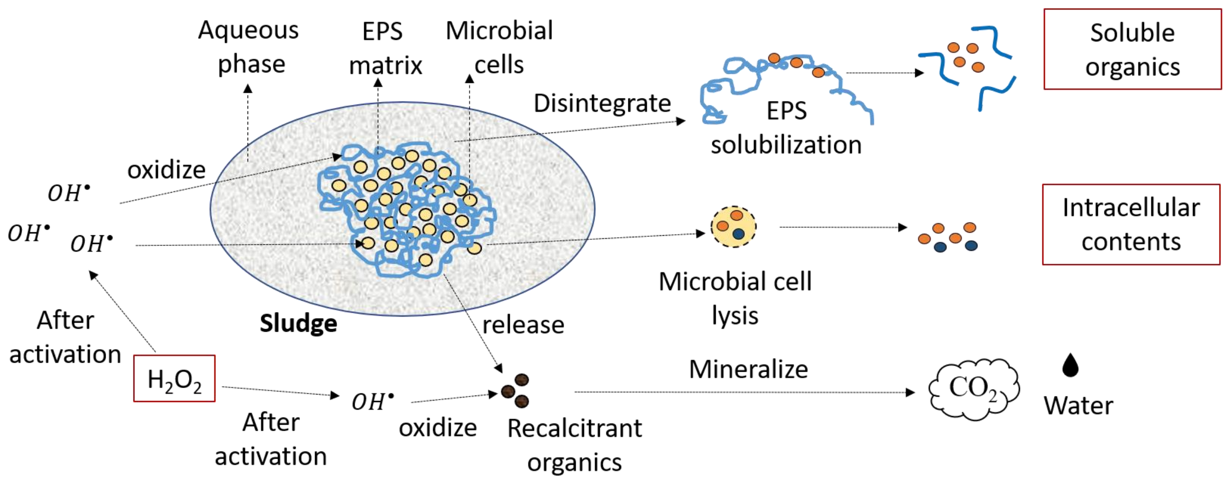


Figure 2.9: Sludge disintegration mechanism by H_2O_2 based AOP

The sludge disintegration achieved by H_2O_2 based AOP can be determined by the decrease of total suspended solids (TSS), volatile suspended solids (VSS), tCOD, MLSS and increase in sCOD (Guan et al., 2018; E. Neyens et al., 2003b). In the Photo-Fenton process, where light is used to assist Fenton's reaction, 40 % MLSS reduction was achieved with 60 mg Fe^{2+}/ l and 4000 mg H_2O_2/ l after 2 days reaction time (Tokumura et al., 2009). Furthermore, an increase in sCOD concentration was observed from 179 mg/l to 1023 mg/ l with Fenton pre-treatment (Dewil et al., 2005). Similarly, at different H_2O_2/ Fe^{2+} concentrations, an increase in COD and BOD concentrations were observed in the soluble phase of secondary sludge (Dewil et al., 2007), indicating the solubilization efficiency of the Fenton mechanism. However, the dosage of H_2O_2 needs to be optimized, as excessive H_2O_2 may lead to the formation of hydroperoxyl radical ($HO_2^{\bullet}$) (Guan et al., 2018).

Various authors have reported increased sludge biodegradation and biogas production with H_2O_2 based AOP (Dewil et al., 2007; Dhar et al., 2011b; Erden and Filibeli, 2010, 2011; Lee and

Shoda, 2009). The solubilization of intracellular components, EPS matrix and oxidization of recalcitrant substances are the reasons for improved anaerobic biodegradation. However, sometimes solubilization of organics does not entirely reflect in biomethanation, as they can be converted to refractory soluble organics that are difficult to biodegradation (D. H. Kim et al., 2013). Besides, H_2O_2 doses higher than optimum can also lead to a decrease in biodegradation and low methane yield (T. Zhang et al., 2015). The optimum dosage for efficient biodegradation varies among different studies, as the sludge composition is different. Erden and Filibili reported 19.4 % higher methane production with a treatment of 4 g Fe^{2+}/ kg TS and 60 g H_2O_2/ kg TS for 60 min (Erden and Filibeli, 2011). Whereas, Dewil et al. observed a maximum of 75 % increase in methane production with a treatment of 0.07 g Fe^{2+}/ g H_2O_2 and 50 g H_2O_2/ kg DS (Dewil et al., 2007). Besides, the author also reported a proportional increase in biogas production with an increase in H_2O_2 concentration.

Furthermore, Fenton's reaction increases the sludge dewaterability (Buyukkamaci, 2004; Dewil et al., 2005; Lu et al., 2001, 2003; E. Neyens et al., 2003b; Tony et al., 2008). The improved dewaterability is exhibited through (1) reduction of EPS and water interaction (2) changes in interparticle interactions between sludge flocs and their components (Pilli et al., 2015). Consequently, the interstitial water entrapped between the EPS and sludge flocs is released from the bonds, as the treatment break down sludge flocs. In addition, the oxidation of organic substances and microbial cell walls by ($OH^{\bullet}$) radicals results in breakage of aromatic ring structures and conjugated bonds in chain structures, thereby weakens the water interaction (Jun Liu et al., 2016; Liu et al., 2011). Buyukamacci et al. reported a 93 % reduction in specific resistance to filtration (SRF) with a treatment of 5 g H_2O_2/l and 6 g Fe^{2+}/l at pH 4 (Buyukkamaci, 2004). Similarly, a 74 % reduction in SRF was reported with a treatment of 373 mg H_2O_2/ g DS and 288 mg Fe^{2+} / g DS at pH 2 (He et al., 2015).

While ozonation provides enhanced biogas production, it also requires 50 % of energy demand for producing pure oxygen as an input element (Müller et al., 2004). Based on energy requirement, the sole addition of H_2O_2 is beneficial over ozonation and Fenton's reagents (Gonzalez et al., 2018). Furthermore, the activation of H_2O_2 by physical methods (ultrasound, microwave) gave rise to various hybrid technologies which showed better biodegradation compared to sole H_2O_2 treatment (Carrère et al., 2010; Gonzalez et al., 2018).

b) Alkaline/ acids

The disintegration of sludge can be achieved through the increase or decrease in pH, through an alkali or acid, respectively. In alkaline pre-treatment, an alkali such as NaOH or $Ca(OH)_2$ is added to the activated sludge. Consequently, the structure, physicochemical properties, electrostatic charge and flocculation ability of the biomolecules in the EPS matrix are modified (Wang et al., 2012). The microbial flocculation in the activated sludge is closely related to its EPS composition. In particular, the ionization state of functional groups on various biomolecules present in the EPS fractions significantly influence the flocculation (Wang et al., 2012). These functional groups may be protonated or deprotonated at a given pH condition, dependent on their pKa values. For instance, the carboxylic and sulfate groups remain deprotonated at pH above 4 (pKa ≈ 4), whereas amino groups remain protonated at pH below 7 to 9 (pKa ≈ 7.0-9.0) (Wang et al., 2012). Hence, a difference in pH caused by the addition of alkaline pre-treatment would significantly influence the ionization state of these functional groups, thereby affect the structure of WAS matrix. At alkaline pH, the acidic groups get dissociated from EPS causing electrostatic repulsion between the negatively charged EPS, which increases its solubilization (Wingender et al., 1999). Moreover, protein denaturation and the release of intracellular contents at extreme pH also causes solubilization (Vaclavik and Christian, 2008). Therefore, high alkaline pH causes the break-up of floc structures and increases sludge solubilization, which is good for anaerobic biodegradation, however, it worsens the dewaterability (Doğan and Sanin, 2009; J. Kim et al., 2003; Xiao et al., 2015). Furthermore, the type of reagents used for the pre-treatment also influences the solubilization and VS reduction (Gonzalez et al., 2018). For instance, NaOH treatment achieved higher COD solubilization compared to $Ca(OH)_2$ while using the same dose (Li et al., 2008). Similarly, a higher VS removal was observed with NaOH pre-treatment compared to $Ca(OH)_2$ (Ray et al., 1990).

Quite like alkaline pre-treatment, the pre-treatment with acids such HCl, H_2SO_4, H_3PO_4 and HNO_2 works on the principle of alteration in pH, thereby affecting the physicochemical properties of the functional groups present in EPS and microbial cell walls (Gonzalez et al., 2018). The pH ranges from 1-5.5 in an acid pre-treatment. In contrast to other acids, HNO_2 also exhibits the mechanism of lipid peroxidation and disruption of cell envelope (Horton and Philips, 1973; Zahedi et al., 2016). During acid pre-treatment, the protonated state of EPS functional groups results in compact aggregated structures (Wang et al., 2012). Besides, the ionization of acid groups is suppressed at acidic pH, thereby results in aggregation. Accordingly, the highest flocculation is achieved at low pH near the isoelectric point (Wang et al., 2012).

Microbial cell disruption is an important parameter to evaluate the efficiency of various pre-treatments. In the pH range of 9.0-12.0, disruption of cell walls, cell membranes, cell nuclei and EPS was observed in an alkaline pre-treatment (NaOH) of WAS (Xiao et al., 2015). Similarly, with NaOH alkali pre-treatment at pH 12.30, disruption of *Flavobacterium aquatile* cells was observed and consequently results in improved compactibility of biological sludge (Erdincler and Vesilind, 2000). Furthermore, under acidic pre-treatment at about pH 0.98 by the addition of H_2SO_4, approximately 20 % of damaged and damaging cells were observed compared to 12.99 % in the control activated sludge (Guo et al., 2014). Whereas, an acidic pre-treatment at 2.02 mg HNO_2-N/ l (pH 6/ treatment time- 48 h), enhanced the biocidal effect significantly resulting in only 20 % viable cells compared to 80 % in control secondary sludge (Pijuan et al., 2012). A significant reduction in viable cell count was also observed with HNO_2 treatment in a similar study (Wu et al., 2018).

During alkaline/ acid pre-treatment, the pH of the activated sludge is altered beyond the optimum of an efficient anaerobic digestion process. Methanogenesis is inhibited at pH below 6 and beyond 8 (Jiunn-Jyi et al., 1997). Therefore, pH neutralization is considered a requisite in such chemical pre-treatments. A maximum of 15.4 % increase in biogas production was observed with NaOH treatment (pH 10) for 24 h compared to control in mesophilic anaerobic digestion of activated sludge (Shao et al., 2012). However, the biogas production decreased by 18.1 % at pH 12 NaOH treatment compared to the control in the same study. Despite the pH neutralization post-treatment, Shao et al. observed this inhibiting effect of high alkaline pre-treatment in anerobic digestion (Shao et al., 2012). Similarly, no increment in the biogas production was observed compared to control in a pH 12 alkaline pre-treatment, wherein substrate: inoculum ratio was kept 1:10 instead of a post neutralization step (Valo et al., 2004). Furthermore, acid treatment (HCl) at pH 2 (pH neutralized after 24 h treatment) yielded maximum biogas yield in batch studies and a 14.3 % increase in methane yield in the semi-continuous study compared to untreated activated sludge (Devlin et al., 2011). Similarly, an increase in biogas production was observed with HNO_2 pre-treatment (Li et al., 2016; Wang et al., 2014).

Both acid and alkaline pre-treatment require chemical reagents for reaching extreme pH conditions and also for neutralization, which increases the energy and cost of this pre-treatment method (Gonzalez et al., 2018). Besides, it also costs capital investment for digesters to withstand such extreme pH conditions in large scale WWTPs.

2.2.1.3 Biological pre-treatment

This classification includes enzymatic and microbial pre-treatments that catalyze the sludge disintegration (Carrere et al., 2016). Biological pre-treatments work on the principle of enzyme catalysis, that cause hydrolysis of macromolecules such as carbohydrates and proteins present in the EPS fraction and microbial cell walls. Biological treatment can be carried out in two ways. (1) Direct addition of isolated endogenous enzymes before anaerobic digestion (Odnell et al., 2016; Romano et al., 2009; Yang et al., 2010) (2) addition of specific aerobic/ anaerobic microbes that can secrete extracellular hydrolyzing enzymes (De Laclos et al., 2008; Fdez.-Güelfo et al., 2011; Li et al., 2009; Tang et al., 2012).

Biological pre-treatments can be carried out at milder pH and temperature conditions without the production of hazardous by-products, compared to the physical and chemical pre-treatment methods (Parawira, 2012). Within biological pre-treatments, commercial enzymatic preparation is expensive for application in huge volume at WWTPs (Parmar et al., 2001). Besides, free enzymes are prone to quick degradation even before the start of enzymatic hydrolysis (Müller, 2001). Hence, enzymes immobilized in the EPS matrix or cellulosome (multi-enzyme containing catalytic complex) are preferable for their stability and catalytic activity (Burgess and Pletschke, 2008; Matsumoto and Ohashi, 2003). On the other hand, endogenous hydrolytic enzymes through bioaugmentation (cultivation of microorganisms) can be a better alternative for enzyme stability and cost-effectiveness (Yu et al., 2013).

Limited studies have been reported with enzymatic pre-treatment of activated sludge for anaerobic digestion. In a study by Yu et al. endogenous amylases and proteases were found to have little influence on floc sizes, however, the former increased COD solubilization by 78.2 % after 7 h of pre-treatment (Yu et al., 2013). Besides, endogenous enzymes did not cause significant cell lysis indicating that the enzymatic activity was limited to EPS disintegration (Yu et al., 2013). Similarly, further reports suggest that enzymatic action (proteinase, amylase, cellulase) on cell lysis has been insignificant (Sesay et al., 2006; Yi et al., 2014). Regarding biogas production, Ushani et al. reported a significant improvement in hydrolysis rate and methane yield from mesophilic anaerobic digestion of bacterial (protease secreting) pre-treated and deflocculated sludge (Ushani et al., 2017). Whereas, amylase and protease combined enzyme pre-treatment yielded a 23 % increase in biogas production compared to control in mesophilic anaerobic digestion of activated sludge (Yu et al., 2013). On the contrary, enzymatic (Accelerase enzyme with endoglucanase activity and β-glucosidase activity) pre-treatment alone did not improve methane yield in thermophilic anaerobic digestion of paper mill secondary sludge (Bayr et al., 2013).

The efficiency of mono- enzymatic pre-treatment in enhancing anaerobic biodegradation is still uncertain, as previous studies have reported contradicting observations (Bayr et al., 2013; Yu et al., 2013). Besides, enzymatic treatment requires optimum pH and temperature conditions to maintain the catalytic ability. Furthermore, enzymatic pre-treatment have shown improved performance when applied in combination with other pre-treatments (Bayr et al., 2013).

2.2.2 Hybrid/ combined pre-treatment

The combination of two or more mono pre-treatment techniques resulted in “hybrid” technologies. In hybrid pre-treatment, synergism between physical, chemical and biological treatment strategies enhances the overall treatment performance. Besides, it also helps to overcome some of the drawbacks of individual pre-treatments. The combination of physical treatment with chemical treatment has been a topic of wide interest and reported by many. The following section discusses some of the potential hybrid pre-treatment technologies.

2.2.2.1 Thermal combined pre-treatment

Thermal hydrolysis, as a mono pre-treatment has yielded enhanced sludge solubilization and biogas production. The combination of thermal hydrolysis with chemical treatment technologies have been reported by various authors (Anjum et al., 2016; Carrère et al., 2010; Tyagi and Lo, 2011).

Earlier studies on chemical addition with thermal treatment stated that the “thermochemical” treatment can achieve sludge disintegration under normal pressures (Alsop and Conway, 1982; Hiraoka et al., 1985). Furthermore, Stuckey and McCarty reported a thermochemical pre-treatment with NaOH and observed a 27 % increase in methane production in anaerobic digestion of WAS (Stuckey and McCarty, 1984). Similarly, in another study, thermochemical (thermal + NaOH) pre-treatment yielded a 200 % increase in methane production in batch anaerobic digestion studies (Tanaka et al., 1997). Also, the combined pre-treatment accelerated the solubilization of COD and VSS. Penaud et al. observed 85 % COD solubilization with a thermochemical (26.1 g NaOH/ l and 140 °C/ 30 min) pre-treatment, however, limited biodegradability (Penaud et al., 2000). The decrease in methane production could be the result of refractory compounds generated at higher pH conditions. On the other hand, with a reduced NaOH concentration (7g/ l) and temperature (121 °C/ 30 min), Park et al. observed a significant improvement in anaerobic digestion (Park et al., 2005). The study reported an 88.9 % reduction in tCOD, 77.5 % reduction in VS and 79.5 % increase in methane yield. Various other studies also supported the efficiency of thermochemical pre-treatment in enhancing anaerobic digestion efficiency and solid reduction (Feki et al., 2019; Heo et al., 2003; J. Kim et al., 2003; E Neyens et al., 2003; Rocher et al., 1999; Takashima and Tanaka, 2010; Valo et al., 2004; Vlyssides and Karlis, 2004; Xu et al., 2014). Besides, thermochemical pre-treatment have also shown efficient pathogen reduction (Na et al., 2011; E Neyens et al., 2003).

Thermal hydrolysis can also be combined with AOP, however, very little researches have gone through in this field. Abelleira et al. reported a combination of thermal pre-treatment (86-164 °C) with H_2O_2 (oxidant coefficient < 1), in a process termed "advanced thermal hydrolysis" (Abelleira et al., 2012). The author reported improved dewaterability, sludge solubilization and biogas production as a result of synergism between peroxidation and direct steam injection. Furthermore, the treatment operates at mild conditions, which overcomes the energy requirement in either of the individual pre-treatments (Abelleira et al., 2012). Further, the thermo-oxidative pre-treatment conducted at 60 °C with 0.6 mg H_2O_2 + 1.5 $FeCl_2$/ mg S^{2-} yielded significant improvement in biogas production in continuous anaerobic digestion (Dhar et al., 2011a). Similarly, thermo-oxidative pre-treatments have shown enhanced sludge solubilization and biogas production compared to individual treatments (Hallaji et al., 2019; Hassan et al., 2016).

The major advantages of thermochemical pre-treatments are the enhancement in biodegradation with lesser chemical consumption and milder treatment conditions (Tyagi and Lo, 2011). However, the optimization of combined pre-treatment is essential as extreme temperature or pH conditions can lead to the generation of refractory compounds.

2.2.2.2 Microwave combined pre-treatment

Microwave technology is considered a potential alternative to thermal pre-treatment due to the reduced reaction time, better control and heating efficiency. Combining microwave with chemical pre-treatments is an attractive development and has been under research focus in recent years.

Like thermochemical pre-treatment, microwave-alkaline was studied for sludge solubilization and dewaterability by various authors (Chi et al., 2011; Doğan and Sanin, 2009; Qiao et al., 2008; Tyagi and Lo, 2012; Yang et al., 2013; Yu et al., 2017). The solubilization of suspended solids and COD significantly increased with the hybrid microwave-alkaline pre-treatment. Qiao et al. observed 50-70 % and 80 % solubilization of VSS and COD, respectively with the hybrid pre-treatment (120-170 °C and 0.2 g NaOH/ g DS) of secondary sludge (Qiao et al., 2008). Similarly, Dogan and Sanin observed an increase in solubilization from 0.5 % in control to 34 % with the hybrid pre-treatment (160 °C and pH 12) and also, 22 % increment in dewaterability in the anaerobic digestate (Doğan and Sanin, 2009). Further, 72 % maximum solubilization degree of WAS was reported with the hybrid pre-treatment (170 °C and 60 meq NaOH/ l) and maximum biogas production was observed at 135 °C and 20 meq NaOH/ l (Jang and Ahn, 2015). Again, the optimization of microwave condition and alkaline concentration is crucial to achieving maximum biogas production. Despite, maximum solubilization, anaerobic digestion performance could be limited by other factors associated with the pre-treatment.

Regarding biogas production, Dogan and Sanin observed a 19 % and 53 % increment in biogas production by a microwave-alkaline treatment (160 °C and pH 12/ NaOH) in batch and semi-continuous anaerobic digestion, respectively (Doğan and Sanin, 2009). Further, the hybrid pre-treatment achieved 10 % higher methane production than a mono microwave pre-treatment. In another study, microwave-alkaline pre-treatment (110-210 °C/ 1-51 min and 0-2.5 g NaOH/ g SS) achieved a 27 % increment in cumulative methane production in thermophilic batch anaerobic digestion of TWAS (Chi et al., 2011).

2.2.2.2.1 Microwave-hydrogen peroxide (MW-H_2O_2) pre-treatment

In microwave enhanced AOP, the oxidation power of the oxidants can be enhanced by the application of microwave irradiation (Özön and Erdinçler, 2019). As mentioned earlier, the activation of H_2O_2 to highly reactive ($OH^{\bullet}$) radicals can be accelerated by microwave irradiation

(Eswari et al., 2016) (equation 2.20). The mechanism of sludge disruption achieved by combined microwave and hydrogen peroxide pre-treatment has been presented in Figure 2.10. The strong oxidative ($OH^{\bullet}$) radicals can oxidize EPS fractions and break down complex organics, thereby promote sludge solubilization (Guan et al., 2018). The effects of MW-H_2O_2 pre-treatment on sludge solubilization is given in Table 2.5.

$$Microwave + H_2O_2 \rightarrow 2OH^{\bullet} \qquad \text{(equation 2.20)}$$

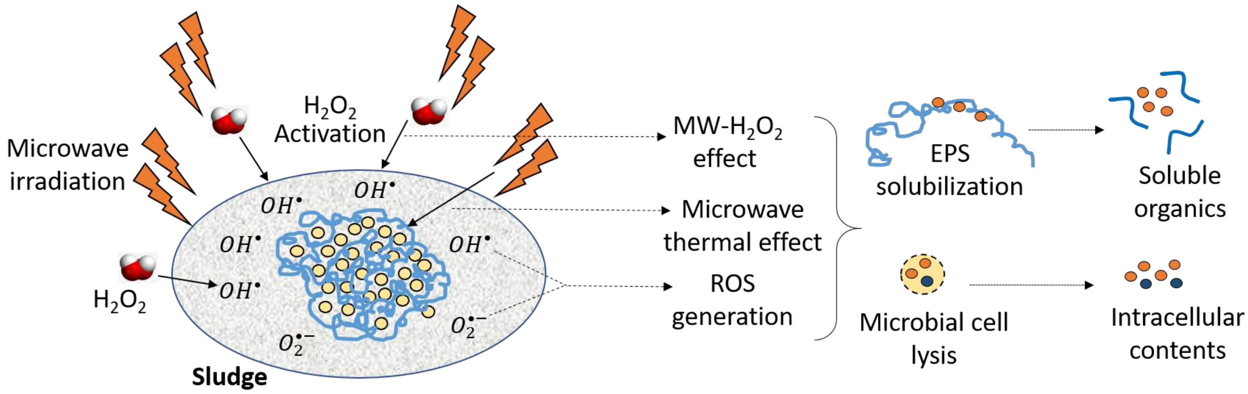


Figure 2.10: Sludge disruption mechanism of MW-H_2O_2 pre-treatment

As a pioneer, Eskicioglu et al. studied the synergetic effects of microwave and H_2O_2 (MW-H_2O_2) in sludge solubilization and biogas production in subsequent anaerobic digestion (Eskicioglu et al., 2008). The author reported enhanced solubilization of particulate COD with 1 g H_2O_2/ g TS oxidant addition and microwave irradiation (> 80 °C), however, limited biodegradation was observed in the anaerobic digestion. The decreased biodegradation could be attributed to the generation of refractory soluble organics from the combined pre-treatment (Eskicioglu et al., 2008). Later, the effects of H_2O_2 (0 to 4 H_2O_2/ tCOD) dosing strategy in MW-H_2O_2 pre-treatment was evaluated by (Wang et al., 2009). The author suggested the following important considerations while conducting MW-H_2O_2 pre-treatment.

1. The sludge pre-treatment with MW-H_2O_2 should consider the impact of the enzyme catalase in the degradation of H_2O_2. The enzyme catalase catalyzes the following reaction 2.21 and thereby reduces the generation of ($OH^{\bullet}$) radicals.

$$2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2 \qquad \text{(equation 2.21)}$$

2. The inactivation of catalase can be achieved by raising the temperature to 60-80 °C. The WAS can be initially heated to a temperature of 80 °C and then optimum H_2O_2 can be added to achieve maximum efficiency of MW-H_2O_2 pre-treatment.

Table 2.5: Effects of MW-H_2O_2 pre-treatment on sludge solubilization (Tyagi and Lo, 2013)

Sl. No	Treatment conditions	Observation	Reference
1	MW (20 °C/ min) for 5 min and 71 ml/l H_2O_2	96 % of tCOD was solubilized	(Liao et al., 2007)
2	80 °C (5 min) , 1 ml H_2O_2 (30 %)/ 1 % TS	25 % increase in sCOD	(Kenge et al., 2008)
3	MW/ H_2O_2 (1 %)-AOP, 80 °C- 3 min	18 % increase in sCOD	(Lo et al., 2008)
4	120 °C- 10 min, 1 g H_2O_2 (30 % v/v)/ g TS	COD solubilization increased from 3 % to 24 %	(Eskicioglu et al., 2008)
5	80 °C- 3 min with 2 % H_2O_2	sCOD reached 87 % of tCOD	(Yin et al., 2008)
6	70 °C- 0.1 % H_2O_2	1000 mg/l of maximum sCOD achieved	(Yu et al., 2010)

Furthermore, Xiao et al. optimized the H_2O_2 dosage for MW-H_2O_2 pre-treatment as 0.2 H_2O_2/ MLSS ratio and followed a similar dosing strategy proposed by (Wang et al., 2009), to inactivate catalase (Xiao et al., 2012). Later, Liu et al. investigated the effects of MW-H_2O_2 pre-treatment on the rheological properties of sewage sludge with 600 W microwave irradiation and 0.2 g H_2O_2/ g TS (Jibao Liu et al., 2016b). The author observed improved sludge flowability and weakened viscoelastic properties as a result of the MW-H_2O_2 pre-treatment. Lo et al. investigated the performance of a microwave-oxidation system with oxidants such as ozone and H_2O_2 in various combinations and concluded that ozonation followed by MW-H_2O_2 pre-treatment yielded maximum sludge solubilization and excellent settling property (Lo et al., 2015). Eswari et al. reported a 30 to 50 % increment in sludge solubilization and maximum methane production in a MW-H_2O_2 pre-treatment conducted at acidic (pH 5) condition (Eswari et al., 2016). Further, the author optimized 0.3 mg H_2O_2/ g SS as the optimum dosage and emphasized the role of pH in increasing the efficiency of pre-treatment. To achieve maximum digestion efficiency, usage of optimum dosage of H_2O_2 is essential in a combined MW-H_2O_2 pre-treatment. The generation of refractory compounds and the presence of residual H_2O_2 at higher H_2O_2 dosage can inflict biogas production through anaerobic digestion (Eskicioglu et al., 2008; Liu et al., 2017).

In addition, Liu et al. investigated the presence of residual H_2O_2 during anaerobic digestion after MW-H_2O_2 pre-treatment (Liu et al., 2017). The author reported that MW-H_2O_2 pre-treatment achieved high solubilization (35-50 %), however, large amounts of residual H_2O_2 remained in the digester at high dosage (0.6 and 1.0 g H_2O_2/ g TS) and inhibited both acidogenesis and methanogenesis with the generation of refractory compounds. Recently, Zhang et al. reported that MW-H_2O_2 pre-treatment could reduce the gene copies and relative abundance of ARGs (antibiotic resistance genes) in subsequent anaerobic digestion (Zhang et al., 2017). Besides, the combined MW-H_2O_2 pre-treatment also improves the rheological properties of sludge and reduces pathogen level in the digested sludge (Jibao Liu et al., 2016b; Liu et al., 2018; Zhang et al., 2017).

The microwave-induced AOP is beneficial in terms of faster reaction time, targeted heating effect and efficient oxidation. However, the optimization of H_2O_2 dosage is paramount in achieving efficient sludge solubilization and anaerobic digestion. Most of the reported works on hybrid MW-H_2O_2 pre-treatment were carried out for mesophilic anaerobic digestion. The research on thermophilic digestion will expand the scope of hybrid MW-H_2O_2 pre-treatment in wastewater treatment and also throws light on its mechanism of action at different operational conditions. In addition, the mechanism of ROS generation through the hybrid MW-H_2O_2 treatment and their effects on anaerobic digestion are yet to be explored.

2.2.2.3 Ultrasound combined pre-treatment

Ultrasonic pre-treatment enhances sludge homogenization by the formation of cavitation in a liquid medium by ultrasound waves. The efficiency of this pre-treatment can be enhanced by combining it with other pre-treatment methods such as acid, alkali, ozone and microwave (Anjum et al., 2016). The combination of ultrasound with alkaline (NaOH) pre-treatment was reported to yield 84 % tVFA/ tCOD in the anaerobic biotransformation of organic matter to VFAs (Chiu et al., 1997). Further, a combination of ultrasound with acid (H_3PO_4) achieved the same sludge disintegration efficiency as obtained by a mono-ultrasound pre-treatment but with shorter sonication times (Sahinkaya, 2015). The optimized treatment conditions attained by Sahinkaya et al. are 1.0 W/ ml ultrasound power density for a duration of 10 min and pH 2.0 with H_3PO_4. However, high solids concentration can limit the efficiency of this hybrid pre-treatment in sludge solubilization (Liu et al., 2008; Sahinkaya, 2015). Moreover, the EPS disintegration is enhanced by the addition of alkali in the hybrid pre-treatment, which is limited in mono ultrasonication (Jin et al., 2009). In addition, ultrasonication with AOP has also shown a synergetic effect in sludge solubilization. Xu et al. observed a huge increase in sCOD from 83 to 2483 mg/l through a combined ultrasound-ozone pre-treatment (0.26 W/ml and 0.6 gO_3/ h) (Xu et al., 2010). The synergetic effect of ultrasound-ozone pre-treatment (≤ 12 KJ/ g TS and 0.048 g O_3/ g TS) induced 41.3 % VSS solubilization compared to pure ozone treatment (Tian et al., 2015). Further, the solubilized components in the hybrid pre-treatment yielded low molecular weight products, whereas high molecular weight soluble products were observed in ultrasonication treatment alone. Besides, the maximum methane production in the study was also achieved in the hybrid pre-treatment (Tian et al., 2015).

Further, ultrasonication has combined with microwave and significant improvement in sludge solubilization and biogas production has been observed (Yeneneh, 2014). The combined pre-treatment (2450 MHz/ 3 min microwave and 0.4 W/ ml ultrasonication) enhanced the cumulative methane production to a greater degree compared to either of the individual pre-treatments. In brief, integrating ultrasonication with other pre-treatments enhances the solubilization effect and overcomes some of the limitations in individual treatments.

2.2.2.4 Chemical combined pre-treatment

Apart from the combination of physical methods with chemical methods, few studies have gone through the chemical-chemical combination in search of an efficient and cost-effective sludge pre-treatment strategy. AOP with H_2O_2 is a promising technology, as it yields effective sludge disintegration with environment-friendly by-products. To further enhance the efficiency of AOP, Kim et al. studied the H_2O_2-alkali combination (1.6 M H_2O_2 and pH 11/ NaOH) (Kim et al., 2009). The author observed a 49 % and 69.1 % decrease in TS content and viscosity, respectively and a 57.4 % increase in the sCOD/tCOD ratio. Besides, improved settleability was also observed. In another study, Dhar et al. studied combinations of H_2O_2 and $FeCl_2$ along with mechanical pre-treatment (Dhar et al., 2011b). The author reported that no significant improvement was observed with different chemical combinations in sludge solubilization or biogas production compared with a mono-mechanical pre-treatment. However, the combinations of chemicals achieved a significant decrease in dissolved sulfide concentration (Dhar et al., 2011b).

2.3 Non-pre-treatment strategies

Although various pre-treatment technologies have emerged in recent years for the improvement of anaerobic digestion, certain challenges like high cost, harmful by-products and high energy requirement are associated with them. To overcome these challenges, some innovative approaches have been conceived as process modifications in conventional WWTPs. Some of the novel ideas for the improvement of biogas production, cost-cutting and better management of wastes are classified in this section (Patinvoh et al., 2017).

2.3.1 Integrated biogas production

In any chemical manufacturing or engineering industry, process integration is an important strategy followed for efficient utilization of resources, while reducing the cost and environmental emissions (Hallale, 2001). Process integration connects various processes in a platform, where cost and energy reduction can be achieved through interlinked resources management. In the case of WWTPs, energy consumption for the treatment of a large volume of wastewater and the management of excess sludge production are the major challenges. For instance, an integrated energy/ biofuel production facility in WWTPs to face the energy need and shared logistics to meet the transport and disposal expenses in WWTPs are developments that help in cost-cutting (Patinvoh et al., 2017). Dererie et al. used thermochemically treated oat straw for the production of ethanol and the by-product of ethanol fermentation was subjected to biogas production (Dererie et al., 2011). The energy yield from the integrated process was 28-34 % higher than the direct biogas yield from the digestion of oat straw. Further, biogas production from the by-products of ethanol fermentation was faster, indicating that the enzymatic saccharification has acted as a pre-treatment which made the feedstock more digestible (Dererie et al., 2011). Similarly, Pourbafrani et al. integrated the production of ethanol, pectin, limonene and biogas from citrus wastes, thereby making the process more economically attractive (Pourbafrani et al., 2010). The citrus wastes were acid-hydrolyzed for the extraction of pectin, followed by vapour condensation in the expansion tank for limonene recovery. The remaining sugars were used for ethanol fermentation and the stillage was used for biogas production. The author demonstrated that from a ton of citrus waste, 39 l of ethanol, 45 m^3 of methane, 8.9 l of limonene and 39 kg of pectin can be produced (Pourbafrani et al., 2010).

Moreover, anaerobic digestion can be integrated with nutrient recovery or gas treatment units that minimize eutrophication or the emission of GHGs (Greenhouse Gases) into the environment, respectively (Beavis and Lundie, 2003; Piao et al., 2016; Rashid et al., 2020; Roldán et al., 2020). Arias et al. reported that a combination of high-rate activated sludge and partial nitrification anammox process can reduce the environmental impacts by 70 % in the climate change category and 50 % in the eutrophication potential category (Arias et al., 2020). Also, the integrated process could reduce the cost by 35-45 % depending on the size of the plant. In another study, the biogas produced from anaerobic digestion was used as a fuel for Solid Oxide Fuel Cell (SOFC) and the emitted CO_2 was fixed in the form of carbon in a photobioreactor culturing micro-algae (Rillo et al., 2017). Hence, process integration is vital in improving the economics and also, from an environmental perspective in the operation of WWTPs.

2.3.2 Innovative reactor design

In WWTPs, the design and operational conditions of anaerobic digesters could be simple or detailed depending on the scale of application and the nature of the feedstock. Many large scale WWTPs operate with conventional mesophilic anaerobic digesters to reduce the cost of operation and maintenance (Gebreeyessus and Jenicek, 2016; Ruffino et al., 2020). Nevertheless, the design of anaerobic digesters aims to provide sufficient contact between the microorganisms and substrate for the digestion to take place. In some cases where the anaerobic digestion is restricted due to the production of inhibitors or the by-products of chemical treatment (Bolado-Rodríguez et al., 2016), innovative reactor design could conceal the microbial load from inhibition thereby enhancing the biogas production.

Ezeji et al. operated a continuous one-stage fermentation and gas stripping product recovery (Ezeji et al., 2013). To avoid growth suppression by the accumulation of inhibiting chemicals and products, the bioreactor was bled semi-continuously, however, maintaining the cell concentration constant at 3-7 g/ l. The innovative design achieved 25 times higher butanol than the conventional bioreactor used as control (Ezeji et al., 2013). In another study, an upflow anaerobic sludge-fixed film (UASFF) reactor, a hybrid design with upflow fixed film (UFF) part on top of an upflow anaerobic sludge blanket (UASB) was designed for the treatment of palm oil mill effluent (POME) (Najafpour et al., 2006). The novel design helps to overcome the long start-up period associated with UASB reactors and helps in achieving an 89 % reduction in COD at 23.15 g COD/ d OLR with 1.5 days HRT. This remarkable design is essential for POME digestion, as it contains suspended, colloidal compounds, proteins and fats which would result in bed failure or washout of microorganisms in conventional UASB reactors (Zinatizadeh et al., 2007).

2.3.2.1 Two-stage anaerobic digestion

Another important evolution in the reactor design of anaerobic digesters is the advent of the "two-stage" or "multi-stage" set-up (Patinvoh et al., 2017; Zhang et al., 2019). The separation of distinct microbial communities for acidogenesis and methanogenesis in two separate digesters facilitates the respective microbial growth and metabolism (Guerrero et al., 1999). As hydrolytic-acidogenic and methanogenic microorganisms have different growth characteristics, phase separation helps to achieve methane percentage up to 80 %, thereby assists in gas purification (Kumanowska et al., 2017). Besides, for wastewaters with a high concentration of organic solids two-stage digestion helps to overcome the inhibitory effect of accumulated VFAs by the acidogenic microorganisms (Mata-Alvarez, 1987). Hence, two-stage digestion shows better process stability and control over high OLRs than the conventional single-stage digesters (Demirel and Yenigün, 2002). Typically, a two-stage digester set-up consists of two reactors (phase-I and phase-II) connected in series, phase-I being acid stage and phase-II, the gas stage (Pérez-Elvira et al., 2006) (Figure 2.11).

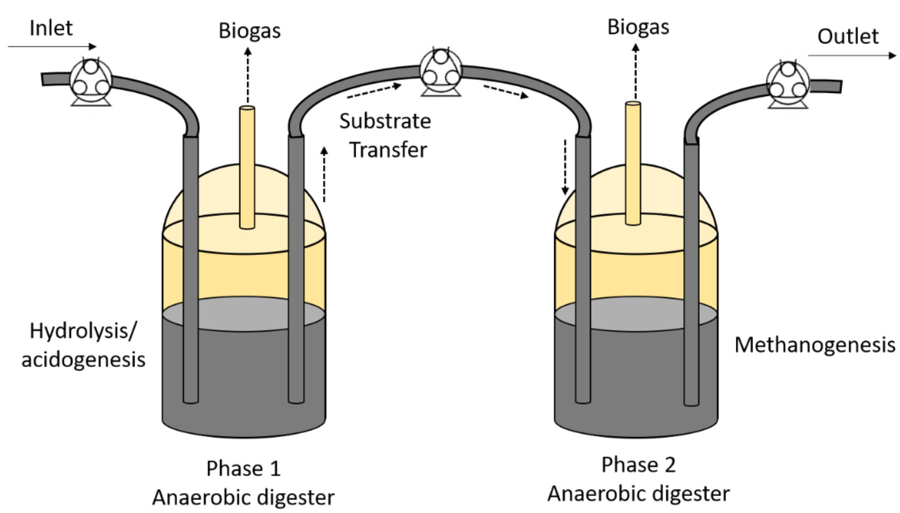


Figure 2.11: A simplified representation of two-stage anaerobic digestion

In two-stage digestion, the optimization of pH, temperature and retention time for the respective microbial population is essential to enhance the specific activity of microorganisms within the reactor (Demirel and Yenigün, 2002; Guerrero et al., 1999). Besides, the concentration of the microbial population and the subsequent changes during the start-up phase also influence the efficiency of two-stage digestion (Anderson et al., 1994). Theoretically, the first digester performs hydrolysis/ acidogenesis with the release of VFAs into the solution, while the second digester converts the VFAs and other biodegradable organic substances into methane and carbon dioxide (Figure 2.11) (Ince, 1998).

Various authors have performed two-stage anaerobic digestion for the improvement in biodegradation of WAS (Baldi et al., 2019; Bolzonella et al., 2007; Han et al., 1997; Jeihanipour et al., 2013; Kang et al., 2016; Kumanowska et al., 2017; Liu et al., 2019; Maspolim et al., 2015). Recently, alterations in the temperature range in both stages have been studied which gave rise to Temperature Phased Anaerobic Digestion (TPAD), wherein the phase-I and phase-II are maintained at two different temperatures (Borowski, 2015; Ge et al., 2011a; Xiao et al., 2018).

Table 2.6: Biodegradation of WAS through TPAD system (Gonzalez et al., 2018)

Sl. No	Acidogenic stage		Methanogenic stage		Change in biodegradation/ *Change in CH_4 production	Reference
	Temp (°C)	Retention Time (d)	Temp (°C)	Retention Time (d)		
1	45	4	35	16	↑ 84 % *	(J. Kim et al., 2013)
2	50	2	37	14	34 %	(Ge et al., 2011b)
3	55	6	35	24	↑ 18 %	(L. J. Wu et al., 2016)
4	55	2	55	18	↑ 11 %	(Leite et al., 2016)
5	60	2	37	14	41 %	(Ge et al., 2011b)
6	65	2	37	14	43 %	(Ge et al., 2011b)
7	65	2	55	18	↑ 5 %	(Bolzonella et al., 2012)
8	70	2	37	14	48 %	(Ge et al., 2011b)
9	70	2	35	14	↑ 15 %	(Ge et al., 2011a)

The biodegradation of WAS through the TPAD system, achieved across various studies is given in Table 2.6. Han et al. reported that a TPAD (thermophilic-mesophilic) system achieved complete destruction of total and faecal coliform over a range of SRTs from 11 to 28 days (Han et al., 1997). Besides, the VS removal was double compared to that of a conventional single-stage system. Furthermore, an increment in biogas yield was observed in a similar study with two-stage digestion with 55 °C phase-I digestion for 12 hours (Roberts et al., 1999). In another study, a two-stage UASB set-up achieved 95 % removal of COD and was able to withstand high OLR (10-15 g/ l.d) (Diamantis and Aivasidis, 2010). Kobayashi et al. observed that during TPAD, a higher rate of hydrolysis happens in the thermophilic digester followed by higher consumption of sCOD in the mesophilic digester (Kobayashi et al., 2009). Furthermore, semi-continuous operations of TPAD (3 days and 15 days SRT) yielded a maximum methane yield of 0.62 l CH_4/ g VS and 10^3 MPN/ g TS faecal coliform (Riau et al., 2010). Apart from enhanced biodegradation, TPAD has shown a significant reduction in pathogen levels and thereby achieves class-A biosolids to be used as a soil conditioner (De León and Jenkins, 2002; Riau et al., 2010). Also, with hyper-thermophilic (70 °C) (phase-I) acidifier, significant improvement in COD solubilization and biogas yield has been documented (Ge et al., 2010; Lee et al., 2009; L.-J. Wu et al., 2016). Although TPAD systems have shown remarkable improvement in biogas production and pathogen removal, the reduced dewaterability of the final digestate is still a drawback (Novak and Bivins, 2011; Riau et al., 2010).

2.3.3 Co-digestion

Another interesting strategy to improve the performance of anaerobic digestion is to co-digest different substrates to overcome their respective limitations in mono-digestion. Mono-digestion of recalcitrant substrates, fruit and vegetable wastes or feedstocks with harmful substances/ inhibitors results in a slow digestion process with low biogas yield (Patinvoh et al., 2017). These limitations can be overcome by co-digestion with complementary substrates that could maintain the optimum balance between biochemical reactions in anaerobic digestion. For instance, the mono-digestion of fruit and vegetable wastes (FVW) faces severe acidification issues and nitrogen depletion due to its high sugar content and C/ N (carbon: nitrogen) ratio (Ji et al., 2017; Scano et al., 2014). To overcome this, co-digestion of FVW with a low C/ N ratio like sewage sludge is encouraged (Gómez et al., 2006). Co-digestion is an effective strategy in reducing ammonia inhibition, diluting harmful compounds and favoring the synergism between different substrates in anaerobic digestion (Mata-Alvarez et al., 2014).

Furthermore, sewage sludge is characterized by higher buffer capacity due to its low C/ N ratio (Astals et al., 2013), which makes it suitable for substrates with high biodegradable compounds and low alkalinity values. Many investigations on co-digestion of sludge with OFMSW (Organic Fraction of Municipal Solid Waste), fats, oils and greases and FVW have shown improved anaerobic biodegradation compared to mono-digestion with either of the individual substrates (Mata-Alvarez et al., 2014). Krupp et al. evaluated the efficiency of OFMSW co-digestion with sludge and concluded that co-digestion is advantageous over mono-digestion and OFMSW composting (Krupp et al., 2005). However, in a bench-scale anaerobic co-digestion study, accumulation of heavy metals was observed in mechanically biologically treated OFMSW when added as a co-substrate to primary sewage sludge (Pahl et al., 2008). Furthermore, co-digestion of WAS with biowaste (a mixture of OFMSW and FVW) achieved a maximum biogas production when operated at 8.0 kg VS/ m^3 d (Liu et al., 2013).

Despite numerous studies in the field of anaerobic digestion and wastewater treatment, the number of researches on the combination of different technologies is limited. For instance, studies on co-digestion within two-stage anaerobic digestion are less reported. Combined technologies increase the overall performance of the process overcoming some of their limitations. Two-stage operation sustains the microbial activity in separate digesters, whereas co-digestion provides nutrient balance for stable anaerobic digestion. Therefore, dependent on substrate characteristics and nutrient requirements, the combination of different non-pre-treatment strategies can be carried out to enhance the performance efficiency of anaerobic digestion.

2.4 Conclusion

Anaerobic digestion is an efficient technology in the stabilization of excess sludge produced in WWTPs. However, anaerobic digestion of WAS is slowed down due to the complex nature of biological flocs and hence the poor hydrolysis rate. To overcome this difficulty, various pre-treatment methods have been studied previously (physical, chemical and biological) that break down EPS fractions and cause microbial cell rupture. Although many pre-treatments achieve sludge solubilization, the efficiency of pre-treatments can be enhanced by combining two or more pre-treatments. Among various hybrid technologies, microwave enhanced AOP (MW-H_2O_2) is of research interest, as it achieves thermal heating through microwave irradiation and also enhanced oxidation of organics through hydrogen peroxide. Besides, MW-H_2O_2 pre-treatment can be done with a short reaction time and no harmful by-products are formed, making it an environment-friendly pre-treatment. However, pre-treatments with microwave, H_2O_2 and microwave combined strategies have been found to produce recalcitrant products at high temperature or dosage (H_2O_2), which decreased biogas production. Hence, the optimization of microwave treatment conditions and H_2O_2 dosage is essential to avoid the formation of such recalcitrant soluble molecules. Furthermore, the phase separation of acidogenic and methanogenic microbial populations have been found to increase the specific activity of microorganisms. Besides, two-stage anaerobic digestion helps to overcome the inhibitory effects of VFAs and other by-products that are produced during the acidogenic phase. However, limited semi-continuous studies have been reported with two-stage anaerobic digestion where phase-I is maintained in a thermophilic condition and phase-II is maintained in a mesophilic condition. In addition, the evolution of oxidative stress through superoxide radicals have not been quantified along with anaerobic digestion in the previous researches. Therefore, in the current thesis work, experimental validation of the synergetic effect of MW-H_2O_2 pre-treatment has been done in two-stage anaerobic digestion with the study of oxidative stress through the quantification of reactive oxygen species.

CHAPTER 3

MATERIALS, METHODOLOGY AND CHARACTERIZATION TOOLS

3.1 Introduction

The current thesis work encompasses the effects of MW-H_2O_2 pre-treatment on sludge solubilization, single-stage/ two-stage anaerobic digestion, anaerobic co-digestion, and EPS composition of Waste Activated Sludge (WAS). The experimental work was conducted at both IIT Madras, Chennai (India) and Curtin University, Perth (Western Australia) laboratories at different timelines. The sample collection, experimental setup and analytical methods were designed as per the convenience and availability of equipment/ lab facilities at both places. For research purpose, real sewage sludge was collected from three WWTPs, namely Beenyup Wastewater Treatment Plant (BWWTP), Nesapakkam sewage treatment plant (NSTP) and IITM sewage treatment plant (IITMSTP). The optimization of hybrid MW-H_2O_2 parameters was conducted with sludge samples collected from both BWWTP and NSTP based on sludge solubilization, for the subsequent anaerobic digestion.

This chapter comprises details on sample collection, experimental strategies and analytical techniques for measurement and characterization. However, a brief materials and methods section is discussed in the subsequent research chapters. The overall schematic of the experimental work is given in Figure 3.1. The WAS samples were collected from NSTP and BWWTP and characterized at Environmental and Water Resources Engineering (EWRE), IIT Madras and WASM, Curtin University, respectively (Stage 1, Figure 3.1). Followed by, the effects of MW-H_2O_2 pre-treatment on sludge solubilization and oxidative stress (Stage 2). The single-stage and two-stage anaerobic digestion studies were carried out at B300, Curtin University (Stage 3). Subsequently, co-digestion of WAS with FVW in two-stage anaerobic digestion was conducted (Stage 4). Further, the effects of combined treatment on EPS distribution was studied at both Curtin University and IIT Madras (Stage 5). In addition, the current chapter gives a brief overview of the water treatment processes at BWWTP, NSTP and IITMSTP.

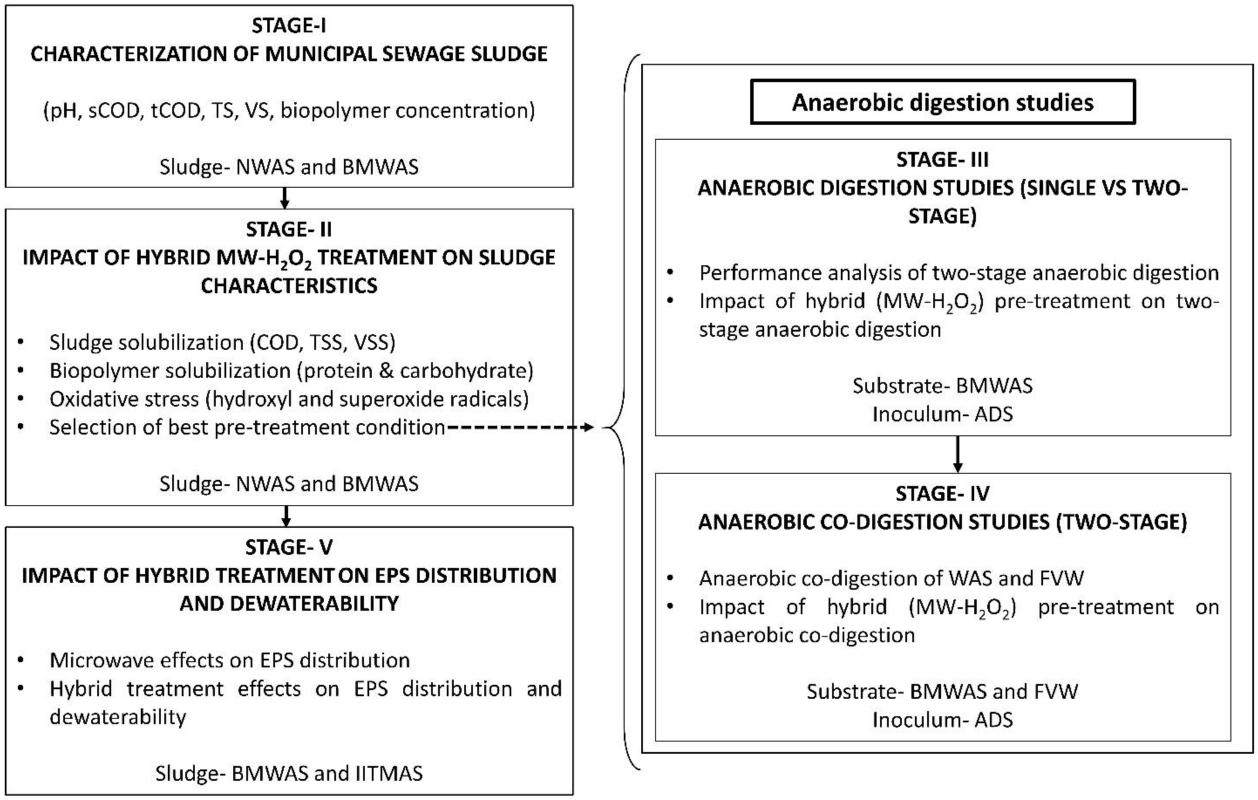


Figure 3.1: The overall schematic of the experimental framework of current thesis

3.2 Wastewater treatment process at BWWTP

The WWTP at Beenyup is one of the three biggest WWTPs in Perth (Western Australia), operated by Water Corporation. The other locations are Subiaco and Woodman Point, WA. The BWWTP has a treatment capacity of 135 million liters per day (MLD) which serves around 650,000 inhabitants in the northern suburbs (Water-technology, 2020). The BWWTP influent contains approximately 98 % domestic wastewater and 2 % light industrial wastewater (PLOOM, 2016). Furthermore, BWWTP discharges approximately 116 MLD of secondary treated wastewater into the sea through the ocean reef outlet (PLOOM, 2016).

The overall process schematic of municipal wastewater treatment at BWWTP is given in Figure 3.2 (Yeneneh, 2014). The preliminary treatment involves a bar screen, that removes larger solids such as plastics and rags from the influent. Followed by the grit chamber, which removes larger inorganic material (grit) through the settling process, and the organic substances stay suspended in the wastewater. The primary treatment consists of six rectangular sedimentation tanks that allow 90 % of solids to settle down and the oil and grease are collected at the top of the tank. The settled solids are further processed in aeration tanks for secondary treatment.

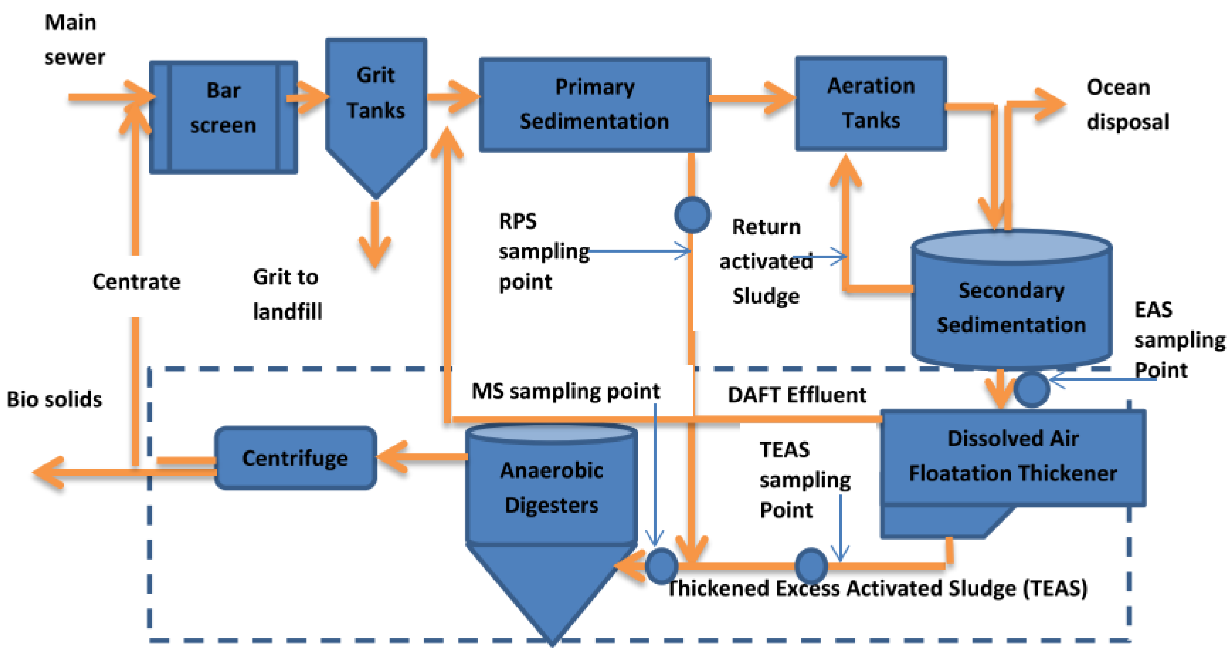


Figure 3.2: The overall process schematic of municipal sewer treatment at BWWTP (Yeneneh, 2014).

EAS- Excess activated sludge; RPS- Raw primary sludge; TEAS- Thickened excess activated sludge; MS- Mixed sludge

The secondary treatment involves the activated sludge process that results in the production of excess activated sludge. A fraction of activated sludge is feedback to the aeration tanks as the return activated sludge. Further, the thickened excess activated sludge (TEAS) from the dissolved air floatation thickeners is mixed with raw primary sludge in the ratio of 3:1 and transferred to the two-stage heated anaerobic digestion process. After anaerobic digestion, the sludge is subjected to dewatering through the centrifugation process, and the resultant biosolids are excellent soil conditioner for agricultural use. Besides, the biogas generated through anaerobic digestion is used as a fuel to meet the energy requirement for the digester operation. For the current research, samples were collected from mixed sludge and TEAS sampling points (Figure 3.2). The mixed sludge sampling point is located after the sludge break tank, where the mixing of RPS and TEAS takes place. The mixed waste activated sludge (MWAS) is composed of RPS and TEAS in the ratio of 65: 35. The MWAS originated from BWWTP was termed Beenyup Mixed Waste

Activated Sludge (BMWAS). The BMWAS samples collected from BWWTP varied widely in its characteristics such as COD and TS compared to other samples collected in the current study due to the presence of TEAS and RPS in its composition. The anaerobically digested sludge (ADS) used in the current study was collected from the centrifuge of the dewatering section. The ADS was used as an inoculum for anaerobic digestion and EPS distribution studies.

3.3 Wastewater treatment process at NSTP

The Nesapakkam sewage treatment plant (NSTP) is located in the western part of Chennai city (India) and treats the wastewater generated in zone 4, among 5 zones operated by Chennai Metropolitan Water Supply and Sewerage Board (CMWSSB) (CMWSSB, 2020). The plant was commissioned in 1974 and with a treatment capacity of 23 MLD. With an upgrade, the plant is currently running three treatment trains with a combined capacity of 117 MLD (23 MLD, 40 MLD and 54 MLD) (NSTP Case study, 2018). All the STPs at Nesapakkam operate with activated sludge process technology. The overall schematic of the NSTP is given in Figure 3.3 (Pitta et al., 2010).

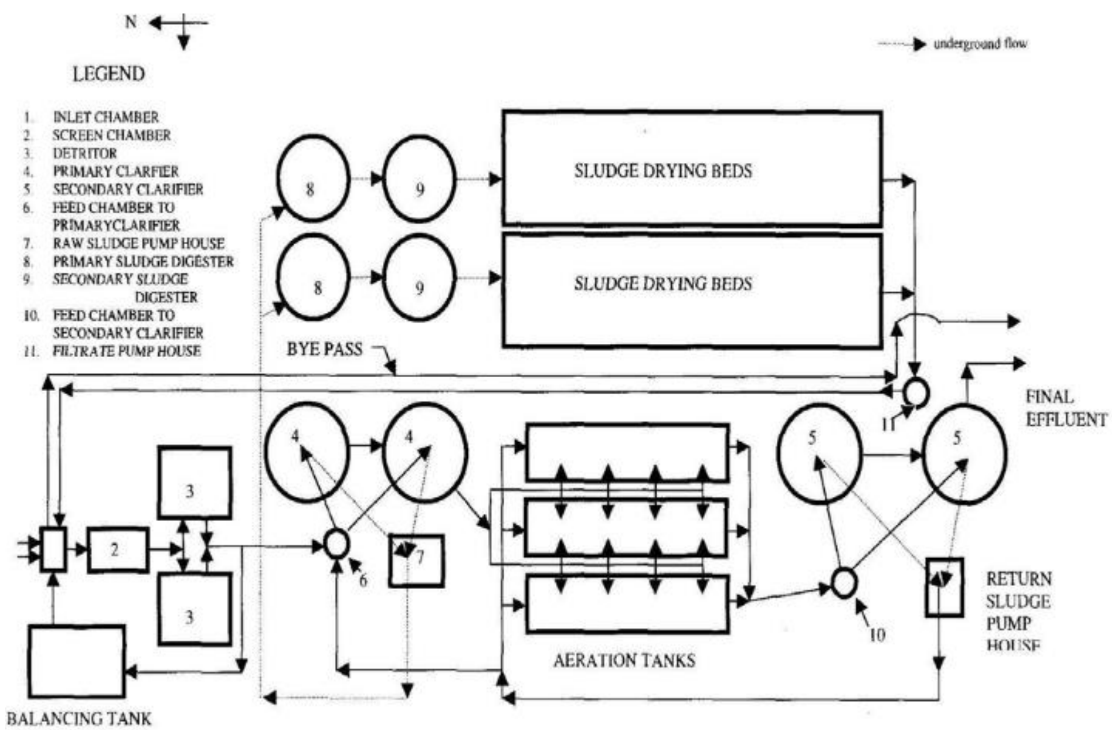


Figure 3.3: The overall schematic of municipal sewer treatment at NSTP (Zone 4).

The preliminary screening of influent wastewater is done with a screen chamber (width 2.41 m; depth 1.37 m; bar spacing 25 mm) (Pitta et al., 2010). After screening, the wastewater is transported to the detritus tank for grit removal. Further, two identical circular primary clarifiers are used for primary sedimentation, whence primary sludge is produced. Followed by three aeration tanks to carry out the activated sludge process. The tanks are provided with 12 fixed aerators with a capacity of 12.5 HP each. The wastewater is then transported to two secondary sedimentation tanks for the separation of activated sludge. The primary and secondary sludge are digested separately in primary and secondary digesters, respectively as shown in Figure 3.3. The activated sludge samples were collected from the sample collection ports after the aeration tank. These samples were termed Nesapakkam Waste Activated Sludge (NWAS). The digested sludge is collected from the outlet of anaerobic digesters before transportation to the dewatering process.

3.4 Wastewater treatment process at IITM STP

The Indian Institute of Technology Madras Sewage Treatment Plant (IITMSTP) is located inside the campus of IIT Madras, Chennai (India). The campus is about 620 acres with over 10,000 residents, which includes students, faculty members and academic staffs. The IITMSTP was commissioned by Aqua Designs Pvt Ltd (Aqua Designs, n.d.). The plant has a treatment capacity of 4.0 MLD with sequential batch reactor technology (SBR). A short schematic of IITMSTP is given in Figure 3.4.

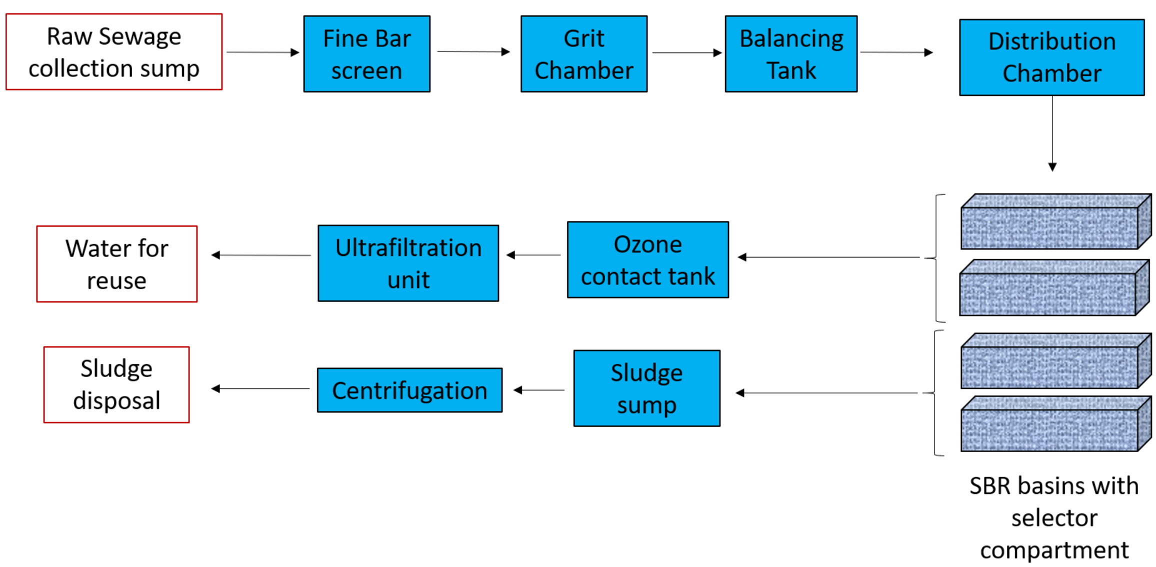


Figure 3.4: A short schematic of sewage treatment at IITMSTP

In IITMSTP, 2.0 MLD of treated sewage will be disposed of and the balance 2.0 MLD will be treated by tertiary treatment through ultrafiltration for recycling. The treated wastewater will be used for gardening, flushing of toilets and construction on the campus. For the current study, the activated sludge sample was collected from the outlet of SBR. The activated sludge originated from IITMSTP was termed Indian Institute of Technology Madras Activated Sludge (IITMAS)

3.5 Sludge characterization and analytical methods

The waste activated sludge samples collected from BWWTP, NSTP and IITMSTP were characterized based on various physical, chemical and biological parameters. After the collection of samples from the respective plants, the samples were immediately characterized for pH, COD (soluble and total), total solids (TS) and volatile solids (VS). Once the characterization is done, the samples were immediately refrigerated at 4 °C and stored until experimentation. The equipment and methods used for the sample characterization and analysis are listed in Table 3.1.

3.5.1 pH, temperature, TDS- conductivity

The pH, temperature, TDS- conductivity were measured using WP-90 and WP-81 pH/ temperature/ TDS-conductivity meter, equipped with a glass electrode, according to the standard methods (APHA, 1999). The pH measurement was also carried out using ADWA AD 11 and Mettler Toledo pH meters. The pH was measured during sample characterization, before and after pre-treatment and on daily basis during anaerobic digestion. Besides, the analysis was done immediately after the collection of samples at room temperature, to minimize the contact of the sample with air. The electrode was rinsed with water between measurements and the instrument was calibrated periodically using buffer solutions pH 4, pH 7 and pH 9 (Yeneneh, 2014).

3.5.2 Total and soluble chemical oxygen demand (tCOD and sCOD)

The tCOD and sCOD of the sample were measured by oxidation using HACH COD reagent, followed by colorimetric analysis in ORION UV-spectrometer, at Curtin University. For the measurement of tCOD, the samples collected were diluted 50 or 100 times with distilled water depending on the solid concentration. 2 ml of diluted sample was transferred into HACH COD vial and invert mixed slowly. The vials were transferred to the COD reactor and digested for 2 hours at 150 °C. After cooling down the vials to room temperature, the COD was measured using ORION UV- spectrometer. For blank, distilled water was added instead of sample and the succeeding steps were followed. For the measurement of sCOD, the collected samples were centrifuged at 5000 rpm for 10 minutes to separate the supernatant from residual sludge. The supernatant was filtered using 0.45-micron Whatman filter paper and analyzed following the steps for tCOD measurement.

Table 3.1: Parameters analyzed for characterization and anaerobic digestion performance

Parameter	**Equipment (or) analytical technique**
COD	(1) Oxidation with HACH COD reagent and colorimetric analysis on ORION UV- spectrometer (2) Oxidation with $K_2Cr_2O_7$ and titration against ferrous ammonium sulfate
pH and temperature	pH/ temperature meter
Conductivity/ TDS	TDS/ conductivity meter
VFA	Direct titration technique (Dillalo and Albertson, 1961)
TS, VS	APHA standard method (APHA, 1999)
TSS, VSS	APHA standard method (APHA, 1999)
Specific intracellular superoxide s$[O_2^{\bullet-}]$	Using fluorescent dye Dihydroethidium (DHE) and quantitative measurement in a fluorescent spectrometer
Protein	Bicinchoninic acid (BCA) assay
Carbohydrate	Phenol-sulfuric acid method (Masuko et al., 2005)
Dehydrogenase activity	Triphenyl Tetrazolium Chloride (TTC-DHA)
Biogas composition	GA 2000 Gas Analyser
Rheology	Rheometer
Functional group analysis	Fourier Transform Infrared Spectroscopic Technique (FTIR)
Particle size distribution	Mastersizer 2000
Elemental composition	Elemental analyzer
Pathogen level	Coliform assay
Dewaterability	Capillary Suction Timer (CST equipment)

The measurement of COD in samples collected from NSTP and IITMSTP was carried out using the closed reflux method as per APHA standard methods (APHA, 1999). In this method, 2.5 ml of sample was taken in COD tubes and 1.5 ml of 0.016 M $K_2Cr_2O_7$ solution was added. To this mixture, 3 ml of sulfuric acid was slowly added. The tubes were sealed tightly with caps and invert mixed gently. Then, the tubes were placed in a COD reactor and digested for 2 h at 150 °C. After digestion, the tubes were cooled down to room temperature. The solution is transferred to 150 ml conical flasks and 1 or 2 drops of Ferroin indicator was added and mixed. The solution was titrated against 0.1 M Ferrous Ammonium Sulfate (FAS). In the same manner, titration was done with a blank sample. The COD of the sample was determined using the following equation.

$$COD\ \left(\frac{mg}{l}\right) = \frac{(A-B)*M*8000}{V} \qquad \text{(equation 3.1)}$$

Where,

A= ml FAS used for blank,

B= ml FAS used for the sample,

M= Molarity of FAS

8000= milliequivalent weight of O_2 * 1000 ml/ l

3.5.3 Measurement of solids and suspended solids (TS, VS, TSS and VSS)

The TS, TSS, VS and VSS were measured according to the standard methods for the examination of water and wastewater (APHA, 1999). For the measurement of solids, the liquid content was evaporated using a muffle furnace or oven and the remaining solids are measured using a physical balance. “Everflow” muffle furnace and Semco Scientific hot air oven were used for this purpose. The detailed description of the measurement of TS and VS are as follow.

a) TS measurement:

1) The evaporating dish was cleaned through ignition at 550 °C for 1 h in a muffle furnace.
2) The dish was cooled down in a desiccator and the empty dish weight was measured (B mg).
3) A sample volume of 2 to 10 ml (that would account for 2.5 mg to 200 mg of solid residue) was poured into the dish and weight was measured (C mg).
4) The dish was dried in an oven at 103-105 °C for 1 h.

5) The dish was cooled down in a desiccator and the weight was measured (A mg). The solid residue at this stage is denoted as residue A.
6) The cycle was repeated with fresh samples until a constant weight was obtained or until the weight loss in < 4 % of the previous weight.

The TS content is calculated based on the following formula

$$Total\ solids\ \left(\frac{mg}{l}\right) = \frac{(A-B)*1000}{Sample\ volume,ml} \qquad \text{(equation 3.2)}$$

$$Total\ solids\ (\%) = \frac{(A-B)*100}{(C-B)} \qquad \text{(equation 3.3)}$$

b) VS measurement:

The VS were measured as a continuation to TS measurement of respective samples as follow. The VS content is measured based on equations 3.4 and 3.5.

7) The residue A is heated in a muffle furnace at 550 °C for 1 h.
8) After cooling down for a while in the air, the dish was transferred to a desiccator and further cooled down.
9) The weight of the dish was measured (D). The cycle was repeated with fresh samples until a constant weight was obtained or until the weight loss in < 4 % of the previous weight.

$$Volatile\ solids\ \left(\frac{mg}{l}\right) = \frac{(A-D)*1000}{Sample\ volume,ml} \qquad \text{(equation 3.4)}$$

$$Volatile\ solids\ (\%) = \frac{(A-D)*100}{(A-B)} \qquad \text{(equation 3.5)}$$

c) TSS measurement:

The total suspended solids (TSS) and volatile suspended solids (VSS) measurement are similar to the TS and VS measurements. In this measurement, the suspended solids are filtered using a filtration apparatus and the weight of residual solids are measured similar to the previous section. The detailed description of the method is as follow.

1) The filter paper used for the measurement was cleaned, dried in the oven and the initial weight was measured (B mg).
2) A sample of 5 ml to 10 ml was added into the filter apparatus and filtered through vacuum suction.

3) After filtration, the filter paper along with the residue was placed in an inert crucible/ dish and dried in an oven at 103-105 °C for 1 h.
4) The crucible/ dish with the filter paper is cooled down in a desiccator and the final dried weight was measured (A mg). The cycle was repeated with fresh samples until a constant weight was obtained. The TSS was measured using equation 3.6.

$$Total\ suspended\ solids\ \left(\frac{mg}{l}\right) = \frac{(A-B)*1000}{Sample\ volume,ml} \qquad \text{(equation 3.6)}$$

d) VSS measurement:

The VSS measurement was done as a continuation of TSS measurement. The VSS content was calculated from equation 3.7

5) The filter paper along with the residue (from step 4) is placed in an inert crucible and ignited in a muffle furnace at 550 °C for 1 h.
6) After cooling down for a while in the air, the crucible was transferred to a desiccator and further cooled down.
7) The final weight of the filter paper was measured (D mg). The cycle was repeated with fresh samples until a constant weight was obtained. The VSS was calculated according to equation 3.7.

$$Volatile\ suspended\ solids\ \left(\frac{mg}{l}\right) = \frac{(A-D)*1000}{Sample\ volume,ml} \qquad \text{(equation 3.7)}$$

3.5.4 VFAs (Volatile fatty acids) and alkalinity

The volatile fatty acids/ alkalinity is an important digester control test; hence it was monitored periodically during the operation of anaerobic digestion. A direct titration method was followed for the determination of VFAs/ alkalinity (Dillalo and Albertson, 1961). The measurement of VFAs and alkalinity are done as below.

1) The pH meter was standardized with buffer 4 and 7 before the analysis. The sample was collected from the digester and diluted for the measurement of VFAs/ alkalinity.
2) The diluted sample was transferred into a beaker and the pH was measured. With the meter inserted in the solution, the sample was titrated with H_2SO_4 to reach pH 4.0. The amount of acid used (ml) was noted.
3) The titration was continued till it attains pH 3.5 and the sample temperature was noted.

4) The sample was boiled for at least 3 minutes on a hot plate and then cooled down to the original temperature using a cold-water bath.
5) The sample was then titrated back to pH 4 with NaOH and the volume of alkali added was noted. Further, it was titrated to pH 7 and the respective volume of NaOH used was noted.

The total alkalinity was measured based on the amount of acid required to titrate the sample to pH 4, according to equation 3.8.

$$Total\ alkalinity\ \left(\frac{mg}{l}\right) = \frac{(mls\ of\ acid\ used)*N\ (normality)of\ acid*50000}{mls\ of\ sample\ used} \qquad \text{(equation 3.8)}$$

The volatile acid alkalinity was measured based on the amount of alkali used to titrate the sample from pH 4 to pH 7, according to equation 3.9

$$Volatile\ acid\ alkalinity\ \left(\frac{mg}{l}\right) = \frac{(mls\ of\ NaOH\ used)*N\ (normality)of\ NaOH*50000}{mls\ of\ sample\ used} \qquad \text{(equation 3.9)}$$

The volatile fatty acids are calculated by the multiplication of volatile acid alkalinity by:

- 1, if the volatile acid alkalinity is less than 180 mg/l
- 1.5, if the volatile acid alkalinity is greater than 180 mg/l

$$Volatile\ fatty\ acids\ \left(\frac{mg}{l}\right) = 1\ (or)1.5 * volatile\ acid\ alkalinity \qquad \text{(equation 3.10)}$$

In addition, the stability of anaerobic digester is determined by the ratio of volatile fatty acids to alkalinity, often mentioned as FOS/ TAC ratio, determined by the equation 3.11

$$\frac{FOS}{TAC} = \frac{Volatile\ fatty\ acids\ (\frac{mg}{l})}{Alkalinity\ (\frac{mg}{l})} \qquad \text{(equation 3.11)}$$

3.5.5 Reactive oxygen species (ROS)

The intracellular ROS production was monitored through the quantification of intracellular hydroxyl ($OH^{\bullet}$) and superoxide radicals ($O_2^{\bullet -}$). The measurement of ROS was carried out by the application of fluorescent dyes and quantitative analysis in a fluorescent spectrometer.

The specific intracellular superoxide radicals s[$O_2^{\bullet -}$] were measured using the fluorescent dye Dihydroethidium (DHE). The fluorescent probe DHE is freely permissible across cell membranes, is suitable for the in-situ production of ($O_2^{\bullet -}$) radicals (Hayyan et al., 2016). In this method, DHE gets oxidized by superoxide into a fluorescent product called hydroxy ethidium which was measured at 485/20 nm excitation and 590/35 nm emission in the Synergy HTX multi-mode microplate reader (Chen et al., 2013; Zhao et al., 2003). To measure s[$O_2^{\bullet -}$], 1 ml of the sample was centrifuged at 5000 rpm for 10 min at 4 °C to separate the cell pellet. The pellet was washed and resuspended in 1 ml phosphate buffer (pH 7.4). To this solution, DHE was added to a final concentration of 10 µM. The reaction was incubated in dark at room temperature for 30 min and then measured in a fluorescent spectrometer. The specific intracellular hydroxyl radicals s[$OH^{\bullet}$] were measured using Amino Phenyl Fluorescein (APF) dye (Setsukinai et al., 2003). The fluorescent product was measured at 490 nm excitation and 515 nm emission. The reaction was incubated at 25 °C for 30 minutes, followed by measurement in the Jasco V-630 fluorescent spectrophotometer. The specific intracellular reactive oxygen species were measured according to the equation 3.12.

$$Specific \text{ intracellular } ROS \ \ (\mu moles/\text{TS}) = \frac{[ROS]\mu M}{Total\ solids\ (\frac{mg}{l})} \qquad \text{(equation 3.12)}$$

3.5.6 TTC- DHA activity

The sludge bioactivity was measured using the TTC-DHA activity (Triphenyl Tetrazolium Chloride- Dehydrogenase), with a modified protocol of Sun et al. (2012) (Burdock et al., 2011; Sun et al., 2012). The assay involves the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TF) by free electrons, that are produced as a result of oxidation reactions catalyzed by dehydrogenase enzymes (Ghaly and Mahmoud, 2006). The red formazan salt produced during the TTC-DHA assay was measured colorimetrically at 485 nm. In this measurement, with 1 ml of sludge sample, 500 µl of Na_2SO_3, 500 µl of 0.0577 % $CoCl_2$, 1 ml of tris Hcl and 1ml of TTC-glucose were added and mixed. The reaction mixture was incubated at 37 °C for 1 h in a water bath. After incubation, the reaction mix was centrifuged at 5000 rpm for 10 minutes and the supernatant was discarded. With the pellet, 2 ml of methanol was added and vortex mixed. Two extractions with methanol were done followed by centrifugation at 5000 rpm for 10 minutes and the extractions were pooled together for measurement. The combined extractions were measured at 485 nm in the Synergy HTX multi-mode microplate reader. A standard curve was developed to determine the concentration of TF (µmol/ ml) corresponding to an absorbance measurement at 485 nm.

3.5.7 Biopolymer measurement

In the current study, proteins and carbohydrates were measured in total, soluble and extracted EPS fractions in the samples collected from anaerobic digestion. The measurement of protein was carried out using the BCA assay (Pierce^{TM} BCA Protein Assay Kit). In this method, the reduction of Cu^{2+} to Cu^{1+} by proteins is detected colorimetrically using a unique reagent, Bicinchoninic acid. The assay was optimized for analysis in a microplate reader as follow. Briefly, 25 µl of the sample was added to a 96-well plate. To this, 200 µl of working reagent (50:1 ratio of reagent A: B) was added and incubated at 37 °C for 30 min. After incubation, the plate was read in Synergy HTX multi-mode reader at 562 nm. A standard plot for the determination of protein concentration was developed using the albumin standard ampules provided in the kit. Furthermore, the carbohydrate estimation was carried out using the phenol-sulfuric acid method, according to (Masuko et al., 2005). In this method, 50 µl of the sample was added to the 96-well plate. To this, 150 µl of H_2SO_4 was added rapidly and mixed. Followed by, the addition of 30 µl of 5 % phenol and then, incubated at 90 °C for 5 min. The plate was cooled down and read in Synergy htx multi-mode reader at 490 nm.

3.5.8 Biogas composition

The biogas composition (CH_4/ O_2/ CO_2) was measured using a GA 2000 plus gas analyzer. The analysis was conducted by pumping the collected gas into the gas inlet port of the analyzer at a rate of 8 ml/ s. The analyzer is equipped with an internal suction pump for this purpose. The gas chromatographic technique was used to confirm the accuracy and precision of the measurement done through the gas analyzer. The concentration of ammonia and H_2S was monitored by a meter in addition to other components of biogas.

3.5.9 Particle size distribution

The particle size distribution of untreated, treated and digested sludge was studied using Malvern Mastersizer Laser Diffraction Particle Size Analyzer (Hydro 2000S). The instrument uses laser diffraction technique to quantify particle size as d(0.1), d(0.5) and d(0.9), which indicates that 10 %. 50 % and 90 % of particles were less than or equal to the sizes mentioned, respectively (Yeneneh, 2014). For analysis of sludge, the particle refractive index was set as 1.538 in settings. The sample was loaded in the tank area and the measurement was initiated. A cleaning sequence was set between different sample loadings in the "tank functions" tab, to enable subsequent sample measurement. After the measurement, the tank was emptied and refilled with distilled water for subsequent sample loading.

3.5.10 Elemental analysis

The elemental composition (C, H, N and O) of sludge samples was measured using EA 2400 CHNS/ O elemental analyzer. For analysis, the sludge samples were prepared by drying at 105 °C for 2 h in a hot air oven, followed by overnight desiccation. Less than 2 mg of sample was measured using a microbalance and loaded in tin or aluminium vials for measurement. The data is generated as the percentage composition of carbon, hydrogen, nitrogen and sulfur for the sample analyzed. The element “O” was obtained assuming C+H+N+O=99.5 % on a volatile solid basis.

3.5.11 Rheological measurement

The rheological measurement of sludge samples was carried out using a DHR-2 Rheometer from TA instruments. The TRIOS software was opened and connected to the instrument for measurement. The instrument uses concentric-cylinder geometry (diameter 30 mm) for the measurement of the stress-strain relationship of the sample. An appropriate volume of the sample was loaded in the cup and connected with the rheometer for measurement. The measurement was initiated through TRIOS software and the data was generated as shear stress vs shear rate and viscosity vs shear rate for the applied samples.

3.5.12 Functional group analysis

The functional group analysis was carried out using Fourier Transform InfraRed (FTIR) Spectroscopy (PerkinElmer Spectrum). The dried samples were prepared as mentioned in 3.4.10. The samples were loaded in the loading slot and the scan was started through the PerkinElmer spectrum software. The data was generated as % transmittance or absorbance graphs for the applied samples.

3.5.13 Pathogen level

The pathogen level was measured using coliform assay done by Coliscan Easygel kit. Coliscan is a nutrient medium containing two chromogenic substrates. One is cleaved by the enzyme galactosidase (produced by all coliforms) and the other by the enzyme glucuronidase (produced by E. Coli). The former produces pink/ red colonies and the latter produces blue/ purple colonies. The samples for measurement were diluted appropriately to provide a differentiable colony count in the petri dish. Briefly, 1 ml of sample was mixed with the Coliscan easy gel and poured into the petri dish and spread equally. The plates were closed and incubated at 35 °C for 24-48 h in an incubator. The colonies were counted after incubation and colony-forming units (CFU) per 100 ml was calculated based on the colony count.

3.5.14 Dewaterability

The dewaterability of sludge samples was determined through capillary suction time, measured using Capillary Suction Timer (Type 304 CST) equipment. The samples were incubated at room temperature for 1-2 hours, to ensure precision and accuracy of the testing across different samples. In this method, the CST paper was placed between the contacting sensors and a stainless-steel cylinder was placed in the circular slot provided at the centre. 5 ml of sample was loaded on to the cylinder and the time required for the liquid to diffuse through the paper and reach the second sensor was recorded as CST (s).

3.5.15 EPS extraction

The EPS fraction of sludge samples was isolated using a modified protocol of Huang et al. (Huang et al., 2016). Briefly, 5 ml of the sample was collected and centrifuged at 2500 rpm for 5 minutes. The supernatant was collected as the "slime" fraction. The pellet was completely resuspended to its original volume using Milli-Q water. The sample was sheared by a vortex mixer for 1 min. The sample was centrifuged at 5000 rpm for 10 min. The supernatant was collected as "loosely-bound" EPS (LB-EPS). The pellet was resuspended to its initial volume using phosphate buffer (pH 7.4), followed by ultrasound treatment. The sample was centrifuged at 10,000 rpm for 15 min and the supernatant was collected as "tightly-bound" EPS (TB-EPS).

3.5.16 Zeta potential

The Zeta potential of sludge samples was measured using Nanoparticle size- Zeta potential analyzer SZ-100 Horiba Scientific. The samples were diluted appropriately/ undiluted EPS fractions were analyzed using this instrument. The samples were placed in the Zeta potential cuvettes and kept in the analysis slot of the instrument. The measurement was initiated using the Horiba Scientific SZ 100 software. Multiple measurements were taken with each sample and the cuvette was cleaned using distilled water between measurements.

3.5.17 Total organic carbon

The total organic carbon (TOC) of the samples were measured using a TOC-V_{CPH} analyzer (Shimadzu). The samples were prepared by filtration through a 0.45 µm Whatman filter paper. The samples were placed in the autosampler and the analysis was initiated through the TOC-Control V software.

3.6 Pre-treatment of sludge samples (Equipment and conditions)

In the current thesis work, a hybrid (MW-H_2O_2) pre-treatment was applied for the disintegration of sludge floc structure and microbial cell walls. The dielectric heating mechanism of microwave irradiation causes rapid heating of sludge, as it contains more than 90 % of water and organics that are susceptible to microwave (Pino-Jelcic et al., 2006). Besides, the addition of chemical oxidants such as H_2O_2 along with the microwave enhances the sludge solubilization by induction of ($OH^{\bullet}$) radicals, that subsequently oxidize most of the organics in the sludge (Eswari et al., 2016; Özön and Erdinçler, 2019). However, the dosage of H_2O_2, intensity of microwave irradiation and combined treatment strategy need to be carefully optimized to avoid evaporation, generation of refractory components and enhance the anaerobic biodegradation (Liu et al., 2017; Wang et al., 2009). In the current research, the hybrid (MW-H_2O_2) pre-treatment was optimized in BWWTP and NSTP for COD and biopolymer solubilization. The details on pre-treatment equipment, condition and concentration of chemical reagent used have been discussed in detail in the following section.

3.6.1 Microwave (MW) pre-treatment

A Panasonic microwave oven with inverter technology was used as the equipment for microwave pre-treatment with samples collected from BWWTP. The oven was operated with 2450 MHz frequency and provided with variable power (110 W to 1100 W) and time settings. The samples for microwave treatment were kept in a glass beaker and the top was covered with a microwave-safe plastic wrap to restrict water loss due to evaporation. Throughout the experiment, the same beaker dimension was used to avoid the differences in microwave efficiency. The sample volume was measured before and after treatment, to ensure treatment without loss of water due to evaporation. For the samples collected from NSTP and IITMSTP, a Samsung (MW73V/ 2450 MHz) microwave oven was used. Its microwave power output range varied from 100 W to 1100 W with adjustable time settings.

In the present study, the microwave exposure time, power output and intensity have been considered independent variables of the process and the optimization of respective conditions have been dealt with in chapter 4. The initial concentration of sludge has been maintained as a constant in both optimization experiments and anaerobic digestion studies.

3.6.2 Hydrogen peroxide (H_2O_2) pre-treatment

The H_2O_2 is a strong oxidant that causes microbial cell lysis, mediated by highly reactive ($OH^{\bullet}$) radicals. For the destruction of sludge microbial flocs, an AOP was conducted with H_2O_2 pre-treatment. A 30 % (w/w) H_2O_2 (Sigma Aldrich) solution was used for this purpose. The dosage of H_2O_2 was optimized on the total solid concentration (per unit TS) of the sludge sample. Further, the addition of H_2O_2 to sludge was carried out in dark, to avoid the decomposition of the reagent in light.

3.6.3 Hybrid (MW-H_2O_2) pre-treatment

The hybrid (MW-H_2O_2) pre-treatment was carried out with optimized microwave condition (power output and time) and optimum H_2O_2 dosage for single-stage and two-stage anaerobic digestion. While carrying out the combined pre-treatment, the impact of catalase and residual H_2O_2 was considered, hence, a sequential (MW-H_2O_2-MW) treatment was followed (Wang et al., 2009). The equipment and conditions for the hybrid treatment are the same as discussed in sections 3.6.1 and 3.6.2 for microwave and H_2O_2, respectively.

3.7 Two-stage anaerobic digestion

In the current study, a two-stage semi-continuous anaerobic digestion was followed in which, the first stage (phase-I) carries out hydrolysis-acidogenesis and the second stage (phase-II) carries out methanogenesis. The transcendency of two-stage digestion over conventional single-stage anaerobic digestion has been discussed in detail in section 2.3.2.1. For the current study, two-stage digester set-ups were assembled with two 1-liter reactors connected in a semi-continuous fashion as shown in Figure 3.5.

Figure 3.5: Two-stage anaerobic digestion set-up used in the current thesis work. Reactors (a) and (c) are connected semi-continuously in two-stage set-up. Similarly, reactors (b) and (d) are connected to form a control two-stage set-up. Reactors (a) & (b) serve as phase-I and reactors (c) & (d) are operated as phase-II digesters. The reactors are maintained in their respective temperature conditions by water circulation through a jacket heating system.

The reactors are jacketed vessels provisioned with sampling ports for liquid sludge and gas collection. The phase-I reactor was maintained in a constant thermophilic condition (55 ± 2 °C) and the phase-II reactor was maintained in a constant mesophilic (37 ± 2 °C) condition, by connecting to two separate water baths. Masterflex LS peristaltic pumps were used to drain the phase-I and phase-II reactors after respective hydraulic retention times (HRTs). The retention time of the phase-I reactor was maintained shorter and the phase-II reactor was maintained longer based on the observation from previous studies (Ge et al., 2011a; Riau et al., 2010). The biogas generated was collected in inverted cylinders with water displacement technique, operated at room temperature. Besides, condenser bottles were placed between the reactors and gas collection system, to remove any condensate that is carried on.

CHAPTER 4

IMPACT OF MICROWAVE AND HYDROGEN PEROXIDE TREATMENTS ON WASTE ACTIVATED SLUDGE CHARACTERISTICS

Abstract

This chapter discusses the impact of microwave and hydrogen peroxide treatment on sludge characteristics of two different waste activated sludges collected from different WWTP sources. A comparison was made between the two sludges (NWAS and BMWAS) based on the optimum treatment conditions to achieve maximum sludge solubilization. The effects of pre-treatment process variables on EPS fraction, cellular oxidative stress and solubilization of both sludges were evaluated to understand the impact of sludge complexity in pre-treatment. It was observed that both the sludges vary in composition and optimum treatment condition. For a constant 2 min microwave irradiation, NWAS and BMWAS achieved maximum COD/ biopolymer solubilization at 450 W + 1 % H_2O_2/TS and 660 W + 1 % H_2O_2/TS, respectively. A 30-40 % higher sludge solubilization (sCOD/tCOD) was observed in hybrid (MW-H_2O_2) treatment compared to individual treatment in NWAS. The combined treatment produced 8- and 4.1-fold higher s[$OH^{\bullet}$] and s[$O_2^{\bullet -}$] respectively compared to the untreated fraction. It has been found that higher oxidative stress has corresponded to a significant reduction in volatile suspended solids and chemical oxygen demand. Besides, the solubilization of proteins and carbohydrates in EPS fraction was observed to be increased with an increase in microwave intensity in the hybrid pre-treatment. In addition, the hybrid treatment was observed to increase the flocculation tendency of NWAS compared to individual treatment, which is advantageous in the view of dewaterability in WWTPs.

4.1 Introduction

Various physical, chemical and biological pre-treatment techniques for anaerobic digestion have been investigated over the past two decades (Sen, 2015; Tyagi and Lo, 2011). Among such techniques, microwave and hydrogen peroxide pre-treatments have exhibited enhanced

This chapter has been published. Ambrose, H.W., Philip, L., Sen, T.K., Suraishkumar, G.K., 2019. The Effect of Combined Microwave and Hydrogen Peroxide Pretreatment on Sludge Characteristics and Oxidation Status of Waste Activated Sludge, in: Lefèbvre, B. (Ed.), The Activated Sludge Process: Methods and Recent Developments. Nova Science Publishers, pp. 105–141.

solubilization and anaerobic biodegradation due to their unique mechanism of action. The combination of both pre-treatments has been recently studied by few researchers to establish the synergism in their mode of action (Eskicioglu et al., 2008; Eswari et al., 2016; Kenge et al., 2008; Liu et al., 2017). Although most of the reported results suggested attainment of major sludge solubilization in the process, contradicting results on anaerobic biodegradation have also been observed by few researchers (Eskicioglu et al., 2008; Liu et al., 2017). The decrease in biodegradation is attributed to the generation of refractory compounds at a higher temperature and H_2O_2 dosage (Liu et al., 2017; Wang et al., 2009). Hence, the optimization of MW-H_2O_2 treatment for the WAS is essential to achieve maximum treatment efficiency and also, improved anaerobic digestion performance.

In this chapter, the effect of MW-H_2O_2 treatment on COD, suspended solids and biopolymer solubilization have been studied on WAS samples collected from BWWTP and NSTP. Furthermore, the oxidative stress exerted by the hybrid treatment has been evaluated by the measurement of intracellular hydroxyl and superoxide radical measurement. The study with two different sludges from different sources gives more insight on sludge behavior towards pre-treatments and its effects on anaerobic digestion.

4.1 Materials and methods

4.2.1 Sludge sampling and characterization

For the current study, activated sludge samples were collected from BWWTP of Water Corporation, Perth, Australia and NSTP, Chennai, India (section 3.2 and 3.3). The samples were characterized, and pre-treatment studies were carried out with microwave and H_2O_2. Beenyup Mixed waste activated sludge (BMWAS) was collected from the mixed sludge sampling point in BWWTP. Similarly, the Nesapakkam Waste Activated sludge (NWAS) was collected from the secondary effluent of the aeration tank in NSTP. The samples vary largely in composition, as BMWAS consists of both TEAS and RPS. After collection, the sludge samples were immediately characterized for pH, COD and solid concentration which is presented in Table 4.1. The samples were then stored at 4 °C until further experimentation.

Table 4.1: Initial sludge characterization of NWAS and BMWAS

Sl. No	Parameter	NWAS	BMWAS
1	pH	7.37	6.8
2	tCOD (mg/l)	8448 ± 135	39000 ± 150
3	sCOD (mg/l)	785 ± 90	4000 ± 190
4	TS (mg/l)	8630 ± 75	27200 ± 220
5	VS (mg/l)	7909 ± 95	21900 ± 180
6	TSS (mg/l)	8350 ± 70	26065 ± 165
7	VSS (mg/l)	7600 ± 290	20050 ± 75

4.2.2 Pre-treatment strategies

The details on pre-treatment equipment and strategies have been discussed in section 3.6, of the previous chapter. Briefly, 30 ml of NWAS and BMWAS were taken in beakers of constant dimension and treated with microwave and H_2O_2. For microwave treatment, the samples were irradiated with variable microwave power outputs (100 W to 1100 W) at different contact time (30s, 60s, 120s and 180s). Furthermore, the samples were treated with H_2O_2, at 0.5 % H_2O_2/TS and 1 % H_2O_2/TS. In the combined pre-treatment, H_2O_2 was preceded by microwave to ensure deactivation of catalase (Wang et al., 2009). The pre-treatment methods and conditions followed in this chapter are given in Table 4.2.

4.2.3 Analytical techniques

The sludge samples were analyzed for total solids, suspended solids, pH, tCOD, sCOD, soluble protein/ carbohydrate in EPS, ROS and zeta potential using the standard methods and instruments discussed in section 3.5, of the previous chapter.

Table 4.2: Sludge pre-treatment methods and conditions followed in chapter 4

Sample	Pre-treatment method	Conditions
NWAS	Microwave	2450 MHz, 100 W to 800 W, 2 min
NWAS	Microwave and hydrogen peroxide (MW-H_2O_2)	Microwave: 2450 MHz, 450 W, 2 min H_2O_2: 0.5 % and 1 % (w/w) H_2O_2/TS
BMWAS	Microwave and hydrogen peroxide (MW-H_2O_2)	Microwave: 2450 MHz, 220 W to 1100 W, 1 min, 2 min and 3 min Microwave intensity: 3.94 W/cm^2 (220 W), 7.88 W/cm^2 (440 W), 11.82 W/cm^2 (660 W), 15.76 W/cm^2 (880 W) and 19.70 W/cm^2 (1100 W) H_2O_2: 1 % (w/w) H_2O_2/TS

4.2 Results and Discussion

4.3.1 Effect of microwave irradiation on sludge solubilization

The effects of different microwave power output for 2 min contact time on the solubilization of COD and suspended solids in NWAS, were studied. Microwave irradiation causes a rapid realignment of water dipoles towards oscillating electromagnetic wave and produces energy in the form of heat. Exposure of sludge to extreme microwave irradiation is detrimental as it vaporizes water and thickens the sludge. In the current study, a decrease in both tCOD and sCOD was observed at 800 W compared to 600 W and 450 W microwave treatment of sludge, which suggested decreased dissolution of biomolecules at higher temperature due to thickening of sludge material. The higher power output also causes vaporization of organic molecules and releases toxic fumes into the environment, which is undesirable. For 2 minutes of microwave treatment of NWAS, 450 W showed least evaporation with 22.5 % sludge solubilization (sCOD/tCOD) (Figure 4.1b).

Previous studies have reported that the breakdown of microbial cells is majorly caused by thermal effects caused by microwave exposure (Vela and Wu, 1979). Although certain studies have reported athermal cell lysis, the mechanism of athermal cell lysis is not well established

(Eskicioglu et al., 2007c). Various studies have shown enhancement in anaerobic digestion, dewaterability and pathogen destruction through microwave irradiation of waste activated sludge (Eskicioglu et al., 2007c; Park et al., 2004; Wang and Wang, 2016; Yeneneh et al., 2015b). Some research works have also reported that microwave irradiation does not have a significant effect on biosolids solubilization and anaerobic digestion performance compared to conventional heating operated at the same temperature (80-160^{o}C) (Mehdizadeh et al., 2013).

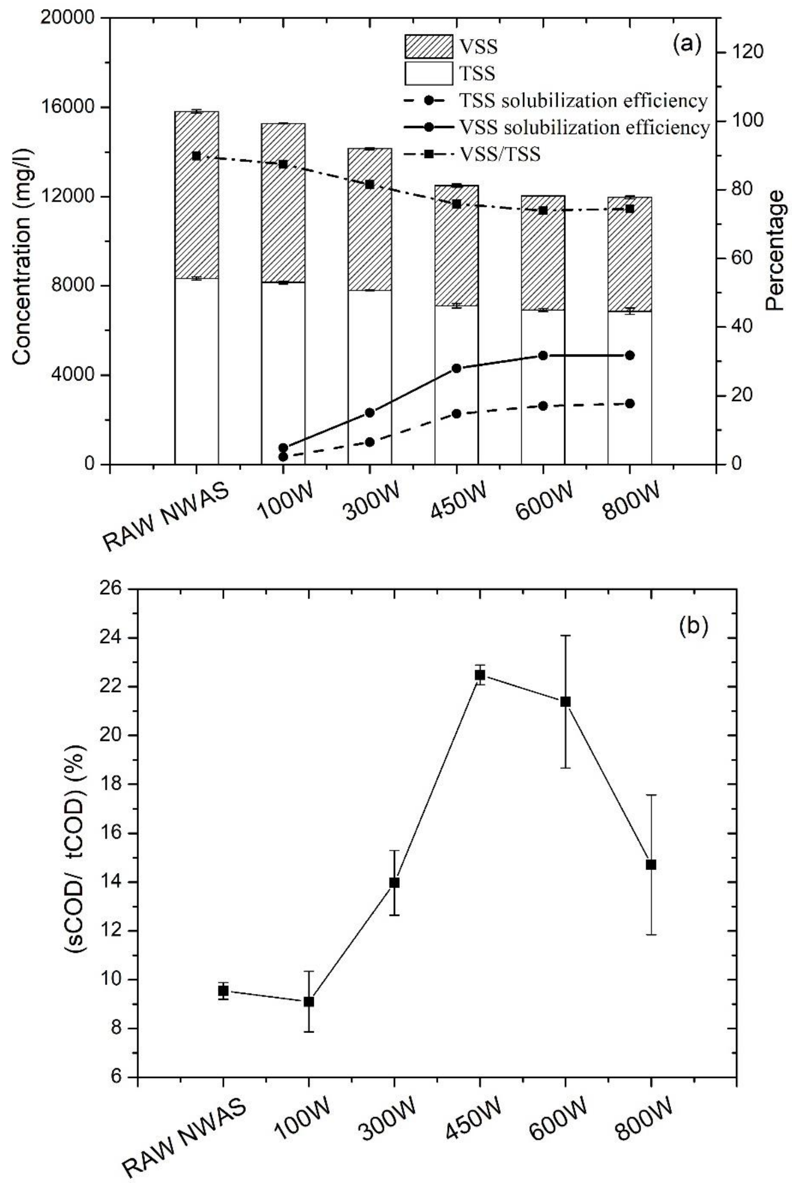


Figure 4.1: Effect of different microwave power outputs (100 W, 300 W, 450 W, 600 W and 800 W) for 2 min contact time on NWAS solubilization.

(a) TSS & VSS solubilization profile of NWAS (b) COD solubilization profile of NWAS.

In the current research work, 20-24 % COD solubilization was observed with mono-microwave treatment at 450 W and 600 W power outputs. No significant increase in COD solubilization was observed in NWAS treated with 100 W microwave, compared to control. The increase in COD solubilization from 100 W to 450 W microwave output is almost linear and concurrent with previous research data (Yeneneh et al., 2017a). Yeneneh et al., have shown 18 % of COD solubilization at 640 W microwave treatment for 3 minutes (Yeneneh et al., 2017a). Eskicioglu et al. reported linear sCOD/tCOD ratios upon microwave heating at 50, 75 and 95 ^{o}C (Eskicioglu et al., 2007c). Microwave power of 700 W for 3 minutes duration yielded 21 % COD solubilization in a palm oil mill effluent, which has an initial COD concentration of 50000 mg/l and also 4000 mg/l of oil & grease (Saifuddin and Fazlili, 2009). Yu et al., have reported that 1.5 to 2 % sludge disintegration was achieved by 900W microwave irradiation for 60s in a secondary sludge. Further treatment time has caused a decrease in dewaterability (Yu et al., 2009). The optimum microwave treatment for maximum COD solubilization differs depending on various sludge characteristics. Comparing previous studies, one can infer that sludge with a higher COD load, may take more microwave power output or time to break down complex sludge flocs that could be achieved with lesser microwave power in a lesser COD load.

The effect of microwave irradiation in sludge solubilization on VSS/TSS is given in Figure 4.1a. A minimum of 74 % VSS/TSS is achieved from 90 % VSS/TSS in raw NWAS, with 600 W microwave power. Haug et al., reported 55.2 % VSS/TSS from 64.2 % raw waste activated sludge upon thermal pre-treatment at 175 °C (Haug et al., 1978). The effect of microwave irradiation time on suspended solid solubilization has been studied previously (Byun et al., 2014, 2018). A higher time of exposure leads to boiling and evaporation, which is undesirable. In the present work, the effect of various microwave power outputs in sludge solubilization for a constant time was studied. The solubilization of NWAS was observed to increase linearly from 100 W to 600 W. Beyond 600 W, a saturation of TSS and VSS solubilization was observed. Hence, the trend in sCOD and VSS/TSS suggest that 800 W microwave irradiation does not improve solubilization. Exposure of NWAS to a high microwave output (800 W) causes a boiling effect and leads to the evaporation of water and volatile organics. This could be the reason for saturation in suspended solid solubilization at 800 W. The optimum microwave power is the one wherein, maximum solubilization of particulate organics occurs without evaporation. Yet another disadvantage of treating sludge at boiling temperature is odor emission.

4.3.2 Effect of hybrid (MW-H_2O_2) treatment on sludge solubilization

To enhance sludge solubilization and biogas production, microwave treatment has been studied in combination with acid, alkali, ultrasonication and H_2O_2 (Doğan and Sanin, 2009; Eskicioglu et al., 2008; Jibao Liu et al., 2016a; Yeneneh et al., 2015b). Previous researchers were confined to study the synergistic effect of combined treatment in overall anaerobic digestion performance and biogas production. In the current research, the effect of hybrid (MW-H_2O_2) treatment on sludge characteristics were evaluated in both NWAS and BMWAS. NWAS solubilization by combined microwave (450 W for 120s) and H_2O_2 (0.5 % and 1 % per TS) treatment was studied. The dosage of hydrogen peroxide in the hybrid pre-treatment is crucial to achieving maximum solubilization of particulate organic substances without causing the production of refractory compounds that would mitigate anaerobic digestion. Liu et al. achieved 35-45 % sludge solubilization with a microwave power output of 600 W (100 °C) and with varying hydrogen peroxide dosage (0.2g, 0.6g and 1g H_2O_2/g TS) (Liu et al., 2017). This study reported the inhibitory effect of residual hydrogen peroxide in overall anaerobic digestion and biogas production. Previously, Eskicioglu et al. reported that combined microwave (120 °C) and H_2O_2 (1g H_2O_2/g TS) dosage resulted in a 24.4 % sCOD/tCOD ratio and 20 % reduction in TS compared to control. However, the hybrid pre-treatment resulted in lower first-order mesophilic biodegradation and methane yield compared to mono- microwave treatment and control waste activated sludge (Eskicioglu et al., 2008). The study claimed that the decrease in anaerobic biodegradation observed in hybrid pre-treatment could be due to the generation of slowly biodegradable refractory compounds. Wang et al. studied the effects of H_2O_2 dosing strategy in a combined microwave- oxidant treatment with H_2O_2 doses ranging from 0.1-4 H_2O_2/tCOD (Wang et al., 2009). Recently, Inan et al. reported 2.5 % (w/v) H_2O_2 combined with 300 W (2.5 minutes) microwave treatment in the degradation of lignocellulosic biomass (Inan et al., 2016). Furthermore, Eswari et al. observed 50 % TS solubilization in a combined pre-treatment (MW-0.1 to 1 mg H_2O_2/g SS) in dairy waste activated sludge (Eswari et al., 2016). Zhang et al. used a dosage of 0.2 g H_2O_2/g TS in combination with 600 W microwave and studied anaerobic digestion efficiency (Zhang et al., 2017). Neyens et al. studied the peroxidation effect in sludge dewatering by using a dosage of 25 g H_2O_2/kg DS (E. Neyens et al., 2003b). Lo et al. reported 39 % VS reduction and 34-45 % TCOD reduction with 1.2 % H_2O_2/ %TS (Lo et al., 2017).

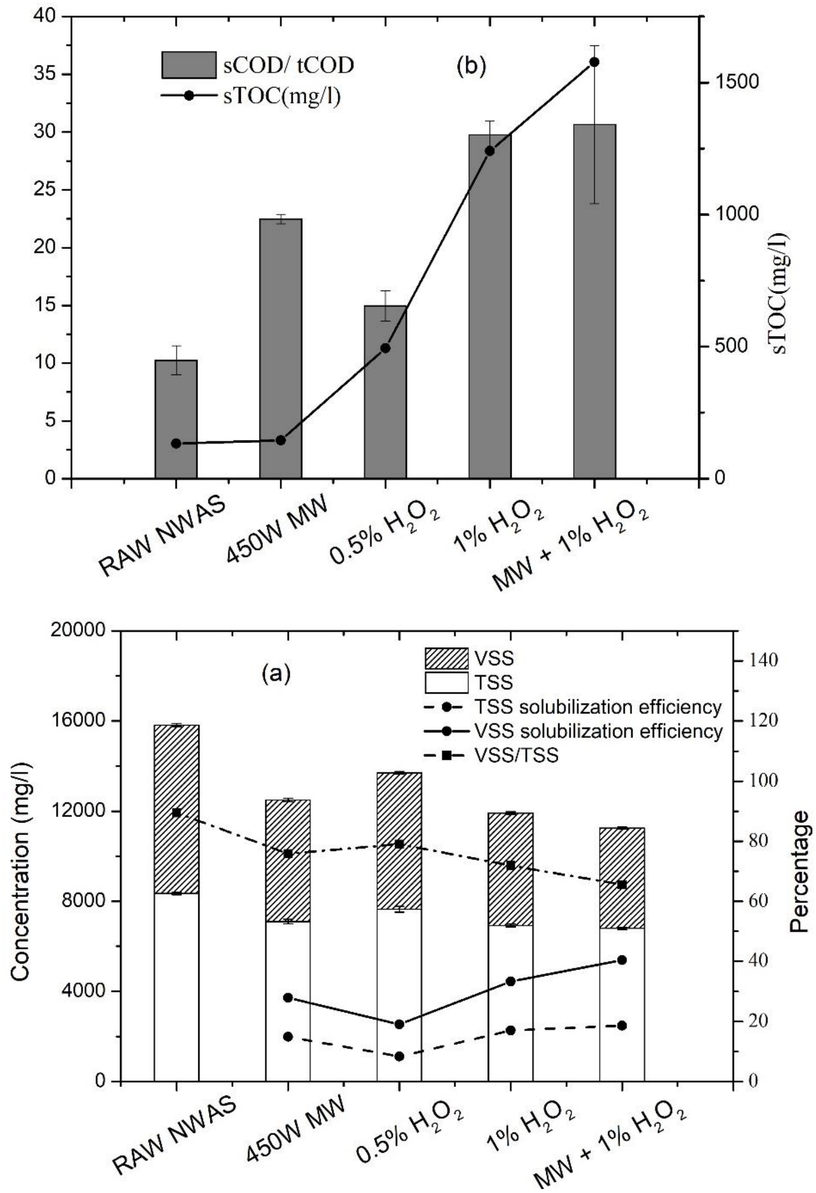


Figure 4.2: Effect of microwave (450 W/120s), 0.5 % & 1 % H_2O_2/TS & combined (450 W + 1 % H_2O_2/TS) on NWAS solubilization.

(a) COD solubilization profile of NWAS (b) TSS & VSS solubilization profile of NWAS.

Most of the researchers used total solids as a standard for optimizing H_2O_2 dosing. Some of them have also reported negative effects of residual H_2O_2 and reduction in anaerobic digestion performance (Eskicioglu et al., 2008; Liu et al., 2017). This is possibly due to the high dosage. In the current study, 0.5 % & 1 % (w/w) hydrogen peroxide per total solids were examined as suggested by (Lo et al., 2017). By combined treatment (MW + 1 % H_2O_2/TS), 30-40 % higher sCOD solubilization was observed compared to 22 % solubilization in mono- microwave treatment (Figure 4.2 a). Also, the TSS solubilization was also observed to be saturated with the combined treatment as seen from the Figure 4.2 b. Further increment in microwave intensity and H_2O_2 concentration might lead to caramelization and generation of refractory compounds, respectively.

Higher efficiency of AOP is dependent on the inactivation of the catalase enzyme, before subjecting it to oxidative stress (Liu et al., 2017). Catalase enzyme catalyzes the conversion of hydrogen peroxide to water and oxygen (equation 2.20). It plays an important role in protecting cells from the formation of ROS under oxidative stress. For the inactivation of catalase, H_2O_2 treatment was preceded by microwave treatment in the current study. Furthermore, the highest VSS solubilization efficiency (40 %) was observed in combined (MW + 1 % H_2O_2/TS) treatment (Figure 4.2b). 0.5 % H_2O_2/TS & 1 % H_2O_2/TS treatment yielded 19 % and 33 % VSS solubilization. The synergistic effect of combined pre-treatment has caused the generation of ROS species such as hydroxyl and superoxide radicals, therefore increasing solubilization.

The effect of combined microwave and 1 % H_2O_2/TS treatment on BMWAS solubilization is given in Figure 4.3. A significant difference in the optimum condition for pre-treatment is observed in BMWAS compared to NWAS. This could be due to a higher COD load and composition of BMWAS. The highest COD solubilization (24.34 %) was observed in 660 W microwave treatment, followed by 880 W (Figure 4.3b). The 1100 W treatment achieved 17 % COD solubilization. A decrease in solubilization at higher microwave intensities has been observed in both NWAS and BMWAS. The possible reason for this could be the ‘boiling’ effect caused by microwave treatment and subsequent evaporation of organics. The TSS and VSS solubilization along different microwave treatment has been given in Figure 4.3a. A maximum of 61 % VSS solubilization was observed in 1100 W + 1 % H_2O_2/TS treatment. The 660 W + 1 % H_2O_2/TS microwave treatment achieved 54 % VSS solubilization. Comparing the sludge solubilization profile of NWAS and BMWAS, the latter requires high microwave output (660 W) at a constant 1 % H_2O_2/TS concentration, to achieve maximum solubilization. This indicates that sludge with high solid content and COD load (Table 4.1) require more microwave power to achieve the desired sludge solubilization compared to sludge with less complexity.

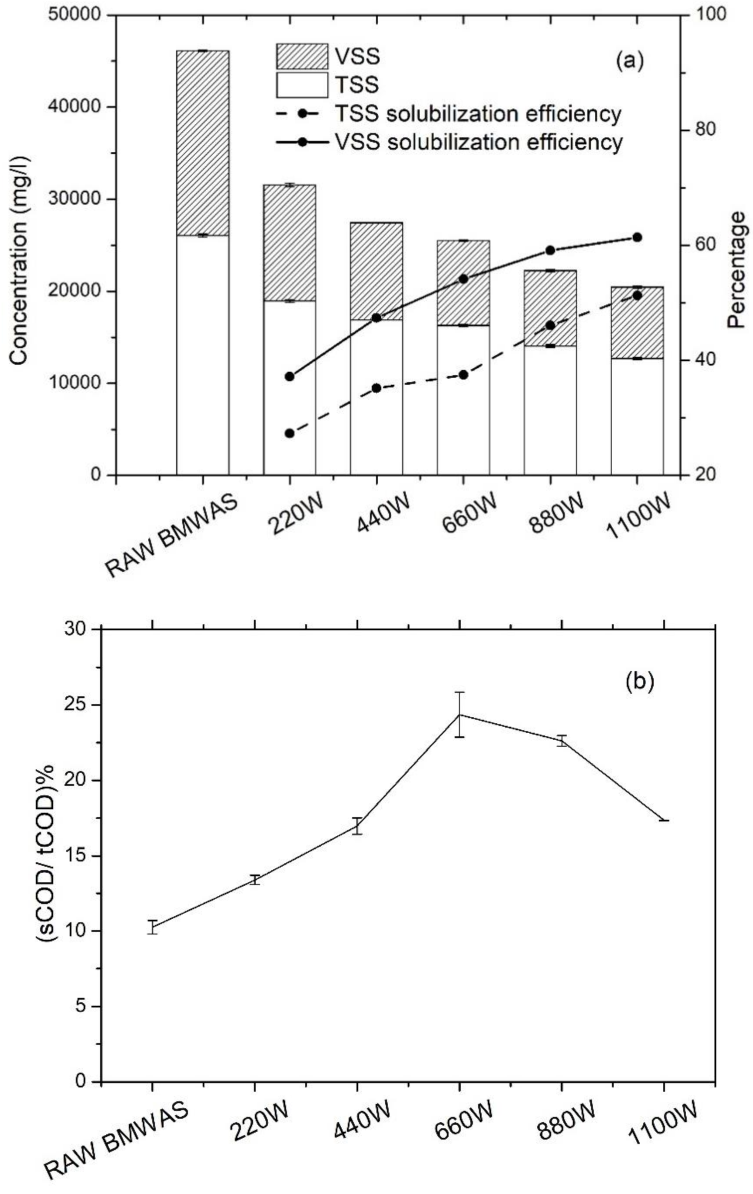


Figure 4.3: Effect of hybrid (MW/120s + 1 % H_2O_2) treatment with variable power outputs on BMWAS solubilization.

(a) TSS & VSS solubilization profile of BMWAS for constant contact time (2 min) (b) COD solubilization profile of BMWAS

4.3.3 Effect of Pre-Treatment on biopolymeric compound Solubilization

Extra-cellular polymeric substances (EPS) of activated sludge are composed of a complex mixture of biopolymers such as carbohydrates, proteins, lipids, humic substances and nucleic acids. The presence of EPS in feed sludge to anaerobic digester strongly influences the digester performance (Jibao Liu et al., 2016b). EPS are excreted by aerobic and anaerobic microorganisms, produced from cellular metabolism, cellular lysis and hydrolysis of macromolecules.

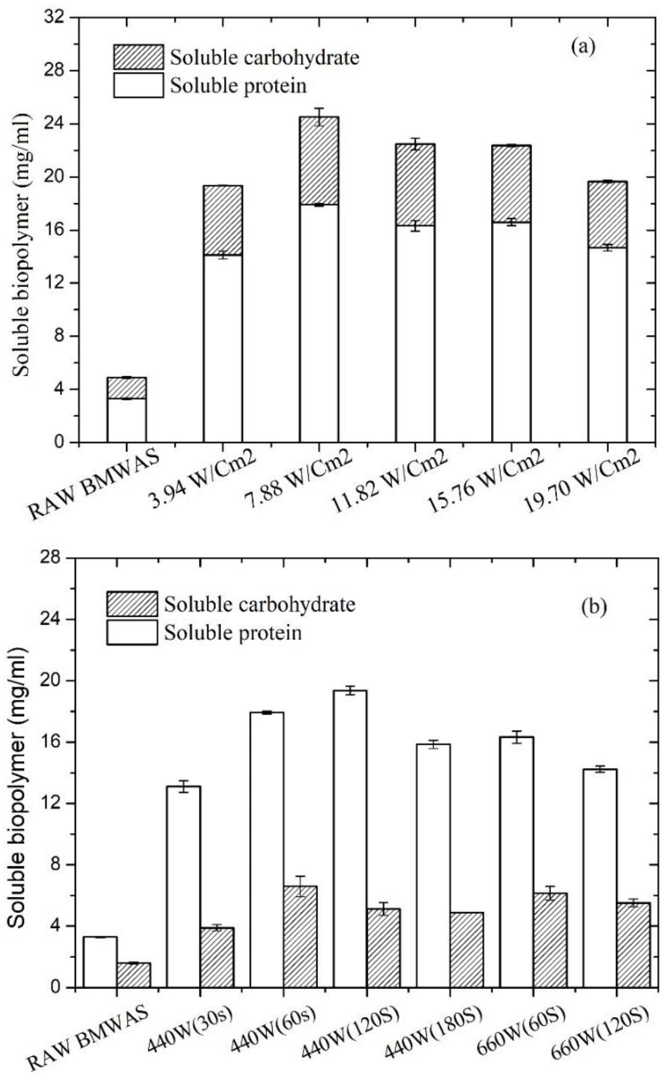


Figure 4.4: Effect of hybrid (MW + 1 % H_2O_2/TS) pre-treatment with variable power intensities on EPS solubilization.

(a) Biopolymer solubilization at various power intensities (b) Biopolymer solubilization at various microwave contact time of BMWAS.

EPS forms a complex 3-D matrix around sludge flocs and affect its physicochemical properties, substrate/product mass transfer, surface charge and flocculation properties of microbial aggregates. Carbohydrates and proteins are the major components of EPS.

In the current research, the solubilization of carbohydrates and proteins upon microwave irradiation and oxidative treatment in BMWAS was analyzed (Figure 4.4). BMWAS was treated with varying microwave intensities at variable time (60s, 120s and 180s) along with a constant 1 % H_2O_2/TS addition. Soluble carbohydrate and soluble protein fraction were extracted from EPS. As seen in Figure 4, the amount of protein release was 3 times higher than the amount of carbohydrate released. The highest amount of protein release was observed at 7.88 W/cm^2 (440 W/60s) combined with 1 % H_2O_2/TS. As the treatment intensity is increased beyond this point, there is a slight decrease in soluble protein concentration. The same trend is observed in carbohydrate release. 6.6 mg/ml carbohydrate release was achieved in the combined treatment 7.88 W/cm^2 (440 W/60s) with 1 % H_2O_2/TS, and further power intensity has decreased the carbohydrate release. EPS forms a colloidal interphase between microbial aggregates and the bulk of the liquid. The production of EPS particularly proteins is elevated in presence of toxic heavy metals and higher BOD load, as organisms produce more EPS for their protection against the harsh environment. The production of a higher fraction of protein in BMWAS goes in accordance with Sheng et al’s report on EPS behavior in sludge (Sheng et al., 2010) and solubilization of biopolymers by microwave and conventional heating by Mehdizadeh et al. (Mehdizadeh et al., 2013).

The decrease in protein and carbohydrate concentration at higher microwave intensity could be due to the Maillard reaction and caramelization (Eskicioglu et al., 2007c). At temperatures above 100 °C, reducing sugars and amino acids can polymerize to form melanoidins that transform into particulate phase (Lund and Ray, 2017). Maillard reaction occurs when cooking food rich in protein and carbohydrate at a higher temperature with sudden dehydration. With higher COD and BOD load, the complexity of waste activated sludge increases. This causes EPS fractions to be rich in proteins and carbohydrates as seen in BMWAS. In the current study, evaporation of water was observed at higher microwave intensities at a longer treatment time in BMWAS, which lead to thickened and caramelized giant flocs. A similar decrease in biopolymer concentration at higher microwave intensities has been reported by other studies (Eskicioglu et al., 2007c; Toreci et al., 2010). The formation of Maillard products can subsequently affect anaerobic digestion efficiency.

Dwyer et al. has reported that the thermal hydrolysis process operated at 165 °C had poor biodegradability in anaerobic digestion because of the formation of melanoidins (Dwyer et al., 2008). Despite high solubilization by pre-treatment, the overall anaerobic digestion performance can decline because of Maillard reactions. Therefore, optimization of pre-treatment techniques to avoid melanoidin formation is essential. At microwave intensities 11.82 W/cm^2 (660 W & 1 % H_2O_2/TS) & 15.76 W/cm^2 (880 W & 1 % H_2O_2/TS), equivalent amount of biopolymer solubilization was observed (Figure 4.4 a). At 1100 W (& 1 % H_2O_2/TS), the solubilization of biopolymer has decreased. A concurrent result is observed in COD solubilization at this pre-treatment. Therefore, 1100 W (& 1 % H_2O_2/TS) may not be suitable for achieving maximum BMWAS solubilization without harming anaerobic digestion. Therefore, in the perspective of higher COD solubilization and biopolymer solubilization, the treatment condition 660 W & 1 % H_2O_2/TS has achieved good results followed by 880 W & 1 % H_2O_2/TS for a 2 min treatment time. Another important parameter to be addressed in microwave irradiation is contact time. Koupaie et al., have reported that slow heating ramp rates (3 °C/min) to a temperature of 160 °C solubilized significantly higher amount of biopolymers compared to fast heating ramp rates (6 °C /min) & (11 °C /min) (Koupaie and Eskicioglu, 2016). Eskicioglu et al., reported a 1.3 °C /min ramp rate to have better solubilization capacity compared to 1.4 °C/min & 1.2 °C/min (Eskicioglu et al., 2007c). In the current study, decreased solubilization was observed at 1100 W (& 1 % H_2O_2/TS) treatment (Figure 4b), which suggests that a faster heating rate is not advantageous. As seen from the Figure, the release of protein increased with an increase in treatment time and attained a maximum at 440 W for 2 minutes irradiation time. A maximum of 6.6 mg/ml soluble carbohydrate is released in (440 W/60s) with 1 % H_2O_2/TS treatment. There is a non-linearity in the release of proteins and carbohydrates into the soluble phase through pre-treatment. Treatment at 180 s caused a sharp decline in both biopolymer solubilization. With a combined treatment of 660 W & 1 % H_2O_2/TS, maximum dissolution of protein was observed in 60s, further treatment time decreased dissolution.

4.3.4 Effect of pre-treatment on oxidative stress of sludge

In the advanced oxidation process (AOP), the generation of ($OH^{\bullet}$) and ($O_2^{\bullet -}$) radicals can degrade sludge flocs, disrupt EPS and thus improve sludge settleability and dewaterability. Hydrogen peroxide is a strong oxidant, and microwave exposure accelerates its dissociation into hydroxyl radicals. The highly reactive hydroxyl radicals can oxidize target compounds with reaction rates of the order of 10^9 M^{-1} s^{-1}. A significant role is played by another radical- superoxide;

it replenishes the concentration of hydroxyl radicals through hydrogen peroxide formation (equation 2.19). The specific levels of intracellular reactive species, hydroxyl and superoxide, are direct indicators of oxidative stress (Menon et al., 2013). Therefore, to understand the oxidative stress effects in sludge microbes, intracellular quantification of both hydroxyl & superoxide radical is required. The role of ROS in oxidative pre-treatment of activated sludge has been previously reported (Gong et al., 2015; A. Zhang et al., 2015). The generation of hydroxyl radicals in sludge because of microwave treatment has been studied earlier, but the quantification is limited. Quantification of ROS species by fluorescent probes is an attractive technology in the case of biology and medicine. Fluorescent probes are highly specific to target reactive species and can even help real-time monitoring in living cells (Zhuang et al., 2014). Radical scavengers THIO and DABCO were used to study the effect of ($OH^{\bullet}$) and ($O_2^{\bullet -}$) radicals in removal of endocrine-disrupting compounds in waste activated sludge by calcium peroxide treatment (A. Zhang et al., 2015). Zhang et al. reported that ($OH^{\bullet}$) and ($O_2^{\bullet -}$) radicals are the functional ROS species involved in EDC removal. Also, ($OH^{\bullet}$) radical was reported to be the most potent oxidizer (A. Zhang et al., 2015). Gong et al., quantified hydroxyl radical concentration by terephthalic acid (TA) trapping in an ultrasonic-assisted Fenton oxidation of excess sludge (Gong et al., 2015). Sarkar and Suraishkumar (2011) quantified intracellular superoxide radicals using the hydroxyethidium fluorescence in *Bacillus subtilis* under pH and temperature stress. In the current research, the intracellular hydroxyl and superoxide radicals were quantified using aminophenyl fluorescein (APF) and dihydroethidium (DHE), respectively based on established reports (Jose et al., 2018; Sarkar and Suraishkumar, 2011). The fast-reacting hydroxyl radicals have a half-life of about 1ns, and direct detection is highly impossible (Buxton et al., 1988). However, the pseudo-steady state levels of reactive species can give significant insights, and are measurable (Menon et al., 2013). The quantification of the fluorescent product (APF), helps us to quantify $[OH^{\bullet}]$ radicals. The effect of microwave (450 W/120s) and oxidant treatment (0.5 % and 1 % H_2O_2 per TS) on sROS (specific intracellular reactive oxygen species) in NWAS was studied (Figure 4.5). The specific ROS measurement determines the absolute oxidative stress on microbial cells in an activated sludge upon treatment with oxidant and microwave (equation 3.12).

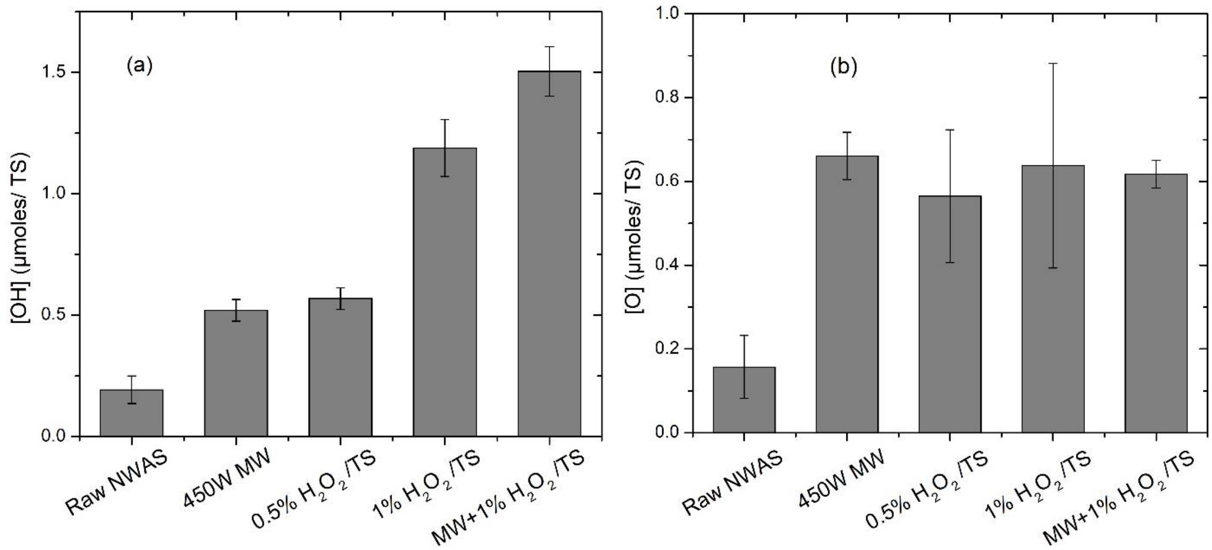


Figure 4.5: Effect of microwave (450 W/120s), 0.5 % & 1 % H_2O_2/TS & combined (450 W/120s & MW+ 1 % H_2O_2/TS) on sROS generation in NWAS

(a) s[$OH^{\bullet}$] for control NWAS, microwave, H_2O_2 & combined (MW + H_2O_2) treatment (b) s[$O_2^{\bullet-}$] for control NWAS, microwave, H_2O_2 & combined (MW + H_2O_2) treatment.

Highest s[$OH^{\bullet}$] concentration (1.5 µmole/g cells) was observed in combined treatment, which was 1.3-fold higher than 1 % H_2O_2/TS and 3-fold higher than 0.5 % H_2O_2/TS (Figure 4.5 a). The microwave treatment accelerates the decomposition of H_2O_2 to hydroxyl radicals. Microwave treatment and 0.5 % H_2O_2/TS treatment, almost have an equivalent amount of s[$OH^{\bullet}$]. In a comparison of s[$O_2^{\bullet-}$] levels, the highest concentration (0.66 µmole/g cells) was observed in pure microwave treatment (Figure 4.5b). This could be a reason for higher flux in s[$OH^{\bullet}$] concentration in microwave treatment, as superoxide can eventually form hydroxyl radical as per equation 2.19. Shaoying et al. reported elevated superoxide scavenging enzyme activity in apple juice treated at 720 W and 900 W from 75-100s (Zhang and Zhang, 2014). The study also reported that microwave induction can enhance the 'chelating' and 'reducing' property of apple juice. Besides, the study also suggests the Maillard reaction to being a reason for the reduction in amino nitrogen in the samples with microwave treatment. In the current study, a decrease in biopolymer and sCOD release was observed at elevated microwave treatment (880 W & 1100 W), which could also suggest the presence of Maillard reaction (Figure 4.4b) (Eskicioglu et al., 2007c). The evaluation of ROS under microwave and H_2O_2 treatment could establish more insights into the oxidative status of sludge. Sludge solubilization in NWAS goes concurrent with the oxidative stress caused by hydroxyl and superoxide radicals. The combined treatment, H_2O_2 (0.5 %), H_2O_2 (1 %) and MW have produced 8-, 3-, 6.2- & 2.7-fold higher s[$OH^{\bullet}$] respectively compared to control. The combined treatment, H_2O_2 (0.5 %), H_2O_2 (1 %) and MW have produced 4.1-, 3.7-, 4.2- & 4.4-fold higher s[$O_2^{\bullet-}$] respectively compared to control.

4.3.5 Effect of sludge pre-treatment on settleability

Surface forces at the interface between sludge and suspending liquid become an important factor for understanding the flocculation phenomenon. Microbial suspension in sludge forms a colloidal phase interacting with the surrounding liquid fraction, mostly water. Activated sludge flocs carry negative surface charge in a range between -10 mV to -20 mV, due to ionization of anionic functional groups (X. S. Jia, 1996). Zeta potential (ZP) is the potential difference between the surface of solids (immersed in a liquid) and the bulk of the liquid. ZP is measured by the particle velocity induced when a potential difference is applied along a capillary tube containing the sample. ZP helps understand sedimentation and flocculation phenomenon and is largely dependent on the electrical surface charge on microorganisms. Dependent on the cell wall structure, microbial cells have a higher or lower surface charge which determines their flocculation properties. ZP of microbial cells vary according to species, for example, Escherichia coli -24.96 mV, *Alcaligenes faecalis* -34.24 mV and *Leucobacter sp* -12.78 mV (Xie et al., 2010). To understand the effects of treatment conditions on the surface charge of different phases of activated sludge, NWAS was partitioned into 3 fractions, *viz* NWAS (solid fraction), slime fraction and LB-EPS (Loosely bound EPS). The solid fraction was obtained by resuspension of dry solids in distilled water. The zeta potential of untreated NWAS (solid fraction) was -52.37 ± 4.21 mV. This may be due to heavy BOD load in treatment plants, or the process technology or the constituents of wastewater (Bennoit, 2014). The absorption of charged ions by sludge flocs due to its large surface area can be another reason for the high negative ZP (Tang and Zhang, 2014). The higher the zeta potential, the poorer is the flocculating tendency of sludge. Increased negative surface charge leads to greater repulsive electrostatic force according to DLVO theory (Xie et al., 2010).

Pre-treatments have yielded -44.87 mV, -37.9 mV, -30.06 mV and -22.41 mV for microwave, 0.5 % H_2O_2/TS, 1 % H_2O_2/TS and combined (MW + 1 % H_2O_2/TS) for NWAS (Figure 4.6). As seen from Figure 4.6, the combined pre-treatment has decreased the absolute zeta potential from ~ -52 mV to -22 mV. Treatment imparts a positive charge on the microbial surface which was highly negative (~ -52 mV) in raw NWAS. This could be because of the release of divalent cations (Ca^{2+} and Mg^{2+}), due to floc breakage (Chu et al., 2001). These divalent cations are major bridging elements in the floc structure. Microwave and oxidative treatment cause major floc deterioration which results in the release of these divalent cations. Huang et al. has reported neutralization of negative cell surface charge by Mg^{2+} ions from $Mg(OH)_2$ pre-treatment (Huang et al., 2016). The pre-treatment techniques break down chemical bonds and dissolve complex organic matrix, thereby destabilizing the suspended particles and bring down their surface charge. The higher the oxidative stress, the higher is the disaggregation and flocculating tendency of particulate matter in solid fraction of sludge.

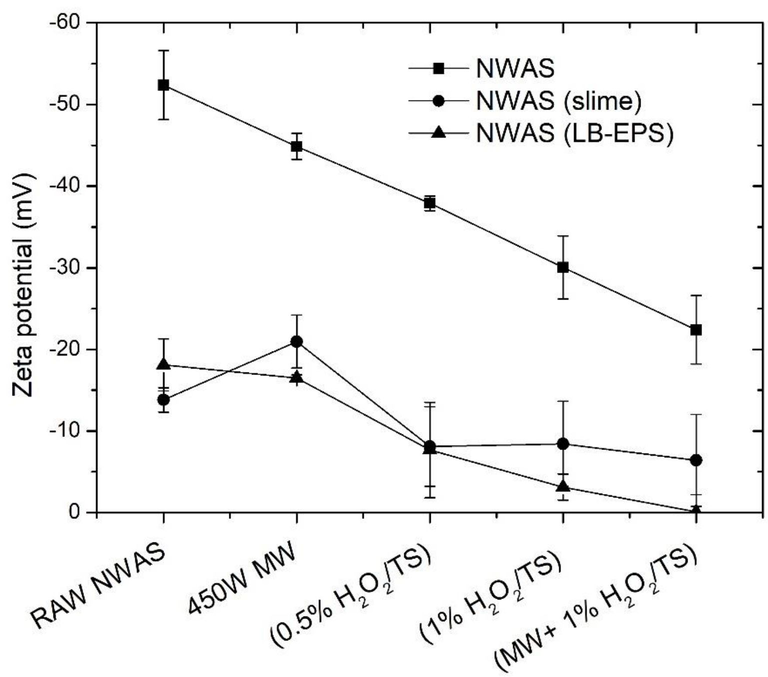


Figure 4.6: Effect of microwave (450 W/120s), 0.5 % H_2O_2/TS, 1 % H_2O_2/TS & combined (450 W/120s & MW+ 1 % H_2O_2/TS) on Zeta Potential of NWAS.

The extracted LB- EPS and slime fraction have -13mV and -18mV ZP respectively. These fractions are majorly composed of proteins and carbohydrates. Zhang et al. have reported a detailed analysis of various EPS proteins present in aerobic, anaerobic and anoxic sludges and their characteristics by shotgun proteomics (P. Zhang et al., 2015). The isoelectric points and molecular mass analysis in the report indicate that most of the EPS proteins carry a negative charge. Approximately 73 % of these proteins were identified as binding and catalytic proteins. The disruption of sludge flocs through pre-treatment releases the bound biopolymers thereby results in flocculation. The EPS architecture of sludge varies based on various factors such as sludge age, protein diversity, microbial population, pollutant level and aerobic/anaerobic condition. According to the current study, the pre-treatments resulted in charge neutralization as observed through the decrease in absolute Zeta Potential. A detailed study on the effects of pre-treatment on EPS architecture of activated and anaerobically digested sludge has been presented in chapter 7.

4.3 Conclusion

In the current study, the effects of microwave and hydrogen peroxide treatment were analyzed in individual and combination on various parameters like sludge solubilization, oxidative status and EPS behavior in two separate sludges (NWAS & BMWAS). According to the composition and sludge characteristics, the treatment effects can vary across different sludges. For a constant treatment time of 2 min, maximum solubilization in NWAS and BMWAS was achieved at 450 W + 1 % H_2O_2/TS and 660 W + 1 % H_2O_2/TS, respectively. And in both treatment conditions, the combined (MW & H_2O_2) treatment showed enhanced performance compared to individual treatments, in terms of solubilization efficiency and oxidative stress. Apart from the well-established role of hydroxyl radicals in causing oxidation, superoxide radicals also play a crucial role in the oxidation process. Furthermore, an effective treatment is the one that causes maximum solubilization without causing evaporation of organics. ‘Boiling’ temperatures can be detrimental as they lead to the formation of refractory compounds that will cause difficulty in the hydrolysis step of anaerobic digestion. In addition, pre-treatments impact the surface charge of various fractions of sludge differently. The fraction containing microbial aggregates responded linear to the treatment regime, whereas EPS fraction and slime responded differently due to the presence of proteins and carbohydrates. This work widens the scope of pre-treatment optimization which can help achieve maximum anaerobic digestion performance.

CHAPTER 5

EFFECT OF HYBRID (MW-H_2O_2) SLUDGE PRE-TREATMENT ON ANAEROBIC DIGESTION EFFICIENCY

Abstract

This chapter deals with the anaerobic digestion studies with hybrid (MW-H_2O_2) sludge pre-treatment. The impact of feed sludge pre-treatment on performance efficiency of conventional single-stage and a novel two-stage semi-continuous anaerobic digestion of mixed waste activated sludge were studied to enhance biogas production and digestate quality. Untreated two-stage anaerobic digestion (thermophilic followed by mesophilic) achieved 76.4 ml/g tCOD methane yield compared to 40.4 ml/g tCOD achieved through conventional single-stage mesophilic anaerobic digestion, with an increase in methane percentage. Application of hybrid (MW-H_2O_2) sludge pre-treatment in the two-stage digestion enhanced initial sludge hydrolysis/solubilization and consequently achieved 143.4 ml/g tCOD methane yield. Also, the highest methane percentage of 71 % was achieved during the peak methanogenesis stage in this process. The synergetic effects of hybrid pre-treatment were also confirmed by the higher release of extracellular polymeric substances. Oxidative stress exerted by the pre-treatment resulted in the accumulation of superoxide radicals in the initial thermophilic phase; followed by increased sludge activity and biomethanation in the later phase of two-stage digestion. Hybrid feed sludge pre-treatment in the two-stage system achieved a 73 % volatile solids reduction and more than 90 % reduction of faecal coliform. The various kinetic model parameters were determined by the application of the modified Gompertz model. Besides, the two-stage anaerobic digestion with pre-treatment also showed enhanced rheological properties by weakening its viscoelastic properties. These results illustrate that a novel semi-continuous two-stage anaerobic digester with hybrid feed sludge pre-treatment improved the overall anaerobic digestion performance compared to conventional single-stage digestions in terms of biogas production, solid reduction and digestate quality.

This chapter has been published. Ambrose, H.W., Chin, C.T.L., Hong, E., Philip, L., Suraishkumar, G.K., Sen, T.K., Khiadani, M., 2020. Effect of hybrid (microwave-H_2O_2) feed sludge pretreatment on single and two-stage anaerobic digestion efficiency of real mixed sewage sludge. Process Saf. Environ. Prot. 136, 194–202. https://doi.org/10.1016/j.psep.2020.01.032

5.1 Introduction

The previous chapter (chapter 4) has shown the effects of hybrid pre-treatment on sludge characteristics such as EPS dissolution, COD and suspended solids solubilization in BMWAS. Furthermore, the efficiency of pre-treatment on anaerobic digestion was studied in the current chapter. As hydrolysis is considered the major rate-limiting step of anaerobic digestion (Algapani et al., 2017; Seng et al., 2010), pre-treatment strategies were followed to break down the complex sludge flocs in WAS. In the current chapter, the BMWAS was treated with hybrid (MW-H_2O_2) pre-treatment and studied in a novel semi-continuous two-stage anaerobic digestion and compared with conventional mesophilic digestion. The purpose of the two-stage digester operation is to improve the overall anaerobic digestion performance by adding a pre-methanizer step oriented towards hydrolysis/ acidogenesis. To achieve good phase separation, optimization of operational parameters such as pH, temperature and HRT is required (Guerrero et al., 1999). Phase separation of hydrolysis and methanogenesis processes through temperature-controlled systems have shown improvement in biomethanation (Borowski, 2015). Furthermore, thermophilic regimes (55 ± 2 °C) are preferred over mesophilic regimes (37 ± 2 °C) as the former yields higher organic solids destruction, increased pathogen removal and improved solid-liquid separation (M. Kim et al., 2003). Therefore, the mesophilic phase-II anaerobic digestion was preceded by a short phase-I pre-methanizer (acidifier) maintained at thermophilic condition in the current study.

The efficiency of anaerobic digestion was evaluated based on various factors such as biogas production, solids reduction, pathogen removal, rheological characteristics and dewaterability. Furthermore, the evolution of superoxide radicals during anaerobic digestion was determined to distinguish the oxidative stress in pre-treated vs untreated anaerobic digestion. Besides, the anaerobic digestion kinetics of two-stage digestion was evaluated using the modified Gompertz equation in the current chapter.

5.2 Materials and methods

5.2.1 Sludge sampling and characterization

The anaerobic digestion of BMWAS was studied in single-stage and two-stage anaerobic digesters in the current chapter. For this purpose, BMWAS and anaerobically digested sludge (ADS) were collected from BWWTP, Water Corporation, Perth, Australia. A detailed description on sample collection has been given in the previous section 3.2. Both the sludge samples were characterized immediately after collection, which is presented in Table 5.1.

Table 5.1: Characterization of raw BMWAS, pre-treated BMWAS and ADS

Parameters	Raw BMWAS	Pre-treated BMWAS	ADS
pH	6	6.8	6.8
tCOD	39000 ± 163	35900 ± 648	5500 ± 85
sCOD	3900 ± 21	7950 ± 37	1400 ± 16
Total solids (mg/l)	27200 ± 244	27000 ± 408	15433 ± 47
Volatile solids (mg/l)	21900 ± 81	21600 ± 216	11066 ± 124
(VS/TS) %	80.5 ± 0.62	80 ± 0.52	71.6 ± 0.79

5.2.2 Sludge Pre-treatment

A sequential hybrid (MW-H_2O_2-MW) pre-treatment was carried out as detailed in section 3.6.3. 450 ml of BMWAS with a solid concentration of 27.2 g TS/l was taken in a 500 ml beaker. The sludge was initially treated to 80 °C by microwave irradiation at 660 W for 2 minutes in a laboratory-scale microwave oven with a power density of 1.46 W/ml to inhibit catalase activity in aerobic living cells of BMWAS (Liu et al., 2017). The beaker was covered on top with a microwave-safe plastic wrap and the treatment was carried out with an intermittent break to avoid water loss by evaporation. After cooling to room temperature, it was subjected to hydrogen peroxide treatment (1 % w/w H_2O_2/TS) with constant agitation. The sludge was again continued with microwave irradiation at 660 W for 3 minutes. The characteristics of pre-treated BMWAS is given in Table 5.1.

5.2.3 Anaerobic digestion studies

For anaerobic digestion studies, the pre-treated sample of BMWAS was introduced into stage-I of two-stage digesters with 10 % ADS as inoculum, making up to an operating volume of 500 ml. Similarly, the untreated BMWAS was studied in the two-stage set-up. Besides, two mesophilic single-stage digesters were operated with the same pre-treatment regimen for comparison. The reactors were named conventional single-stage and single-stage (MW-H_2O_2)

treated digester for untreated and hybrid microwave- oxidative treatment, respectively. The detailed description of the experimental setup has been discussed in section 3.7. Briefly, four anaerobic digestion studies were conducted in the current chapter (Figure 5.1).

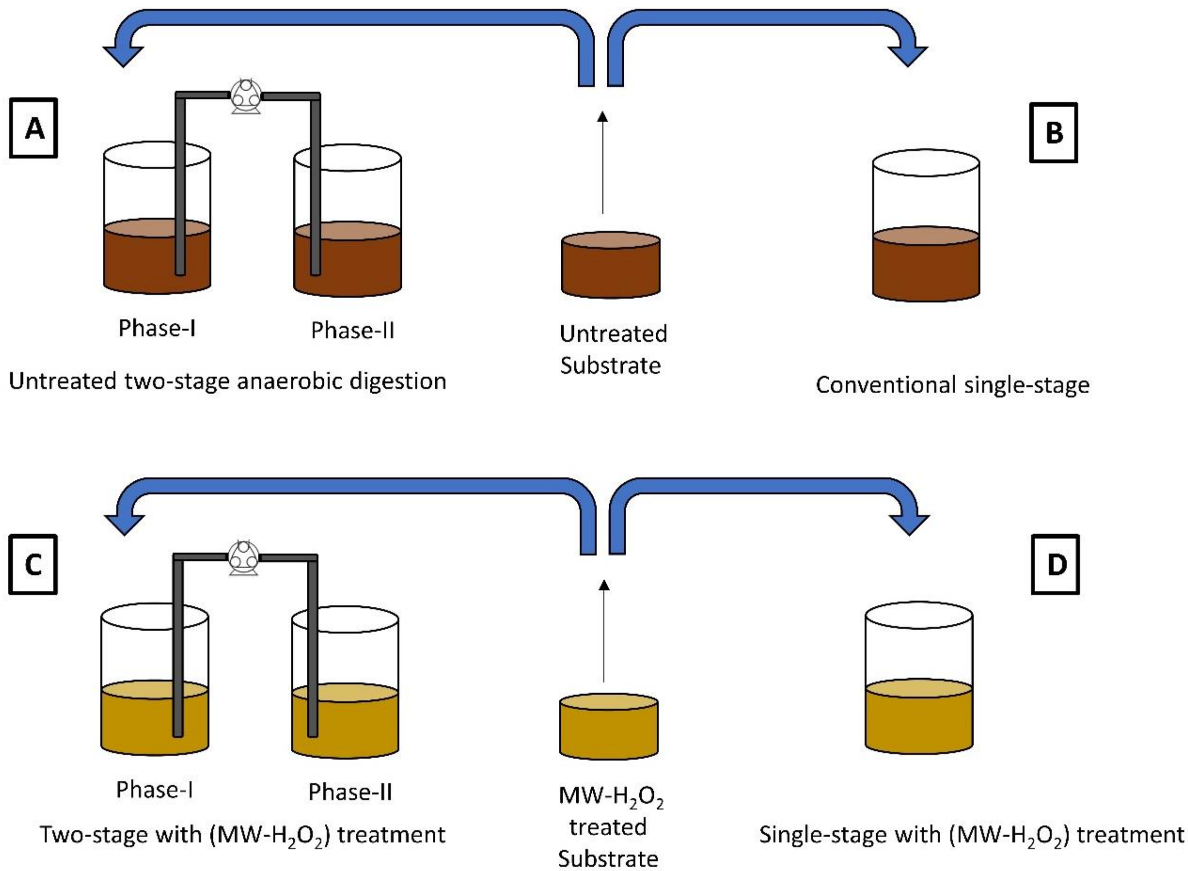


Figure 5.1: A schematic of anaerobic digestion studies conducted in chapter 5

Both thermophilic and mesophilic reactors were acclimatized with BMWAS feedstock and ADS inoculum at respective temperatures for 3 weeks before the start of experimentation. During experimentation, the thermophilic digesters were maintained at 4.16 g VS/l.day for 5 days of retention time. The short thermophilic phase ensures faster acidogenesis, pathogen control and effective organic matter degradation and, the retention time was arrived based on similar studies with two-phase anaerobic digestion (Ge et al., 2010; Riau et al., 2010). The mesophilic digesters were constantly maintained at 1 g VS/l.day for 20 days of retention time. The total retention time of BMWAS in the digestion set-up is 25 days (SRT- 5 days in phase-I and 20 days in phase-II). The gas produced during the anaerobic digestion were collected in the water displacement- gas collection system. The gas composition was measured periodically using a gas analyzer and recorded.

5.2.4 Analytical methods

For the current study, various parameters such as pH, temperature, tCOD, sCOD, VFAs, DHA, superoxide, elemental analysis, FTIR and rheology of sludge were measured. A detailed description of the analysis and equipment used for the measurement has been given in section 3.5.

5.2.5 Calculations

VS reduction percentage is calculated based on initial and final volatile solids concentration in digesters.

$$\text{Volatile solids (VS)reduction } \% = \frac{\text{VS}in - \text{VS}fin}{\text{VS}in} \times 100 \qquad \text{(equation 5.1)}$$

Where VS_{in} = Initial volatile solids concentration (mg/l); VS_{fin}= Final volatile solids concentration (mg/l)

The specific energy input for the microwave pre-treatment was calculated according to the following equation.

$$\text{Specific energy (kJ/kg TS)} = \frac{\text{Microwave power (kW)} * \text{irradiation time (s)}}{\text{Sample volume (L)} * \text{total solids (kg/l)}}$$

(equation 5.2)

5.2.5.1 Gompertz sludge hydrolysis kinetic model parameters calculations and theoretical methane yield

Sludge hydrolysis is the major rate-limiting step of the anaerobic digestion process. There are several model equations used to determine the hydrolysis rate constant. In the current study, the maximum methane production, methane production potential and lag phase are determined using the following modified Gompertz equation (Yeneneh et al., 2015a).

$$M = P \times \exp\left(-\exp\left[\frac{Rm.e}{P}\,(\lambda - t) + 1\right]\right) \qquad \text{(equation 5.3)}$$

Where, M= cumulative methane production (ml), Rm= maximum methane production rate (ml/day), P= methane production potential (ml), λ= lag phase (days) and t= time (days). The hydrolysis rate constant (k) is determined by assuming the first-order kinetics of cumulative methane production from hydrolysis of organic matter as per Veeken and Hamelers model (Veeken and Hamelers, 1999).

$$M = P(1 - e^{-kt}) \qquad \text{(equation 5.4)}$$

Theoretical methane yield (TMY) of anaerobic digestion is calculated based on elemental compositions of organic substrates using the Buswell formula (Buswell and Mueller, 1952).

$$C_nH_aO_bN_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right)CO_2 + cNH_3$$

$$TMY\left(\frac{mlCH_4}{gVS}\right) = \frac{(22.4).(1000).\left(\frac{n}{2}+\frac{a}{8}-\frac{b}{4}-\frac{3c}{8}\right)}{12n+a+16b+14c} \qquad \text{(equation 5.5)}$$

$$Anaerobic\ digestibility = \frac{EMY}{TMY} \times 100 \qquad \text{(equation 5.6)}$$

Where EMY is experimental methane yield.

5.2.5.2 Rheological models

The flow pattern and rheological properties of anaerobic sludge are important parameters in WWTPs as they influence pumping cost, mixing hydrodynamics, polymer consumption and dewaterability (Yeneneh et al., 2016). Therefore, the rheological properties of the anaerobically digested effluent sludge were characterized in the current study. The rheological behavior of Non-Newtonian fluids exhibits a non-linear trend when applied shear stress, due to its complex structure and deformation effects (Yeneneh et al., 2016). Various rheological models have been generated to better represent the Non-Newtonian behavior and determine parameters such as shear stress, yield stress, infinite and zero-rate viscosities (Sokolov, 2013). In the current study, Herschel-Bulkley and Carreau-Yasuda models have been used to fit the experimental data for the determination of various rheological parameters (Hong et al., 2017; Ratkovich et al., 2013), which has a strong implication on digester hydrodynamics/mixing and transportation.

Herschel- Bulkley $$\tau = \tau_y + k\dot{Y}^n \qquad \text{(equation 5.7)}$$

Carreau-Yasuda $$\frac{\mu-\mu_\infty}{\mu_0-\mu_\infty} = \left(1 + \left(\lambda\dot{Y}\right)^2\right)^{n-1/2} \qquad \text{(equation 5.8)}$$

Where, τ= shear stress (Pa), τ_y= yield stress (Pa), k= consistency index (Pa s^n), n= flow index, $\dot{Y}$ = shear rate (s^{-1}), μ_∞= infinite-rate apparent viscosity (Pa s), μ_0= zero-shear apparent viscosity (Pa s), λ= time constant (s).

5.3 Results and Discussion

5.3.1 Effect of dual digestion configuration: Conventional single stage vs two-stage anaerobic digestion

The purpose of dual digestion is to enhance anaerobic digestion performance by the addition of a pre-digestion step to improve sludge hydrolysis rate. Anaerobic digestion is a multi-phase process governed by distinct microbial communities at different time frames along the process. Independent optimization of hydrolysis and methanogenesis phases in two separate reactors helps in increasing the biochemical methane potential of the substrate (Guerrero et al., 1999). Thermophilic (50-60 °C) anaerobic digestion has been reported to have accelerated process efficiency and higher degradation rates of organics compared to mesophilic digestion (Carrère et al., 2010). Conventional single-stage (mesophilic) and two-stage (thermophilic-mesophilic) anaerobic digestion efficiencies were compared based on cumulative methane production and biogas quality which is presented in Figure 5.2. It was found that two-stage digester achieved 76.4 ml/g tCOD cumulative methane yield, whereas conventional mesophilic single-stage digester reached 40.4 ml/g tCOD at 24 days of SRT (Figure 5.2a). Besides, the two-stage digester showed a significant improvement in biogas quality compared to conventional single-stage digester, as the substrate is transferred to the methanogenic reactor at day 5 (Figure 5.2b). On average, phase II (methanogenic reactor of two-stage digester) maintained 51 % methane content. The maximum methane percentage achieved by single-stage digester was 49 ± 1.6 on days 16 to 18; whereas two-stage digester achieved 58 % methane content within day 10 (Figure 5.2b).

The efficiency of the thermophilic condition in the solubilization of particulate organics in waste activated sludge is enhanced compared to mesophilic condition for the same feedstock (Ge et al., 2011a). The initial thermophilic phase (55 ± 2 °C) of two-stage digester significantly accelerated COD solubilization compared to single-stage digester within 2 days which is shown in Figure 5.3a. sCOD/tCOD % of thermophilic phase was 24 ± 4 % at days 2 to 4, in two-stage digester (Figure 5.3 b). Whereas commonly used mesophilic single-stage digester attained a maximum sCOD/tCOD % of 30 ± 1 % from days 4 to 6 (Figure 5.3b). Sludge disintegration is observed to be slow in conventional single-stage digester compared to the two-stage dual digester system.

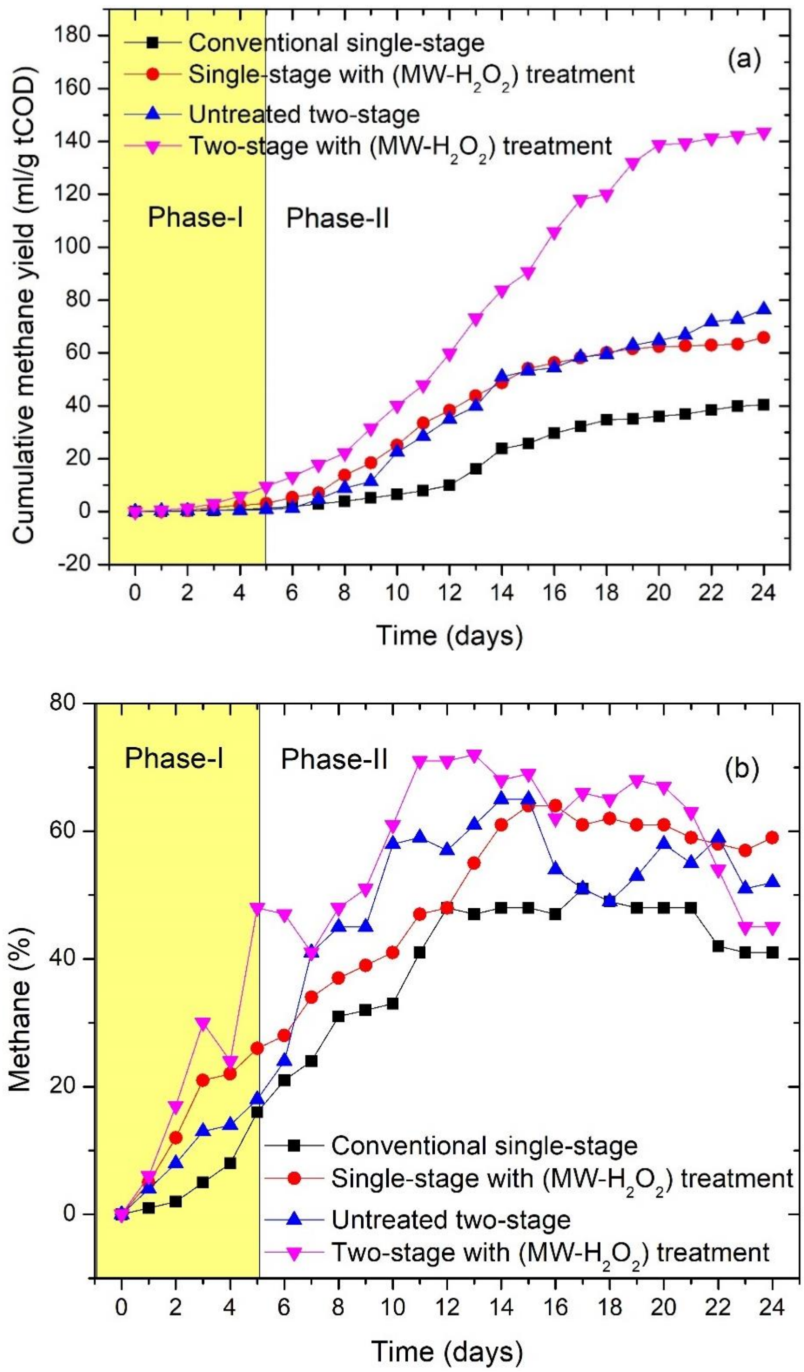


Figure 5.2: Comparison of anaerobic digestion performance of all four digester set-ups based on (a) cumulative methane yield (ml/g tCOD) (b) methane percentage

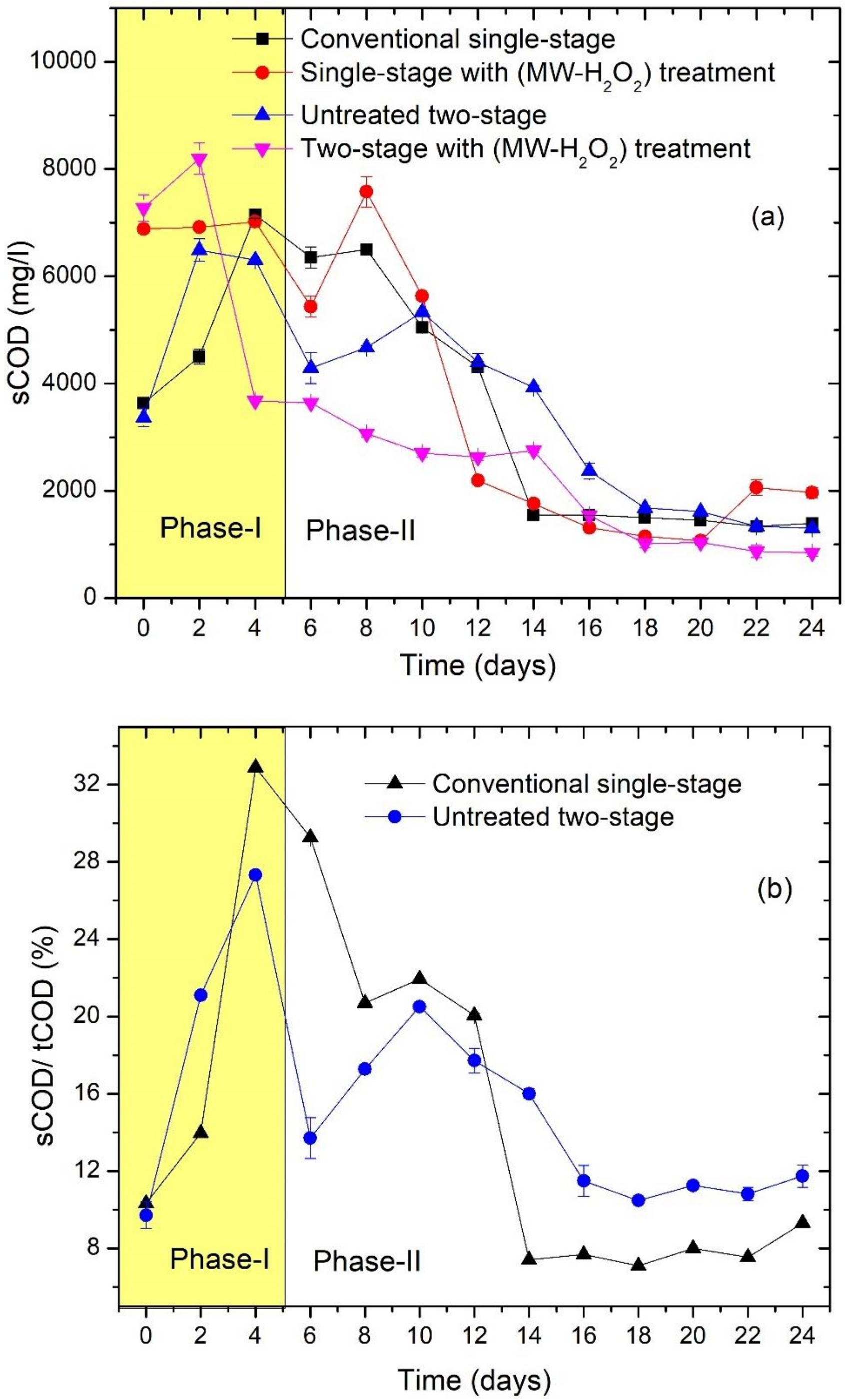


Figure 5.3: Effect of hybrid (MW-H_2O_2) pre-treatment and phase-separation on (a) sCOD concentration and (b) sCOD/tCOD % respectively.

Accelerated sludge hydrolyzation in the two-stage digester facilitates acidogenic bacteria to form simpler organic volatile acids and subsequently utilized by methanogenic bacteria for methane production in phase II. This is evident from the amount of VFAs accumulated across anaerobic digestion of the conventional single-stage and dual two-stage digesters which is presented in Figure 5.4. VFAs accumulation at the end of phase-I thermophilic digestion was observed to be 2700 ± 60 mg/l, which was significantly higher than the corresponding single-stage mesophilic digestion at day 4 (Figure 5.4). The organic acids conversion rate is lower in the single-stage digester as the amount of solubilization was low. Further, at 24 days of SRT, single-stage and dual two-stage digesters achieved 43 % and 61 % of VS reduction which is presented in Table 5.2.

Based on Buswell's formula (equation 5.5 & 5.6), a 1.5-fold increase in anaerobic biodegradability is observed in dual two-stage digestion compared to conventional single-stage digestion. Comparative anaerobic digestion efficiency concerning biogas yield, biogas quality, % VS reduction, % TS reduction etc between conventional single-stage and two-stage digesters are presented in Table 5.2. The poor biodegradability of mixed sludge (BMWAS) samples is due to the complex nature of TEAS. TEAS originates from dissolved air flotation thickeners and it is characterized with higher floc complexity and microbial biomass that are difficult to biodegrade in anaerobic digestion.

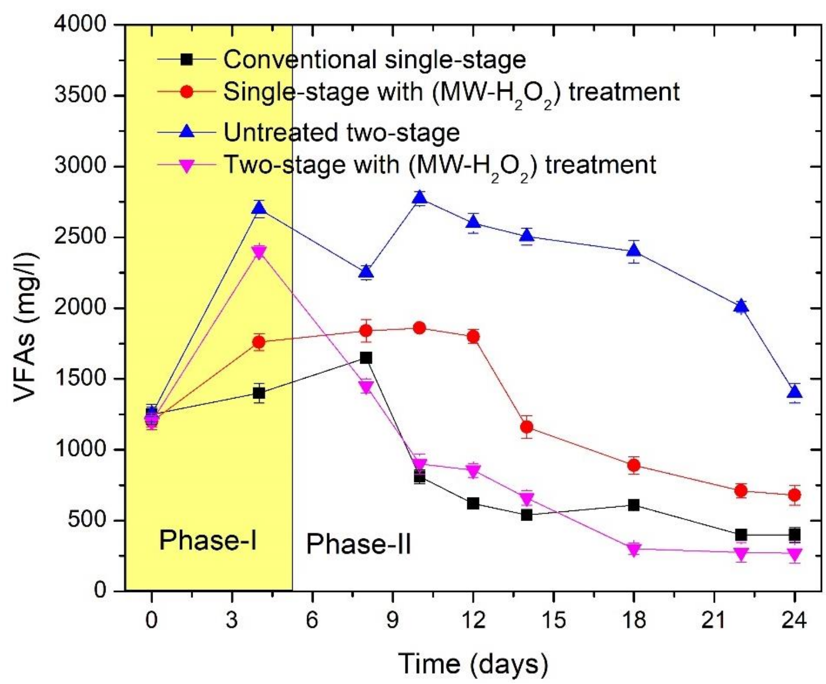


Figure 5.4: Evolution of volatile fatty acids (VFAs) along the anaerobic digestion process of all four digester set-ups.

The partial hydrolysis achieved in the thermophilic phase (of two-stage digestion) can be due to both physical heating and chemical/ biological activity. Intracellular superoxide radical levels were studied to understand the oxidative stress condition of both the digesters (Figure 5.5a). The s$[O_2^{\bullet-}]$ levels were observed to be 23 to 93 % higher in thermophilic two-stage digestion compared to the respective initial phase of the single-stage digester. This indicates that thermophilic (55 °C) treatment induces temperature mediated oxidative stress on micro-organisms.

Table 5.2: Characteristics of one-stage and two-stage anaerobic digestion

Parameters	Conventional single-stage (without pre-treatment)	Single-stage (with $MW\text{-}H_2O_2$) pre-treatment	Two-stage (without pre-treatment)	Two-stage (with $MW\text{-}H_2O_2$) pre-treatment
pH	6.1-7.3	6.1-7.1	5.5-7.7	6-7.5
VS reduction %	43.7 ± 1.5 [a]	53.3 ± 3.8 [a]	61.6 ± 1.2 [a]	73.4 ± 2.2 [a]
TS reduction %	40.6 ± 1.8 [a]	50 ± 2 [a]	52.1 [a]	58 ± 2 [a]
COD removal %	57.61 ± 1.2 [a]	60.46 ± 0.6 [a]	68 ± 1.3 [a]	81.2 ± 0.18 [a]
Methane yield (ml/ g VS)	58.81	91.08	117.37	234.57
Methane yield (ml/ g tCOD)	40.41	65.89	76.44	143.45
Maximum methane % achieved	51	64	65	71
Fecal coliform (CFU/ml) [b]	>2.7×10^6	1.12×10^5	1.19×10^6	0.064×10^6

[a] Data are averages ± standard deviation calculated from the results of triplicate measurements.

[b] Faecal coliform results are presented in the colony-forming unit (CFU) which represents the viable bacterial count in the tested sample.

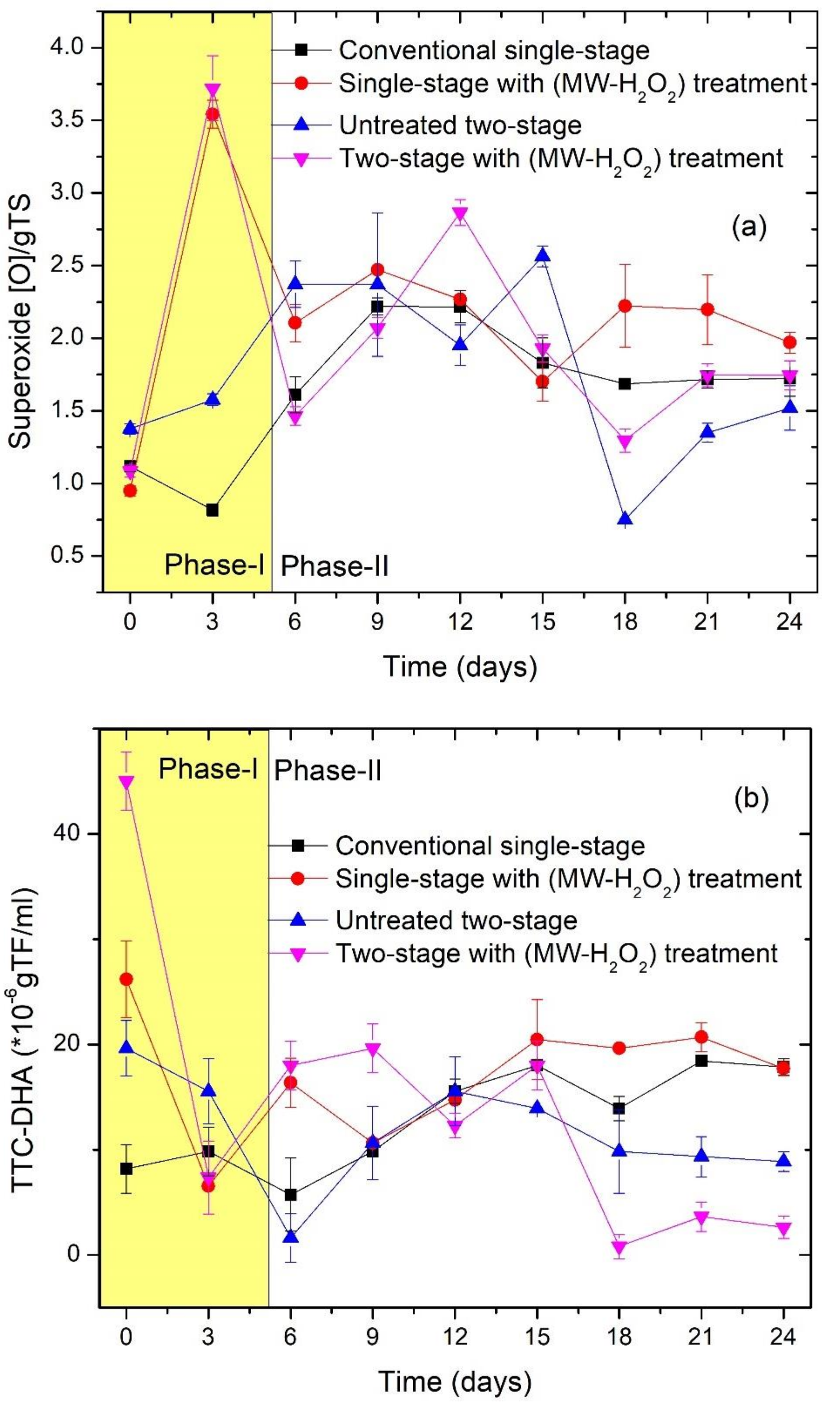


Figure 5.5: Evolution of (a) intracellular superoxide radical concentration s$[O_2^{\bullet -}]$ and (b) TTC-DHA activity on all four digester set-ups.

Sarkar and Suraishkumar (2011) have reported that *Bacillus subtilis* has shown 314 % higher s$[O_2^{\bullet -}]$ levels upon exposure to 55 °C for 1 hour compared to 37 °C. Although the author reported that temperature mediated superoxide did not cause DNA fragmentation, rather leads to lipid peroxidation and subsequent necrotic cell death. However, in the current research, 17 ± 2 (x 10^{-6} gTF/ml) sludge bioactivity (TTC-DHA) was observed in II-stage digester compared to 9 ± 0.8 (x 10^{-6} gTF/ml) I-stage digester at days 0 to 3 which is shown in Figure 5.5b. This implies that the initial thermophilic phase enhances the metabolic activity of the microbial community through oxidative stress in the dual two-stage digesters. This also goes concurrent with the enhanced solubilization and acidification in the initial thermophilic phase. The TTC-DHA activity is observed to decrease at day 6 in both the digesters (Figure 5.5b). The decrease in TTC-DHA activity corresponds to the accumulation of VFAs in the digester (Chung and Neethling, 1989). The initial thermophilic phase helps to achieve maximum acidification in two-stage digestion. The s$[O_2^{\bullet-}]$ is maintained at 2.19 ± 1 µmoles/g TS at peak methanogenic phase (days 9 to 15) in both digesters. Although the TTC-DHA activity remains similar in both reactors, methane production is heightened in the two-stage digester compared to the single-stage digester during this period (Figure 5.2); which is possibly due to enhanced solubilization and acidification achieved in phase-I of the two-stage digester system.

Disposal of activated sludge undergoes strict environmental regulations due to the presence of faecal coliform. One of the targets of current research work is the destruction of faecal coliforms and *Escherichia coli* in effluent anaerobic digestate. The two-stage mode of operation caused a 0.35 log (CFU/ml) decrease in faecal coliform levels compared to the one-stage digester at the end of 24 days SRT (Table 5.2). Two-stage digestion has a significant effect on pathogen removal, as a consequence of the initial thermophilic phase, which achieves high levels of pathogen destruction and helps in producing “class-A” biosolids, as per the requirement of US EPA (United States Environmental Protection Agency) (Riau et al., 2010). Overall, the dual two-stage mode of operation has shown improved initial sludge hydrolysis, biogas production and quality, %VS reduction, COD reduction and also achieved a significant pathogen removal.

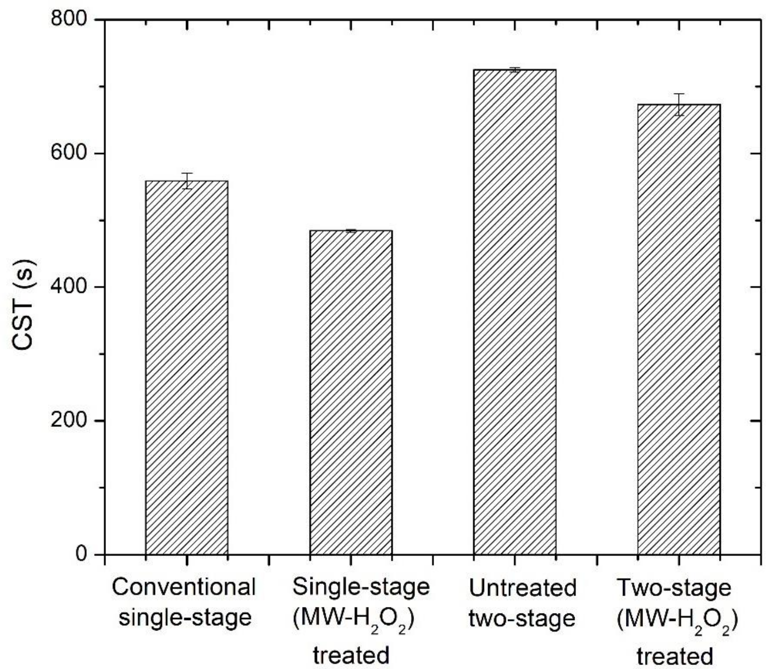


Figure 5.6: Comparison of dewaterability of effluent digestate from all four anaerobic digesters based on capillary suction time (CST)

Although the two-stage mode of operation improved inherent anaerobic digestion efficiencies and pathogen destruction, dewaterability has been a concern over the years. The dewaterability of dual anaerobic digestate was studied by capillary suction time (CST) measurement which is presented in Figure 5.6. Many factors influence the dewaterability of digested sludge including EPS, particle size, floc structure and also the digestion temperature (Zhou et al., 2014). It was found from Figure 5.6 that the dewaterability of digestate deteriorated in the two-stage mode of operation compared to conventional single-stage. This could be due to the EPS fractions released during the initial thermophilic phase of the two-stage operation (Riau et al., 2010).

5.3.2 Effect of hybrid (MW-H_2O_2) feed sludge pre-treatment on anaerobic digestion efficiency

The efficiency of hybrid microwave-oxidative (MW-H_2O_2) pre-treatment in sludge anaerobic digestion is studied in conventional single-stage and dual two-stage anaerobic digesters. No significant difference in TS and VS (%TS) after treatment suggests that no organic compound was vaporized during pre-treatment of sludge. Also, no significant amount of water was vaporized during the pre-treatment. MW-H_2O_2 pre-treatment is considered to be an efficient technology in enhancing sludge solubilization, thereby helps in overcoming the slow-hydrolysis rate barrier in the anaerobic digestion process (Eskicioglu et al., 2008), which was also witnessed in the studies presented in section 4.3.2 of chapter 4. Besides, the hybrid pre-treatment increases the release of biopolymer from the EPS matrix and influences the dewatering capability of the sludge as discussed in chapter 4. However, various contradictory results on its efficiency in subsequent anaerobic digestion and biogas production were reported. Reduction in methane yield and inhibition of anaerobic digestion have been observed with MW-H_2O_2 pre-treatment, and this is mainly due to the H_2O_2 dosage strategy (Eskicioglu et al., 2008; Liu et al., 2017). In the current research, microwave-oxidative (MW-H_2O_2) feed sludge pre-treatment significantly increased cumulative methane gas yield in both the conventional single-stage and dual two-stage anaerobic digesters which is presented in Figure 5.2. At 12 days, pre-treated single-stage digester operation yielded a cumulative methane yield of 40 ml/g tCOD equivalent to 24 days operation without pre-treatment (Figure 5.2a). By the end of 24 days of digestion, sludge pre-treated digester yielded 65.8 ml/g tCOD of cumulative methane yield. Apart from biogas production, the quality of biogas was also improved in treated digesters (Figure 5.2b). The treated digester achieved a maximum of 64 % methane content at day 15 (Figure 5.2b). A similar increase in methane production was also observed in dual two-stage digesters wherein, treated digester produced 143.4 ml/g tCOD cumulative methane yield at the end of 24 days of SRT (Figure 5.2a). Also, the biogas quality is greatly improved as the substrate is transferred into the methanogenic phase. The methane content achieved a maximum of 71 % by day 11 in the treated two-stage digester, and the methane production decelerated from day 13 (Figure 5.2b). The average methane content of two-stage digester in phase II is 59.1 %, which is 1.25-fold higher than the single-stage treated digester. The increase in biogas quality in initial days of phase-II (mesophilic) operation could be due to a syntrophic association of hydrogenotrophic methanogens and acetoclastic methanogens (Ghasimi et al., 2015).

Furthermore, the decrease in biogas production in previous studies was reasoned with the generation of refractory compounds and poor biodegradability of soluble organics (Eskicioglu et al., 2008; Liu et al., 2017). The biochemical methane potential of anaerobic digestion is mainly associated with its initial hydrolysis efficiency. Ozon et al, reported a 517 % increase in initial sCOD concentration and consequently, 38 % increase in methane yield achieved by H_2O_2/MW

(H_2O_2 dosage- 1 g H_2O_2/ g TS) pre-treatment, whereas MW treatment resulted in 158 % and 64 % increase in sCOD solubilization and methane yield respectively (Özön and Erdinçler, 2019). Although higher H_2O_2 dosage (>0.6 g H_2O_2/g TS) increases sludge solubilization, it does not aid in sludge biodegradability and ultimate methane production (Liu et al., 2017; Özön and Erdinçler, 2019). During thermal pre-treatment, higher treatment temperatures lead to the formation of melanoidins through Maillard reactions that affect sludge degradability (Carrère et al., 2010). To avoid the formation of such refractory compounds, the current pre-treatment was done with 1 % H_2O_2/TS. Also, inactivation of catalase was taken care of by initial microwave irradiation at 80 °C (Liu et al., 2017). Hybrid sludge pre-treatment achieved a COD solubilization of 22.12 % compared to 10.3 % in the conventional single-stage digester (Figure 5.7). A comparison of COD solubilization with microwave specific energy input has been presented in Table 5.3. Comparatively, higher COD solubilization was observed in the current study with lesser specific energy, for a sludge characterized with relatively higher TS content.

Table 5.3: Comparison of microwave irradiation energy with different pre-treatments (Ebenezer et al., 2015)

Treatment method	sCOD/ tCOD %	Specific energy (kJ/ kg TS)	Total solids (g/l)	References
Ultrasonication	10	108000	17.81	(Salsabil et al., 2009)
Microwave	19	23000	26	(Ahn et al., 2009)
Microwave	18.6	82400	11.6	(Uma Rani et al., 2013)
Ultrasonication	22	18000	14.38	(Feng et al., 2009)
Microwave	31	14000	14.4	(Ebenezer et al., 2015)
Microwave (MW-H_2O_2)	22.12	16176	27	This study

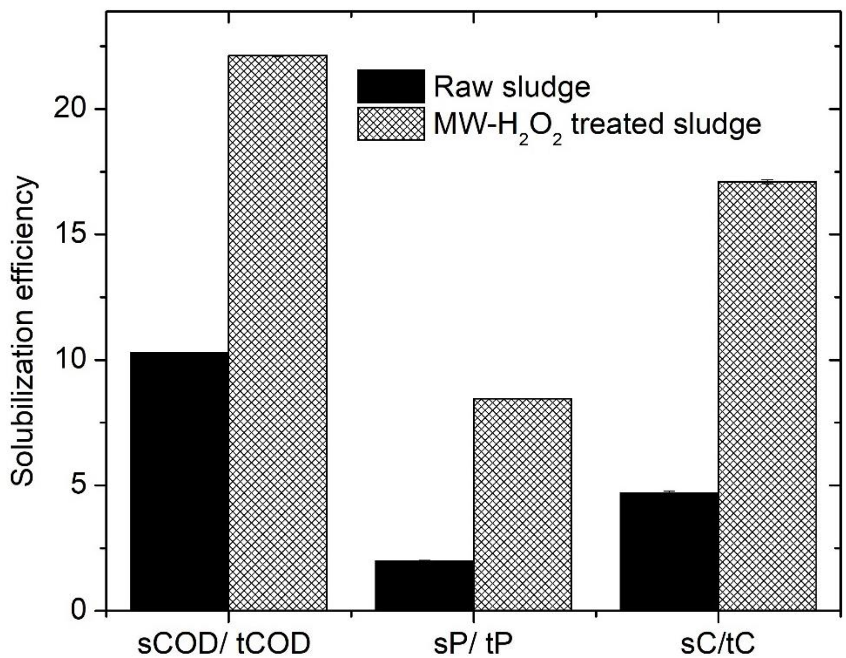


Figure 5.7: Comparison of solubilized fraction percentage of raw sludge and MW-H_2O_2 treated sludge.

sCOD/tCOD= ratio of soluble chemical oxygen demand to total chemical oxygen demand; sP/tP=ratio of soluble protein to total protein; sC/tC= ratio of soluble carbohydrate to total carbohydrate.

As protein and carbohydrate constitute the major fraction of waste activated sludge, hydrolysis efficiency can be expressed by the ratio of soluble to total fractions of protein and carbohydrates. The release of carbohydrates into soluble fraction was observed to be higher than protein by hybrid pre-treatment which is shown in Figure 5.7. This result is concurrent with previous researches where carbohydrate solubilization is higher compared to protein by microwave treatment (Zhou et al., 2010). This implies that MW-H_2O_2 pre-treatment breaks down microbial cells and releases intracellular substances as soluble organics into the surrounding medium. The enhanced initial sludge solubilization achieved by hybrid pre-treatment results in improved biodegradability of organics and subsequent biogas production. The consumption of sCOD in the two-stage treated digester is faster compared to the single-stage treated digester at days 2 to 14; indicating enhanced acidogenesis and methanogenesis (Figure 5.3a). This is evident from increasing VFAs production in two-stage treated digester compared to the single-stage treated digester at the initial thermophilic phase (Figure 5.4). After the transfer of substrate to the methanogenic reactor at day 5, rapid consumption of VFAs is observed in the two-stage treated digester, which reflects in enhanced methane yield (Figure 5.4 & 5.2). Hybrid pre-treatment followed by two-stage anaerobic digestion favors the evolution of methanogenic population across both phases, thereby facilitating both acidogenesis and methanogenesis (Liu et al., 2018). At the end of 24 days SRT, two-stage (MW-H_2O_2) digester achieved 73 % VS reduction, which was the maximum VS reduction achieved throughout this study.

Microwave enhanced advanced oxidation process induces oxidative stress during anaerobic digestion (Eswari et al., 2016). An acute spike in s$[O_2^{\bullet-}]$ levels is observed in both single-stage and two-stage treated digesters at day 3, which indicates prolonged oxidative stress exerted by hybrid (MW-H_2O_2) treatment on sludge (Figure 5.5a). This phase is also characterized by increased COD solubilization and decreased TTC-DHA activity (Figure 5.3a and 5.5b). The prolonged oxidative-stress mediated by s$[O_2^{\bullet-}]$ radicals lead to solubilization of sludge matrix and damage microbial cells, which could result in decreased TTC-DHA activity. The insignificant s$[O_2^{\bullet-}]$ levels at day 0 could be due to one of two reasons. (a) Inactivation of $[O_2^{\bullet-}]$ by superoxide dismutase generated by active anaerobic population as an oxidative stress response (b) rapid conversion of $[O_2^{\bullet-}]$ to $OH^{\bullet}$ that ultimately causes microbial death according to equation 2.19. On account of enhanced TTC-DHA activity at day 0 in both digesters, it can be interpreted that superoxide levels are subdued through oxidative stress response exhibited by the active microbial population which is shown in Figure 5.5b. Enhanced biological oxidation increases sludge activity as measured by TTC-DHA (Liu et al., 2005). The TTC-DHA activity is observed to achieve a maximum of 18.5 ± 1 (x 10^{-6}gTF/ml) in the peak methanogenesis phase (days 6 to 15) except day 12, in the two-stage treated digester. Whereas, the maximum activity achieved in the one-stage treated digester is 19.63 ± 1.5 (x 10^{-6}gTF/ml) on days 19 to 24. The sludge activity declines from day 15 in the two-stage digester and a concurrent decline in biogas quality is also observed in methane production (Figure 5.5 b & 5.2 b). The hybrid treatment in two-stage anaerobic digestion reduces SRT to 15 days compared to 24 days of SRT in digesters without treatment.

Hybrid (MW-H_2O_2) sludge pre-treatment is an efficient sludge conditioning method before disposal of excess sludge. The synergetic effect of both treatments causes cellular level damage and greater pathogen destruction. Yu et al reported that microwave irradiation of 70 °C with 0.08 % or higher H_2O_2 (w/w) eliminated faecal coliform without regrowth in municipal sewage sludge

(Yu et al., 2010). In addition, acidifiers in two-stage anaerobic digestion can achieve significant pathogen reduction along with hybrid MW-H_2O_2 treatment (Zhang et al., 2017). In the current study, the effect of MW-H_2O_2 treatment and two-stage anaerobic digestion on pathogen levels were detected using the coliform assay as shown in figure 5.8. As seen from table 5.2, the single-stage (MW-H_2O_2) treated digester showed a 1.38 log (CFU/ml) reduction of faecal coliform compared to untreated conventional single-stage. Two-stage (MW-H_2O_2) treated digester showed 1.27 log (CFU/ml) and 1.62 log (CFU/ml) reduction of faecal coliforms compared to untreated two-stage and untreated single-stage digester respectively (Table 5.2). In comparison with various systems, dual two-stage (MW-H_2O_2) treated digester has enhanced anaerobic digestion efficiency, maximum methane yield, greater solid reduction and also eliminates faecal coliform over 90 %.

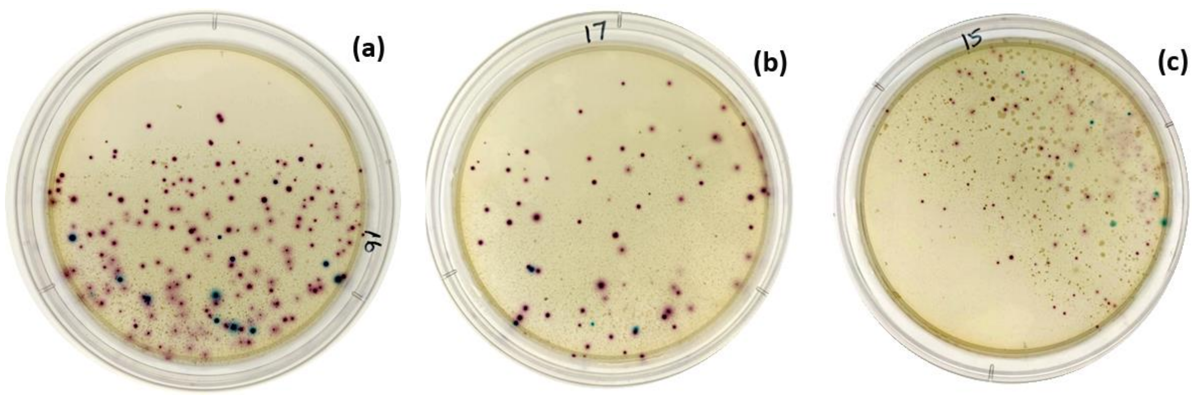


Figure 5.8: Measurement of faecal coliform through coliform assay.

(a) untreated two-stage (b) single-stage (MW-H_2O_2) treated and (c) two-stage (MW-H_2O_2) treated. The samples were appropriately diluted for the measurement.

The dewaterability of pre-treated single-stage and two-stage digesters are shown in Figure 5.6. It was observed that pre-treatment improved the dewatering capability of both single-stage and two-stage treated digesters compared to their respective untreated systems. The solubilization of proteins and polysaccharides of EPS by hybrid pre-treatment may weaken the viscoelastic properties of the digested sludge, thus assisting treatments such as sludge conditioning and dewaterability (Jibao Liu et al., 2016b). However, pre-treatment in the two-stage digester failed to recover the dewatering capability lost during the initial thermophilic phase of the operation.

5.3.3 Application of kinetic Gompertz model and determination of kinetic parameters

The experimental data obtained in the current study were used to fit with the modified Gompertz model as shown in Figure 5.9 and the kinetic parameters were tabulated in Table 5.4. The parameters P, Rm and λ of the Gompertz equation are estimated by applying a least-squares fit of equation 5.3 to experimental data. The experimental data fitted with the Gompertz model with a high correlation coefficient of 0.99. The first-order hydrolysis rate constant was determined by model fitting of Veeken and Hamelers equation 5.4.

Model-based analysis confirms that the improved performance in the dual two-stage mode of operation was due to an increased hydrolysis rate constant (Table 5.4). Also, the two-stage mode of operation has shown a significant reduction in lag-phase compared to conventional single-stage digestion. Comparing methane potential and hydrolysis rate constant, it is observed that two-stage digestion with ($MW-H_2O_2$) treatment has higher anaerobic digestion efficiency than other digesters in this study.

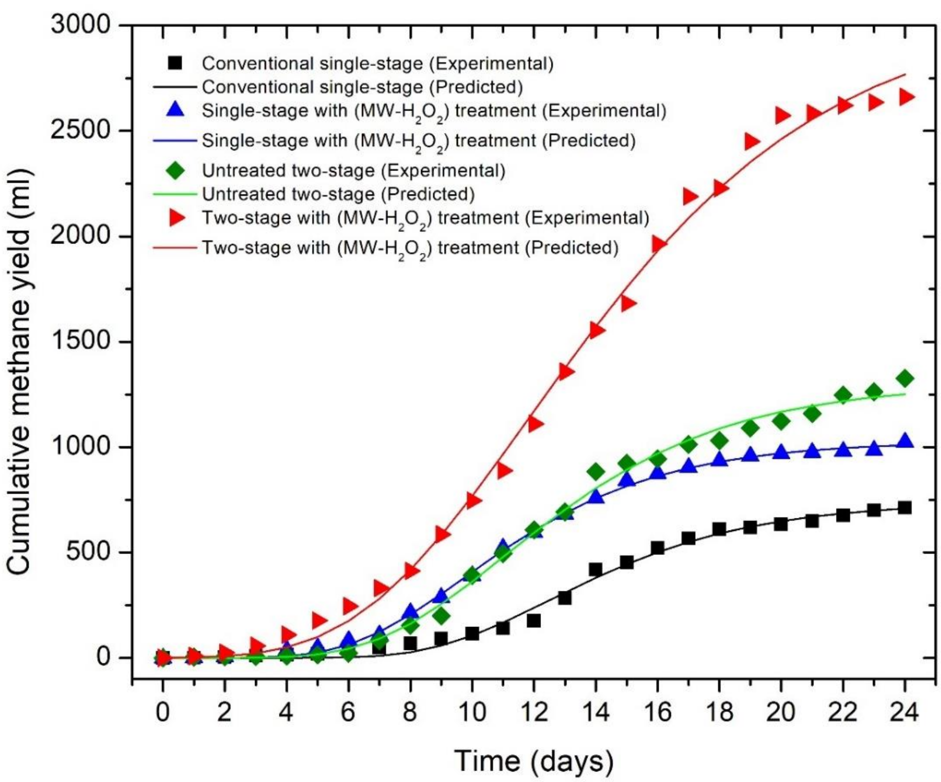


Figure 5.9: Plot showing experimental data of cumulative methane yield (ml) fit with the modified Gompertz equation for all four digester set-ups.

Table 5.4: Theoretical prediction of kinetic parameters

Model	Kinetic Parameters	Conventional single-stage digestion	One- stage ($MW-H_2O_2$) digestion	Two-stage digestion	Two-stage ($MW-H_2O_2$) digester
Modified Gompertz model	Methane potential P (ml)	742.86	1028.7	1305.7	3093.2
	Maximum daily rate Rm (ml/day)	72.76	104.9	117.1	205.8
	Lag phase λ (day)	8.7	6.1	6.9	6.3
	R^2	0.9929	0.9988	0.9952	0.9967
Veeken and Hamelers	Hydrolysis rate	0.0540	0.1207	0.1327	0.2605
Buswell formula	Anaerobic biodegradability (%)	15	23.1	30	60

5.3.4 Effluent sludge characteristics of two-stage anaerobic digestion

5.3.4.1 Functional group analysis- FTIR

The functional group analysis of anaerobic digestates was carried out through FTIR spectroscopy to determine the predominant organic substances present. The FTIR spectroscopic plots of effluents collected from untreated and pre-treated two-stage anaerobic digestions are presented in Figure 5.10.

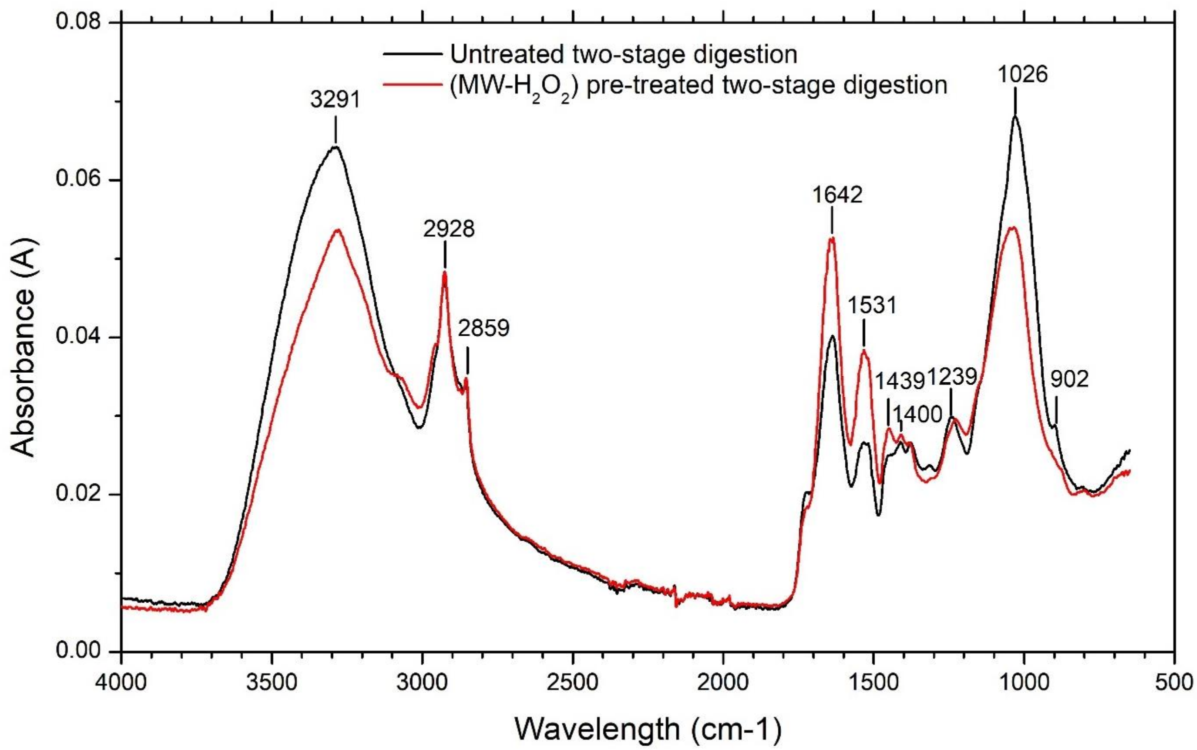


Figure 5.10: FTIR spectra of effluent digestates from untreated and pre-treated two-stage anaerobic digestions.

In the bandwidth of 3100-3500 cm^{-1}, a strong broad peak was observed in both the samples indicating -OH functional groups (alcohols, carboxylic acids, phenols and water) and a medium peak at 2928 cm^{-1} due to -CH stretching vibrations (alkanes and aldehydes) (Gómez-Ordóñez and Rupérez, 2011; Hong et al., 2017). The peak intensity at 3291 cm^{-1} was higher in the untreated digestion compared to the treated fraction. Similarly, a higher relative intensity was observed in the untreated digestion in the bandwidth 1250-1020 cm^{-1} which corresponds to -CN stretching (amines). This may reflect the presence of undigested carbohydrates and proteins in the untreated digestion. The peak in 2859 cm^{-1} may signify the symmetric stretch vibration of CH in -CH_2 exhibited by alkanes and fatty acids. Besides, the peak at 2928 cm^{-1} may also correspond to the decomposed fatty acids and lipids (Hong et al., 2017). The slightly higher peak intensity in untreated digestion at 1700 to 1725 cm^{-1} (C=O stretching) may correspond to free fatty acids left in the untreated digestion (Koca et al., 2007). On the other hand, the relatively intense bands at 1439 cm^{-1} and 1400 cm^{-1} in the treated digestion may correspond to aliphatic -CH deformation of structures such as fatty acids and -OH deformation, respectively (Lguirati et al., 2005).

Furthermore, the band 1642 cm^{-1} may correspond to aromatic C=C bonds and C=O stretching of primary amides and ketone, which signify the amount of amino acids and peptides (Cuetos et al., 2010; Hong et al., 2017; Wassate et al., 2014). Similarly, the strong band at 1531 cm^{-1} may correspond to C=N stretching in secondary amides (Cuetos et al., 2010). When comparing with untreated digestion enrichment of amides and nitrogen-containing compounds were observed in pre-treated digestion. This may be due to the difference in carbon lost in the microbial decomposition of alcohols, acids and biogas (CH_4 and CO_2) produced during the anaerobic digestion (Cuetos et al., 2010).

5.3.4.2 Rheological behavior

The rheological behavior of sludge is dependent on various factors such as solid concentration, sludge pre-treatment method, temperature and sludge age (Hong et al., 2016; Ratkovich et al., 2013). In the current study, the rheological properties of the four anaerobic digestions were investigated to understand the effects of sludge pre-treatment (MW-H_2O_2) and two-stage anaerobic digestion on the flow behavior of effluent sludge. The effluent sludge samples were collected on the final day of single-stage and two-stage mode of operations. The stored samples were brought down to room temperature for consistency before the measurement. The rheograms of the effluent sludge were generated as shown in Figure 5.11. It was observed that both two-stage mode of operation and pre-treatment had influenced the rheological behavior of the digested sludge compared to conventional mesophilic digestion (untreated single stage). The shear stress increased non-linearly with the shear rate in all conditions, indicating the non-Newtonian behavior of digested sludge samples (Figure 5.11a). In addition, a decrease in apparent viscosity was observed at low shear rates demonstrating a shear-thinning property of the digested sludge samples (Figure 5.11b). On comparison of all samples, (MW-H_2O_2) pre-treated anaerobic digestions showed decreased viscosity, indicating improved sludge flowability (Figure 5.11b). Besides, the increased rate index (n>1) indicates weakened internal structures of the sludge (Jibao Liu et al., 2016b), which was predominantly observed in the (MW-H_2O_2) pre-treated anaerobic digestions (Table 5.5). This is concurrent to the effects of hybrid pre-treatment on biopolymer solubilization and microbial floc breakdown before anaerobic digestion (Figure 5.7).

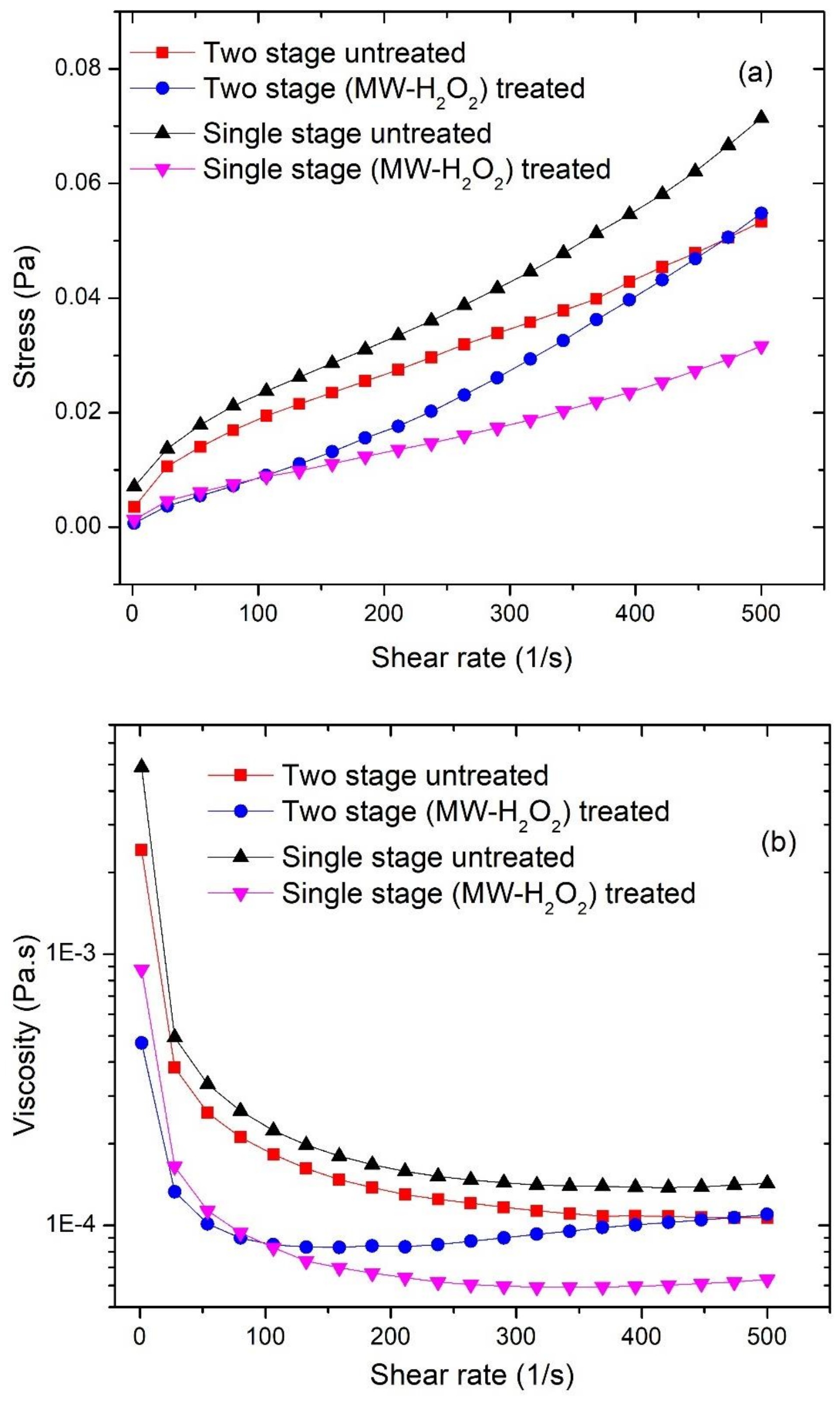


Figure 5.11: Rheograms- (a) Shear stress vs shear rate and (b) viscosity vs shear rate of effluent digestate from all four anaerobic digestions.

Furthermore, the rheograms were fitted to rheological models such as Herschel-Bulkley (equation 5.7) and Carreau-Yasuda (equation 5.8) to determine the yield stress (Pa) and apparent viscosity (Pa.s) (Table 5.5). The hybrid (MW-H_2O_2) two-stage digestion yielded the lowest viscosity (7.93 x 10^{-6} Pa s) determined by the Herschel-Bulkley model. The zero-rate viscosity determined by the Carreau-Yasuda model also showed a similar trend with pre-treated two-stage digestion being the lowest among all four. The improved rheological characteristics of two-stage and pre-treated conditions could be due to the improved TS reduction achieved in both compared to the conventional single-stage digestion (Table 5.2). Higher TS concentration could result in a stronger network of sludge floc structure which contributes to an increase in yield stress and viscosity (Cao et al., 2016; Yeneneh et al., 2016). Besides, smaller TS concentration favors the dewatering process due to its rheological characteristics (Baudez et al., 2011; Sanin, 2002; Yeneneh et al., 2016). Similarly, improved dewaterability was observed in the current research in digestates with (MW-H_2O_2) pre-treatment (Figure 5.6).

Table 5.5: Rheological parameters determined through models

Rheological parameters		Single-stage (untreated)	Single-stage with (MW-H_2O_2) treatment	Two-stage (untreated)	Two-stage with (MW-H_2O_2) treatment
Herschel-Bulkley	Yield stress (Pa) (*10^{-3})	10.85	3.14	5.45	2.55
	Viscosity (Pa. s) (*10^{-6})	73.10	25.87	268.05	7.93
	Rate index (n)	1.07	1.11	0.82	1.41
	R^2	0.9918	0.9926	0.9935	0.9987
Carreau-Yasuda	Zero-rate viscosity (*10^{-4}) (Pa. s)	48.95	36.98	24.17	5.35
	Infinity-rate viscosity (*10^{-5}) (Pa. s)	10.99	5.77	7.35	9.26
	R^2	0.9981	0.9977	0.9991	0.9442

5.4 Conclusion

The present study reports the effects of hybrid (MW-H_2O_2) feed sludge pre-treatment and dual two-stage anaerobic digestion processes on performance enhancement of commonly used single-stage anaerobic digester. Anaerobic digestion efficiency of mixed activated sludge shows that higher methane production and improved biogas quality is achieved in two-stage anaerobic digestion with pre-treatment. The enhanced anaerobic digestion is attributed to initial sludge solubilization achieved through pre-treatment and heightened microbial activity in the thermophilic phase of two-stage digestion. Furthermore, the two-stage mode of operation helps in optimizing acidification and methanogenesis in the anaerobic digestion process. The research also identifies that superoxide radicals of Fenton type oxidation play a significant role in maintaining oxidative stress in the system and also, they are reliable indicators of the oxidative status of the system. Also, pre-treatment followed thermophilic anaerobic digestion corresponds to maximum pathogen reduction and helps in producing “Class-A” biosolids for disposal. However, the two-stage mode of operation with pre-treatment fails to retain good dewaterability of effluent sludge. Overall, dual two-stage anaerobic digestion integrated with hybrid feed sludge system promotes the hydrolysis of feedstocks and acidogenesis in thermophilic range and ensures higher syntrophic acetogenesis and methanogenesis in the subsequent mesophilic stage and therefore may be an effective strategy in performance enhancement of conventional mesophilic anaerobic digestion and overcome some of its limitations.

CHAPTER 6

IMPACT OF PRE-TREATMENT ON ANAEROBIC CO- DIGESTION OF SEWAGE SLUDGE WITH FRUIT AND VEGETABLE WASTES

Abstract

The findings of the previous chapter confirm that the two-stage mode of operation along with hybrid pre-treatment is efficient in enhancing the biodegradation of mixed WAS. The combined strategy was further validated with anaerobic co-digestion of fruit and vegetable waste (FVW) with WAS. The current study involves the optimization of the mixing ratio of FVW and BMWAS for process stability and biogas production in two-stage anaerobic digestion. A mixing ratio of 75 % BMWAS and 25 % FVW showed a 1.6-fold increase in overall methane yield and achieved 0.38 volatile fatty acids to alkaline buffer capacity ratio (FOS/TAC) compared to a mixture of 50 % BMWAS and 50 % FVW. Application of hybrid (MW-H_2O_2) pre-treatment in the former mixing ratio increased sludge solubilization by 33 % and consequently enhanced overall methane yield by 2.17-fold. The treated digester showed increased process stability with a FOS/TAC ratio of 0.26 as a consequence of buffer capacity offered by released biopolymers during pre-treatment. The generation of superoxide radicals during anaerobic digestion was studied and found to negatively correlate with sludge bioactivity. Two-stage digestion also minimizes the issue of high acidification caused by FVW.

6.1 Introduction

In the previous chapter (5), the mono-digestion of activated sludge in two-stage anaerobic digestion was discussed with the merits of hybrid (MW-H_2O_2) pre-treatment. The two-stage operation enhanced the biomethanation of WAS with increment in methane percentage and effluent digestate quality. However, the stability of the two-stage digester under acidifying substrate conditions was yet to be understood. As seen from the previous chapter, the assimilation of VFAs in the phase-I digester and their subsequent utilization in the phase-II digester are crucial

This chapter has been published. Ambrose, H.W., Philip, L., Suraishkumar, G.K., Karthikaichamy, A., Sen, T.K., 2020. Anaerobic co-digestion of activated sludge and fruit and vegetable waste: Evaluation of mixing ratio and impact of hybrid (microwave and hydrogen peroxide) sludge pre- treatment on two-stage digester stability and biogas yield. J. Water Process Eng. 37. https://doi.org/10.1016/j.jwpe.2020.101498

in maintaining both the digester stability and biogas production. Besides, the ability of the two-stage digester to co-digest complementary substrates with pre-treatment conditions was yet to be explored. Therefore, the performance efficiency of two-stage operation was further studied by anaerobic co-digestion of WAS with fruit and vegetable waste (FVW) in the current chapter.

FVW constitute one of the major organic wastes produced from different sources and in Western Australia, around 30 % of production end up as waste (Ghosh et al., 2016). The disposal of FVW in large quantities in landfills is difficult due to its high biodegradable organic content and subsequent environmental impacts (Ji et al., 2017; Mata-Alvarez et al., 2000). Therefore, one of the alternatives to landfills of these large quantities of FVW is the utilization and valorization of the residue in the production of biogas and high-quality organic-rich digestate through the anaerobic co-digestion process.

Fermentation of FVW in anaerobic digestion faces severe acidification issues that inhibit the growth of the methanogenic population due to its high sugar content and VS fraction (Scano et al., 2014). Moreover, the high C/N ratio of FVW leads to nitrogen depletion in anaerobic digestion and requires an additional substrate that can sustain fermentation efficiency (Ji et al., 2017). For these reasons, co-digestion of FVW with complementary substrates such as kitchen waste, agricultural waste and sewage sludge have been tried out (Ji et al., 2017). The co-digestion of sewage sludge with FVW have shown improvement in biogas yield and agronomic digestate quality (Di Maria et al., 2014; Gómez et al., 2006). Besides, the acidic nature of FVW has the potential to enhance the effects of hybrid (MW-H_2O_2) pre-treatment in anaerobic co-digestion with activated sludge (Eswari et al., 2016). And also, phase separation of the microbial population by two-stage operation offers better control over the respective phases and thereby help overcome the adverse effects of acidification.

Therefore, the co-digestion of FVW with activated sludge was studied in the two-stage operation and the effects of hybrid (MW-H_2O_2) pre-treatment on digestion efficiency was evaluated. In the current chapter, two different mixing ratios of FVW and mixed activated sludge were evaluated based on biomethanation and process stability, followed by the studies of hybrid (MW-H_2O_2) pre-treatment. The impact of hybrid (MW-H_2O_2) pre-treatment on anaerobic digestibility of co-digestion is evaluated based on biogas production, biogas quality, sludge hydrolyzation and process stability. In addition, the effects of pre-treatment on the oxidative status of anaerobic digestion mediated by superoxide radicals were studied along with sludge bioactivity to establish knowledge on reactive oxygen species in anaerobic co-digestion.

6.2 Materials and methods

6.2.1 Sludge sampling and characterization

The FVW was collected from Spudshed supermarket in Perth, WA. The FVW for experimentation was prepared by blending the components given in Table 6.1. The composition of FVW was kept constant in the study for the reproducibility of the experiment.

Table 6.1: Percentage composition of fruit and vegetable waste (FVW)

Component	**Percentage**
Cabbage	25
Eggplant	20
Zucchini	25
Potato	20
Broccoli	5
Tomato	1
Nectarine	4
Total	100

Table 6.2: Initial characterization of BMWAS and FVW

Parameters	**BMWAS[a]**	**FVW[a]**
pH	6.6	5.0
tCOD (mg/l)	36566 ± 94	50800 ± 81
sCOD (mg/l)	3500 ± 163	29100 ± 216
TS (mg/l)	27000 ± 81	37000 ± 282
VS (mg/l)	21333 ± 94	35810 ± 94
VS (%TS)	79.01	96.78

[a] Data are averages ± standard deviation calculated from the results of triplicate measurements.

The BMWAS used in the current study was withdrawn from the mixed sludge sampling point from BWWTP as detailed in the previous section 3.2. All the samples were freshly collected/

prepared and used for experiments to avoid any microbial contamination during storage. All samples were characterized immediately for pH, chemical oxygen demand, total solids and volatile solids (Table 6.2).

6.2.2 Feedstock pre-treatment

A sequential hybrid (MW-H_2O_2-MW) pre-treatment was carried out as detailed in section 3.6.3. Briefly, 450 ml of feedstock with a constant solid concentration of 28.6 ± 1 g/l TS was taken in a 500 ml beaker and subjected to microwave treatment with a power density of 1.46 W/ml. The feedstock was initially heated at 80 °C by microwave irradiation at 660 W for 2 minutes. Followed by, the addition of hydrogen peroxide (1 % w/w H_2O_2/TS). The sludge was again microwave irradiated at 660 W for 3 minutes, with an intermittent break to avoid water evaporation.

6.2.3 Anaerobic digestion studies

The two-stage set-up used in the current study consists of phase-I thermophilic and phase-II mesophilic reactors connected in a semi-continuous fashion as mentioned in section 3.7. Both phases were acclimatized with feedstock (BMWAS+FVW) and 10 % ADS as inoculum, under respective temperature conditions over a period of 4 weeks. The hydraulic retention time (HRT) of phase-I and phase-II digestions were maintained as 3 days and 17 days, respectively. Two different feedstocks F_1 (1:1, BMWAS: FVW) and F_2 (3:1, BMWAS: FVW) were produced and evaluated for anaerobic process stability. Both feedstocks were developed with a constant 28.6 ± 1 g/l TS concentration. Followed by a study on hybrid (MW-H_2O_2) pre-treatment on anaerobic co-digestion with feedstock F_2 was conducted. In brief, three two-stage anaerobic digestions were conducted with mixed feedstock (BMWAS+FVW) as represented in Figure 6.1.

6.2.4 Analytical methods

For the current study, various parameters such as pH, temperature, tCOD, sCOD, TS/VS, protein, carbohydrate, VFAs, alkalinity, DHA and superoxide concentration of the sludge were measured. A detailed description of the analysis and equipment used for the measurement has been given in section 3.5.

6.2.5 Calculation

The VS reduction percentage was calculated according to equation 5.1. The process stability of anaerobic digestion was measured by FOS/TAC ratio, as per equation 6.1.

$$FOS/TAC = Volatile\ fatty\ acids\ (\frac{mg}{l})/Alkalinity\ (\frac{mg}{l}) \qquad \text{equation (6.1)}$$

6.2.6 Statistical analysis

The statistical significance of differences in variation to different mixing ratios and pre-treatment conditions was tested by two-way ANOVA and Tukey's/ Sidak's multiple comparisons test. The significant difference between average FOS/TAC values was calculated using a two-tailed t-test. Pearson's correlation coefficient was computed to find the correlation between TTC-DHA and superoxide (untreated and treated). All the analyses were carried out using the statistical software GraphPad Prism 8 (GraphPad Software, Inc., CA, USA), with a level of significance $P < 0.05$.

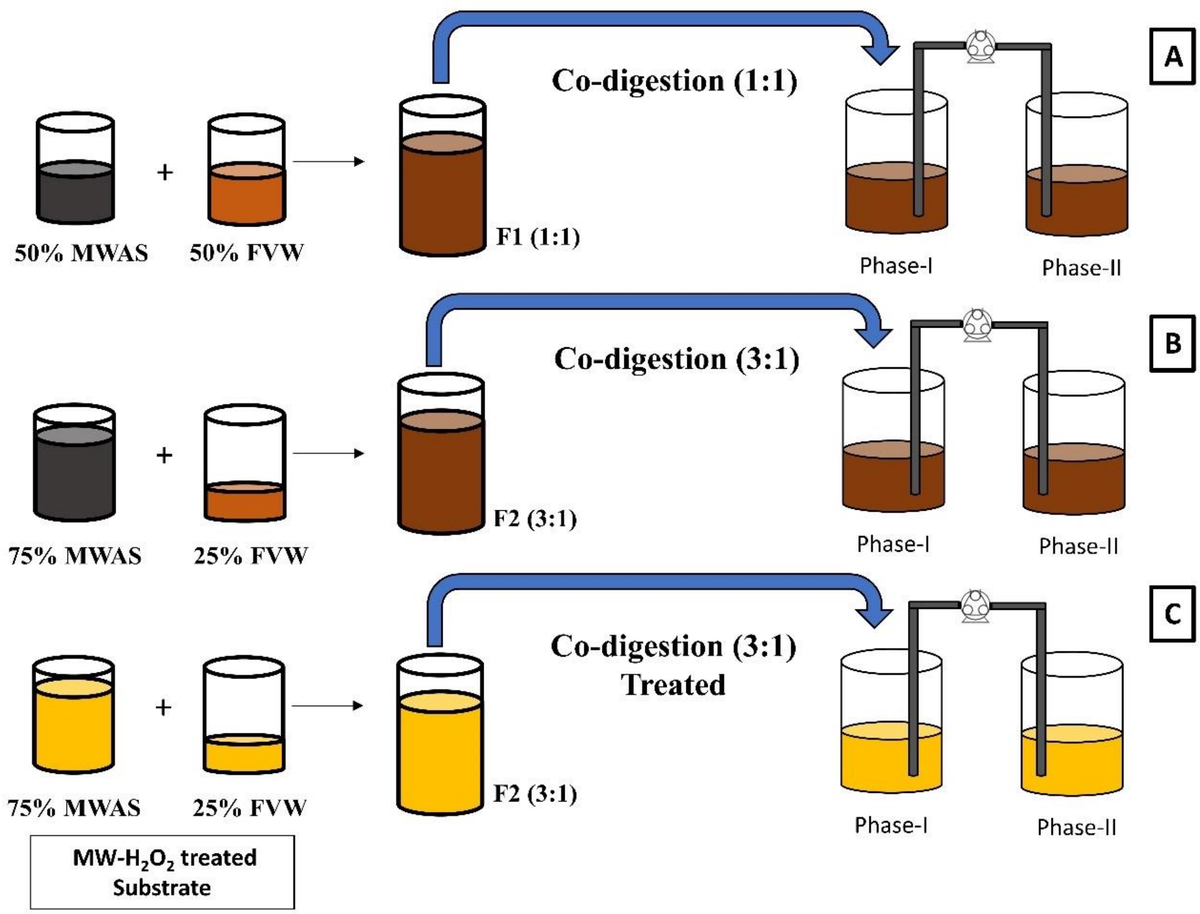


Figure 6.1: A schematic representation of anaerobic digestion studies conducted in chapter 6

6.3 Results and Discussion

6.3.1 Effect of mixing ratio in anaerobic digestibility of FVW in co-digestion

Various authors have investigated the efficiency of co-digestion of sewage sludge with FVW (Ji et al., 2017). The higher COD, TS and VS content of FVW compared to BMWAS (Table 6.2), assures higher biodegradable organic content and makes it suitable for anaerobic digestion. However, the high VS content of FVW favors quick hydrolysis, consequently followed by increased acidification which could threaten methanogenesis (Bouallagui et al., 2005). In the current study, the mixing ratio of BMWAS with FVW was optimized in two-stage anaerobic digesters at constant HRT. Co-digestion (3:1) achieved 1.6 times higher cumulative methane yield compared to co-digestion (1:1) (Figure 6.2a). In comparison with untreated mono-digestion of BMWAS in chapter 5, the co-digestion (3:1) of the current study achieved 1.27-fold higher biogas production under similar experimental conditions at 20 days retention time. Therefore, co-digestion of FVW with sludge improves the biomethanation in anaerobic digestion in accordance with earlier research work (Arhoun et al., 2019).

Cumulative methane yield of untreated co-digestion was significantly influenced by the mixing ratio of FVW and BMWAS ($P=0.0086$). A similar phenomenon of reduction in biogas yield was observed when the amount of FVW was increased from 10-20 % to 30-40 % in co-digestion of FVW with waste mixed sludge (Di Maria et al., 2014). Furthermore, the methane percentage in both digestions was observed to be low during the initial thermophilic phase (Figure 6.2b). This could be due to the increased production of carbon dioxide, hydrogen and acetate by the acidogenic microbial population in the thermophilic phase during anaerobic digestion of FVW (Park et al., 2012). The amount of methane production was significantly lower in co-digestion (1:1) compared to co-digestion (3:1) ($P=0.002$), signalling unsuitable conditions for the methanogenic population inside the digester. Compared to (1:1), co-digestion (3:1) achieved 9.1-fold higher methane production during the initial thermophilic phase, as a courtesy of improved pH stability (Figure 6.3).

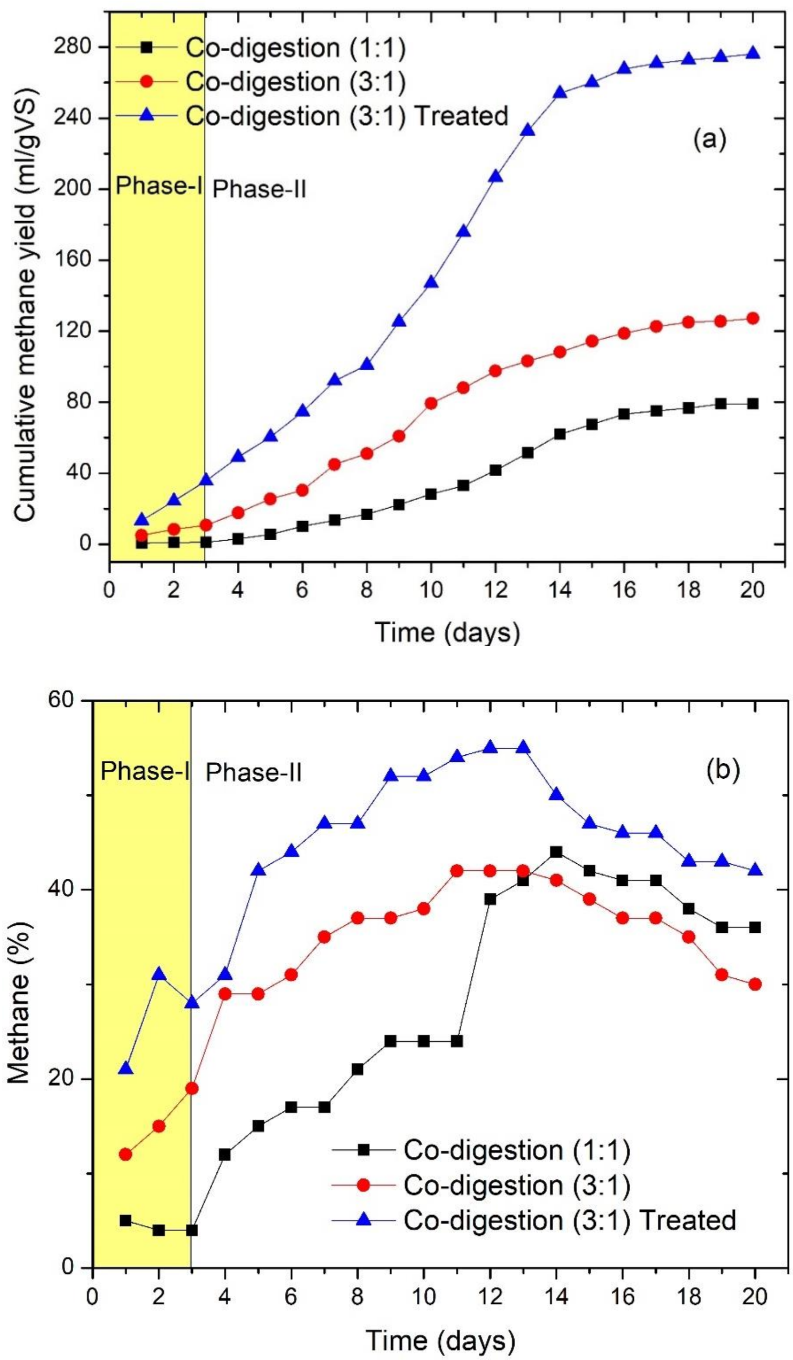


Figure 6.2: Evolution of (a) cumulative methane yield (ml/g VS) and (b) methane percentage in anaerobic co-digestion.

Co-digestion (1:1- BMWAS:FVW); co-digestion (3:1- BMWAS:FVW) and co-digestion (3:1- BMWAS:FVW) treated with hybrid (MW-H_2O_2) pre-treatment.

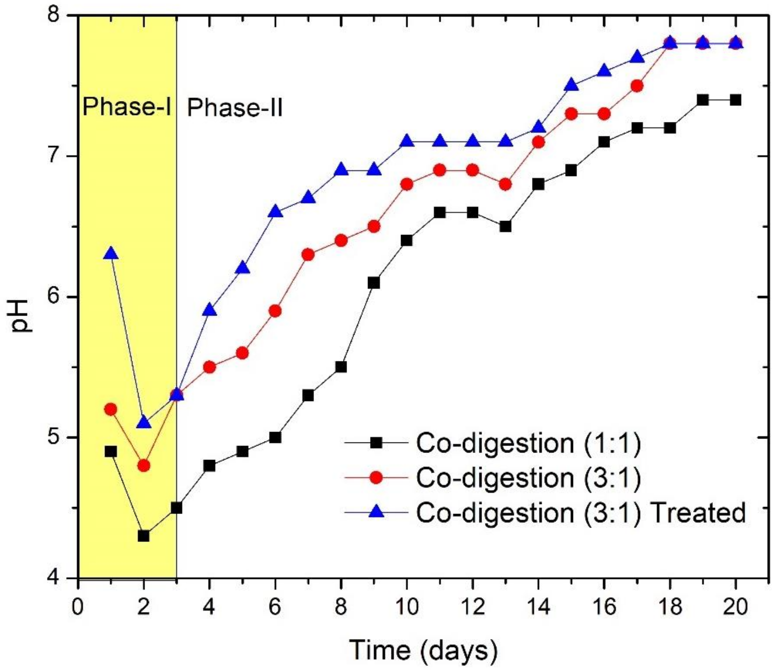


Figure 6.3: Evolution of pH in anaerobic co-digestion.

However, phase-II anaerobic digestion increased methane production by 1.49-fold in co-digestion (3:1), compared to (1:1) (Figure 6.2a). Besides, during this phase methane percentage increased significantly and a greater reduction of carbon dioxide compared to co-digestion (1:1) was observed (Figure 6.2b) (P=0.002). The average methane percentage during peak methanogenesis (days 9 to 15) was observed to be 34 % and 40 % in phase-II of co-digestion (1:1) and (3:1) respectively. This could be due to the conversion of VFAs and other metabolic products into biogas by active methanogenic population in phase-II, thereby improving the digestion efficiency. Phase separation of microbial communities in two separate digesters enables pH stabilization and also enhances the methane conversion rate of FVW (Bouallagui et al., 2005). Consequently, a 33 % higher VS removal was observed in co-digestion (3:1) compared to co-digestion (1:1), in the mesophilic phase II. Furthermore, the FVW fraction and OLR have been reported to have a significant effect on biogas production and process stability in co-digestion with sludge (Arhoun et al., 2019; Bolzonella et al., 2006; Di Maria and Barratta, 2015; Liu et al., 2012a). The overall OLR of the digestions were 1.3 g VS/l.day and 1 g VS/l.day in co-digestion (1:1) and co-digestion (3:1), respectively. Di Maria et al. reported a higher OLR (>2 kg VS/m^3.day) with an excessive fraction of FVW in co-digestion with waste mixed sludge resulted in microorganism washout and a decrease in biogas production under mesophilic condition (Di Maria et al., 2014). Whereas, in the present study it was observed that 50 % of FVW in co-digestion with sludge with an OLR of 1.3 g/l/day had a negative effect on the cumulative methane production (Table 6.3). This could be due to the exposure of the methanogenic population to unfavorable acidifying conditions in the initial days of the phase-II digester (Figure 6.3). Even though thermophilic condition favors hydrogenotrophic methanogens (methane production from H_2 and CO_2), poor methanogenesis was observed in phase-I anaerobic digestion due to the accumulation of VFAs and poor buffering capability of FVW (Figure 6.4a and b).

Table 6.3: Comparison of co-digestion (BMWAS: FVW)

Parameters	**Co-digestion (1:1)**	**Untreated Co-digestion (3:1)**	**(MW-H_2O_2) treated co-digestion (3:1)**
pH	4.3-7.4	4.8-7.8	5.1-7.8
VS reduction %	55 ± 3	63 ± 1.5	69 ± 2.5
TS reduction %	51 ± 3.2	51 ± 2	56.2 ± 1.7
COD removal %	73.8 ± 1.2	74.4 ± 0.1	81.6 ± 0.2
Methane yield (ml/g VS)	79.11	127.14	276.09
Methane yield (ml/g tCOD)	51.60	77.66	169.18

FOS/TAC is the ratio of volatile organic acid to alkaline buffer capacity, and it is often widely used to assess the process stability of an anaerobic digester. Several reports have stated that FOS/TAC values of stable anaerobic digestion lie in the range of 0.2-0.6 (Nkuna et al., 2019; Scano et al., 2014; Wan et al., 2011). FOS/TAC values beyond 0.6 indicate unsuitable operating conditions for anaerobic microorganisms and results in a decrease in biogas production. In the current study, the average values of FOS/TAC in the phase-I thermophilic reactor were 1.91 and 1.1 in co-digestion (3:1) and co-digestion (1:1) respectively (Figure 6.4b). As the phase-I digester serves as an acidifier for the phase-II methanizer, the high FOS/TAC rather indicates the acidification achieved in respective digesters, than instability. Considering that waste activated sludge is characterized by low C/N ratios and high buffer capacity, an increase in WAS fraction in co-digestion has improved the FOS/TAC ratio as indicated by Mata-Alvarez et al (Mata-Alvarez et al., 2014).

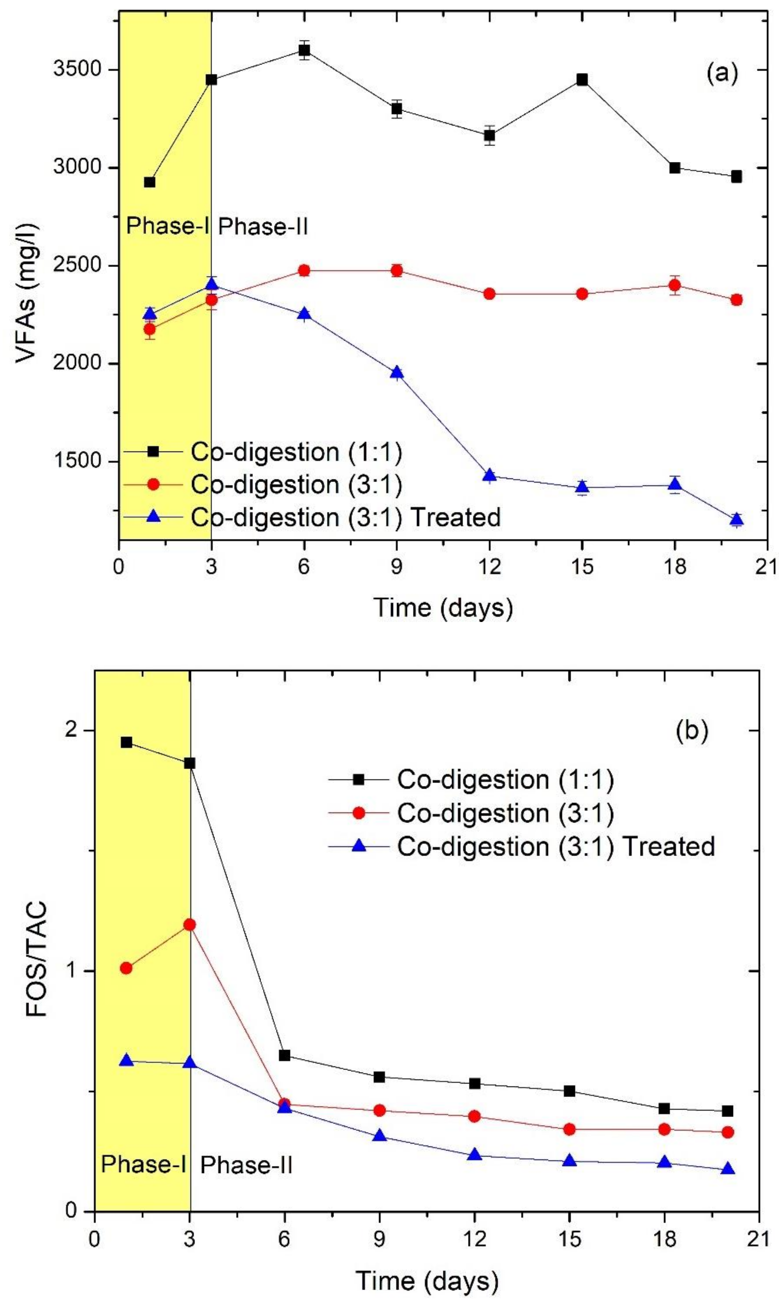


Figure 6.4: Comparison of acidification and digester stability in anaerobic co-digestion.

Evolution of (a) VFAs (mg/l) and (b) FOS/TAC ratio in co-digestion (1:1- BMWAS:FVW), co-digestion (3:1- BMWAS:FVW) and co-digestion (3:1- BMWAS:FVW) treated with hybrid (MW-H_2O_2) pre-treatment.

As the thermophilic condition has higher organic degradation rates, along with increased organic loading, the phase-I anaerobic digestion caused an accumulation of VFAs resulting in unstable anaerobic digestion in co-digestion (1:1) (Figure 6.4a). However, transfer of substrate to Phase-II significantly improved the stability of methanogenic digestion and resulted in average FOS/TAC values of 0.51 and 0.38 in co-digestion (3:1) and co-digestion (1:1) respectively (Figure 6.4b) ($P=0.0027$). At the end of 20 days of HRT, improved process efficiency was observed in co-digestion (3:1) in phase-II, with a 21 % higher reduction in VFAs and a 1.6-fold increase in methane yield compared to co-digestion (1:1).

6.3.2 Effect of hybrid (MW-H_2O_2) pre-treatment in anaerobic co-digestion

6.3.2.1 Biogas production

The efficiency of hybrid (MW-H_2O_2) feed sludge pre-treatment in enhancing anaerobic digestibility of co-digestion (BMWAS+FVW) was studied in two-stage anaerobic digesters with the co-digestion ratio 3:1 (BMWAS: FVW). The application of hybrid (MW-H_2O_2) pre-treatment was observed to enhance the sludge solubilization and consequently biogas production in the mono-digestion of BMWAS in chapter 5. In the current study, hybrid (MW-H_2O_2) pre-treatment was observed to significantly improve the biogas production ($P<0.0001$) and biogas quality ($P<0.0001$) in co-digestion (3:1). The pretreated co-digestion (3:1) yielded 2.17-fold higher cumulative methane yield compared to untreated co-digestion (3:1) at the end of 20 days (Figure 6.2a). Higher methane production (2.91-fold) was achieved in thermophilic phase-I of treated digester compared to untreated co-digestion (3:1). Besides, the biogas quality achieved in pre-treated sludge fed thermophilic digester was also significantly improved. The average methane percentage achieved in the treated phase-I digester was 26 %. Contrarily in the untreated phase-I digester, the average methane percentage was 13.5 %, which indicates the accelerated methanogenic activity in treated co-digestion. MW-H_2O_2 pre-treatment increases the relative abundance of the methanogenic community in digesters due to the availability of abundant biodegradable substrates for microbial growth (Liu et al., 2018). On average, the phase-II biogas quality was improved with methane content 52.14 % in the treated digester, in comparison to 40.14 % in untreated digester during peak methanogenesis (days 9 to 15).

Several reports suggest that pre-treatments enhance the anaerobic digestibility of FVW by accelerated hydrolyzation and breakdown of macromolecules (Liu et al., 2012b; Ruggeri et al., 2013; Zhou et al., 2013). Zhou et al. reported 115 % increased anaerobic digestion performance and a 2-fold increase in digestion rate in thermally pre-treated co-digestion involving FVW compared to untreated digestion (Zhou et al., 2013). Recently, Shanthi et al. reported a 31 % increase in biomethane yield in anaerobic digestion of FVW by combined (Dimethyl sulfoxide and ultrasonication) pre-treatment (Shanthi et al., 2018). Moreover, combined (MW-H_2O_2) pre-treatment achieved an 8.72-fold increase in biogas production in anaerobic digestion of dairy wastewater (Eswari et al., 2016). The study also reported that combined (MW-H_2O_2-acid) pre-treatment enhanced biogas production by 9.78-fold, emphasizing the efficiency of combined treatment under acidic conditions. In the current study, the hybrid (MW-H_2O_2) pre-treated FVW co-digestion increased methane production from the organic content-rich substrate. The untreated digestion showed a lag phase in biogas production during the thermophilic condition, whereas the treated digestion exhibited an exponential increase due to the availability of soluble substrate for the methanogenic population (Figure 6.2). Most of the studies on pretreated anaerobic digestion suggest that enhancement in biogas production is mostly due to the initial hydrolyzation achieved through pre-treatment (Liu et al., 2018, 2017; Özön and Erdinçler, 2019). Besides, phase separation in anaerobic digestion of FVW has been highly efficient due to the process stability and enhanced biomethanation (Bouallagui et al., 2005; Ravi et al., 2018). Moreover, the improved methane production in both phases in treated digestion could be due to hydrogenotrophic and syntrophic acetate oxidizing microbes, as the latter has a significant effect at high VFA (>1000 mg/l) concentration (Di Maria and Barratta, 2015).

6.3.2.2 Feedstock solubilization and digester performance

The FVW was initially blended and homogenized to facilitate anaerobic digestion. Consequently, the homogenized co-digested sludge achieved an initial COD solubilization of 22 % which was 2.2-fold higher than BMWAS reported in chapter 5. Application of hybrid (MW-H_2O_2) pre-treatment on mixed sludge further enhanced COD solubilization by 33 % facilitating the availability of soluble organic content for subsequent utilization by acidogenic microbial population (Figure 6.5 and 6.6).

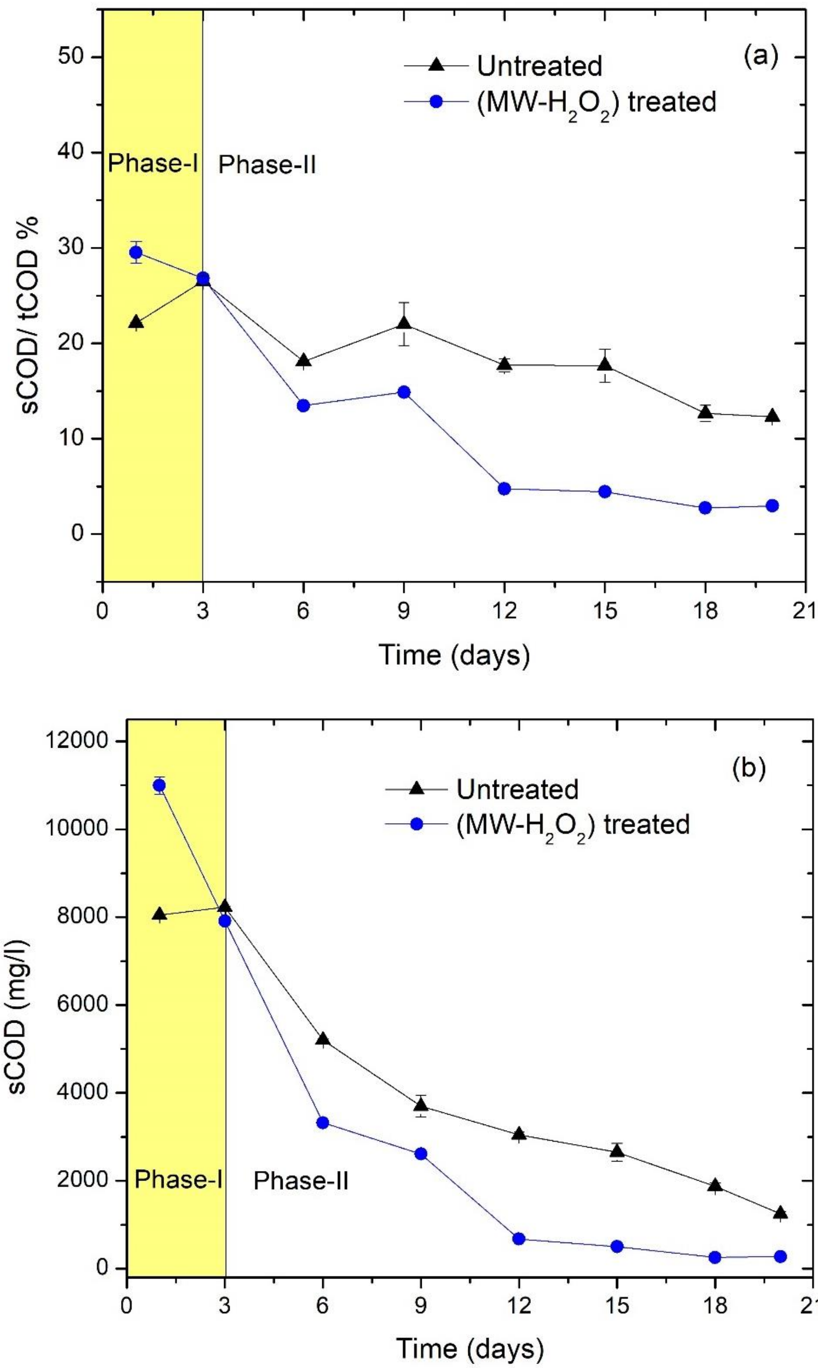


Figure 6.5: Comparison of COD solubilization between untreated and hybrid (MW-H_2O_2) treated co-digestion (3:1- BMWAS:FVW).

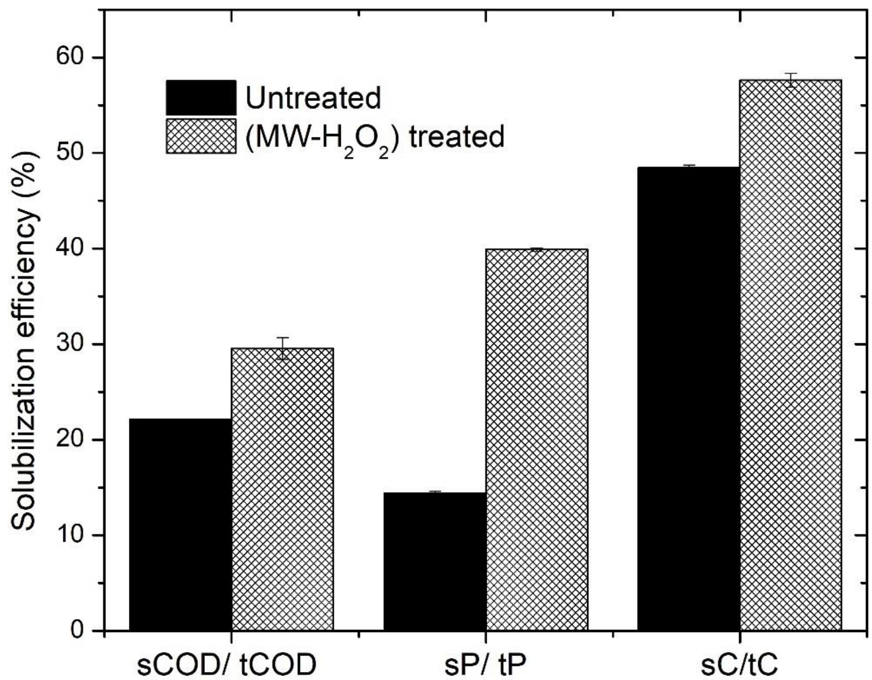


Figure 6.6: Comparison of COD solubilization, protein and carbohydrate solubilization in untreated and hybrid (MW-H_2O_2) treated co-digestion (3:1- BMWAS:FVW).

sCOD/tCOD= ratio of soluble chemical oxygen demand to total chemical oxygen demand; sP/tP=ratio of soluble protein to total protein; sC/tC= ratio of soluble carbohydrate to total carbohydrate.

The microwave enhanced oxidation process exhibits oxidative stress on anaerobic digestion by the generation of reactive oxygen species (ROS), which oxidizes organic microbial fractions and causes cell wall breakdown. The efficiency of hybrid (MW-H_2O_2) pre-treatment on optimum sludge solubilization of organic compounds without the formation of recalcitrant products is highly dependent on the dosage of hydrogen peroxide (Liu et al., 2017, 2015). Optimum sludge solubilization involves the disintegration of EPS (extracellular polymeric substances) matrix and dissolution of protein and carbohydrate fractions of activated sludge, without the formation of melanoidins through Maillard reaction as discussed in section 4.3.3. In the current research, the dissolution of proteins and carbohydrates was consistent with the COD solubilization as per the equivalent relationships: 1.5 g COD/g Protein and 1.06 g COD/g Carbohydrate (Lu et al., 2012). Accordingly, the soluble protein was observed to be 18.35 % and 39.40 % of sCOD in the untreated and treated digester respectively; soluble carbohydrate constituted 40 % and 42 % of sCOD in the untreated and treated digester, respectively. As the feedstock constitutes 25 % FVW, the solubilized fraction of carbohydrates was observed to be significantly higher than the solubilized fraction of proteins ($P<0.0001$) (Figure 6.6). Application of pre-treatment enhanced protein solubilization by 177 % and carbohydrate solubilization by 18 % (Figure 6.6). This indicates that the application of (MW-H_2O_2) pre-treatment affects EPS disintegration more than the organic constituents of FVW, as EPS constitutes more than 43 % protein and 10-18 % of carbohydrate in its composition (Liu et al., 2015). The higher COD solubilization and EPS disintegration would accelerate the subsequent anaerobic digestion in the two-stage digesters.

The digester performance of the two-stage anaerobic digestion with and without hybrid treatment was compared using VFA accumulation and FOS/TAC ratio (Figure 6.4). VFAs are common inhibitors of anaerobic digestion and their accumulation within the digesters lead to process instability (Chung and Neethling, 1989). In the current study, VFA accumulation in treated digester was significantly higher than untreated digester during the initial thermophilic phase ($P<0.0001$) (Figure 6.4a). However, the FOS/TAC ratio was 77 % higher in the untreated digester compared to the treated digester, during this phase (Figure 6.4b). The improved digester performance in the thermophilic phase of treated digester could be due to the buffering capability of microbial intracellular compounds released during the hybrid (MW-H_2O_2) pre-treatment. Zhou et al, reported a similar phenomenon, wherein despite an increase in VFA accumulation by thermal hydrolysis pre-treatment, the digester maintained stable pH and VFA/alkalinity levels compared to the untreated digester, indicating the buffering ability achieved during the pre-treatment (Zhou et al., 2013). Besides, the increased nitrogen mineralization with co-digestion (3:1) along with the pre-treatment could also increase the buffering capability of the system (Mahdy et al., 2019). The mesophilic phase-II anaerobic digestion significantly improved FOS/TAC ratio in both the systems with the treated digester achieving an average of 0.26, which is well within the acceptable range of stable anaerobic digestion. Moreover, the improved consumption of VFAs and digester stability have also been observed in the hybrid (MW-H_2O_2) pre-treated mono-digestion of BMWAS in the chapter 5, which further demonstrates the efficiency of pre-treatment in improving the buffering ability within the anaerobic digesters.

6.3.2.3 Oxidative status and sludge activity

The oxidative status of anaerobic digestion after hybrid (MW-H_2O_2) pre-treatment was observed to impact sludge activity in the mono-digestion of BMWAS in chapter 5. Similarly, in the current chapter, the oxidative stress induced by hybrid (MW-H_2O_2) pre-treatment on anaerobic co-digestion was studied through the quantification of superoxide radicals and correlated with the sludge bioactivity (Figure 6.7).

Surprisingly, on day 1, the intracellular superoxide s[$O_2^{\bullet -}$] levels of the untreated digester were observed to be 81 % higher than the hybrid (MW-H_2O_2) pre-treated digester (Figure 6.7a). A plausible explanation of this phenomenon could be the formation of more reactive hydroxyl radicals [$OH^{\bullet}$] through Haber-Weiss type reaction (equation 6.2), in the treated digester that could account for the decrease in superoxide levels (Kehrer, 2000).

$$O_2^{\bullet -} + H_2O_2 \rightarrow OH^{\bullet} + HO^{-} + O_2 \qquad \text{equation (6.2)}$$

The mechanism of the Haber-Weiss reaction makes use of Fenton chemistry and the catalysis requires the presence of ferric ion in sludge. Mixed sludge being a source of various indigenous metal ions becomes a suitable target for Haber-Weiss reaction and accelerates the production of the highly reactive $OH^{\bullet}$ radicals (X. Zhou et al., 2015). The initial s[$O_2^{\bullet -}$] levels in untreated digester in day 1 could be due to the oxidative stress experienced by FVW and metazoans of untreated feedstock when they are exposed to anoxic conditions (Biemelt et al., 2000; Hernansanz-Agustín et al., 2014; Martín-Cereceda et al., 2001). In the subsequent days, reduction of s[$O_2^{\bullet -}$] levels were observed in the untreated digester, suggesting declined oxidative stress under anaerobic condition (Liochev, 2014). A delayed increase in s[$O_2^{\bullet -}$] levels was observed on day 3, in the treated digester similar to chapter 5, which confirms that the oxidative stress exerted by hybrid (MW-H_2O_2) pre-treatment has a prolonged effect in anaerobic digestion (Figure 6.7a). The following reasons are speculated for this effect in the treated digester, (1) the presence of residual hydrogen peroxide from the pre-treatment (Liu et al., 2017) (2) the presence of oxidative intermediates as a result of pre-treatment, which might give rise to superoxide radicals later (3) thermophilic-temperature mediated induction of superoxide radical generation (Sarkar and Suraishkumar, 2011). The molecular mechanism involved in the generation of free radicals under oxidative pre-treatment in anaerobic digestion can be established through future studies. With reference to the TTC-DHA activity, the treated digester exhibited 127 % higher bioactivity during the initial thermophilic phase, which is concurrent with the increased methane production and process stability achieved in the treated digester (Figure 6.2, 6.4a and 6.7b).

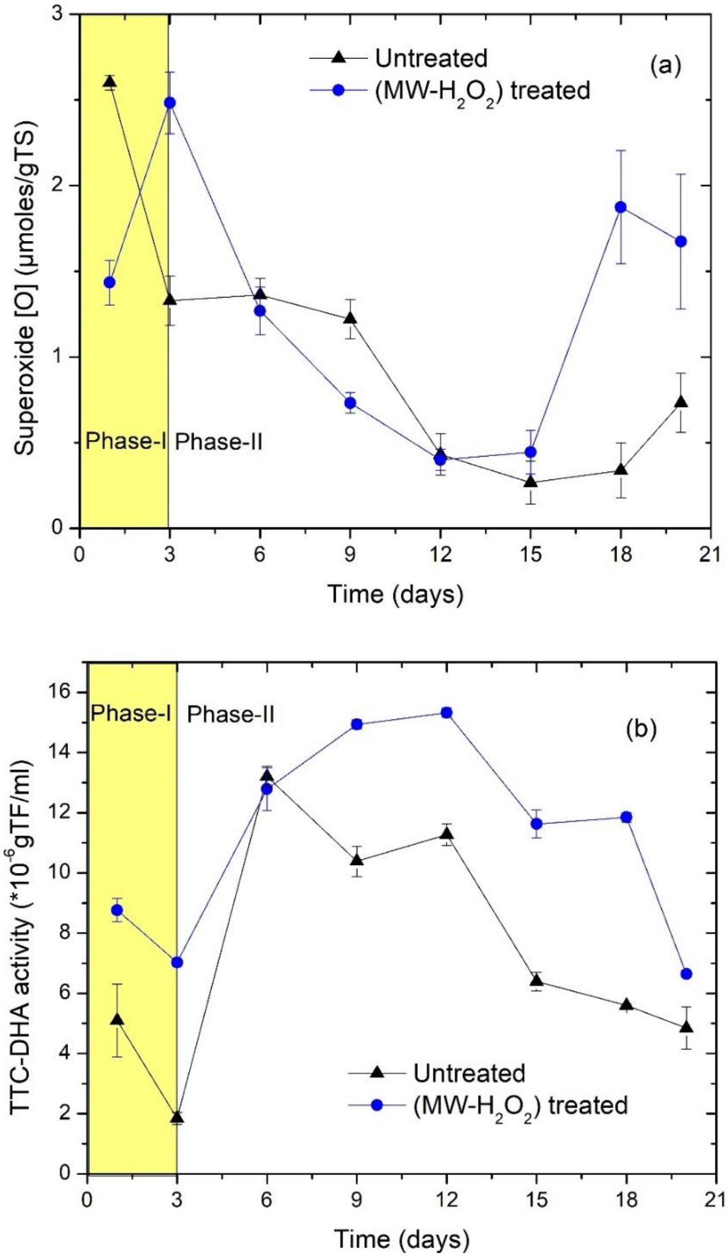


Figure 6.7: Comparison of (a) specific intracellular superoxide s[$O_2^{\bullet-}$] (µmoles/ g TS) and (b) TTC-DHA activity (x10^{-6} gTF/ml) in untreated and hybrid (MW-H_2O_2) treated co-digestion (3:1-BMWAS:FVW)

On transfer to the mesophilic anaerobic digester, an improvement in methanogenic activity is observed in both systems (Figure 6.7b). Also, s[$O_2^{\bullet-}$] levels were diminished during active methanogenesis in both the systems (Figure 6.7a). Moreover, an increasing s[$O_2^{\bullet -}$] trend was observed as the digestion nears completion which is marked by decreasing TTC-DHA activity (Figure 6.7). In treated digester, a significant decrease in s[$O_2^{\bullet -}$] levels was observed in correlation with an increase in TTC-DHA activity (Pearson's correlation coefficient r = -0.7503, P=0.032). The untreated system followed a similar trend in the mesophilic phase-II digester, wherein the superoxide radical levels were subdued during active methanogenesis followed by an increase towards the end of digestion. Furthermore, the increasing superoxide radical levels at the end of digestion could indicate the physiological stress of microorganisms due to the decrease in substrate concentration inside the digester. Which is concurrent with the declining biological activity in both the digesters. Menon et al, reported a 10-15 -fold increase in s[$O_2^{\bullet -}$] levels on shifting microalgae to a nutrient deficient medium (Menon et al., 2013). The free radical chain reactions that decide the fate of superoxide radicals in anaerobic digestion could be an interesting study for the future.

6.4 Conclusion

The performance efficiency of the two-stage anaerobic digester was studied by co-digestion of highly acidifying FVW with WAS. The stability of the two-stage operation was evaluated with two mixing ratios of BMWAS and FVW. The two-stage anaerobic digester showed better process stability and biogas production with 75 % BMWAS and 25 % FVW. Increased acidification and poor buffer capability were observed in thermophilic phase-I of co-digestion (1:1) which led to process instability and low methane production in subsequent mesophilic phase-II. Application of hybrid (MW-H_2O_2) pre-treatment increased the solubilization of biopolymers and enhanced buffer capability in phase-I and ultimately increased the overall biogas production. In addition, it was observed that the hybrid pre-treatment induced prolonged oxidative stress through superoxide radical generation, and it was downregulated by sludge bioactivity in the course of anaerobic digestion. It can be understood from the current chapter that the two-stage operation with hybrid (MW-H_2O_2) pre-treatment can improve the overall anaerobic digestion performance of FVW and WAS through improved sludge solubilization and process stability.

CHAPTER 7

IMPACT OF MICROWAVE AND HYBRID (MW-H_2O_2) TREATMENT ON EPS DISTRIBUTION AND DEWATERABILITY

Abstract

The advantages of hybrid (MW-H_2O_2) sludge pre-treatment in enhancing sludge solubilization and subsequent anaerobic digestion have been dealt with in previous chapters. More insights into the effects of microwave and hybrid (MW-H_2O_2) treatment in the distribution of biopolymers in EPS layers and their corresponding dewaterability have been tested and discussed in this chapter. Both treatments were found to gradually increase the release of proteins and carbohydrates into slime and LB-EPS layers with increasing microwave intensities, accompanied by improved dewaterability. However, beyond an optimum point, the release of biopolymers decreased and a corresponding fall in dewaterability was observed. And these effects of treatments were observed to be similar in different sludge types, namely activated sludge (IITMAS) and anaerobically digested sludge (ADS). Moreover, exposure to higher microwave power (990 W and 1100 W) did not improve dewaterability even at a shorter contact time (20s). In digested sludge, the maximum dewaterability was achieved in 880 W and 660 W + 1 % H_2O_2 with microwave and hybrid treatment, respectively at 30s contact time. Furthermore, the hybrid treatment was observed to accelerate the dewatering capacity at lower microwave intensities (220 W to 660 W). The decrease in dewaterability at higher microwave intensities in hybrid treatment could be due to the generation of fine particles that facilitate water adhesion. In most of the experiments, the release of carbohydrate fraction into the slime layer was observed to peak at conditions where enhanced dewaterability was observed. Therefore, the current chapter establishes the importance of EPS distribution in different layers of sludge matrix and their corresponding effects on sludge dewaterability.

7.1 Introduction

The previous chapters have discussed the efficiency of hybrid pre-treatment and two-stage operation in anaerobic biodegradation of mixed sludge and organic fruit wastes. Although the effluent digestate of the two-stage operation had enhanced pathogen removal, the dewaterability was observed to be lessened (chapter 5). This effect could be due to the distribution of polymers in the EPS matrix as a result of pre-treatment and two-stage operation. Therefore, the current study was aimed to understand the effects of microwave and hybrid (MW-H_2O_2) treatment on EPS distribution of sewage sludge and consequently its role in dewaterability.

For achieving dewaterability in WWTPs, various conditioning agents such as polyelectrolytes, surfactants, Fenton's reagent and other chemicals have been studied (Chen et al., 2001; Fakhru'l-Razi and Molla, 2007; Hong et al., 2017; Sen, 2015; Sun et al., 2014; Yu et al., 2009). These flocculants help to release the bound water trapped in flocs and cells of sludge matter, which cannot be removed otherwise through conventional dewatering methods (Sun et al., 2014; Zhou, 2015). The entrapment of water in sludge flocs is highly dependent on the nature and characteristics of EPS composition (Liu et al., 2015; Neyens et al., 2004). Moreover, the EPS composition contributes to the structural, surface charge, mass transfer and settling properties of the activated sludge flocs (Houghton et al., 2001; Sheng et al., 2010). The major components of EPS are carbohydrates and proteins, followed by humic substances, nucleic acids and lipids (Sheng et al., 2010). There are varied opinions on the role of EPS composition in sludge dewaterability, as some researchers have observed strong relationship and others did not (Cetin and Erdincler, 2004; Higgins and Novak, 1997; Mikkelsen and Keiding, 2002; Nielsen et al., 1996; Shao et al., 2012). Yu et al. introduced the stratification of the EPS matrix into 3 layers namely, slime, loosely-bound and tightly-bound to better understand the structural properties of sludge flocs (Figure 7.1) (Yu et al., 2008). The integrity of these layers determines the various physicochemical properties of the sludge. Therefore, the study on the distribution of proteins and carbohydrates in EPS layers is inherent in understanding the implications of pre-treatment in sludge dewaterability.

In the current study, the distribution of proteins and carbohydrates in the EPS fractions were studied in digested sludge from BWWTP and activated sludge from IITMSTP, along with different treatment conditions. The corresponding dewaterability achieved in the samples were analyzed to understand the effects of EPS distribution in sludge dewaterability.

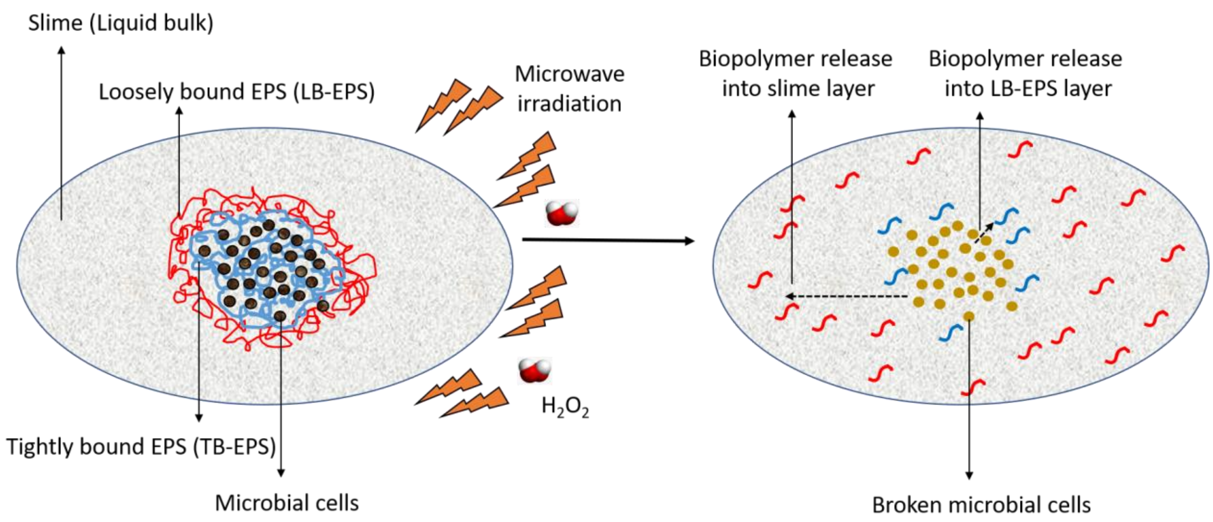


Figure 7.1: Breakdown of sludge matrix and microbial cells, and release of biopolymers into slime and LB-EPS layers with microwave and hydrogen peroxide treatments.

7.2 Materials and methods

7.2.1 Sludge sampling and characterization

The anaerobically digested sludge (ADS) used in the current study was collected from the centrifuge unit after anaerobic digestion in BWWTP as mentioned in section 3.2. The secondary activated sludge (IITMAS) was collected from the outlet of SBR from the IITMSTP as mentioned in section 3.4. Both the samples were characterized as presented in Table 7.1 and stored at 4 °C until experimentation.

Table 7.1: Characterization of ADS and IITMAS

Parameters	**ADS**	**IITMAS**
pH	6.8	6.91
tCOD (mg/l)	5500 ± 85	11520 ± 672
sCOD (mg/l)	1400 ± 16	-
TS (mg/l)	15433 ± 47	11330 ± 60
VS (mg/l)	11066 ± 124	8215 ± 25
VS/TS %	71.6	72.5

Note: ‘-‘ means ‘not measured’

7.2.2 Sludge treatment

In the current study, microwave and hybrid (MW-H_2O_2) treatment studies were conducted on the ADS and IITMAS. Briefly, 30 ml of sludge sample was taken in beakers of constant dimension for the treatments. A detailed discussion on treatment equipment and strategies have been mentioned in section 3.6. The treatment methods and conditions followed in the current chapter are mentioned in Table 7.2.

Table 7.2: Sludge treatment methods and conditions followed in chapter 7

Sample	**Treatment method**	**Conditions**
ADS	Microwave	2450 MHz, 220 W to 1100 W, 20s and 30s treatment time
ADS	Microwave and hydrogen peroxide (MW-H_2O_2)	MW: 2450 MHz, 220 W to 1100 W, 30s treatment time H_2O_2: 1 % (w/w) H_2O_2/ TS
IITMAS	Microwave	2450 MHz, 100 W to 800 W, 30s treatment time
IITMAS	Microwave and hydrogen peroxide (MW-H_2O_2)	MW: 2450 MHz, 100 W to 800 W, 30s treatment time H_2O_2: 1 % (w/w) H_2O_2/ TS

7.2.3 Analytical methods

The sludge samples were analyzed for pH, total solids, COD, zeta potential and CST. The EPS extraction was carried out as discussed in section 3.5.15. The zeta potential measurement was conducted on the slime fraction collected through EPS extraction in IITMAS. The sludge settling experiment was conducted in 50 ml centrifuge tubes by allowing the samples to settle in 15 minutes resting time. The microscopic examination was conducted using a Labomed Vision 2000 binocular microscope under 4x magnification.

7.2.4 Calculation

The fraction of proteins and carbohydrates in different extractions were determined to evaluate their distribution in the EPS matrix. The fraction of proteins in slime and loosely-bound EPS extractions is given by,

$$Fp\ (Slime) = \frac{Protein\ concentration\ in\ Slime}{Total\ protein\ concentration}$$

$$Fp\ (LB\ EPS) = \frac{Protein\ concentration\ in\ loosely - bound\ EPS}{Total\ protein\ concentration}$$

The fraction of carbohydrates in slime and loosely-bound EPS extractions is given by,

$$Fc\ (Slime) = \frac{Carbohydrate\ concentration\ in\ Slime}{Total\ carbohydrate\ concentration}$$

$$Fc\ (LB\ EPS) = \frac{Carbohydrate\ concentration\ in\ loosely - bound\ EPS}{Total\ carbohydrate\ concentration}$$

7.3 Results and Discussion

7.3.1 Effect of microwave treatment in EPS distribution and dewaterability

In anaerobically digested sludge:

Microwave irradiation causes the breakdown of microbial cell walls through thermal effect which results in the release of bound water (Yu et al., 2009). Further, it leads to changes in the physicochemical properties of the sludge thereby enhancing its bio-flocculation capacity (Liu et al., 2015; Neyens and Baeyens, 2003b). In the current study, the dewatering capability of digested sludge upon microwave treatment was evaluated at contact times 20s and 30s, through capillary suction time (CST) measurement (Figure 7.2). For a contact time of 30s, 880 W microwave treatment yielded the least CST suggesting improved dewaterability. However, when the microwave power was extended further, the dewaterability was worsened. A similar result was observed at 20s contact time, where the lowest CST was attained at 990 W treatment. The effect of microwave at different contact time is apparent, as larger microwave power requires shorter contact time and vice versa (Yeneneh et al., 2017a). The current observation is similar to the effects of microwave power on CST reported by (Yu et al., 2009), wherein the dewaterability decreased beyond an optimum microwave power and contact time. The improved dewaterability could be due to the re-flocculation of smaller particles derived as a result of microwave treatment. Besides, the changes in the EPS matrix caused by microwave irradiation could also influence sludge dewaterability.

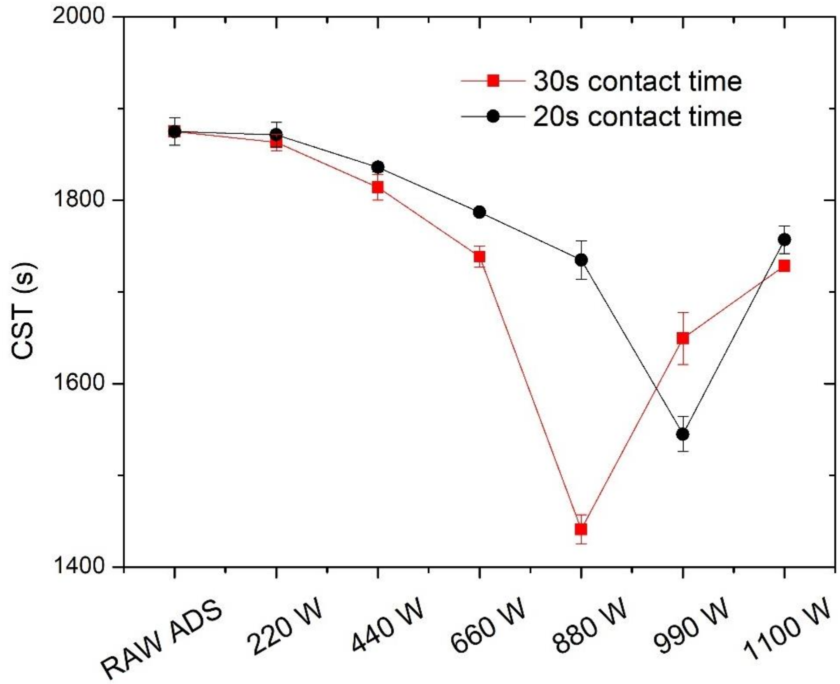


Figure 7.2: Effect of different microwave power outputs (220 W, 440 W, 660 W, 880 W, 990 W and 1100 W) on sludge dewaterability of ADS

To establish the effects of microwave treatment in sludge floc structure, the distribution of proteins and carbohydrates in EPS layers was studied at 30s contact time (Figure 7.3). Similar to the previous study with BMWAS (chapter 4), the proteins were dominant over carbohydrates in the slime fraction of the raw anaerobically digested sludge. With increasing microwave power output, the concentration of both biopolymers was observed to increase in both slime and LB-EPS fractions. This phenomenon is due to the diffusion of biopolymers from the core of sludge flocs towards the bulk of liquid, as the treatment disrupts sludge flocs (Zhen et al., 2019). A schematic of this phenomenon has been presented in Figure 7.1. The maximum dissolution of proteins and carbohydrates were observed in 880 W and 990 W microwave irradiation. Furthermore, the maximum release of proteins into the LB-EPS layer and carbohydrates into the slime layer was observed at 880 W, which achieved the maximum dewaterability as represented by the CST measurement (Figure 7.2). The increasing dewaterability with increasing Fc (Slime) goes in accordance with the previous study, where carbohydrate concentration in EPS was correlated with dewaterability (Cetin and Erdincler, 2004). Nevertheless, the relationship between EPS protein concentration and dewaterability have been contrasting among various studies (Buntner et al., 2014; Cetin and Erdincler, 2004; Li and Yang, 2007; W. Zhang et al., 2015a). A strong correlation between soluble EPS (SB-EPS) protein and dewaterability has been observed in recent studies, where an increase in protein concentration has influenced higher dewaterability (Liang et al., 2020; W. Zhang et al., 2015b, 2015a). In the current study, an increase in protein concentration was observed in slime fraction at both 880 W and 990 W conditions, where maximum dewaterability has been observed. Also, the SB-EPS extraction followed in the above-mentioned studies are similar to slime extraction adapted in the current study. Therefore, an increase in both biopolymers in the bulk of slime during microwave treatment has influenced the flocculating behavior of digested sludge.

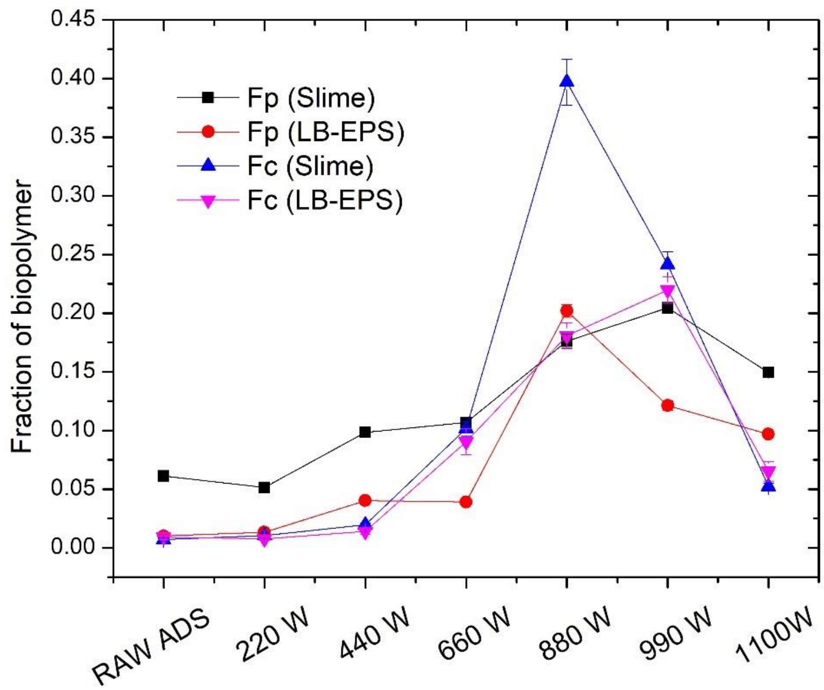


Figure 7.3: Effect of different microwave power outputs (220 W, 440 W, 660 W, 880 W, 990 W and 1100 W) at 30s contact time on EPS distribution in slime and LB-EPS layers of ADS

In activated sludge:

Furthermore, the effects of microwave irradiation on EPS distribution was studied in the activated sludge samples collected from IITMSTP. The microwave treatment was conducted at 30s contact time, and the respective biopolymer distribution in EPS layers has been given in Figure 7.4.

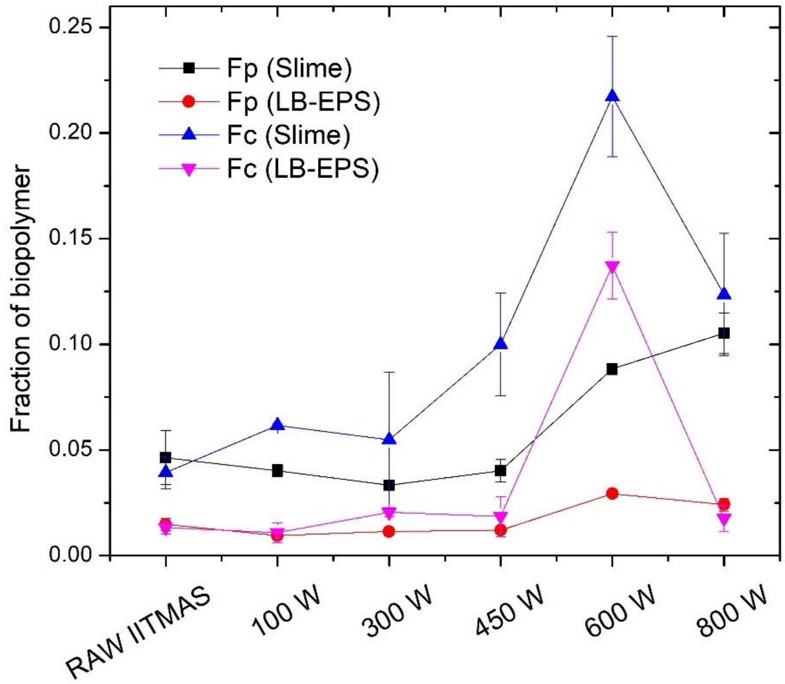


Figure 7.4: Effect of different microwave power outputs (100 W, 300 W, 450 W, 600 W and 800 W) at 30s contact time on EPS distribution in slime and LB-EPS layers of IITMAS

Similar to the study conducted in anaerobically digested sludge, the release of proteins and carbohydrates increased with microwave energy and declined beyond an optimum point. The maximum carbohydrate release into EPS and slime layers was observed at 600 W and the maximum protein dissolution into the slime layer was at 800 W. Consequently, the settling property of microwaved sludge samples was enhanced, particularly in the range 450 W to 800 W compared to the untreated IITMAS (Figure 7.5).

The enhanced dewaterability could be due to the flocculation of coarsened sludge particles as a result of microwave treatment. The microwave irradiation weakens the equilibrium of hydrophilic colloidal particles with the surrounding water, which results in aggregation and flocculation (Yu et al., 2009). Moreover, the decrease in absolute zeta potential with microwave treatment in the range 300 W to 600 W may indicate the neutralization of negatively charged microbial surfaces (Chu et al., 2001; Grubel and MacHnicka, 2011; Yu et al., 2009) (Table 7.3). The morphology of activated sludge and digested sludge are different in terms of microbial composition, EPS architecture and molecular content (Liu et al., 2021). In addition, the digested sludge is characterized by low divalent cations which are responsible for bridge formation among negatively charged sludge flocs, thereby enhance sludge dewaterability (Huang et al., 2020; Liu et al., 2021; Yu et al., 2001). However, both sludges have shown flocculating behavior upon microwave irradiation in the current study, which indicates the effect of microwave in enhancing dewaterability among different sludge types.

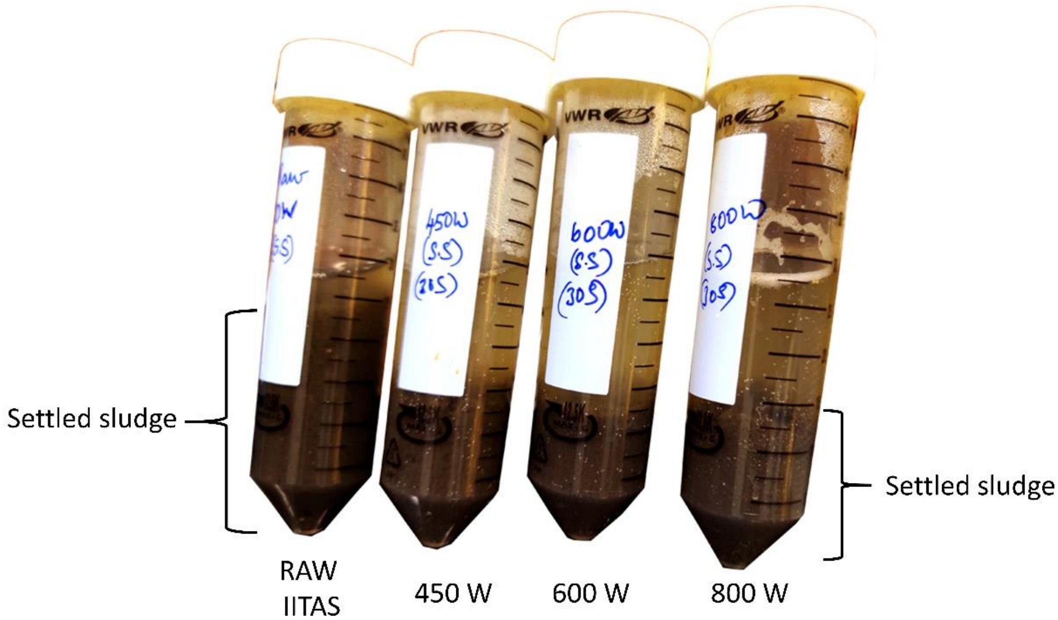


Figure 7.5: Comparison of sludge settling in microwave treated samples to the untreated IITMAS

Table 7.3: Effects of microwave and hybrid treatment on Zeta potential of IITMAS

Treatment	Treatment condition	Zeta potential (mV)	
		Microwave treated IITMAS	Hybrid (MW-H_2O_2) treated IITMAS
Microwave	100 W	-16 ± 0.20	-13.4 ± 0.32
	300 W	-13.86 ± 0.49	-12.53 ± 0.77
	450 W	-13.06 ± 0.04	-10.63 ± 0.44
	600 W	-12.03 ± 0.41	-13.4 ± 0.32
	800 W	-13.1 ± 0.86	-14.6 ± 0.94
H_2O_2	1 % H_2O_2/ TS	-	-12.2 ± 1.77
Untreated	-	-15 ± 0.37	-15 ± 0.37

Note: '-' means 'not applicable'

7.3.2 Effect of hybrid (MW-H_2O_2) treatment in EPS distribution and dewaterability

In anaerobically digested sludge:

The effects of hybrid (MW-H_2O_2) pre-treatment on sludge solubilization and anaerobic digestion have been discussed in the previous chapters. Furthermore, the hybrid treatment also influences sludge flocculation as discussed in section 4.3.3. In the current section, the effects of hybrid treatment in EPS distribution and dewaterability of digested and activated sludge have been discussed. As the hybrid (MW-H_2O_2) treatment exhibits synergy between the individual techniques, both the thermal effect and production of ($OH^{\bullet}$) radicals together can improve sludge dewaterability (Bilgin Oncu and Akmehmet Balcioglu, 2013; Chan et al., 2010; J. Zhang et al., 2016). In the current study, the dewaterability of digested sludge treated with hybrid (MW-H_2O_2) treatment at varying microwave intensities has been presented in Figure 7.6.

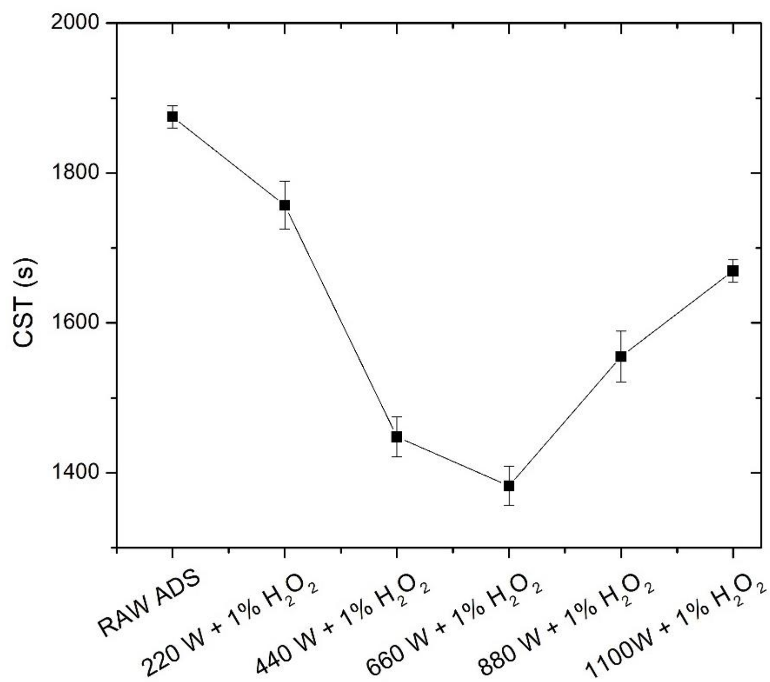


Figure 7.6: Effect of hybrid (MW-H_2O_2) treatment at 30s contact time on dewaterability of ADS

Similar to the effects of microwave (Figure 7.2), the hybrid treatment accelerated sludge dewaterability with increasing microwave power, however, beyond an optimum point the dewaterability was found to decrease. A similar observation on dewaterability was reported with microwave initiated Fe(II)-persulfate oxidation treatment on activated sludge, wherein longer contact time or higher microwave power worsened sludge dewaterability (Zhen et al., 2019). In the current study, the minimum CST was achieved at 660 W + 1 % H_2O_2, which was 0.95-fold lesser than pure microwave treatment (880 W). Furthermore, the average CST in the range 220-660 W in hybrid treatment was accelerated by 0.84-fold compared to its counterpart in pure microwave treatment. Hence, hybrid treatment considerably increases sludge dewaterability compared to pure microwave treatment, at an optimum microwave range. The enhanced dewaterability observed in the hybrid treatment could be due to changes in surface charge and particle size (Chan et al., 2010; Liu et al., 2015). Further, the effects of hybrid treatment in EPS distribution have been presented in Figure 7.7.

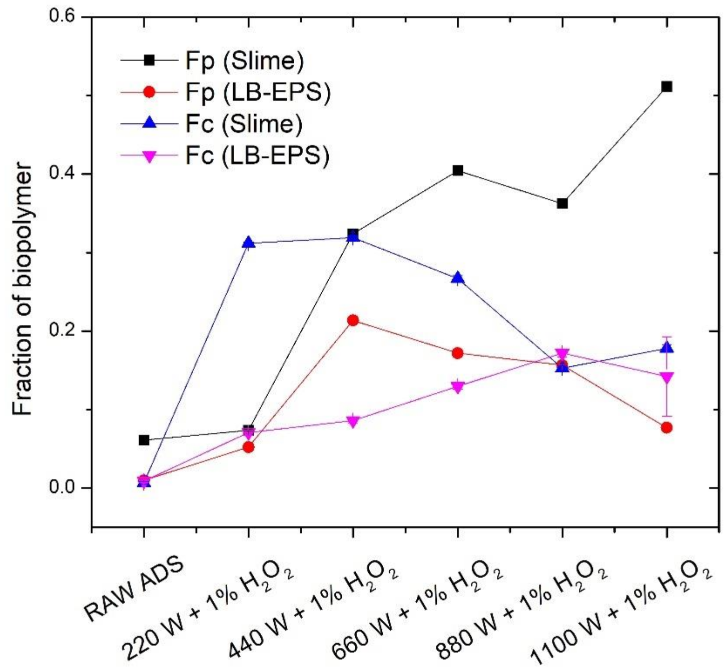


Figure 7.7: Effect of hybrid (MW-H_2O_2) treatment on EPS distribution of ADS.

The release of carbohydrates in slime fraction was observed to peak around 220-440 W and decline beyond that point. Also, the Fc (slime) at 440 W in hybrid treatment was 16-fold higher than the carbohydrate release in 440 W pure microwave treatment, which further validates the efficiency of hybrid treatment in achieving sludge disintegration. Besides, it also suggests the significance of carbohydrate release in sludge flocculation. The average Fc (slime) was 0.29 ± 0.02 in the range 220-660 W, where the fall in CST was observed to be accelerated. The faster decline in carbohydrate concentration in slime and EPS after 440 W may signify the oxidizing capability of hybrid treatment at higher treatment conditions (Zhen et al., 2019). Besides, the formation of smaller particles at higher treatment conditions could also be the reason for the decrease in dewaterability (Chan et al., 2010).

In activated sludge:

The effect of hybrid (MW-H_2O_2) treatment in the EPS distribution of IITMAS has been presented in Figure 7.8. The distribution of EPS fractions upon treatment was observed to be similar to the previous studies. An almost linear increase in biopolymer release was detected from the 300 W to 600 W range in the hybrid treatment. Also, a decline of both biopolymer fractions in the LB-EPS layer was observed beyond 600 W + 1 % H_2O_2. However, a linear increase in Fp (slime) was noticed beyond 300 W (Figure 7.8), which is similar to the increased protein release observed in digested sludge (Figure 7.7). Hence, it can be inferred that protein release into the slime layer has been enhanced at higher microwave intensities in hybrid treatment. This could be attributed to the enhanced disruption of the EPS matrix and microbial cells by synergetic action at higher microwave intensities in the hybrid treatment (Eskicioglu et al., 2008). But, higher microwave intensities could result in an increased fraction of smaller particles that facilitate water adhesion, thereby weakening dewaterability (Zhen et al., 2019). The increased turbidity observed at higher microwave intensities in the current study could be due to the generation of such smaller particles through treatment (Figure 7.9). Also, the absolute zeta potential of 600 W and 800 W in hybrid treatment was observed to increase which may indicate stabilization of particles in suspension (Table 7.3). The effect of different hybrid treatment conditions on sludge settling has been presented in Figure 7.9. The settling was observed to improve along with increasing microwave intensities in the study.

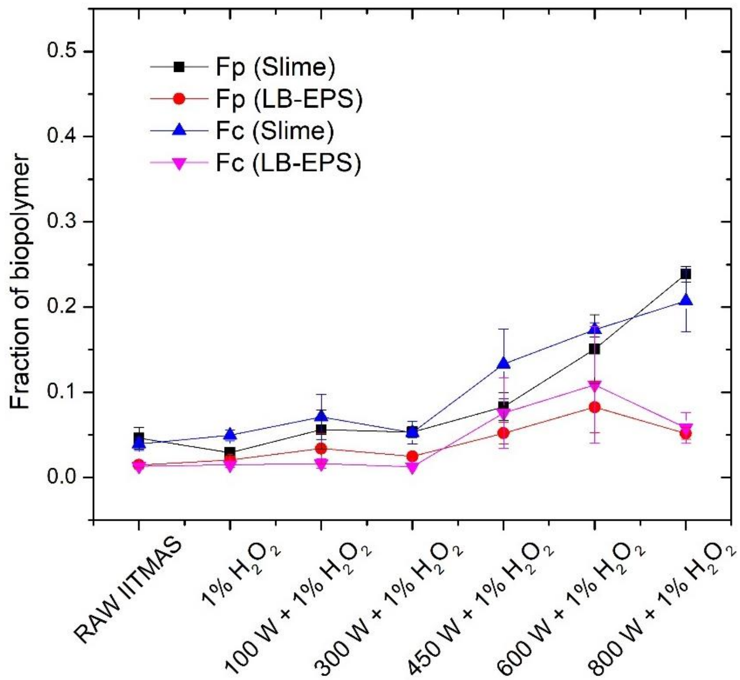


Figure 7.8: Effect of hybrid (MW-H_2O_2) treatment on EPS distribution of IITMAS

Furthermore, a microscopic examination of hybrid treated IITMAS samples has been presented in Figure 7.10. As shown in the figure, the sludge particles are found to be highly aggregated in the 450 W hybrid treatment compared to the untreated IITMAS. This explains the flocculation behavior of sludge under microwave and hydrogen peroxide treatments. As mentioned earlier, the phenomenon of flocculation under these treatment conditions rely on the disruption of the equilibrium between sludge colloidal particles and water, thereby leaving the particles to re-flocculate and sediment. Besides, the aggregation of particles was not greatly enhanced at 800 W + 1 % H_2O_2, which again confirms the deteriorating mechanism of higher treatment conditions.

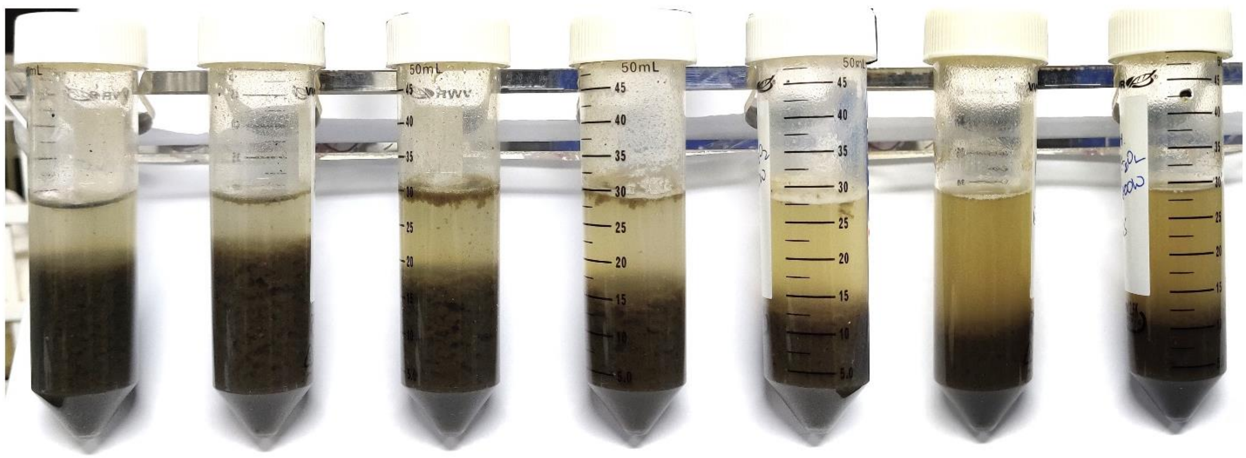


Figure 7.9: Comparison of sludge settling in samples with hybrid (MW-H_2O_2) treatment to the untreated IITMAS

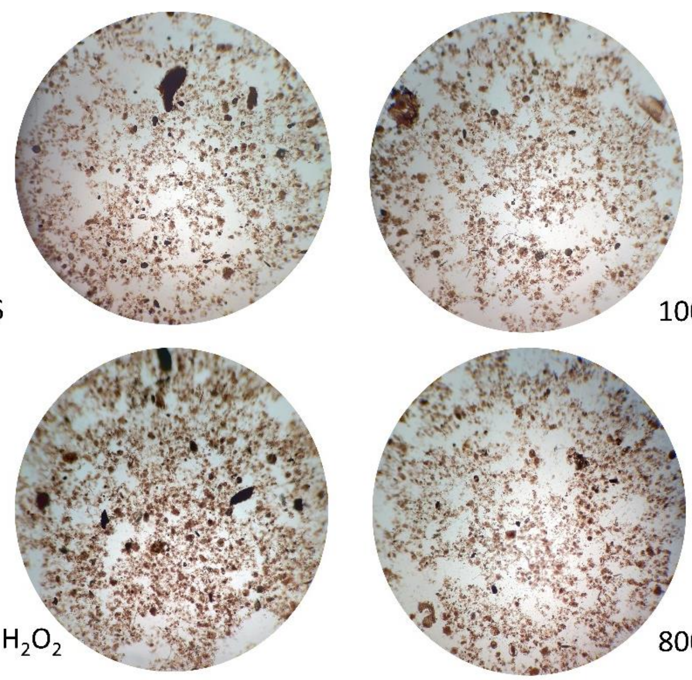


Figure 7.10: Microscopic examination of IITMAS after different hybrid treatments.

7.4 Conclusion

The effects of microwave and hybrid (MW-H_2O_2) treatment on dewaterability and EPS distribution of anaerobically digested and activated sludge have been studied in the current chapter. The release of proteins and carbohydrates into the LB-EPS and slime layer was enhanced along with increasing microwave intensities in both the sludges. However, beyond an optimum point, the dewaterability was observed to decline, which was concurrent with a decrease in the biopolymer release into slime fraction. The release of carbohydrates into the slime layer was predominant in samples that exhibit good dewaterability. The application of hybrid (MW-H_2O_2) treatment accelerated the biopolymer release into slime and LB-EPS layer, consequently improving the dewaterability. Similar to the effects of the microwave, the hybrid treatment also resulted in a decrease in dewaterability at higher treatment conditions. These findings suggest that either as an individual or combined with H_2O_2, microwave treatment has a significant effect on the disruption of sludge flocs and release of biopolymers from the core to the bulk of the liquid. And this phenomenon will be beneficial in various aspects of sludge treatment at WWTPs, in terms of activated floc breakage, solubilization of particulate organics and dewaterability.

CHAPTER 8

CONCLUSION AND RECOMMENDATION

8.1 Conclusion

The current thesis work involves the various aspects of hybrid (MW-H_2O_2) pre-treatment and two-stage anaerobic digestion in improving the anaerobic biodegradation, pathogen removal, methanogenic bioactivity and dewaterability. The following conclusions are made through various observations obtained in this thesis work.

- The hybrid pre-treatment enhanced sludge solubilization significantly compared to the individual microwave and hydrogen peroxide treatments. However, higher treatment conditions resulted in the decrease of soluble organics, also it might lead to the generation of refractory compounds which is detrimental. Therefore an optimum treatment condition is preferred wherein the solubilization of organics/ breakdown of activated sludge flocs is achieved without the negative impact.
- The hybrid pre-treatment leads to the generation of ($OH^{\bullet}$) and ($O_2^{\bullet-}$) radicals. Increased production of ($OH^{\bullet}$) radicals were observed in hybrid pre-treatment compared to the individual treatments. The ($O_2^{\bullet-}$) radicals also play an important role in oxidizing the organic compounds and replenishing the concentration of ($OH^{\bullet}$) radicals in the system. Besides, the generation of these oxidizing species has enhanced the efficiency of sludge solubilization compared to the individual treatments.
- The hybrid pre-treatment enhanced overall anaerobic digestion in conventional mesophilic operation. The study showed that improved sludge solubilization through pre-treatment increased biogas production and biogas quality compared to untreated digestion. Furthermore, the hybrid pre-treated digestion resulted in improved dewaterability of resultant digestate.
- The two-stage anaerobic digestion showed better performance compared to conventional single-stage digestion. Also, the application of hybrid pre-treatment enhanced the efficiency of two-stage digestion in terms of biogas production, biogas quality, pathogen removal and methanogenic activity. This enhancement is ascribed to the initial solubilization of sludge flocs through pre-treatment and enriched methanogenic activity in the thermophilic phase of

two-stage operation. However, the dewaterability of final digestate was lessened in this operation, even though the pre-treatment had a positive effect in increasing dewaterability.

- The digestion of highly acidifying FVW was studied in the two-stage mode of operation. The digester showed better performance in terms of biogas production and quality with 75 % activated sludge and 25 % FVW, than 50 % of each substrate. Furthermore, the application of hybrid pre-treatment increased the buffer capacity of digesters through the release of biopolymers, thereby improving the cumulative biogas production.
- The evolution of ($O_2^{\bullet-}$) radicals were analyzed during the anaerobic digestion. The hybrid pre-treatment showed a prolonged generation of ($O_2^{\bullet-}$) radicals during the initial thermophilic phase. The radical generation was downregulated in the subsequent methanogenic phase with an increase in sludge bioactivity.
- Microwave and hybrid pre-treatments were observed to increase dewaterability in activated sludge and effluent digestate with increasing microwave intensity till a threshold, and further irradiation resulted in a decrease in dewaterability. This phenomenon was accompanied by the release of biopolymers into slime and LB-EPS layers. Besides, the role of carbohydrate release into the slime layer was observed to be significant where dewaterability was enhanced.

These findings add valuable knowledge in the anaerobic digestion of activated sludge and subsequent dewaterability in WWTPs. Moreover, the study on the evolution of ROS in anaerobic digestion advances the understanding of oxidative stress in anaerobic microorganisms and encourages further research in this field.

8.2 Recommendations for future research

The outcome of this research revealed the impact of hybrid pre-treatment in two-stage anaerobic digestion and various advantages of the two-stage operation. Also, the effects of hybrid pre-treatment in sludge architecture and oxidative status have been studied. With this background, the following research directions are recommended for further investigations.

- A pilot-scale study of two-stage anaerobic digestion with further investigations on the effect of hybrid pre-treatment on sludge characteristics is recommended. The economic aspects of the pre-treatment and two-stage operation can be evaluated through the pilot-scale study for their implementation at WWTPs.

- Rheological characteristics of various sludge type during hybrid pre-treatment and anaerobic digestion can be studied. Sludge rheology plays a major role in digester hydrodynamics and mixing pattern. In the current study, the rheology of effluent digestate was characterized. However, the investigation of sludge rheology during pre-treatment and the course of anaerobic digestion will help understand the flow and mixing behavior. As the impact of hybrid pre-treatment is dependent on substrate characteristics, the rheology of different sludge types can be studied in pre-treated two-stage anaerobic digestion in the future.
- The molecular mechanism involved in the generation and control of ROS during anaerobic digestion needs to be explored. The current study elucidated the generation of ROS during hybrid (MW-H_2O_2) pre-treatment through the quantification of ($OH^{\bullet}$) and ($O_2^{\bullet -}$) radicals. Future studies can demonstrate the molecular mechanisms and pathways involved in the evolution of ROS and the control mechanisms in anaerobic microorganisms.
- The structure-function relationship of biopolymers released during pre-treatment and their implications in dewaterability can be investigated. As the release of biopolymers and sludge dewaterability are correlated in the current research, further studies are recommended to elucidate the characteristics of released biopolymers and their functionality in dewaterability.

REFERENCES

AB32, 2006. Global warming solutions act of 2006, California State Assembly.

Abelleira, J., Pérez-Elvira, S.I., Sánchez-Oneto, J., Portela, J.R., Nebot, E., 2012. Advanced Thermal Hydrolysis of secondary sewage sludge: A novel process combining thermal hydrolysis and hydrogen peroxide addition. Resour. Conserv. Recycl. 59, 52–57. https://doi.org/10.1016/j.resconrec.2011.03.008

Achinas, S., Euverink, G.J.W., 2016. Theoretical analysis of biogas potential prediction from agricultural waste. Resour. Technol. 2, 143–147. https://doi.org/10.1016/j.reffit.2016.08.001

Ahn, J.H., Shin, S.G., Hwang, S., 2009. Effect of microwave irradiation on the disintegration and acidogenesis of municipal secondary sludge. Chem. Eng. J. 153, 145–150. https://doi.org/10.1016/j.cej.2009.06.032

Alfaro, N., Cano, R., Fdz-Polanco, F., 2014. Effect of thermal hydrolysis and ultrasounds pretreatments on foaming in anaerobic digesters. Bioresour. Technol. 170, 477–482. https://doi.org/10.1016/j.biortech.2014.08.013

Algapani, D.E., Qiao, W., Su, M., di Pumpo, F., Wandera, S.M., Adani, F., Dong, R., 2016. Bio-hydrolysis and bio-hydrogen production from food waste by thermophilic and hyperthermophilic anaerobic process. Bioresour. Technol. 216, 768–777. https://doi.org/10.1016/j.biortech.2016.06.016

Algapani, D.E., Wang, J., Qiao, W., Su, M., Goglio, A., Wandera, S.M., Jiang, M., Pan, X., Adani, F., Dong, R., 2017. Improving methane production and anaerobic digestion stability of food waste by extracting lipids and mixing it with sewage sludge. Bioresour. Technol. 244, 996–1005. https://doi.org/10.1016/j.biortech.2017.08.087

Alqaralleh, R.M., Kennedy, K., Delatolla, R., 2020. Molecular weight distribution of pretreated thickened waste activated sludge and fat, oil, and grease. Environ. Sci. Pollut. Res. 27, 13227–13236. https://doi.org/10.1007/s11356-020-07906-1

Alqaralleh, R.M., Kennedy, K., Delatolla, R., 2019. Microwave vs. alkaline-microwave pretreatment for enhancing Thickened Waste Activated Sludge and fat, oil, and grease solubilization, degradation and biogas production. J. Environ. Manage. 233, 378–392.

https://doi.org/10.1016/j.jenvman.2018.12.046

Alsop, G.M., Conway, R.A., 1982. Improved thermal sludge conditioning by treatment with acids and bases. J. Water Pollut. Control Fed. 54, 146–152.

Alvarez, J.G., Touchstone Joseph C, Luis, B., Storey, B.T., 1978. Spontaneous Hydrogen Superoxide Enzyme Lipid Peroxide Dismutase Peroxidation and Production in Human of Spermatozoa and Superoxide as Major Against Oxygen Toxicity. J. Androl. 338–348. https://doi.org/10.1002/j.1939-4640.1987.tb00973.x

Anderson, G.K., Kasapgil, B., Ince, O., 1994. Microbiological study of two-stage anaerobic digestion during start-up. Water Res. 28, 2383–2392.

Anderson, N.J., Dixon, D.R., Harbour, P.J., Scales, P.J., 2002. Complete characterisation of thermally treated sludges. Water Sci. Technol. 46, 51–54. https://doi.org/10.2166/wst.2002.0287

Andreozzi, R., 1999. Advanced oxidation processes (AOP) for water purification and recovery. Catal. Today 53, 51–59. https://doi.org/10.1016/S0920-5861(99)00102-9

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., Van Lier, J.B., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. Water Sci. Technol. 59, 927–934. https://doi.org/10.2166/wst.2009.040

Anjum, M., Al-Makishah, N.H., Barakat, M.A., 2016. Wastewater sludge stabilization using pre-treatment methods. Process Saf. Environ. Prot. 102, 615–632. https://doi.org/10.1016/j.psep.2016.05.022

Anukam, A., Mohammadi, A., Naqvi, M., Granström, K., 2019. A Review of the Chemistry of Anaerobic Digestion: Methods of Accelerating and Optimizing Process Efficiency. Processes 7, 504. https://doi.org/10.3390/pr7080504

APHA, 1999. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington DC.

Appels, L., Degrève, J., Van der Bruggen, B., Van Impe, J., Dewil, R., 2010. Influence of low temperature thermal pre-treatment on sludge solubilisation, heavy metal release and anaerobic digestion. Bioresour. Technol. 101, 5743–5748. https://doi.org/10.1016/j.biortech.2010.02.068

Appels, L., Houtmeyers, S., Degreve, J., Van Impe, J., Dewil, R., 2013. Influence of microwave pre-treatment on sludge solubilization and pilot scale semi-continuous anaerobic digestion. Bioresour. Technol. 128, 598–603. https://doi.org/10.1016/j.biortech.2012.11.007

Apul, O.G., Atalar, I., Zorba, G.T., Sanin, F.D., 2010. The dewaterability of disintegrated sludge samples before and after anaerobic digestion. Dry. Technol. 28, 901–909. https://doi.org/10.1080/07373937.2010.490764

Aqua Designs, n.d. Engineering Procurement and Construction Division.

Arhoun, B., Villen-Guzman, M., Gomez-Lahoz, C., Rodriguez-Maroto, J.M., Garcia-Herruzo, F., Vereda-Alonso, C., 2019. Anaerobic co-digestion of mixed sewage sludge and fruits and vegetable wholesale market waste: Composition and seasonality effect. J. Water Process Eng. 31, 100848. https://doi.org/10.1016/j.jwpe.2019.100848

Arias, A., Behera, C.R., Feijoo, G., Sin, G., Moreira, M.T., 2020. Unravelling the environmental and economic impacts of innovative technologies for the enhancement of biogas production and sludge management in wastewater systems. J. Environ. Manage. 270, 110965. https://doi.org/10.1016/j.jenvman.2020.110965

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., Lens, P.N.L., 2014. Pretreatment methods to enhance anaerobic digestion of organic solid waste. Appl. Energy 123, 143–156. https://doi.org/10.1016/j.apenergy.2014.02.035

Astals, S., Esteban-Gutiérrez, M., Fernández-Arévalo, T., Aymerich, E., García-Heras, J.L., Mata-Alvarez, J., 2013. Anaerobic digestion of seven different sewage sludges: A biodegradability and modelling study. Water Res. 47, 6033–6043. https://doi.org/10.1016/j.watres.2013.07.019

Aylin Alagöz, B., Yenigün, O., Erdinçler, A., 2016. Ultrasound assisted biogas production from co-digestion of wastewater sludges and agricultural wastes: Comparison with microwave pre-treatment. Ultrason. Sonochem. 40, 193–200. https://doi.org/10.1016/j.ultsonch.2017.05.014

Baldi, F., Pecorini, I., Iannelli, R., 2019. Comparison of single-stage and two-stage anaerobic co-digestion of food waste and activated sludge for hydrogen and methane production. Renew.

Energy 143, 1755–1765. https://doi.org/10.1016/j.renene.2019.05.122

Banik, S., Bandyopadhyay, S., Ganguly, S., 2003. Bioeffects of microwave - a brief review. Bioresour. Technol. 87, 155–159. https://doi.org/10.1016/S0960-8524(02)00169-4

Bao, H., Jiang, L., Chen, C., Yang, C., He, Z., Feng, Y., Cai, W., Liu, W., Wang, A., 2015. Combination of ultrasound and Fenton treatment for improving the hydrolysis and acidification of waste activated sludge. RSC Adv. 5, 48468–48473. https://doi.org/10.1039/c5ra05791d

Bartram, J., Pedley, S., 1996. Water Quality Monitoring- A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes. United Nations Environ. Program. World Heal. Organ. 1–27. https://doi.org/10.1002/ejoc.201200111

Baudez, J.C., Markis, F., Eshtiaghi, N., Slatter, P., 2011. The rheological behaviour of anaerobic digested sludge. Water Res. 45, 5675–5680. https://doi.org/10.1016/j.watres.2011.08.035

Bayr, S., Kaparaju, P., Rintala, J., 2013. Screening pretreatment methods to enhance thermophilic anaerobic digestion of pulp and paper mill wastewater treatment secondary sludge. Chem. Eng. J. 223, 479–486. https://doi.org/10.1016/j.cej.2013.02.119

Beavis, P., Lundie, S., 2003. Integrated environmental assessment of tertiary and residuals treatment - LCA in the wastewater industry. Water Sci. Technol. 47, 109–116. https://doi.org/10.2166/wst.2003.0678

Bennoit, H., 2014. Improvement of separation processes in waste water treatment by controlling the sludge properties m, 1–9. https://doi.org/10.13140/2.1.1086.3685

Biemelt, S., Keetman, U., Mock, H.P., Grimm, B., 2000. Expression and activity of isoenzymes of superoxide dismutase in wheat roots in response to hypoxia and anoxia. Plant, Cell Environ. 23, 135–144. https://doi.org/10.1046/j.1365-3040.2000.00542.x

Bilgin Oncu, N., Akmehmet Balcioglu, I., 2013. Microwave-assisted chemical oxidation of biological waste sludge: Simultaneous micropollutant degradation and sludge solubilization. Bioresour. Technol. 146, 126–134. https://doi.org/10.1016/j.biortech.2013.07.043

Bjerg-Nielsen, M., Ward, A.J., Møller, H.B., Ottosen, L.D.M., 2018. Influence on anaerobic digestion by intermediate thermal hydrolysis of waste activated sludge and co-digested wheat straw. Waste Manag. 72, 186–192. https://doi.org/10.1016/j.wasman.2017.11.021

Bolado-Rodríguez, S., Toquero, C., Martín-Juárez, J., Travaini, R., García-Encina, P.A., 2016. Effect of thermal, acid, alkaline and alkaline-peroxide pretreatments on the biochemical methane potential and kinetics of the anaerobic digestion of wheat straw and sugarcane bagasse. Bioresour. Technol. 201, 182–190. https://doi.org/10.1016/j.biortech.2015.11.047

Bolzonella, D., Battistoni, P., Susini, C., Cecchi, F., 2006. Anaerobic codigestion of waste activated sludge and OFMSW: The experiences of Viareggio and Treviso plants (Italy). Water Sci. Technol. 53, 203–211. https://doi.org/10.2166/wst.2006.251

Bolzonella, D., Cavinato, C., Fatone, F., Pavan, P., Cecchi, F., 2012. High rate mesophilic, thermophilic, and temperature phased anaerobic digestion of waste activated sludge: A pilot scale study. Waste Manag. 32, 1196–1201. https://doi.org/10.1016/j.wasman.2012.01.006

Bolzonella, D., Pavan, P., Zanette, M., Cecchi, F., 2007. Two-phase anaerobic digestion of waste activated sludge: Effect of an extreme thermophilic prefermentation. Ind. Eng. Chem. Res. 46, 6650–6655. https://doi.org/10.1021/ie061627e

Borowski, S., 2015. Temperature-phased anaerobic digestion of the hydromechanically separated organic fraction of municipal solid waste with sewage sludge. Int. Biodeterior. Biodegrad. 105, 106–113. https://doi.org/10.1016/j.ibiod.2015.08.022

Bouallagui, H., Touhami, Y., Ben Cheikh, R., Hamdi, M., 2005. Bioreactor performance in anaerobic digestion of fruit and vegetable wastes. Process Biochem. 40, 989–995. https://doi.org/10.1016/j.procbio.2004.03.007

Bouda, M., Hammy, F., Mercier, G., Blais, J.-F., 2009. Chemical Leaching of Metals from Wastewater Sludge: Comparative Study by Use of Three Oxidizing Agents [H2O2 , FeCl3 , and Fe2(SO4)3]. Water Environ. Res. 81, 523–531. https://doi.org/10.2175/106143008x390825

Bougrier, C., Albasi, C., Delgenès, J.P., Carrère, H., 2006. Effect of ultrasonic, thermal and ozone pre-treatments on waste activated sludge solubilisation and anaerobic biodegradability. Chem. Eng. Process. Process Intensif. 45, 711–718. https://doi.org/10.1016/j.cep.2006.02.005

Bougrier, C., Battimelli, A., Delgenes, J.P., Carrere, H., 2007. Combined ozone pretreatment and anaerobic digestion for the reduction of biological sludge production in wastewater treatment. Ozone Sci. Eng. 29, 201–206. https://doi.org/10.1080/01919510701296754

Bougrier, C., Delgenès, J.P., Carrère, H., 2008. Effects of thermal treatments on five different waste activated sludge samples solubilisation, physical properties and anaerobic digestion. Chem. Eng. J. 139, 236–244. https://doi.org/10.1016/j.cej.2007.07.099

Bozkurt, Y.C., Apul, O.G., 2020. Critical review for microwave pretreatment of waste-activated sludge prior to anaerobic digestion. Curr. Opin. Environ. Sci. Heal. 14, 1–9. https://doi.org/10.1016/j.coesh.2019.10.003

Braguglia, C.M., Carozza, N., Gagliano, M.C., Gallipoli, A., Gianico, A., Rossetti, S., Suschka, J., Tomei, M.C., Mininni, G., 2014. Advanced anaerobic processes to enhance waste activated sludge stabilization. Water Sci. Technol. 69, 1728–1734. https://doi.org/10.2166/wst.2014.067

Braguglia, C.M., Gianico, A., Mininni, G., 2012. Comparison between ozone and ultrasound disintegration on sludge anaerobic digestion. J. Environ. Manage. 95, S139–S143. https://doi.org/10.1016/j.jenvman.2010.07.030

Bryant, S., Rahmanian, R., Tam, H., Zabetian, S., 2007. Effects Of Microwave Irradiation And Heat On T4 Bacteriophage Inactivation. J. Exp. Microbiol. Immunol. 11, 66–72.

Buntner, D., Spanjers, H., van Lier, J.B., 2014. The influence of hydrolysis induced biopolymers from recycled aerobic sludge on specific methanogenic activity and sludge filterability in an anaerobic membrane bioreactor. Water Res. 51, 284–292. https://doi.org/10.1016/j.watres.2013.10.065

Burdock, T., Brooks, M., Ghaly, A., Dave, D., 2011. Effect of assay conditions on the measurement of dehydrogenase activity of Streptomyces venezuelae using triphenyl tetrazolium chloride. Adv. Biosci. Biotechnol. 02, 214–225. https://doi.org/10.4236/abb.2011.24032

Burgess, J.E., Pletschke, B.I., 2008. Hydrolytic enzymes in sewage sludge treatment: A mini-review. Water SA 34, 343–349. https://doi.org/10.4314/wsa.v34i3.180627

Buswell, A.M., Mueller, H.F., 1952. Mechanism of Methane Fermentation. Ind. Eng. Chem. 44, 550–552. https://doi.org/10.1021/ie50507a033

Buxton, G. V., Greenstock, C.L., Helman, W.P., Ross, A.B., 1988. Critical Review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals (OH/O) in Aqueous Solution. J. Phys. Chem. Ref. Data 17, 513–886. https://doi.org/10.1063/1.555805

Buyukkamaci, N., 2004. Biological sludge conditioning by Fenton's reagent. Process Biochem. 39, 1503–1506. https://doi.org/10.1016/S0032-9592(03)00294-2

Byun, I., Lee, Jaeho, Lim, J., Lee, Jeongmin, Park, T., 2014. Impact of Irradiation Time on the Hydrolysis of Waste Activated Sludge by the Dielectric Heating of Microwave 19, 83–89.

Byun, I.G., Lee, J.H., Lee, J.M., Lim, J.S., Park, T.J., 2018. Evaluation of non-thermal effects by microwave irradiation in hydrolysis of waste-activated sludge 742–749. https://doi.org/10.2166/wst.2014.295

C P Leslie Grady, Daigger, G.T., Love, N.G., Filipe, C.D.M., 2011. Biological Wastewater Treatment, Third. ed. IWA publishing.

Cao, X., Jiang, Z., Cui, W., Wang, Y., Yang, P., 2016. Rheological Properties of Municipal Sewage Sludge: Dependency on Solid Concentration and Temperature. Procedia Environ. Sci. 31, 113–121. https://doi.org/10.1016/j.proenv.2016.02.016

Carrere, H., Antonopoulou, G., Affes, R., Passos, F., Battimelli, A., Lyberatos, G., Ferrer, I., 2016. Review of feedstock pretreatment strategies for improved anaerobic digestion: From lab-scale research to full-scale application. Bioresour. Technol. 199, 386–397. https://doi.org/10.1016/j.biortech.2015.09.007

Carrère, H., Dumas, C., Battimelli, A., Batstone, D.J., Delgenès, J.P., Steyer, J.P., Ferrer, I., 2010. Pretreatment methods to improve sludge anaerobic degradability: A review. J. Hazard. Mater. 183, 1–15. https://doi.org/10.1016/j.jhazmat.2010.06.129

Cella, M.A., Akgul, D., Eskicioglu, C., 2016. Assessment of microbial viability in municipal sludge following ultrasound and microwave pretreatments and resulting impacts on the efficiency of anaerobic sludge digestion. Appl. Microbiol. Biotechnol. 100, 2855–2868. https://doi.org/10.1007/s00253-015-7139-3

Cetin, S., Erdincler, A., 2004. The role of carbohydrate and protein parts of extracellular polymeric substances on the dewaterability of biological sludges. Water Sci. Technol. 50, 49–56.

https://doi.org/10.1111/j.1467-8624.2009.01394.x.Biological

Chacana, J., Alizadeh, S., Labelle, M.A., Laporte, A., Hawari, J., Barbeau, B., Comeau, Y., 2017. Effect of ozonation on anaerobic digestion sludge activity and viability. Chemosphere 176, 405–411. https://doi.org/10.1016/j.chemosphere.2017.02.108

Chan, W.I., Liao, P.H., Lo, K.V., 2010. Effects of Irradiation Intensity and pH on Nutrients Release and Solids Destruction of Waste Activated Sludge Using the Microwave-Enhanced Advanced Oxidation Process. Water Environ. Res. 82, 2229–2238. https://doi.org/10.2175/106143010x12681059116419

Chen, C.K., Lo, S.L., 2003. Treatment of slaughterhouse wastewater using an activated sludge/contact aeration process. Water Sci. Technol. 47, 285–292. https://doi.org/10.2166/wst.2003.0658

Chen, D., Sharma, S.K., Mudhoo, A., 2012. Handbook on Applications of Ultrasound: Sonochemistry for Sustainability. CRC Press.

Chen, J., Rogers, S.C., Kavdia, M., 2013. Analysis of kinetics of dihydroethidium fluorescence with superoxide using xanthine oxidase and hypoxanthine assay. Ann. Biomed. Eng. 41, 327–337. https://doi.org/10.1007/s10439-012-0653-x

Chen, Y., Yang, H., Gu, G., 2001. Effect of acid and surfactant treatment on activated sludge dewatering and settling. Water Res. 35, 2615–2620. https://doi.org/10.1016/S0043-1354(00)00565-0

Cheng, C.J., Andy Hong, P.K., Lin, C.F., 2012. Improved solubilization of activated sludge by ozonation in pressure cycles. Chemosphere 87, 637–643. https://doi.org/10.1016/j.chemosphere.2012.02.001

Chi, Y., Li, Y., Fei, X., Wang, S., Yuan, H., 2011. Enhancement of thermophilic anaerobic digestion of thickened waste activated sludge by combined microwave and alkaline pretreatment. J. Environ. Sci. 23, 1257–1265. https://doi.org/10.1016/S1001-0742(10)60561-X

Chiappero, M., Demichelis, F., Lin, X., Liu, C., Frigon, D., Fiore, S., 2019. Investigation of pre-treatments improving low-temperature anaerobic digestion of waste activated sludge. Process Saf. Environ. Prot. 131, 28–37. https://doi.org/10.1016/j.psep.2019.08.034

Chiu, Y.-C., Chang, C.-N., Lin, J.-G., Huang, S.-J., 1997. Alkaline and ultrasonic pretreatment of sludge before anaerobic digestion. Water Sci. Technol. 36, 155–162.

Chong, S., Sen, T.K., Kayaalp, A., Ang, H.M., 2012. The performance enhancements of upflow anaerobic sludge blanket (UASB) reactors for domestic sludge treatment - A State-of-the-art review. Water Res. 46, 3434–3470. https://doi.org/10.1016/j.watres.2012.03.066

Chu, C.P., Chang, B.V., Liao, G.S., Jean, D.S., Lee, D.J., 2001. Observations on changes in ultrasonically treated waste-activated sludge. Water Res. 35, 1038–1046. https://doi.org/10.1016/S0043-1354(00)00338-9

Chu, L.B., Yan, S.T., Xing, X.H., Yu, A.F., Sun, X.L., Jurcik, B., 2008. Enhanced sludge solubilization by microbubble ozonation. Chemosphere 72, 205–212. https://doi.org/10.1016/j.chemosphere.2008.01.054

Chung, Y.C., Neethling, J.B., 1989. Microbial activity measurements for anaerobic sludge digestion. J. Water Pollut. Control Fed. 61, 343–349.

Cieślik, B.M., Namieśnik, J., Konieczka, P., 2015. Review of sewage sludge management: Standards, regulations and analytical methods. J. Clean. Prod. 90, 1–15. https://doi.org/10.1016/j.jclepro.2014.11.031

Climent, M., Ferrer, I., Baeza, M. del M., Artola, A., Vázquez, F., Font, X., 2007. Effects of thermal and mechanical pretreatments of secondary sludge on biogas production under thermophilic conditions. Chem. Eng. J. 133, 335–342. https://doi.org/10.1016/j.cej.2007.02.020

CMWSSB, 2020. Chennai Metropolitan Water Supply and Sewerage Board: Sewerage System [WWW Document]. URL https://chennaimetrowater.tn.gov.in/stp.html (accessed 9.8.20).

Coelho, N.M.G., 2012. Application of Microwaves and Thermophilic Anaerobic Digestion of Wastewater Sludge Treatment. University of Ottawa, Canada.

Coelho, N.M.G., Droste, R.L., Kennedy, K.J., 2011. Evaluation of continuous mesophilic, thermophilic and temperature phased anaerobic digestion of microwaved activated sludge. Water Res. 45, 2822–2834. https://doi.org/10.1016/j.watres.2011.02.032

Collivignarelli, M.C., Pedrazzani, R., Sorlini, S., Abbà, A., Bertanza, G., 2017. H2O2 based oxidation processes for the treatment of real high strength aqueous wastes. Sustain. 9, 1–14. https://doi.org/10.3390/su9020244

Cuéllar, A.D., Webber, M.E., 2008. Cow power: The energy and emissions benefits of converting manure to biogas. Environ. Res. Lett. 3. https://doi.org/10.1088/1748-9326/3/3/034002

Cuetos, M.J., Gómez, X., Otero, M., Morán, A., 2010. Anaerobic digestion of solid slaughterhouse waste: Study of biological stabilization by Fourier Transform infrared spectroscopy and thermogravimetry combined with mass spectrometry. Biodegradation 21, 543–556. https://doi.org/10.1007/s10532-009-9322-7

De Laclos, H.F., Thiebaut, E., Saint-Joly, C., 2008. Anaerobic digestion of residual municipal solid waste using biological-mechanical pre-treatment: The plant of Varennes Jarcy. Water Sci. Technol. 58, 1447–1452. https://doi.org/10.2166/wst.2008.519

De León, C., Jenkins, D., 2002. Removal of fecal coliforms by thermophilic anaerobic digestion processes. Water Sci. Technol. 46, 147–152. https://doi.org/10.2166/wst.2002.0314

Delmas, H., Le, N.T., Barthe, L., Julcour-Lebigue, C., 2015. Optimization of hydrostatic pressure at varied sonication conditions - Power density, intensity, very low frequency - For isothermal ultrasonic sludge treatment. Ultrason. Sonochem. 25, 51–59. https://doi.org/10.1016/j.ultsonch.2014.08.011

Demir, O., Filibeli, A., 2012. Fate of return activated sludge after ozonation: An optimization study for sludge disintegration. Environ. Technol. (United Kingdom) 33, 1869–1878. https://doi.org/10.1080/09593330.2011.650220

Demirel, B., Yenigün, O., 2002. Two-phase anaerobic digestion processes: A review. J. Chem. Technol. Biotechnol. 77, 743–755. https://doi.org/10.1002/jctb.630

Deng, Y., Zhao, R., 2015. Advanced Oxidation Processes (AOPs) in Wastewater Treatment. Curr. Pollut. Reports 1, 167–176. https://doi.org/10.1007/s40726-015-0015-z

Dererie, D.Y., Trobro, S., Momeni, M.H., Hansson, H., Blomqvist, J., Passoth, V., Schnürer, A., Sandgren, M., Ståhlberg, J., 2011. Improved bio-energy yields via sequential ethanol fermentation and biogas digestion of steam exploded oat straw. Bioresour. Technol. 102, 4449–4455. https://doi.org/10.1016/j.biortech.2010.12.096

Dessì, P., Jain, R., Singh, S., Seder-Colomina, M., van Hullebusch, E.D., Rene, E.R., Ahammad, S.Z., Carucci, A., Lens, P.N.L., 2016. Effect of temperature on selenium removal from wastewater by UASB reactors. Water Res. 94, 146–154. https://doi.org/10.1016/j.watres.2016.02.007

Devlin, D.C., Esteves, S.R.R., Dinsdale, R.M., Guwy, A.J., 2011. The effect of acid pretreatment on the anaerobic digestion and dewatering of waste activated sludge. Bioresour. Technol. 102, 4076–4082. https://doi.org/10.1016/j.biortech.2010.12.043

Dewil, R., Appels, L., Baeyens, J., Degre, J., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge 34, 755–781. https://doi.org/10.1016/j.pecs.2008.06.002

Dewil, R., Appels, L., Baeyens, J., Degrève, J., 2007. Peroxidation enhances the biogas production in the anaerobic digestion of biosolids. J. Hazard. Mater. 146, 577–581. https://doi.org/10.1016/j.jhazmat.2007.04.059

Dewil, R., Baeyens, J., Neyens, E., 2005. Fenton peroxidation improves the drying performance of waste activated sludge. J. Hazard. Mater. 117, 161–170. https://doi.org/10.1016/j.jhazmat.2004.09.025

Dhar, B.R., Elbeshbishy, E., Hafez, H., Nakhla, G., Ray, M.B., 2011a. Thermo-oxidative pretreatment of municipal waste activated sludge for volatile sulfur compounds removal and enhanced anaerobic digestion. Chem. Eng. J. 174, 166–174. https://doi.org/10.1016/j.cej.2011.08.070

Dhar, B.R., Youssef, E., Nakhla, G., Ray, M.B., 2011b. Pretreatment of municipal waste activated sludge for volatile sulfur compounds control in anaerobic digestion. Bioresour. Technol. 102, 3776–3782. https://doi.org/10.1016/j.biortech.2010.12.020

Di Maria, F., Barratta, M., 2015. Boosting methane generation by co-digestion of sludge with fruit and vegetable waste: Internal environment of digester and methanogenic pathway. Waste Manag. 43, 130–136. https://doi.org/10.1016/j.wasman.2015.06.007

Di Maria, F., Sordi, A., Cirulli, G., Gigliotti, G., Massaccesi, L., 2014. Co-treatment of fruit and vegetable waste in sludge digesters . An analysis of the relationship among bio-methane generation , process stability and digestate phytotoxicity. Waste Manag. 34, 1603–1608. https://doi.org/10.1016/j.wasman.2014.05.017

Diak, J., Örmeci, B., 2016. Individual and Combined Effects of Freeze-Thaw and Ferrate(VI) Oxidation for the Treatment and Dewatering of Wastewater Sludges. Water. Air. Soil Pollut. 227. https://doi.org/10.1007/s11270-016-3039-0

Diamantis, V., Aivasidis, A., 2010. Two-stage UASB design enables activated-sludge free treatment of easily biodegradable wastewater. Bioprocess Biosyst. Eng. 33, 287–292. https://doi.org/10.1007/s00449-009-0323-z

Díaz, O., Vera, L., González, E., García, E., Rodríguez-Sevilla, J., 2016. Effect of sludge characteristics on membrane fouling during start-up of a tertiary submerged membrane bioreactor. Environ. Sci. Pollut. Res. 23, 8951–8962. https://doi.org/10.1007/s11356-016-6138-y

Dillalo, R., Albertson, O.E., 1961. Volatile acids by direct tritation. Water Pollut. Control Fed. 33, 356–365.

Doble, M., Geetha, V., 2011. Understand the fundamentals of wastewater treatment. Chem. Eng. Prog. 107, 36–42.

Doğan, I., Sanin, F.D., 2009. Alkaline solubilization and microwave irradiation as a combined sludge disintegration and minimization method. Water Res. 43, 2139–2148. https://doi.org/10.1016/j.watres.2009.02.023

Dohányos, M., Zábranská, J., Kutil, J., Jeníček, P., 2004. Improvement of anaerobic digestion of sludge. Water Sci. Technol. 49, 89–96. https://doi.org/10.2166/wst.2004.0616

Dokulilová, T., Vítez, T., Chovanec, J., Rous, R., Vítezová, M., Kushkevych, I., 2018. Primary and activated sludge biogas production: Effect of temperature. Acta Univ. Agric. Silvic. Mendelianae Brun. 66, 23–28. https://doi.org/10.11118/actaun201866010023

Dong, B., Gao, P., Zhang, D., Chen, Y., Dai, L., Dai, X., 2016. A new process to improve short-chain fatty acids and bio-methane generation from waste activated sludge. J. Environ. Sci. (China) 43, 159–168. https://doi.org/10.1016/j.jes.2015.10.004

Dong, M.M., Rosario-Ortiz, F.L., 2012. Photochemical Formation of Hydroxyl Radical from Effluent Organic Matter. Environ. Sci. Technol. 46, 3788–3794. https://doi.org/10.1021/es2043454

Doran, P.M., 1995. Bioprocess Engineering Principles, Academic Press. https://doi.org/10.1017/CBO9781107415324.004

Du, X., Wang, J., Jegatheesan, V., Shi, G., 2018. Dissolved oxygen control in activated sludge process using a neural network-based adaptive PID algorithm. Appl. Sci. 8. https://doi.org/10.3390/app8020261

Dwyer, J., Starrenburg, D., Tait, S., Barr, K., Batstone, D.J., Lant, P., 2008. Decreasing activated sludge thermal hydrolysis temperature reduces product colour , without decreasing degradability 42, 4699–4709. https://doi.org/10.1016/j.watres.2008.08.019

Ebenezer, A.V., Kaliappan, S., Adish Kumar, S., Yeom, I.T., Banu, J.R., 2015. Influence of deflocculation on microwave disintegration and anaerobic biodegradability of waste activated sludge. Bioresour. Technol. 185, 194–201. https://doi.org/10.1016/j.biortech.2015.02.102

Elagroudy, S., Radwan, A.G., Banadda, N., Mostafa, N.G., Owusu, P.A., Janajreh, I., 2020. Mathematical models comparison of biogas production from anaerobic digestion of microwave pretreated mixed sludge. Renew. Energy 155, 1009–1020. https://doi.org/10.1016/j.renene.2020.03.166

Elliott, A., Mahmood, T., 2012. Comparison of Mechanical Pretreatment Methods for the Enhancement of Anaerobic Digestion of Pulp and Paper Waste Activated Sludge. Water Environ. Res. 84, 497–505. https://doi.org/10.2175/106143012x13347678384602

Ennouri, H., Miladi, B., Diaz, S.Z., Guelfo, L.A.F., Solera, R., Hamdi, M., Bouallagui, H., 2016. Effect of thermal pretreatment on the biogas production and microbial communities balance during anaerobic digestion of urban and industrial waste activated sludge. Bioresour. Technol. 214, 184–191. https://doi.org/10.1016/j.biortech.2016.04.076

Erden, G., Filibeli, A., 2011. Effects of Fenton Pre-Treatment on Waste Activated Sludge Properties. Clean - Soil, Air, Water 39, 626–632. https://doi.org/10.1002/clen.201000199

Erden, G., Filibeli, A., 2010. Improving anaerobic biodegradability of biological sludges by Fenton pre-treatment: Effects on single stage and two-stage anaerobic digestion. Desalination 251, 58–63. https://doi.org/10.1016/j.desal.2009.09.144

Erdincler, A., Vesilind, P.A., 2000. Effect of sludge cell disruption on compactibility of biological sludges. Water Sci. Technol. 42, 119–126. https://doi.org/10.2166/wst.2000.0185

Eskicioglu, C., Droste, R.L., Kennedy, K.J., Eskicioglu, C., Droste, R.L., Kennedy, K.J., 2007a. Performance of Anaerobic Waste Activated Sludge Digesters After Microwave Pretreatment. Water Environ. Res. 79, 2265–2273. https://doi.org/10.2175/106143007X176004

Eskicioglu, C., Kennedy, K.J., Droste, R.L., 2007b. Enhancement of Batch Waste Activated Sludge Digestion by Microwave Pretreatment. Water Environ. Res. 79, 2304–2317. https://doi.org/10.2175/106143007X184069

Eskicioglu, C., Kennedy, K.J., Droste, R.L., 2006. Characterization of soluble organic matter of waste activated sludge before and after thermal pretreatment. Water Res. 40, 3725–3736. https://doi.org/10.1016/j.watres.2006.08.017

Eskicioglu, C., Prorot, A., Marin, J., Droste, R.L., Kennedy, K.J., 2008. Synergetic pretreatment of sewage sludge by microwave irradiation in presence of H2O2 for enhanced anaerobic digestion. Water Res. 42, 4674–4682. https://doi.org/10.1016/j.watres.2008.08.010

Eskicioglu, C., Terzian, N., Kennedy, K.J., Droste, R.L., Hamoda, M., 2007c. Athermal microwave effects for enhancing digestibility of waste activated sludge. Water Res. 41, 2457–2466. https://doi.org/10.1016/j.watres.2007.03.008

Eswari, P., Kavitha, S., Kaliappan, S., Yeom, I.T., Banu, J.R., 2016. Enhancement of sludge anaerobic biodegradability by combined microwave-H2O2pretreatment in acidic conditions. Environ. Sci. Pollut. Res. 23, 13467–13479. https://doi.org/10.1007/s11356-016-6543-2

Ezeji, T.C., Qureshi, N., Blaschek, H.P., 2013. Microbial production of a biofuel (acetone-butanol-ethanol) in a continuous bioreactor: Impact of bleed and simultaneous product removal. Bioprocess Biosyst. Eng. 36, 109–116. https://doi.org/10.1007/s00449-012-0766-5

Fakhru'l-Razi, A., Molla, A.H., 2007. Enhancement of bioseparation and dewaterability of domestic wastewater sludge by fungal treated dewatered sludge. J. Hazard. Mater. 147, 350–356. https://doi.org/10.1016/j.jhazmat.2007.01.060

Fang, W., Zhang, P., Ye, J., Wu, Y., Zhang, H., Liu, J., Zhu, Y., Zeng, G., 2015. Physicochemical properties of sewage sludge disintegrated with high pressure homogenization. Int. Biodeterior. Biodegrad. 102, 126–130. https://doi.org/10.1016/j.ibiod.2015.02.030

Fdez.-Güelfo, L.A., Álvarez-Gallego, C., Sales Márquez, D., Romero García, L.I., 2011. Biological pretreatment applied to industrial organic fraction of municipal solid wastes

(OFMSW): Effect on anaerobic digestion. Chem. Eng. J. 172, 321–325. https://doi.org/10.1016/j.cej.2011.06.010

Fdz-Polanco, F., Velazquez, R., Perez-Elvira, S.I., Casas, C., del Barrio, D., Cantero, F.J., Fdz-Polanco, M., Rodriguez, P., Panizo, L., Serrat, J., Rouge, P., 2008. Continuous thermal hydrolysis and energy integration in sludge anaerobic digestion plants. Water Sci. Technol. 57, 1221–1226. https://doi.org/10.2166/wst.2008.072

Feki, E., Sayadi, S., Loukil, S., Dhouib, A., Khoufi, S., 2019. Comparison between Thermo-Alkaline and Electro-Fenton Disintegration Effect on Waste Activated Sludge Anaerobic Digestion. Biomed Res. Int. 2019. https://doi.org/10.1155/2019/2496905

Feng, X., Lei, H., Deng, J., Yu, Q., Li, H., 2009. Physical and chemical characteristics of waste activated sludge treated ultrasonically. Chem. Eng. Process. Process Intensif. 48, 187–194. https://doi.org/10.1016/j.cep.2008.03.012

Ferreira, L.C., Souza, T.S.O., Fdz-Polanco, F., Pérez-Elvira, S.I., 2014. Thermal steam explosion pretreatment to enhance anaerobic biodegradability of the solid fraction of pig manure. Bioresour. Technol. 152, 393–398. https://doi.org/10.1016/j.biortech.2013.11.050

Ferrer, I., Ponsá, S., Vázquez, F., Font, X., 2008. Increasing biogas production by thermal (70 °C) sludge pre-treatment prior to thermophilic anaerobic digestion. Biochem. Eng. J. 42, 186–192. https://doi.org/10.1016/j.bej.2008.06.020

Foladori, P., Laura, B., Gianni, A., Giuliano, Z., 2007. Effects of sonication on bacteria viability in wastewater treatment plants evaluated by flow cytometry-Fecal indicators, wastewater and activated sludge. Water Res. 41, 235–243. https://doi.org/10.1016/j.watres.2006.08.021

Fountoulakis, M.S., Petousi, I., Manios, T., 2010. Co-digestion of sewage sludge with glycerol to boost biogas production. Waste Manag. 30, 1849–1853. https://doi.org/10.1016/j.wasman.2010.04.011

Gallego-Schmid, A., Tarpani, R.R.Z., 2019. Life cycle assessment of wastewater treatment in developing countries: A review. Water Res. 153, 63–79. https://doi.org/10.1016/j.watres.2019.01.010

Gao, W., 2011. Freezing as a combined wastewater sludge pretreatment and conditioning method. Desalination 268, 170–173. https://doi.org/10.1016/j.desal.2010.10.014

Gao, Z., Zhang, C., Su, H., Tan, T., 2013. Live steam-pretreatment and anaerobic digestion of waste activated sludge. Environ. Eng. Sci. 30, 546–554. https://doi.org/10.1089/ees.2012.0480

Gavala, H.N., Yenal, U., Skiadas, I. V., Westermann, P., Ahring, B.K., 2003. Mesophilic and thermophilic anaerobic digestion of primary and secondary sludge. Effect of pre-treatment at elevated temperature. Water Res. 37, 4561–4572. https://doi.org/10.1016/S0043-1354(03)00401-9

Ge, H., Jensen, P.D., Batstone, D.J., 2011a. Temperature phased anaerobic digestion increases apparent hydrolysis rate for waste activated sludge. Water Res. 45, 1597–1606. https://doi.org/10.1016/j.watres.2010.11.042

Ge, H., Jensen, P.D., Batstone, D.J., 2011b. Increased temperature in the thermophilic stage in temperature phased anaerobic digestion (TPAD) improves degradability of waste activated sludge. J. Hazard. Mater. 187, 355–361. https://doi.org/10.1016/j.jhazmat.2011.01.032

Ge, H., Jensen, P.D., Batstone, D.J., 2010. Pre-treatment mechanisms during thermophilic-mesophilic temperature phased anaerobic digestion of primary sludge. Water Res. 44, 123–130. https://doi.org/10.1016/j.watres.2009.09.005

Gebreeyessus, G., Jenicek, P., 2016. Thermophilic versus Mesophilic Anaerobic Digestion of Sewage Sludge: A Comparative Review. Bioengineering 3, 15. https://doi.org/10.1111/1471-0528.12510

Ghaly, A.E., Mahmoud, N.S., 2006. Optimum Conditions for Measuring Dehydrogenase Activity of Aspergillus niger using TTC. Am. J. Biochem. Biotechnol. 2, 186–194. https://doi.org/10.3844/ajbbsp.2006.186.194

Ghasimi, D.S.M., Tao, Y., de Kreuk, M., Abbas, B., Zandvoort, M.H., van Lier, J.B., 2015. Digester performance and microbial community changes in thermophilic and mesophilic sequencing batch reactors fed with the fine sieved fraction of municipal sewage. Water Res. 87, 483–493. https://doi.org/10.1016/j.watres.2015.04.027

Ghosh, P.R., Fawcett, D., Sharma, S.B., Perera, D., Poinern, G.E.J., 2016. Survey of Food Waste Generated by Western Australian Fruit and Vegetable Producers: Options for Minimization and Utilization. Food Public Heal. 6, 115–122. https://doi.org/10.5923/j.fph.20160605.02

Ghosh, S., Buoy, K., Dressel, L., Miller, T., Wilcox, G., Loos, D., 1995. Pilot- and full-scale two-phase anaerobic digestion of municipal sludge. Water Environ. Res. 67, 206–214. https://doi.org/10.2175/106143095x131367

Gianico, A., Bertanza, G., Braguglia, C.M., Canato, M., Gallipoli, A., Laera, G., Levantesi, C., Mininni, G., 2016. Enhanced Versus Conventional Sludge Anaerobic Processes: Performances and Techno-Economic Assessment. Water Environ. Res. 88, 468–478. https://doi.org/10.2175/106143016X14504669768534

Gogate, Parag R, Pandit, A.B., 2004. A review of imperative technologies for wastewater treatment II : hybrid methods. Adv. Environ. Res. 8, 553–597. https://doi.org/10.1016/S1093-0191(03)00031-5

Gogate, Parag R., Pandit, A.B., 2004. A review of imperative technologies for wastewater treatment I: Oxidation technologies at ambient conditions. Adv. Environ. Res. 8, 501–551. https://doi.org/10.1016/S1093-0191(03)00032-7

Gómez-Ordóñez, E., Rupérez, P., 2011. FTIR-ATR spectroscopy as a tool for polysaccharide identification in edible brown and red seaweeds. Food Hydrocoll. 25, 1514–1520. https://doi.org/10.1016/j.foodhyd.2011.02.009

Gómez, X., Cuetos, M.J., Cara, J., Morán, A., García, A.I., 2006. Anaerobic co-digestion of primary sludge and the fruit and vegetable fraction of the municipal solid wastes. Conditions for mixing and evaluation of the organic loading rate. Renew. Energy 31, 2017–2024. https://doi.org/10.1016/j.renene.2005.09.029

Gong, C., Jiang, J., Li, D., Tian, S., 2015. Ultrasonic application to boost hydroxyl radical formation during Fenton oxidation and release organic matter from sludge. Sci. Rep. 5. https://doi.org/10.1038/srep11419

Gonzalez, A., Hendriks, A.T.W.M., van Lier, J.B., de Kreuk, M., 2018. Pre-treatments to enhance the biodegradability of waste activated sludge: Elucidating the rate limiting step. Biotechnol. Adv. 36, 1434–1469. https://doi.org/10.1016/j.biotechadv.2018.06.001

Gou, C., Yang, Z., Huang, J., Wang, H., Xu, H., Wang, L., 2014. Effects of temperature and organic loading rate on the performance and microbial community of anaerobic co-digestion of waste activated sludge and food waste. Chemosphere 105, 146–151.

https://doi.org/10.1016/j.chemosphere.2014.01.018

Grim, J., Nilsson, D., Hansson, P.A., Nordberg, Å., 2015. Demand-Orientated Power Production from Biogas: Modeling and Simulations under Swedish Conditions. Energy and Fuels 29, 4066–4075. https://doi.org/10.1021/ef502778u

Grubel, K., MacHnicka, A., 2011. Impact of microwave disintegration on activated sludge. Ecol. Chem. Eng. S 18, 75–82.

Guan, R., Yuan, X., Wu, Z., Jiang, L., Li, Y., Zeng, G., 2018. Principle and application of hydrogen peroxide based advanced oxidation processes in activated sludge treatment: A review. Chem. Eng. J. 339, 519–530. https://doi.org/10.1016/j.cej.2018.01.153

Guerrero, L., Omil, F., Méndez, R., Lema, J.M., 1999. Anaerobic hydrolysis and acidogenesis of wastewaters from food industries with high content of organic solids and protein. Water Res. 33, 3281–3290. https://doi.org/10.1016/S0043-1354(99)00041-X

Guo, W.Q., Yang, S.S., Xiang, W.S., Wang, X.J., Ren, N.Q., 2013. Minimization of excess sludge production by in-situ activated sludge treatment processes - A comprehensive review. Biotechnol. Adv. 31, 1386–1396. https://doi.org/10.1016/j.biotechadv.2013.06.003

Guo, X., Liu, J., Xiao, B., 2014. Evaluation of the damage of cell wall and cell membrane for various extracellular polymeric substance extractions of activated sludge. J. Biotechnol. 188, 130–135. https://doi.org/10.1016/j.jbiotec.2014.08.025

Gurjar, B.R., Tyagi, V.K., 2017. Sludge Management, 1st ed, Sludge Management. London:CRC press. https://doi.org/10.1201/9781315375137

Habashi, N., Mehrdadi, N., Mennerich, A., Alighardashi, A., Torabian, A., 2016. Hydrodynamic cavitation as a novel approach for pretreatment of oily wastewater for anaerobic co-digestion with waste activated sludge. Ultrason. Sonochem. 31, 362–370. https://doi.org/10.1016/j.ultsonch.2016.01.022

Hallaji, S.M., Siami, S., Aminzadeh, B., 2019. Improvement of anaerobic digestion of sewage sludge, using combined hydrogen peroxide and thermal pre-treatment. Pollution 5, 487–500. https://doi.org/10.22059/poll.2018.260122.462

Hallale, N., 2001. Burning bright trends in process integration. Chem. Eng. Prog. 97, 30–41.

Han, Y., Sung, S., Dague, R.R., 1997. Temperature-phased anaerobic digestion of wastewater sludges. Water Sci. Technol. 36, 367–374. https://doi.org/10.1016/S0273-1223(97)00544-1

Hanjie, Z., 2010. Sludge Treatment to Increase Biogas Production. Royal Institute of Technology.

Hassam, S., Ficara, E., Leva, A., Harmand, J., 2015. A generic and systematic procedure to derive a simplified model from the anaerobic digestion model No. 1 (ADM1). Biochem. Eng. J. 99, 193–203. https://doi.org/10.1016/j.bej.2015.03.007

Hassan, M., Ding, W., Shi, Z., Zhao, S., 2016. Methane enhancement through co-digestion of chicken manure and thermo-oxidative cleaved wheat straw with waste activated sludge: A C/N optimization case. Bioresour. Technol. 211, 534–541. https://doi.org/10.1016/j.biortech.2016.03.148

Hasted, J.B., Roderick, G.W., 1958. Dielectric Properties of Aqueous Solutions. J. Chem. Phys. 29. https://doi.org/10.1007/978-94-015-8332-9_21

Hattori, S., 2008. Syntrophic acetate-oxidizing microbes in methanogenic environments. Microbes Environ. 23, 118–127. https://doi.org/10.1264/jsme2.23.118

Haug, R.T., Stuckey, D.C., Gossett, J.M., Mccarty, P.L., 1978. Effect of thermal pretreatment on digestibility and dewaterability of organic sludges. Water Pollut. Control Fed. 50, 73–85. https://doi.org/10.2307/25039508

Hayyan, M., Hashim, M.A., Alnashef, I.M., 2016. Superoxide Ion: Generation and Chemical Implications. Chem. Rev. 116, 3029–3085. https://doi.org/10.1021/acs.chemrev.5b00407

He, D.Q., Wang, L.F., Jiang, H., Yu, H.Q., 2015. A Fenton-like process for the enhanced activated sludge dewatering. Chem. Eng. J. 272, 128–134. https://doi.org/10.1016/j.cej.2015.03.034

He, M. he, Wei, C. hai, 2010. Performance of membrane bioreactor (MBR) system with sludge Fenton oxidation process for minimization of excess sludge production. J. Hazard. Mater. 176, 597–601. https://doi.org/10.1016/j.jhazmat.2009.11.071

Held, C., Wellacher, M., Robra, K.H., Gübitz, G.M., 2002. Two-stage anaerobic fermentation of organic waste in CSTR and UFAF-reactors. Bioresour. Technol. 81, 19–24. https://doi.org/10.1016/S0960-8524(01)00108-0

Henze, M., Comeau, Y., 2008. Biological Wastewater Treatment: Principles Modelling and

Design, IWA publishing. https://doi.org/10.2166/9781780408644_001

Heo, N.H., Park, S.C., Lee, J.S., Kang, H., 2003. Solubilization of waste activated sludge by alkaline pretreatment and biochemical methane potential (BMP) tests for anaerobic co-digestion of municipal organic waste. Water Sci. Technol. 48, 211–219. https://doi.org/10.2166/wst.2003.0471

Hernansanz-Agustín, P., Izquierdo-Álvarez, A., Sánchez-Gómez, F.J., Ramos, E., Villa-Piña, T., Lamas, S., Bogdanova, A., Martínez-Ruiz, A., 2014. Acute hypoxia produces a superoxide burst in cells. Free Radic. Biol. Med. 71, 146–156. https://doi.org/10.1016/j.freeradbiomed.2014.03.011

Higgins, M.J., Novak, J.T., 1997. Dewatering and Settling of Activated Sludges : The Case for Using Cation Analysis Dewatering The sludges : and case settling for using of activated analysis cation. Water Environ. Res. 69, 225–232. https://doi.org/10.2175/106143097X125380

Hiraoka, M., Takeda, N., Sakai, S., Yasuda, A., 1985. Highly efficient anaerobic digestion with thermal pretreatment. Water Sci. Technol. 17, 529–539. https://doi.org/10.2166/wst.1985.0157

Hong, C., Si, Y., Xing, Y., Wang, Z., Liu, M., 2015. RSC Advances E ff ect of surfactant on bound water content and extracellular polymers substances distribution in sludge. RSC Adv. 5, 23383–23390. https://doi.org/10.1039/C4RA15370G

Hong, E., Yeneneh, A.M., Sen, T.K., Ang, H.M., Kayaalp, A., 2017. The relationship between physico-chemical and rheological characteristics of digested sludge, biosolid, centrate and the effects on dewatering performance (A case study). J. Water Process Eng. 19, 193–204. https://doi.org/10.1016/j.jwpe.2017.07.022

Hong, E., Yeneneh, A.M., Sen, T.K., Ang, H.M., Kayaalp, A., Kayaalp, M., 2016. Rheological characteristics of mixture of raw primary and thickened excess activated sludge: impact of mixing ratio, solid concentration, temperature and sludge age. Desalin. Water Treat. 57, 20726–20737. https://doi.org/10.1080/19443994.2015.1115374

Hong, S.M., 2002. Enhancement of Pathogen Destruction and Anaerobic Digestibility Using Microwaves. The University of Wisconsin, Madison.

Hong, S.M., Park, J.K., Lee, Y.O., 2004. Mechanisms of microwave irradiation involved in the destruction of fecal coliforms from biosolids. Water Res. 38, 1615–1625. https://doi.org/10.1016/j.watres.2003.12.011

Hong, S.M., Park, J.K., Teeradej, N., Lee, Y.O., Cho, Y.K., Park, C.H., 2006. Pretreatment of Sludge with Microwaves for Pathogen Destruction and Improved Anaerobic Digestion Performance. Water Environ. Res. 78, 76–83. https://doi.org/10.2175/106143005x84549

Horton, D., Philips, K.D., 1973. The Nitrous Acid Deamination of Glycosides and Acetates of 2-Amino-2-Deoxy-D-Glucose. Carbohydr. Res. 30, 367–374.

Hosseini Koupaie, E., Eskicioglu, C., 2015. Below and above boiling point comparison of microwave irradiation and conductive heating for municipal sludge digestion under identical heating/cooling profiles. Bioresour. Technol. 187, 235–245. https://doi.org/10.1016/j.biortech.2015.03.113

Hosseini Koupaie, E., Johnson, T., Eskicioglu, C., 2017. Advanced anaerobic digestion of municipal sludge using a novel and energy-efficient radio frequency pretreatment system. Water Res. 118, 70–81. https://doi.org/10.1016/j.watres.2017.04.017

Houghton, J.I., Quarmby, J., Stephenson, T., 2001. Municipal wastewater sludge dewaterability and the presence of microbial extracellular polymer. Water Sci. Technol. 44, 373–379.

Hu, K., Jiang, J.Q., Zhao, Q.L., Lee, D.J., Wang, K., Qiu, W., 2011. Conditioning of wastewater sludge using freezing and thawing: Role of curing. Water Res. 45, 5969–5976. https://doi.org/10.1016/j.watres.2011.08.064

Huang, C., Lai, J., Sun, X., Li, J., Shen, J., Han, W., Wang, L., 2016. Enhancing anaerobic digestion of waste activated sludge by the combined use of NaOH and Mg(OH)2: Performance evaluation and mechanism study. Bioresour. Technol. 220, 601–608. https://doi.org/10.1016/j.biortech.2016.08.043

Huang, C., Liu, C., Sun, X., Sun, Y., Li, R., Li, J., Shen, J., Han, W., Liu, X., Wang, L., 2015. Hydrolysis and volatile fatty acids accumulation of waste activated sludge enhanced by the combined use of nitrite and alkaline pH. Environ. Sci. Pollut. Res. 22, 18793–18800. https://doi.org/10.1007/s11356-015-4822-y

Huang, Q., Shen, Y., Wang, Y., Xiao, J., Yuan, H., Lou, Z., Zhu, N., 2020. Synergy between denitrification and calcium bridging improves dewaterability of waste activated sludge. J. Clean. Prod. 242, 118438. https://doi.org/10.1016/j.jclepro.2019.118438

Hwang, M.H., Jang, N.J., Hyun, S.H., Kim, I.S., 2004. Anaerobic bio-hydrogen production from ethanol fermentation: The role of pH. J. Biotechnol. 111, 297–309. https://doi.org/10.1016/j.jbiotec.2004.04.024

Inan, H., Turkay, O., Akkiris, C., 2016. Microwave and microwave-chemical pretreatment application for agricultural waste. Desalin. Water Treat. 57, 2590–2596. https://doi.org/10.1080/19443994.2015.1069217

Ince, O., 1998. Performance of a two-phase anaerobic digestion system when treating dairy wastewater. Water Res. 32, 2707–2713. https://doi.org/10.1016/S0043-1354(98)00036-0

Izrail S. Turovskiy, Mathai, P.K., 2005. Wastewater Sludge Processing. Wiley-Interscience. https://doi.org/10.1002/047179161X

Izumi, K., Okishio, Y. ki, Nagao, N., Niwa, C., Yamamoto, S., Toda, T., 2010. Effects of particle size on anaerobic digestion of food waste. Int. Biodeterior. Biodegrad. 64, 601–608. https://doi.org/10.1016/j.ibiod.2010.06.013

Jan, T.W., Adav, S.S., Lee, D.J., Wu, R.M., Su, A., Tay, J.H., 2008. Hydrogen fermentation and methane production from sludge with pretreatments. Energy and Fuels 22, 98–102. https://doi.org/10.1021/ef700278j

Jang, J.H., Ahn, J.H., 2015. High-Temperature Microwave NaOH Pretreatment of Waste-Activated Sludge for Anaerobic Digestion. J. Environ. Eng. (United States) 141, 1–5. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000933

Jaziri, K., Casellas, M., Dagot, C., 2012. Comparing the effects of three pre-treatment disintegration techniques on aerobic sludge digestion: Biodegradability enhancement and microbial community monitoring by PCR-DGGE. Environ. Technol. (United Kingdom) 33, 1435–1444. https://doi.org/10.1080/09593330.2011.632653

Jeihanipour, A., Aslanzadeh, S., Rajendran, K., Balasubramanian, G., Taherzadeh, M.J., 2013. High-rate biogas production from waste textiles using a two-stage process. Renew. Energy 52, 128–135. https://doi.org/10.1016/j.renene.2012.10.042

Ji, C., Kong, C.X., Mei, Z.L., Li, J., 2017. A Review of the Anaerobic Digestion of Fruit and Vegetable Waste. Appl. Biochem. Biotechnol. 183, 906–922. https://doi.org/10.1007/s12010-017-2472-x

Jia, S., Han, H., Hou, B., Zhuang, H., 2015. Advanced treatment of biologically pretreated coal gasification wastewater by a novel integration of three-dimensional catalytic electro-Fenton and membrane bioreactor. Bioresour. Technol. 198, 918–921. https://doi.org/10.1016/j.biortech.2015.09.080

Jiang, H., Liu, T., Ding, J., Nie, H., Zhou, H., 2018. Optimization and performance of moderate combined alkali and microwave pretreatment for anaerobic digestion of waste-activated sludge. Polish J. Environ. Stud. 27, 689–697. https://doi.org/10.15244/pjoes/76138

Jiang, L. bo, Yuan, X. zhong, Liang, J., Zhang, J., Wang, H., Zeng, G. ming, 2016. Nanostructured core-shell electrode materials for electrochemical capacitors. J. Power Sources 331, 408–425. https://doi.org/10.1016/j.jpowsour.2016.09.054

Jin, Y., Li, H., Mahar, R.B., Wang, Z., Nie, Y., 2009. Combined alkaline and ultrasonic pretreatment of sludge before aerobic digestion. J. Environ. Sci. 21, 279–284. https://doi.org/10.1016/S1001-0742(08)62264-0

Jiunn-Jyi, L., Yu-You, L., Noike, T., 1997. Influences of pH and moisture content on the methane production in high-solids sludge digestion. Water Res. 31, 1518–1524. https://doi.org/10.1016/S0043-1354(96)00413-7

Jorand, F., Zartarian, F., Thomas, F., Block, J.C., Bottero, J.Y., Villemin, G., Urbain, V., Manem, J., 1995. Chemical and structural (2D) linkage between bacteria within activated sludge flocs. Water Res. 29, 1639–1647. https://doi.org/10.1016/0043-1354(94)00350-G

Jose, S., Bhalla, P., Suraishkumar, G.K., 2018. Oxidative stress decreases the redox ratio and folate content in the gut microbe , Enterococcus durans (MTCC 3031). Sci. Rep. 8, 1–7. https://doi.org/10.1038/s41598-018-30691-4

Jung, J., Xing, X.H., Matsumoto, K., 2001. Kinetic analysis of disruption of excess activated sludge by Dyno Mill and characteristics of protein release for recovery of useful materials. Biochem. Eng. J. 8, 1–7. https://doi.org/10.1016/S1369-703X(00)00123-6

Kakita, Y., Kashige, N., Murata, K., Kuroiwa, A., Funatsu, M., Watanabe, K., 1995. Inactivation microwave of lactobacillus bacteriophage irradiation PL-1 by microwave irradiation. Microbiol. Immunol. 39, 571–576.

Kang, X., Liu, Y., Li, X., Yuan, Y., Du, M., 2016. Two-stage mesophilic anaerobic digestion from waste activated sludge enhanced by low-temperature thermal hydrolysis. Desalin. Water Treat. 57, 7607–7614. https://doi.org/10.1080/19443994.2015.1025440

Katsoyiannis, A., Samara, C., 2005. Persistent organic pollutants (POPs) in the conventional activated sludge treatment process: Fate and mass balance. Environ. Res. 97, 245–257. https://doi.org/10.1016/j.envres.2004.09.001

Kehrer, J.P., 2000. The Haber-Weiss reaction and mechanisms of toxicity. Toxicology 149, 43–50. https://doi.org/10.1016/S0300-483X(00)00231-6

Kelessidis, A., Stasinakis, A.S., 2012. Comparative study of the methods used for treatment and final disposal of sewage sludge in European countries. Waste Manag. 32, 1186–1195. https://doi.org/10.1016/j.wasman.2012.01.012

Kenge, A.A., Liao, P.H., Lo, K. V., 2008. Role of mixing on microwave-enhanced advanced oxidation process in treating sewage sludge. J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng. 43, 1431–1440. https://doi.org/10.1080/10934520802232170

Kennedy, K.J., Thibault, G., Droste, R.L., 2007. Microwave enhanced digestion of aerobic SBR sludge. Water SA 33, 261–270. https://doi.org/10.4314/wsa.v33i2.49085

Kianmehr, P., Parker, W., Seto, P., 2013. Assessment of waste-activated-sludge pretreatment by ultrasound and applicability of biodegradability indicators. J. Environ. Eng. (United States) 139, 545–553. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000657

Kim, D.H., Cho, S.K., Lee, M.K., Kim, M.S., 2013. Increased solubilization of excess sludge does not always result in enhanced anaerobic digestion efficiency. Bioresour. Technol. 143, 660–664. https://doi.org/10.1016/j.biortech.2013.06.058

Kim, H.J., Nguyen, D.X., Bae, J.H., 2008. The performance of the sludge pretreatment system with venturi tubes. Water Sci. Technol. 57, 131–137. https://doi.org/10.2166/wst.2008.717

Kim, J., Park, C., Kim, T., Lee, M., Kim, S., Eung-wook Kim, S., Lee, J., 2003. Effects of Various Pretreatments for Enhanced Anaerobic Digestion with Waste Activated Sludge. J. Biosci.

Bioeng. 95, 271–275. https://doi.org/10.1016/S1389-1723(03)80028-2

Kim, J., Yu, Y., Lee, C., 2013. Thermo-alkaline pretreatment of waste activated sludge at low-temperatures: Effects on sludge disintegration, methane production, and methanogen community structure. Bioresour. Technol. 144, 194–201. https://doi.org/10.1016/j.biortech.2013.06.115

Kim, M., Gomec, C.Y., Ahn, Y., Speece, R.E., 2003. Hydrolysis and acidogenesis of particulate organic material in mesophilic and thermophilic anaerobic digestion 24, 1183–1190. https://doi.org/10.1080/09593330309385659

Kim, T.H., Lee, S.R., Nam, Y.K., Yang, J., Park, C., Lee, M., 2009. Disintegration of excess activated sludge by hydrogen peroxide oxidation. Desalination 246, 275–284. https://doi.org/10.1016/j.desal.2008.06.023

Kobayashi, T., Li, Y.Y., Harada, H., Yasui, H., Noike, T., 2009. Upgrading of the anaerobic digestion of waste activated sludge by combining temperature-phased anaerobic digestion and intermediate ozonation. Water Sci. Technol. 59, 185–193. https://doi.org/10.2166/wst.2009.510

Koca, N., Rodriguez-Saona, L.E., Harper, W.J., Alvarez, V.B., 2007. Application of fourier transform infrared spectroscopy for monitoring short-chain free fatty acids in swiss cheese. J. Dairy Sci. 90, 3596–3603. https://doi.org/10.3168/jds.2007-0063

Kopp, J., Muller, J., Dichtl, N., Schwedes, J., 1997. Anaerobic digestion and dewatering characteristics of mechanically disintegrated excess sludge.

Koupaie, E.H., Eskicioglu, C., 2016. Conventional heating vs . microwave sludge pretreatment comparison under identical heating / cooling profiles for thermophilic advanced anaerobic digestion. Waste Manag. 53, 182–195. https://doi.org/10.1016/j.wasman.2016.04.014

Krupp, M., Schubert, J., Widmann, R., 2005. Feasibility study for co-digestion of sewage sludge with OFMSW on two wastewater treatment plants in Germany. Waste Manag. 25, 393–399. https://doi.org/10.1016/j.wasman.2005.02.009

Kuglarz, M., Karakashev, D., Angelidaki, I., 2013. Microwave and thermal pretreatment as methods for increasing the biogas potential of secondary sludge from municipal wastewater treatment plants. Bioresour. Technol. 134, 290–297.

https://doi.org/10.1016/j.biortech.2013.02.001

Kumanowska, E., Uruñuela Saldaña, M., Zielonka, S., Oechsner, H., 2017. Two-stage anaerobic digestion of sugar beet silage: The effect of the pH-value on process parameters and process efficiency. Bioresour. Technol. 245, 876–883. https://doi.org/10.1016/j.biortech.2017.09.011

Kumar, P.S., Pandit, A.B., 1999. Modeling Hydrodynamic Cavitation. Chem. Eng. Technol. 12, 1–1. https://doi.org/10.1201/b11330-2

Laurent, J., Pierra, M., Casellas, M., Dagot, C., 2009. Fate of cadmium in activated sludge after changing its physico-chemical properties by thermal treatment. Chemosphere 77, 771–777. https://doi.org/10.1016/j.chemosphere.2009.08.024

Law, C.K.Y., De Henau, R., De Vrieze, J., 2020. Feedstock thermal pretreatment selectively steers process stability during the anaerobic digestion of waste activated sludge. Appl. Microbiol. Biotechnol. 104, 3675–3686. https://doi.org/10.1007/s00253-020-10472-8

Le, N.T., Julcour-Lebigue, C., Barthe, L., Delmas, H., 2016. Optimisation of sludge pretreatment by low frequency sonication under pressure. J. Environ. Manage. 165, 206–212. https://doi.org/10.1016/j.jenvman.2015.09.015

Lee, H., Shoda, M., 2009. Comparison of treatment efficiencies of fenton's oxidation for untreated and biologically treated wastewaters. Environ. Eng. Sci. 26, 891–896. https://doi.org/10.1089/ees.2007.0299

Lee, I., Han, J.I., 2013. The effects of waste-activated sludge pretreatment using hydrodynamic cavitation for methane production. Ultrason. Sonochem. 20, 1450–1455. https://doi.org/10.1016/j.ultsonch.2013.03.006

Lee, M., Hidaka, T., Hagiwara, W., Tsuno, H., 2009. Comparative performance and microbial diversity of hyperthermophilic and thermophilic co-digestion of kitchen garbage and excess sludge. Bioresour. Technol. 100, 578–585. https://doi.org/10.1016/j.biortech.2008.06.063

Lee, W., Park, S., Cui, F., Kim, M., 2019. Optimizing pre-treatment conditions for anaerobic co-digestion of food waste and sewage sludge. J. Environ. Manage. 249, 109397. https://doi.org/10.1016/j.jenvman.2019.109397

Leite, W.R.M., Gottardo, M., Pavan, P., Belli Filho, P., Bolzonella, D., 2016. Performance and energy aspects of single and two phase thermophilic anaerobic digestion of waste activated sludge. Renew. Energy 86, 1324–1331. https://doi.org/10.1016/j.renene.2015.09.069

Leonelli, C., Mason, T.J., 2010. Microwave and ultrasonic processing: Now a realistic option for industry. Chem. Eng. Process. Process Intensif. 49, 885–900. https://doi.org/10.1016/j.cep.2010.05.006

Lguirati, A., Ait Baddi, G., El Mousadik, A., Gilard, V., Revel, J.C., Hafidi, M., 2005. Analysis of humic acids from aerated and non-aerated urban landfill composts. Int. Biodeterior. Biodegrad. 56, 8–16. https://doi.org/10.1016/j.ibiod.2005.03.004

Li, H., Jin, Y., Mahar, R.B., Wang, Z., Nie, Y., 2008. Effects and model of alkaline waste activated sludge treatment. Bioresour. Technol. 99, 5140–5144. https://doi.org/10.1016/j.biortech.2007.09.019

Li, X., Ma, H., Wang, Q., Matsumoto, S., Maeda, T., Ogawa, H.I., 2009. Isolation, identification of sludge-lysing strain and its utilization in thermophilic aerobic digestion for waste activated sludge. Bioresour. Technol. 100, 2475–2481. https://doi.org/10.1016/j.biortech.2008.12.019

Li, X., Zhao, J., Wang, D., Yang, Q., Xu, Q., Deng, Y., Yang, W., Zeng, G., 2016. An efficient and green pretreatment to stimulate short-chain fatty acids production from waste activated sludge anaerobic fermentation using free nitrous acid. Chemosphere 144, 160–167. https://doi.org/10.1016/j.chemosphere.2015.08.076

Li, X.Y., Yang, S.F., 2007. Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. Water Res. 41, 1022–1030. https://doi.org/10.1016/j.watres.2006.06.037

Li, Y., Park, S.Y., Zhu, J., 2011. Solid-state anaerobic digestion for methane production from organic waste. Renew. Sustain. Energy Rev. 15, 821–826. https://doi.org/10.1016/j.rser.2010.07.042

Li, Y., Zhang, A., 2014. Removal of steroid estrogens from waste activated sludge using Fenton oxidation: Influencing factors and degradation intermediates. Chemosphere 105, 24–30. https://doi.org/10.1016/j.chemosphere.2013.10.043

Liang, J., Zhang, S., Huang, J., Ye, M., Yang, X., Huang, S., Sun, S., 2020. Mechanism of zero valent iron and anaerobic mesophilic digestion combined with hydrogen peroxide pretreatment to enhance sludge dewaterability: Relationship between soluble EPS and rheological behavior. Chemosphere 247, 125859. https://doi.org/10.1016/j.chemosphere.2020.125859

Liao, P.H., Lo, K.V., Chan, W.I., Wong, W.T., 2007. Sludge reduction and volatile fatty acid recovery using microwave advanced oxidation process. J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng. 42, 633–639. https://doi.org/10.1080/10934520701244417

Liao, X., Li, H., Zhang, Y., Liu, C., Chen, Q., 2016. Accelerated high-solids anaerobic digestion of sewage sludge using low-temperature thermal pretreatment. Int. Biodeterior. Biodegrad. 106, 141–149. https://doi.org/10.1016/j.ibiod.2015.10.023

Lin, C.Y., Lee, Y.S., 2002. Effect of thermal and chemical pretreatments on anaerobic ammonium removal in treating septage using the UASB system. Bioresour. Technol. 83, 259–261. https://doi.org/10.1016/S0960-8524(01)00223-1

Lin, Y., Lü, F., Shao, L., He, P., 2013. Influence of bicarbonate buffer on the methanogenetic pathway during thermophilic anaerobic digestion. Bioresour. Technol. 137, 245–253. https://doi.org/10.1016/j.biortech.2013.03.093

Liochev, S.I., 2014. Free radicals: How do we stand them? Anaerobic and aerobic free radical (chain) reactions involved in the use of fluorogenic probes and in biological systems. Med. Princ. Pract. 23, 195–203. https://doi.org/10.1159/000357120

Liu, H., He, Y.H., Quan, X.C., Yan, Y.X., Kong, X.H., Lia, A.J., 2005. Enhancement of organic pollutant biodegradation by ultrasound irradiation in a biological activated carbon membrane reactor. Process Biochem. 40, 3002–3007. https://doi.org/10.1016/j.procbio.2005.02.005

Liu, J., Jia, R., Wang, Y., Wei, Y., Zhang, J., Wang, R., Cai, X., 2017. Does residual H2O2 result in inhibitory effect on enhanced anaerobic digestion of sludge pretreated by microwave-H2O2 pretreatment process? Environ. Sci. Pollut. Res. 24, 9016–9025. https://doi.org/10.1007/s11356-015-5704-z

Liu, J., Tong, J., Wei, Y., Wang, Y., 2015. Microwave and its combined processes: an effective way for enhancing anaerobic digestion and dewaterability of sewage sludge? J. Water Reuse Desalin. 5, 264. https://doi.org/10.2166/wrd.2015.120

Liu, Jibao, Wei, Y., Li, K., Tong, J., Wang, Y., Jia, R., 2016a. Microwave-acid pretreatment : A potential process for enhancing sludge dewaterability. Water Res. 90, 225–234.

Liu, J., Yang, M., Zhang, J., Zheng, J., Xu, H., Wang, Y., Wei, Y., 2018. A comprehensive insight into the effects of microwave-H 2 O 2 pretreatment on concentrated sewage sludge anaerobic digestion based on semi-continuous operation. Bioresour. Technol. 256, 118–127. https://doi.org/10.1016/j.biortech.2018.01.126

Liu, Jun, Yang, Q., Wang, D., Li, Xiaoming, Zhong, Y., Li, Xin, Deng, Y., Wang, L., Yi, K., Zeng, G., 2016. Enhanced dewaterability of waste activated sludge by Fe(II)-activated peroxymonosulfate oxidation. Bioresour. Technol. 206, 134–140. https://doi.org/10.1016/j.biortech.2016.01.088

Liu, Jibao, Yu, D., Zhang, J., Yang, M., Wang, Y., Wei, Y., Tong, J., 2016b. Rheological properties of sewage sludge during enhanced anaerobic digestion with microwave-H2O2 pretreatment. Water Res. 98, 98–108. https://doi.org/10.1016/j.watres.2016.03.073

Liu, Q., Li, Y., Yang, F., Liu, X., Wang, D., Xu, Q., Zhang, Y., Yang, Q., 2021. Understanding the mechanism of how anaerobic fermentation deteriorates sludge dewaterability. Chem. Eng. J. 404, 127026. https://doi.org/10.1016/j.cej.2020.127026

Liu, T., Chen, Z. lin, Yu, W. zheng, You, S. jie, 2011. Characterization of organic membrane foulants in a submerged membrane bioreactor with pre-ozonation using three-dimensional excitation-emission matrix fluorescence spectroscopy. Water Res. 45, 2111–2121. https://doi.org/10.1016/j.watres.2010.12.023

Liu, X., Gao, X., Wang, W., Zheng, L., Zhou, Y., Sun, Y., 2012a. Pilot-scale anaerobic co-digestion of municipal biomass waste: Focusing on biogas production and GHG reduction. Renew. Energy 44, 463–468. https://doi.org/10.1016/j.renene.2012.01.092

Liu, X., Li, R., Ji, M., 2019. Effects of Two-Stage Operation on Stability and Efficiency in Co-Digestion of Food Waste and Waste Activated Sludge. Energies 12, 2748. https://doi.org/10.3390/en12142748

Liu, X., Li, R., Ji, M., Han, L., 2013. Hydrogen and methane production by co-digestion of waste activated sludge and food waste in the two-stage fermentation process: Substrate conversion and energy yield. Bioresour. Technol. 146, 317–323.

https://doi.org/10.1016/j.biortech.2013.07.096

Liu, X., Liu, H., Chen, Jinhuan, Du, G., Chen, Jian, 2008. Enhancement of solubilization and acidification of waste activated sludge by pretreatment. Waste Manag. 28, 2614–2622. https://doi.org/10.1016/j.wasman.2008.02.001

Liu, X., Wang, W., Gao, X., Zhou, Y., Shen, R., 2012b. Effect of thermal pretreatment on the physical and chemical properties of municipal biomass waste. Waste Manag. 32, 249–255. https://doi.org/10.1016/j.wasman.2011.09.027

Lo, K.V., Ning, R., Kei, C., Oliveira, Y. De, 2017. Application of Microwave Oxidation Process for Sewage Sludge Treatment in a Continuous-Flow System. J. Environ. Eng. 143, 1–9. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001247.

Lo, K. V, Liao, P.H., Yin, G.Q., 2008. Sewage sludge treatment using microwave-enhanced advanced oxidation processes with and without ferrous sulfate addition. J. Chem. Technol. Biotechnol. 83, 1370–1374. https://doi.org/10.1002/jctb

Lo, K. V, Srinivasan, A., Liao, P.H., Bailey, S., 2015. Microwave oxidation treatment of sewage sludge. J. Environ. Sci. Heal. Part A 50, 882–889. https://doi.org/10.1080/10934529.2015.1019811

Lofrano, G., Brown, J., 2010. Wastewater management through the ages: A history of mankind. Sci. Total Environ. 408, 5254–5264. https://doi.org/10.1016/j.scitotenv.2010.07.062

Lu, D., Sun, F., Zhou, Y., 2018. Insights into anaerobic transformation of key dissolved organic matters produced by thermal hydrolysis sludge pretreatment. Bioresour. Technol. 266, 60–67. https://doi.org/10.1016/j.biortech.2018.06.059

Lu, L., Xing, D., Liu, B., Ren, N., 2012. Enhanced hydrogen production from waste activated sludge by cascade utilization of organic matter in microbial electrolysis cells. Water Res. 46, 1015–1026. https://doi.org/10.1016/j.watres.2011.11.073

Lu, M.C., Lin, C.J., Liao, C.H., Huang, R.Y., Ting, W.P., 2003. Dewatering of activated sludge by Fenton's reagent. Adv. Environ. Res. 7, 667–670. https://doi.org/10.1016/S1093-0191(02)00039-4

Lu, M.C., Lin, C.J., Liao, C.H., Ting, W.P., Huang, R.Y., 2001. Influence of pH on the dewatering of activated sludge by Fenton’s reagent. Water Sci. Technol. 44, 327–332. https://doi.org/10.2166/wst.2001.0654

Lund, M.N., Ray, C.A., 2017. Control of Maillard Reactions in Foods: Strategies and Chemical Mechanisms. J. Agric. Food Chem. 65, 4537–4552. https://doi.org/10.1021/acs.jafc.7b00882

Mahdy, A., Wandera, S.M., Qiao, W., Dong, R., 2019. Biostimulation of sewage sludge solubilization and methanization by hyper-thermophilic pre-hydrolysis stage and the shifts of microbial structure profiles. Sci. Total Environ. 699, 134373. https://doi.org/10.1016/j.scitotenv.2019.134373

Mao, C., Feng, Y., Wang, X., Ren, G., 2015. Review on research achievements of biogas from anaerobic digestion. Renew. Sustain. Energy Rev. 45, 540–555. https://doi.org/10.1016/j.rser.2015.02.032

Martín-Cereceda, M., Pérez-Uz, B., Serrano, S., Guinea, A., 2001. Dynamics of protozoan and metazoan communities in a full scale wastewater treatment plant by rotating biological contactors. Microbiol. Res. 156, 225–238. https://doi.org/10.1078/0944-5013-00105

Maspolim, Y., Zhou, Y., Guo, C., Xiao, K., Ng, W.J., 2015. Comparison of single-stage and two-phase anaerobic sludge digestion systems - Performance and microbial community dynamics. Chemosphere 140, 54–62. https://doi.org/10.1016/j.chemosphere.2014.07.028

Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S.I., Lee, Y.C., 2005. Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. Anal. Biochem. 339, 69–72. https://doi.org/10.1016/j.ab.2004.12.001

Mata-Alvarez, J., 1987. A Dynamic Simulation of a Two- Phase Anaerobic Digestion System for Solid Wastes. Biotechnol. Adv. 30, 844–851. https://doi.org/10.1017/CBO9781107415324.004

Mata-Alvarez, J., Dosta, J., Romero-Güiza, M.S., Fonoll, X., Peces, M., Astals, S., 2014. A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renew. Sustain. Energy Rev. 36, 412–427. https://doi.org/10.1016/j.rser.2014.04.039

Mata-Alvarez, J., Macé, S., Llabrés, P., 2000. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresour. Technol. 74, 3–16. https://doi.org/10.1016/S0960-8524(00)00023-7

Mateo-sagasta, J., Raschid-sally, L., Thebo, A., 2015. Global Wastewater and Sludge Production , Treatment and Use, in: Drechsel Pay, Qadir, M., Dennis Wichelns (Eds.), Wastewater Economic Asset in an Urbanising World. Springer, pp. 15–25. https://doi.org/10.1007/978-94-017-9545-6

Matsumoto, M., Ohashi, K., 2003. Effect of immobilization on thermostability of lipase from Candida rugosa. Biochem. Eng. J. 14, 75–77. https://doi.org/10.1016/S1369-703X(02)00138-9

Meegoda, J.N., Li, B., Patel, K., Wang, L.B., 2018. A review of the processes, parameters, and optimization of anaerobic digestion. Int. J. Environ. Res. Public Health 15. https://doi.org/10.3390/ijerph15102224

Mehdizadeh, S.N., Eskicioglu, C., Bobowski, J., Johnson, T., 2013. Conductive heating and microwave hydrolysis under identical heating profiles for advanced anaerobic digestion of municipal sludge. Water Res. 47, 5040–5051.

Menon, K.R., Balan, R., Suraishkumar, G.K., 2013. Stress Induced Lipid Production in Chlorella vulgaris : Relationship With Specific Intracellular Reactive Species Levels 110, 1627–1636. https://doi.org/10.1002/bit.24835

Mikkelsen, L.H., Keiding, K., 2002. Physico-chemical characteristics of full scale sewage sludges with implications to dewatering. Water Res. 36, 2451–2462. https://doi.org/10.1016/S0043-1354(01)00477-8

Mishra, S., Noronha, S.B., Suraishkumar, G.K., 2005. Increase in enzyme productivity by induced oxidative stress in Bacillus subtilis cultures and analysis of its mechanism using microarray data. Process Biochem. 40, 1863–1870. https://doi.org/10.1016/j.procbio.2004.06.055

Montusiewicz, A., Lebiocka, M., Rozej, A., Zacharska, E., Pawłowski, L., 2010. Freezing/thawing effects on anaerobic digestion of mixed sewage sludge. Bioresour. Technol. 101, 3466–3473. https://doi.org/10.1016/j.biortech.2009.12.125

Mottet, A., Steyer, J.P., Déléris, S., Vedrenne, F., Chauzy, J., Carrère, H., 2009. Kinetics of thermophilic batch anaerobic digestion of thermal hydrolysed waste activated sludge. Biochem. Eng. J. 46, 169–175. https://doi.org/10.1016/j.bej.2009.05.003

Muller, J., G, L., Schwedes, J., S, B., R, N., Kopp, J., Dichtl, N., A, S., R, K., C, H.D., 1998.

Disintegration of sewage sludges and influence on anaerobic digestion.

Müller, J.A., 2001. Prospects and problems of sludge pre-treatment processes. Water Sci. Technol. 44, 121–128. https://doi.org/10.2166/wst.2001.0598

Müller, J.A., Winter, A., Strünkmann, G., 2004. Investigation and assessment of sludge pre-treatment processes. Water Sci. Technol. 49, 97–104. https://doi.org/10.2166/wst.2004.0618

Na, S.H., Shon, H.K., Kim, J.H., 2011. Minimization of excess sludge and cryptic growth of microorganisms by alkaline treatment of activated sludge. Korean J. Chem. Eng. 28, 164–169. https://doi.org/10.1007/s11814-010-0334-0

Najafpour, G.D., Zinatizadeh, A.A.L., Mohamed, A.R., Hasnain Isa, M., Nasrollahzadeh, H., 2006. High-rate anaerobic digestion of palm oil mill effluent in an upflow anaerobic sludge-fixed film bioreactor. Process Biochem. 41, 370–379. https://doi.org/10.1016/j.procbio.2005.06.031

Narasimhan, B., Ghosh, S., Mujumdar, P., Bhallamudi, M., Mondal, A., 2016. Chennai Floods 2015: A Rapid Assessment.

National Research Council, 1996. Use of Reclaimed Water and Sludge in Food Crop Production, National Academy of Sciences. Washington, D. C. https://doi.org/10.17226/5175

Nazari, L., Yuan, Z., Santoro, D., Sarathy, S., Ho, D., Batstone, D., Xu, C.C., Ray, M.B., 2017. Low-temperature thermal pre-treatment of municipal wastewater sludge: Process optimization and effects on solubilization and anaerobic degradation. Water Res. 113, 111–123. https://doi.org/10.1016/j.watres.2016.11.055

Neumann, P., Pesante, S., Venegas, M., Vidal, G., 2016. Developments in pre-treatment methods to improve anaerobic digestion of sewage sludge. Rev. Environ. Sci. Biotechnol. 15, 173–211. https://doi.org/10.1007/s11157-016-9396-8

Neyens, E., Baeyens, J., 2003a. A review of classic Fenton's peroxidation as an advanced oxidation technique. J. Hazard. Mater. 98, 33–50. https://doi.org/10.1016/S0304-3894(02)00282-0

Neyens, E., Baeyens, J., 2003b. A review of thermal sludge pre-treatment processes to improve dewaterability. J. Hazard. Mater. 98, 51–67. https://doi.org/10.1016/S0304-3894(02)00320-

5

Neyens, E, Baeyens, J., Creemers, C., 2003. Alkaline thermal sludge hydrolysis. J. Hazard. Mater. 97, 295–314. https://doi.org/10.1016/S0304-3894(02)00286-8

Neyens, E., Baeyens, J., Dewil, R., De Heyder, B., 2004. Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering. J. Hazard. Mater. 106, 83–92. https://doi.org/10.1016/j.jhazmat.2003.11.014

Neyens, E., Baeyens, J., Weemaes, M., De Heyder, B., 2003a. Hot acid hydrolysis as a potential treatment of thickened sewage sludge. J. Hazard. Mater. 98, 275–293. https://doi.org/10.1016/S0304-3894(03)00002-5

Neyens, E., Baeyens, J., Weemaes, M., De Heyder, B., 2003b. Pilot-scale peroxidation (H2O2) of sewage sludge. J. Hazard. Mater. 98, 91–106. https://doi.org/10.1016/S0304-3894(02)00287-X

Ni, B.J., Fang, F., Xie, W.M., Sun, M., Sheng, G.P., Li, W.H., Yu, H.Q., 2009. Characterization of extracellular polymeric substances produced by mixed microorganisms in activated sludge with gel-permeating chromatography, excitation-emission matrix fluorescence spectroscopy measurement and kinetic modeling. Water Res. 43, 1350–1358. https://doi.org/10.1016/j.watres.2008.12.004

Nie, Y., Qiang, Z., Zhang, H., Ben, W., 2012. Fate and seasonal variation of endocrine-disrupting chemicals in a sewage treatment plant with A/A/O process. Sep. Purif. Technol. 84, 9–15. https://doi.org/10.1016/j.seppur.2011.01.030

Nielsen, P.H., Frølund, B., Keiding, K., 1996. Changes in the composition of extracellular polymeric substances in activated sludge during anaerobic storage. Appl. Microbiol. Biotechnol. 44, 823–830. https://doi.org/10.1007/BF00178625

Nkuna, R., Roopnarain, A., Adeleke, R., 2019. Effects of organic loading rates on microbial communities and biogas production from water hyacinth : a case of mono- and co-digestion. J. Chem. Technol. Biotechnol. 94, 1294–1304. https://doi.org/10.1002/jctb.5886

Novak, J.T., Bivins, J.L., 2011. Changes in Dewatering Properties Between the Thermophilic and Mesophilic Stages in Tpad Systems. Proc. Water Environ. Fed. 2000, 694–705. https://doi.org/10.2175/193864700784544514

NSTP Case study, 2018. Nesapakkam STP , Chennai- Co-treatment.

Odnell, A., Recktenwald, M., Stensén, K., Jonsson, B.-H., Karlsson, M., 2016. Activity, life time and effect of hydrolytic enzymes for enhanced biogas production from sludge anaerobic digestion. Water Res. 103, 462–471. https://doi.org/10.1016/j.watres.2016.07.064

Oosterhuis, M., Ringoot, D., Hendriks, A., Roeleveld, P., 2014. Thermal hydrolysis of waste activated sludge at Hengelo Wastewater Treatment Plant, the Netherlands. Water Sci. Technol. 70, 1–7. https://doi.org/10.2166/wst.2014.107

Owhondah, R.O., Walker, M., Ma, L., Nimmo, B., Ingham, D.B., Poggio, D., Pourkashanian, M., 2016. Assessment and parameter identification of simplified models to describe the kinetics of semi-continuous biomethane production from anaerobic digestion of green and food waste. Bioprocess Biosyst. Eng. 39, 977–992. https://doi.org/10.1007/s00449-016-1577-x

Özön, E., Erdinçler, A., 2019. Effects of microwave, H2O2/MW and H2O2/heat pre-treatments on the methane production from wastewater sludges: experimental and modeling approach. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-019-05190-2

Pabón Pereira, C.P., Castañares, G., Van Lier, J.B., 2012. An OxiTop® protocol for screening plant material for its biochemical methane potential (BMP). Water Sci. Technol. 66, 1416–1423. https://doi.org/10.2166/wst.2012.305

Pahl, O., Firth, A., MacLeod, I., Baird, J., 2008. Anaerobic co-digestion of mechanically biologically treated municipal waste with primary sewage sludge - A feasibility study. Bioresour. Technol. 99, 3354–3364. https://doi.org/10.1016/j.biortech.2007.08.027

Parawira, W., 2012. Enzyme research and applications in biotechnological intensification of biogas production. Crit. Rev. Biotechnol. 32, 172–186. https://doi.org/10.3109/07388551.2011.595384

Park, B., Ahn, J.H., Kim, J., Hwang, S., 2004. Use of microwave pretreatment for enhanced anaerobiosis of secondary slugde. Water Sci. Technol. 50, 17–23.

Park, C., Helm, R.F., Novak, J.T., 2008. Investigating the Fate of Activated Sludge Extracellular Proteins in Sludge Digestion Using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis. Water Environ. Res. 80, 2219–2227. https://doi.org/10.2175/106143008x325791

Park, C., Lee, C., Kim, S., Chen, Y., Chase, H.A., 2005. Upgrading of anaerobic digestion by incorporating two different hydrolysis processes. J. Biosci. Bioeng. 100, 164–167. https://doi.org/10.1263/jbb.100.164

Park, N.D., Thring, R.W., Helle, S.S., 2012. Comparison of methane production by co-digesting fruit and vegetable waste with first stage and second stage anaerobic digester sludge from a two stage digester. Water Sci. Technol. 65, 1252–1257. https://doi.org/10.2166/wst.2012.004

Park, S.K., Jang, H.M., Ha, J.H., Park, J.M., 2014. Sequential sludge digestion after diverse pre-treatment conditions: Sludge removal, methane production and microbial community changes. Bioresour. Technol. 162, 331–340. https://doi.org/10.1016/j.biortech.2014.03.152

Park, W. J., Ahn, J.H., 2011. Optimization of microwave pretreatment conditions to maximize methane production and methane yield in mesophilic anaerobic sludge digestion. Environ. Technol. 32, 1533–1540. https://doi.org/10.1080/09593330.2010.543929

Park, Woon Ji, Ahn, J.H., 2011. Effects of microwave pretreatment on mesophilic anaerobic digestion for mixture of primary and secondary sludges compared with thermal pretreatment. Environ. Eng. Res. 16, 103–109. https://doi.org/10.4491/eer.2011.16.2.103

Parkin, G.F., Owen, W.F., 1986. Fundamentals of anaerobic digestion of wastewater sludges. J. Environ. Eng. (United States) 112, 867–920. https://doi.org/10.1061/(ASCE)0733-9372(1986)112:5(867)

Parmar, N., Singh, A., Ward, O.P., 2001. Enzyme treatment to reduce solids and improve settling of sewage sludge. J. Ind. Microbiol. Biotechnol. 26, 383–386. https://doi.org/10.1038/sj.jim.7000150

Patinvoh, R.J., Osadolor, O.A., Chandolias, K., Sárvári Horváth, I., Taherzadeh, M.J., 2017. Innovative pretreatment strategies for biogas production. Bioresour. Technol. 224, 13–24. https://doi.org/10.1016/j.biortech.2016.11.083

Pavlostathis, S.G., Giraldo-Gomez, E., 1991. Kinetics of anaerobic treatment: A critical review. Crit. Rev. Environ. Sci. Technol. 21, 411–490. https://doi.org/10.1080/10643389109388424

Penaud, V., Delgenes, J.P., Moletta, R., 2000. Influence of thermochemical pretreatment conditions on solubilization and anaerobic biodegradability of a microbial biomass. Environ. Technol. (United Kingdom) 21, 87–96. https://doi.org/10.1080/09593332108618141

Penaud, V., Delgenès, J.P., Moletta, R., 1999. Thermo-chemical pretreatment of a microbial biomass: Influence of sodium hydroxide addition on solubilization and anaerobic biodegradability. Enzyme Microb. Technol. 25, 258–263. https://doi.org/10.1016/S0141-0229(99)00037-X

Pera-Titus, M., Garcia-Molina, V., Banos, M.A., Gimenez, J., Esplugas, S., 2004. Degradation of chlorophenols by means of advanced oxidation processes: A general review. Appl. Catal. B Environ. 47, 219–256. https://doi.org/10.1016/j.apcatb.2003.09.010

Pérez-Elvira, S.I., Ferreira, L.C., Donoso-Bravo, A., Fdz-Polanco, M., Fdz-Polanco, F., 2010. Full-stream and part-stream ultrasound treatment effect on sludge anaerobic digestion. Water Sci. Technol. 61, 1363–1372. https://doi.org/10.2166/wst.2010.893

Pérez-Elvira, S.I., Nieto Diez, P., Fdz-Polanco, F., 2006. Sludge minimisation technologies. Rev. Environ. Sci. Biotechnol. 5, 375–398. https://doi.org/10.1007/s11157-005-5728-9

Piao, W., Kim, Y., Kim, H., Kim, M., Kim, C., 2016. Life cycle assessment and economic efficiency analysis of integrated management of wastewater treatment plants. J. Clean. Prod. 113, 325–337. https://doi.org/10.1016/j.jclepro.2015.11.012

Pijuan, M., Wang, Q., Ye, L., Yuan, Z., 2012. Improving secondary sludge biodegradability using free nitrous acid treatment. Bioresour. Technol. 116, 92–98. https://doi.org/10.1016/j.biortech.2012.04.016

Pilli, S., Bhunia, P., Yan, S., LeBlanc, R.J., Tyagi, R.D., Surampalli, R.Y., 2011. Ultrasonic pretreatment of sludge: A review. Ultrason. Sonochem. 18, 1–18. https://doi.org/10.1016/j.ultsonch.2010.02.014

Pilli, S., Yan, S., Tyagi, R.D., Surampalli, R.Y., 2016. Anaerobic digestion of ultrasonicated sludge at different solids concentrations - Computation of mass-energy balance and greenhouse gas emissions. J. Environ. Manage. 166, 374–386. https://doi.org/10.1016/j.jenvman.2015.10.041

Pilli, S., Yan, S., Tyagi, R.D., Surampalli, R.Y., 2015. Overview of Fenton pre-treatment of sludge aiming to enhance anaerobic digestion. Rev. Environ. Sci. Biotechnol. 14, 453–472. https://doi.org/10.1007/s11157-015-9368-4

Pinnekamp, J., 1989. Effects of thermal pretreatment of sewage sludge on anaerobic digestion.

Water Sci. Technol. 21, 1542–1543. https://doi.org/10.2166/wst.1989.0214

Pino-Jelcic, S.A., Hong, S.M., Park, J.K., 2006. Enhanced Anaerobic Biodegradability and Inactivation of Fecal Coliforms and Salmonella spp. in Wastewater Sludge by Using Microwaves. Water Environ. Res. 78, 209–216. https://doi.org/10.2175/106143005x90498

Pinto, N., Carvalho, A., Pacheco, J., Duarte, E., 2016. Study of different ratios of primary and waste activated sludges to enhance the methane yield. Water Environ. J. 30, 203–210. https://doi.org/10.1111/wej.12188

Pitta, S.K., Kumar, K.S., M, J.R.B., 2010. Performance Evaluation of Wastewater Treatment Plant. Int. J. Eng. Sci. Technol. 2, 7785–7796.

PLOOM, 2016. Perth Long Term Ocean Outlet Monitoring Program.

Poornima Devi, T., Vimala Ebenezer, A., Adish Kumar, S., Kaliappan, S., Rajesh Banu, J., 2014. Effect of deflocculation on the efficiency of disperser induced dairy waste activated sludge disintegration and treatment cost. Bioresour. Technol. 167, 151–158. https://doi.org/10.1016/j.biortech.2014.06.004

Pourbafrani, M., Forgács, G., Horváth, I.S., Niklasson, C., Taherzadeh, M.J., 2010. Production of biofuels, limonene and pectin from citrus wastes. Bioresour. Technol. 101, 4246–4250. https://doi.org/10.1016/j.biortech.2010.01.077

Prorot, A., Julien, L., Christophe, D., Patrick, L., 2011. Sludge disintegration during heat treatment at low temperature: A better understanding of involved mechanisms with a multiparametric approach. Biochem. Eng. J. 54, 178–184. https://doi.org/10.1016/j.bej.2011.02.016

Prousek, J., 2007. Fenton chemistry in biology and medicine *. Pure Appl. Chem. 79, 2325–2338. https://doi.org/10.1351/pac200779122325

Qadir, M., Wichelns, D., Raschid-Sally, L., McCornick, P.G., Drechsel, P., Bahri, A., Minhas, P.S., 2010. The challenges of wastewater irrigation in developing countries. Agric. Water Manag. 97, 561–568. https://doi.org/10.1016/j.agwat.2008.11.004

Qi, X., Zhang, S., Wang, Y., Wang, R., 2005. Advantages of the integrated pig-biogas-vegetable greenhouse system in North China. Ecol. Eng. 24, 175–183. https://doi.org/10.1016/j.ecoleng.2004.11.001

Qiao, W., Wang, W., Xun, R., Lu, W., Yin, K., 2008. Sewage sludge hydrothermal treatment by microwave irradiation combined with alkali addition. J. Mater. Sci. 43, 2431–2436. https://doi.org/10.1007/s10853-007-1957-3

R Kaur, S P Wani, A.K.S. and K.L., 2014. Wastewater production, treatment and use in india 15, 14. https://doi.org/10.1016/j.watres.2014.11.002

Rahmani, A.R., Nematollahi, D., Azarian, G., Godini, K., Berizi, Z., 2015. Activated sludge treatment by electro-Fenton process: Parameter optimization and degradation mechanism. Korean J. Chem. Eng. 32, 1570–1577. https://doi.org/10.1007/s11814-014-0362-2

Rai, C.L., Mueller, J., Struenkmann, G., Rao, P.G., 2008. Microbial growth reduction in sewage sludge by stirred ball mill disintegration and estimation by respirometry. J. Chem. Technol. Biotechnol. 83, 269–278. https://doi.org/10.1002/jctb

Rajeshwari, K. V., Lata, K., Pant, D.C., Kishore, V.V.N., 2001. A novel process using enhanced acidification and a UASB reactor for biomethanation of vegetable market waste. Waste Manag. Res. 19, 292–300. https://doi.org/10.1177/0734242X0101900405

Rapport, J.L., Zhang, R., Jenkins, B.M., Hartsough, B.R., Tomich, T.P., 2011. Modeling the performance of the anaerobic phased solids digester system for biogas energy production. Biomass and Bioenergy 35, 1263–1272. https://doi.org/10.1016/j.biombioe.2010.12.021

Rashid, S.S., Liu, Y.-Q., Zhang, C., 2020. Upgrading a large and centralised municipal wastewater treatment plant with sequencing batch reactor technology for integrated nutrient removal and phosphorus recovery: Environmental and economic life cycle performance. Sci. Total Environ. 749, 141465. https://doi.org/10.1016/j.scitotenv.2020.141465

Ratkovich, N., Horn, W., Helmus, F.P., Rosenberger, S., Naessens, W., Nopens, I., Bentzen, T.R., 2013. Activated sludge rheology: A critical review on data collection and modelling. Water Res. 47, 463–482. https://doi.org/10.1016/j.watres.2012.11.021

Rattier, M., Reungoat, J., Keller, J., Gernjak, W., 2014. Removal of micropollutants during tertiary wastewater treatment by biofiltration: Role of nitrifiers and removal mechanisms. Water Res. 54, 89–99. https://doi.org/10.1016/j.watres.2014.01.030

Ravi, P.P., Lindner, J., Oechsner, H., Lemmer, A., 2018. Effects of target pH-value on organic acids and methane production in two-stage anaerobic digestion of vegetable waste. Bioresour.

Technol. 247, 96–102. https://doi.org/10.1016/j.biortech.2017.09.068

Ray, B.T., Lin, J.G., Rajan, R. V., 1990. Low-level alkaline solubilization for enhanced anaerobic digestion. Res. J. Water Pollut. Control Fed. 62, 81–87.

Rehl, T., Müller, J., 2011. Life cycle assessment of biogas digestate processing technologies. Resour. Conserv. Recycl. https://doi.org/10.1016/j.resconrec.2011.08.007

Riau, V., De la Rubia, M.Á., Pérez, M., 2010. Temperature-phased anaerobic digestion (TPAD) to obtain class A biosolids: A semi-continuous study. Bioresour. Technol. 101, 2706–2712. https://doi.org/10.1016/j.biortech.2009.11.101

Rillo, E., Gandiglio, M., Lanzini, A., Bobba, S., Santarelli, M., Blengini, G., 2017. Life Cycle Assessment (LCA) of biogas-fed Solid Oxide Fuel Cell (SOFC) plant. Energy 126, 585–602. https://doi.org/10.1016/j.energy.2017.03.041

Roberts, R., Le, S., Forster, C.F., 1999. A thermophilic/mesophilic dual digestion system for treating waste activated sludge. J. Chem. Technol. Biotechnol. 74, 445–450. https://doi.org/10.1002/(SICI)1097-4660(199905)74:5<445::AID-JCTB65>3.0.CO;2-0

Rocher, M., Goma, G., Pilas Begue, A., Louvel, L., Rols, J.L., 1999. Towards a reduction in excess sludge production in activated sludge processes: Biomass physicochemical treatment and biodegradation. Appl. Microbiol. Biotechnol. 51, 883–890. https://doi.org/10.1007/s002530051478

Roldán, M., Bouzas, A., Seco, A., Mena, E., Mayor, Barat, R., 2020. An integral approach to sludge handling in a WWTP operated for EBPR aiming phosphorus recovery: Simulation of alternatives, LCA and LCC analyses. Water Res. 175. https://doi.org/10.1016/j.watres.2020.115647

Romano, R.T., Zhang, R., Teter, S., McGarvey, J.A., 2009. The effect of enzyme addition on anaerobic digestion of Jose Tall Wheat Grass. Bioresour. Technol. 100, 4564–4571. https://doi.org/10.1016/j.biortech.2008.12.065

Rombaut, N., Tixier, A.-S., Bily, A., Chemat, F., 2014. Green extraction processes of natural products as tools for biorefinery. Biofuels, Bioprod. Biorefining 8, 530–544. https://doi.org/10.1002/bbb

Ruffino, B., Cerutti, A., Campo, G., Scibilia, G., Lorenzi, E., Zanetti, M., 2020. Thermophilic vs. mesophilic anaerobic digestion of waste activated sludge: Modelling and energy balance for its applicability at a full scale WWTP. Renew. Energy 156, 235–248. https://doi.org/10.1016/j.renene.2020.04.068

Ruggeri, B., Luongo Malave, A.C., Bernardi, M., Fino, D., 2013. Energy efficacy used to score organic refuse pretreatment processes for hydrogen anaerobic production. Waste Manag. 33, 2225–2233. https://doi.org/10.1016/j.wasman.2013.06.024

Sahinkaya, S., 2015. Disintegration of municipal waste activated sludge by simultaneous combination of acid and ultrasonic pretreatment. Process Saf. Environ. Prot. 93, 201–205. https://doi.org/10.1016/j.psep.2014.04.002

Saifuddin, N., Fazlili, S.A., 2009. Effect of Microwave and Ultrasonic Pretreatments on Biogas Production from Anaerobic Digestion of Palm Oil Mill Effleunt. Am. J. Eng. Appl. Sci. 2, 139–146.

Salsabil, M.R., Laurent, J., Casellas, M., Dagot, C., 2010. Techno-economic evaluation of thermal treatment, ozonation and sonication for the reduction of wastewater biomass volume before aerobic or anaerobic digestion. J. Hazard. Mater. 174, 323–333. https://doi.org/10.1016/j.jhazmat.2009.09.054

Salsabil, M.R., Prorot, A., Casellas, M., Dagot, C., 2009. Pre-treatment of activated sludge: Effect of sonication on aerobic and anaerobic digestibility. Chem. Eng. J. 148, 327–335. https://doi.org/10.1016/j.cej.2008.09.003

Salton, M.R.J., Horne, R.W., 1951. Studies of the bacterial cell wall: II. Methods of preparation and some properties of cell walls 7.

Sanin, F.D., 2002. Effect of solution physical chemistry on the rheological properties of activated sludge. Water SA 28, 207–211. https://doi.org/10.4314/wsa.v28i2.4886

Sarkar, P., Suraishkumar, G.K., 2011. PH and temperature stresses in bioreactor cultures: Intracellular superoxide levels. Ind. Eng. Chem. Res. 50, 13129–13136. https://doi.org/10.1021/ie200081k

Sarwar, R., Elbeshbishy, E., Parker, W.J., 2018. Codigestion of high pressure thermal hydrolysis-treated thickened waste activated sludge with primary sludge in two-stage anaerobic digestion. Environ. Prog. Sustain. Energy 37, 425–433. https://doi.org/10.1002/ep.12700

Sato, K., Ochi, S., Mizuochi, M., 2001. Up-to-date modification of the anaerobic sludge digestion process introducing a separate sludge digestion mode. Water Sci. Technol. 44, 143–147. https://doi.org/10.2166/wst.2001.0604

Scano, E.A., Asquer, C., Pistis, A., Ortu, L., Demontis, V., Cocco, D., 2014. Biogas from anaerobic digestion of fruit and vegetable wastes: Experimental results on pilot-scale and preliminary performance evaluation of a full-scale power plant. Energy Convers. Manag. 77, 22–30. https://doi.org/10.1016/j.enconman.2013.09.004

Sen, T.K., 2015. Physical, Chemical and Biological Treatment Processes for Water and Wastewater, 1st ed. nova science publishers, New York.

Seng, B., Khanal, S.K., Visvanathan, C., 2010. Anaerobic digestion of waste activated sludge pretreated by a combined ultrasound and chemical process. Environ. Technol. 31, 257–265. https://doi.org/10.1080/09593330903453236

Sesay, M.L., Özcengiz, G., Dilek Sanin, F., 2006. Enzymatic extraction of activated sludge extracellular polymers and implications on bioflocculation. Water Res. 40, 1359–1366. https://doi.org/10.1016/j.watres.2006.01.045

Setsukinai, K. ichi, Urano, Y., Kakinuma, K., Majima, H.J., Nagano, T., 2003. Development of novel fluorescence probes that can reliably detect reactive oxygen species and distinguish specific species. J. Biol. Chem. 278, 3170–3175. https://doi.org/10.1074/jbc.M209264200

Shanthi, M., Rajesh Banu, J., Sivashanmugam, P., 2018. Effect of surfactant assisted sonic pretreatment on liquefaction of fruits and vegetable residue: Characterization, acidogenesis, biomethane yield and energy ratio. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2018.05.054

Shao, L., Wang, X., Xu, H., He, P., 2012. Enhanced anaerobic digestion and sludge dewaterability by alkaline pretreatment and its mechanism. J. Environ. Sci. (China) 24, 1731–1738. https://doi.org/10.1016/S1001-0742(11)61031-0

Sharma, S.K., Sanghi, R. (Eds.), 2013. Wastewater Reuse and Management, Springer. https://doi.org/10.1007/978-94-007-4942-9_3

Sheng, G.P., Yu, H.Q., Li, X.Y., 2010. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. Biotechnol. Adv. 28, 882–894. https://doi.org/10.1016/j.biotechadv.2010.08.001

Silvestre, G., Ruiz, B., Fiter, M., Ferrer, C., Berlanga, J.G., Alonso, S., Canut, A., 2015. Ozonation as a pre-treatment for anaerobic digestion of waste-activated sludge: Effect of the ozone doses. Ozone Sci. Eng. 37, 316–322. https://doi.org/10.1080/01919512.2014.985817

Skiadas, I. V., Gavala, H.N., Lu, J., Ahring, B.K., 2005. Thermal pre-treatment of primary and secondary sludge at 701 °C prior to anaerobic digestion. Water Sci. Technol. 52, 161–166. https://doi.org/10.2166/wst.2005.0512

Slupphaug, G., Kavli, B., Krokan, H.E., 2003. The interacting pathways for prevention and repair of oxidative DNA damage. Mutat. Res. - Fundam. Mol. Mech. Mutagen. 531, 231–251. https://doi.org/10.1016/j.mrfmmm.2003.06.002

Sokolov, L.I., 2013. Rheology of Sludges and Residues of Effluent Waters. Life Sci. J. 10, 1021–1029.

Sonune, A., Ghate, R., 2004. Developments in wastewater treatment methods. Desalination 167, 55–63. https://doi.org/10.1016/j.desal.2004.06.113

Sotodate, J.I., Inaba, K., Tajima, H., Yamagiwa, K., 2009. Effect of ultrasonication on excess sludge bioavailability for methane production. J. Chem. Eng. Japan 42, 67–72. https://doi.org/10.1252/jcej.08we129

Spellman, F.R., 1997. Wastewater biosolids to compost. Lancaster, PA : Technomic Pub. Co.

Sperandio, M., Paul, E., Bessiere, Y., 2012. Sludge Production: Quantification And Prediction For Urban Treatment Plants And Assessment Of Strategies For Sludge Reduction, in: Paul, E., Liu, Y. (Eds.), Biological Sludge Minimization and Biomaterials/Bioenergy Recovery Technologies. John Wiley & Sons, Inc.

Sperling, M. von, 2008. Wastewater characteristics, treatment and disposal, in: Biological Wastewater Treatment Series. IWA publishing, pp. 45-2633-45–2633. https://doi.org/10.5860/choice.45-2633

Sponza, D.T., 2002. Extracellular polymer substances and physicochemical properties of flocs in steady- and unsteady-state activated sludge systems. Process Biochem. 37, 983–998. https://doi.org/Pii S0032-9592(01)00306-5\rDoi 10.1016/S0032-9592(01)00306-5

Stuckey, D.C., McCarty, P.L., 1984. The effect of thermal pretreatment on the anaerobic biodegradability and toxicity of waste activated sludge. Water Res. 18, 1343–1353. https://doi.org/10.1016/0043-1354(84)90002-2

Sun, S., Guo, Z., Yang, R., Sheng, Z., Cao, P., 2012. Study on the triphenyl tetrazolium chloride – dehydrogenase activity (TTC-DHA) method in determination of bioactivity for treating tomato paste wastewater. African J. Biotechnol. 11, 7055–7062. https://doi.org/10.5897/AJB11.3548

Sun, Y., Zheng, H., Zhai, J., Teng, H., Zhao, Chun, Zhao, Chuanliang, Liao, Y., 2014. Effects of surfactants on the improvement of sludge dewaterability using cationic flocculants. PLoS One 9, 1–10. https://doi.org/10.1371/journal.pone.0111036

Swachh Suvekshan Grameen, 2019. , Ministry of Jal Shakti, GoI.

Szwergold, B.S., 2013. Maillard reactions in hyperthermophilic archaea: Implications for better understanding of non-enzymatic glycation in biology. Rejuvenation Res. 16, 259–272. https://doi.org/10.1089/rej.2012.1401

Taboada-Santos, A., Lema, J.M., Carballa, M., 2019. Energetic and economic assessment of sludge thermal hydrolysis in novel wastewater treatment plant configurations. Waste Manag. 92, 30–38. https://doi.org/10.1016/j.wasman.2019.05.003

Takashima, M., Tanaka, Y., 2010. Application of acidic thermal treatment for one- and two-stage anaerobic digestion of sewage sludge. Water Sci. Technol. 62, 2647–2654. https://doi.org/10.2166/wst.2010.490

Tambone, F., Scaglia, B., D’Imporzano, G., Schievano, A., Orzi, V., Salati, S., Adani, F., 2010. Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. Chemosphere. https://doi.org/10.1016/j.chemosphere.2010.08.034

Tanaka, S., Kamiyama, K., 2002. Thermochemical pretreatment in the anaerobic digestion of waste activated sludge. Water Sci. Technol. 46, 173–179. https://doi.org/10.1016/S0273-1223(97)00169-8

Tanaka, S., Kobayashi, T., Kamiyama, K., Bildan, M.L.N.S., 1997. Effects of thermochemical pretreatment on the anaerobic digestion of waste activated sludge. Water Sci. Technol. 35, 209–215.

Tang, B., Zhang, Z., 2014. Essence of disposing the excess sludge and optimizing the operation of wastewater treatment: Rheological behavior and microbial ecosystem. Chemosphere 105, 1–13. https://doi.org/10.1016/j.chemosphere.2013.12.067

Tang, Y., Yang, Y.L., Li, X.M., Yang, Q., Wang, D.B., Zeng, G.M., 2012. The isolation, identification of sludge-lysing thermophilic bacteria and its utilization in solubilization for excess sludge. Environ. Technol. 33, 961–966. https://doi.org/10.1080/09593330.2011.603754

Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2003. Wastewater Engineering Treatment and Reuse, 4th ed, McGraw Hill. https://doi.org/10.1002/9780470168219.ch8

Thomashow, M.F., 1998. Role of cold-responsive genes in plant freezing tolerance. Plant Physiol. 118, 1–7. https://doi.org/10.1104/pp.118.1.1

Tian, X., Wang, C., Trzcinski, A.P., Lin, L., Ng, W.J., 2015. Interpreting the synergistic effect in combined ultrasonication-ozonation sewage sludge pre-treatment. Chemosphere 140, 63–71. https://doi.org/10.1016/j.chemosphere.2014.09.007

Tiehm, A., Nickel, K., Zellhorn, M., Neis, U., Tiehm, A., 2001. Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization. Water Res. 35, 2003–2009. https://doi.org/10.1016/S0043-1354(00)00468-1

Tokumura, M., Katoh, H., Katoh, T., Znad, H.T., Kawase, Y., 2009. Solubilization of excess sludge in activated sludge process using the solar photo-Fenton reaction. J. Hazard. Mater. 162, 1390–1396. https://doi.org/10.1016/j.jhazmat.2008.06.026

Tony, M.A., Zhao, Y.Q., Fu, J.F., Tayeb, A.M., 2008. Conditioning of aluminium-based water treatment sludge with Fenton’s reagent: Effectiveness and optimising study to improve dewaterability. Chemosphere 72, 673–677. https://doi.org/10.1016/j.chemosphere.2008.03.032

Toreci, I., Droste, R.L., Kennedy, K.J., 2011. Mesophilic Anaerobic Digestion with High-Temperature Microwave Pretreatment and Importance of Inoculum Acclimation. Water

Environ. Res. 83, 549–559. https://doi.org/10.2175/106143010X12780288628651

Toreci, I., Kennedy, K.J., Droste, R.L., 2010. Effect of high-temperature microwave irradiation on municipal thickened waste activated sludge solubilization. Heat Transf. Eng. 31, 766–773. https://doi.org/10.1080/01457630903501039

Toutian, V., Barjenbruch, M., Loderer, C., Remy, C., 2020. Pilot study of thermal alkaline pretreatment of waste activated sludge: Seasonal effects on anaerobic digestion and impact on dewaterability and refractory COD. Water Res. 182, 115910. https://doi.org/10.1016/j.watres.2020.115910

Turkdogan-Aydinol, F.I., Yetilmezsoy, K., Comez, S., 2011. Effect of extracellular enzyme activity on digestion performance of mesophilic UASB reactor treating high-strength municipal wastewater. Bioprocess Biosyst. Eng. 34, 389–401. https://doi.org/10.1007/s00449-010-0482-y

Tyagi, V.K., Lo, S.L., 2013. Microwave irradiation: A sustainable way for sludge treatment and resource recovery. Renew. Sustain. Energy Rev. 18, 288–305. https://doi.org/10.1016/j.rser.2012.10.032

Tyagi, V.K., Lo, S.L., 2012. Enhancement in mesophilic aerobic digestion of waste activated sludge by chemically assisted thermal pretreatment method. Bioresour. Technol. 119, 105–113. https://doi.org/10.1016/j.biortech.2012.05.134

Tyagi, V.K., Lo, S.L., 2011. Application of physico-chemical pretreatment methods to enhance the sludge disintegration and subsequent anaerobic digestion: An up to date review. Rev. Environ. Sci. Biotechnol. 10, 215–242. https://doi.org/10.1007/s11157-011-9244-9

Uma Rani, R., Adish Kumar, S., Kaliappan, S., Yeom, I.T., Rajesh Banu, J., 2013. Impacts of microwave pretreatments on the semi-continuous anaerobic digestion of dairy waste activated sludge. Waste Manag. 33, 1119–1127. https://doi.org/10.1016/j.wasman.2013.01.016

Uma Rani, R., Adish Kumar, S., Kaliappan, S., Yeom, I.T., Rajesh Banu, J., 2012. Low temperature thermo-chemical pretreatment of dairy waste activated sludge for anaerobic digestion process. Bioresour. Technol. 103, 415–424. https://doi.org/10.1016/j.biortech.2011.09.124

UN, 2014. United Nations, Department of Economic ad Social Affairs, Population Division

(2014), World Urbanization Prospects: The 2014 Revision, Highlights (ST/ESA/SER.A/352). https://doi.org/10.4054/DemRes.2005.12.9

Ushani, U., Kavitha, S., Johnson, M., Yeom, I.T., Banu, J.R., 2017. Upgrading the hydrolytic potential of immobilized bacterial pretreatment to boost biogas production. Environ. Sci. Pollut. Res. 24, 813–826. https://doi.org/10.1007/s11356-016-7819-2

Vaclavik, V.A., Christian, E.W., 2008. Essentials of Food Science, Third. ed. Springer.

Val Del Río, Á., Palmeiro-Sanchez, T., Figueroa, M., Mosquera-Corral, A., Campos, J.L., Méndez, R., 2014. Anaerobic digestion of aerobic granular biomass: Effects of thermal pre-treatment and addition of primary sludge. J. Chem. Technol. Biotechnol. 89, 690–697. https://doi.org/10.1002/jctb.4171

Valo, A., Carrère, H., Delgenès, J.P., 2004. Thermal, chemical and thermo-chemical pre-treatment of waste activated sludge for anaerobic digestion. J. Chem. Technol. Biotechnol. 79, 1197–1203. https://doi.org/10.1002/jctb.1106

Vavilin, V.A., Fernandez, B., Palatsi, J., Flotats, X., 2008. Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview. Waste Manag. 28, 939–951. https://doi.org/10.1016/j.wasman.2007.03.028

Vavilin, V.A., Rytov, S. V., Lokshina, L.Y., 1996. A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. Bioresour. Technol. 56, 229–237. https://doi.org/10.1016/0960-8524(96)00034-X

Veeken, A., Hamelers, B., 1999. Effect of temperature on hydrolysis rates of selected biowaste components. Bioresour. Technol. 69, 249–254. https://doi.org/10.1016/S0960-8524(98)00188-6

Vela, G.R., Wu, J.F., 1979. Mechanism of Lethal Action of 2,450-MHz Radiation on Microorganisms. Appl. Environ. Microbiol. 37, 550–553.

Vergine, P., Zábranská, J., Canziani, R., 2014. Low temperature microwave and conventional heating pre-treatments to improve sludge anaerobic biodegradability. Water Sci. Technol. 69, 518–524. https://doi.org/10.2166/wst.2013.735

Vesilind, P.A., Martel, C.J., 1991. Freezing of Water and Wastewater Sludges. J. Environ. Eng.

116, 854–862.

Vlyssides, A.G., Karlis, P.K., 2004. Thermal-alkaline solubilization of waste activated sludge as a pre-treatment stage for anaerobic digestion. Bioresour. Technol. 91, 201–206. https://doi.org/10.1016/S0960-8524(03)00176-7

Wahidunnabi, A.K., Eskicioglu, C., 2014. High pressure homogenization and two-phased anaerobic digestion for enhanced biogas conversion from municipal waste sludge. Water Res. 66, 430–446. https://doi.org/10.1016/j.watres.2014.08.045

Wainaina, S., Lukitawesa, Kumar Awasthi, M., Taherzadeh, M.J., 2019. Bioengineering of anaerobic digestion for volatile fatty acids, hydrogen or methane production: A critical review. Bioengineered 10, 437–458. https://doi.org/10.1080/21655979.2019.1673937

Walter, I., Lugelow, J., 1973. Environmental Policies in Developing Countries. J. Chem. Inf. Model. 8, 102–109. https://doi.org/10.1017/CBO9781107415324.004

Wan, C., Zhou, Q., Fu, G., Li, Y., 2011. Semi-continuous anaerobic co-digestion of thickened waste activated sludge and fat, oil and grease. Waste Manag. 31, 1752–1758. https://doi.org/10.1016/j.wasman.2011.03.025

Wang, L.L., Wang, L.F., Ren, X.M., Ye, X.D., Li, W.W., Yuan, S.J., Sun, M., Sheng, G.P., Yu, H.Q., Wang, X.K., 2012. PH dependence of structure and surface properties of microbial EPS. Environ. Sci. Technol. 46, 737–744. https://doi.org/10.1021/es203540w

Wang, N., Wang, P., 2016. Study and application status of microwave in organic wastewater treatment – A review. Chem. Eng. J. 283, 193–214. https://doi.org/10.1016/j.cej.2015.07.046

Wang, Q., Fujisaki, K., Ohsumi, Y., Ogawa, H.I., 2001. Enhancement of dewaterability of thickened waste activated sludge by freezing and thawing treatment. J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng. 36, 1361–1371. https://doi.org/10.1081/ESE-100104884

Wang, Q., Jiang, G., Ye, L., Yuan, Z., 2014. Enhancing methane production from waste activated sludge using combined free nitrous acid and heat pre-treatment. Water Res. 63, 71–80. https://doi.org/10.1016/j.watres.2014.06.010

Wang, Q., Kuninobu, M., Kakimoto, K., I.-Ogawa, H., Kato, Y., 1999. Upgrading of anaerobic digestion of waste activated sludge by ultrasonic pretreatment. Bioresour. Technol. 68, 309–313. https://doi.org/10.1016/S0960-8524(98)00155-2

Wang, Q., Noguchi, C., Hara, Y., Sharon, C., Kakimoto, K., Kato, Y., 1997. Studies on anaerobic digestion mechanism: Influence of pretreatment temperature on biodegradation of waste activated sludge. Environ. Technol. (United Kingdom) 18, 999–1008. https://doi.org/10.1080/09593331808616619

Wang, Q., Wei, W., Gong, Y., Yu, Q., Li, Q., Sun, J., Yuan, Z., 2017. Technologies for reducing sludge production in wastewater treatment plants: State of the art. Sci. Total Environ. 587–588, 510–521. https://doi.org/10.1016/j.scitotenv.2017.02.203

Wang, Y., Wei, Y., Liu, J., 2009. Effect of H2O2 dosing strategy on sludge pretreatment by microwave-H2O2 advanced oxidation process. J. Hazard. Mater. 169, 680–684. https://doi.org/10.1016/j.jhazmat.2009.04.001

Wang, Z.W., Liu, Y., Tay, J.H., 2007. Biodegradability of extracellular polymeric substances produced by aerobic granules. Appl. Microbiol. Biotechnol. 74, 462–466. https://doi.org/10.1007/s00253-006-0686-x

Wassate, B., Bouabid, G., El Falaki, K., Karhat, Y., El Amrani, B., Azzi, M., 2014. Oxidation of sludge from a wastewater treatment plant of food industry. J. Mater. Environ. Sci. 5, 1792–1798.

Water-technology, 2020. Perth Metropolitan Wastewater Treatment Plant Upgrade [WWW Document]. URL https://www.water-technology.net/projects/perthmetropolitanwas/ (accessed 6.9.20).

Weavers, L.K., Hoffmann, M.R., 2000. Kinetics and Mechanism of Pentachlorophenol Degradation by Sonication , Ozonation , and Sonolytic Ozonation 34, 1280–1285. https://doi.org/10.1021/es980795y

Wei, Y., Van Houten, R.T., Borger, A.R., Eikelboom, D.H., Fan, Y., 2003. Minimization of excess sludge production for biological wastewater treatment. Water Res. 37, 4453–4467. https://doi.org/10.1016/S0043-1354(03)00441-X

WHO/UNICEF, 2017. Progress on Drinking Water, Sanitation and Hygiene - Joint Monitoring Programme 2017 Update and SDG Baselines. Who 66. https://doi.org/10.1111 / tmi.12329

WHO, UNICEF, 2017. Progress on household drinking water, sanitation and hygiene 2000-2017. Special focus on inequalities. Who 1–71.

Wilson, C.A., Novak, J.T., 2009. Hydrolysis of macromolecular components of primary and secondary wastewater sludge by thermal hydrolytic pretreatment. Water Res. 43, 4489–4498. https://doi.org/10.1016/j.watres.2009.07.022

Wingender, J., Neu, T.R., Fleming, H.-C., 1999. What are bacterial extracellular polymeric substances?, in: Microbial Extracellular Polymeric Substances. pp. 1–19.

Wu, J., Jiang, X., Wheatley, A., 2009. Characterizing activated sludge process effluent by particle size distribution, respirometry and modelling. Desalination 249, 969–975. https://doi.org/10.1016/j.desal.2009.06.061

Wu, J., Yang, Q., Luo, W., Sun, J., Xu, Q., Chen, F., Zhao, J., Yi, K., Wang, X., Wang, D., Li, X., Zeng, G., 2018. Role of free nitrous acid in the pretreatment of waste activated sludge: Extracellular polymeric substances disruption or cells lysis? Chem. Eng. J. 336, 28–37. https://doi.org/10.1016/j.cej.2017.11.038

Wu, L.-J., Higashimori, A., Qin, Y., Hojo, T., Kubota, K., Li, Y.-Y., 2016. Comparison of hyper-thermophilic–mesophilic two-stage with single-stage mesophilic anaerobic digestion of waste activated sludge: Process performance and microbial community analysis. Chem. Eng. J. 290, 290–301. https://doi.org/10.1016/j.cej.2016.01.067

Wu, L.J., Higashimori, A., Qin, Y., Hojo, T., Kubota, K., Li, Y.Y., 2016. Upgrading of mesophilic anaerobic digestion of waste activated sludge by thermophilic pre-fermentation and recycle: Process performance and microbial community analysis. Fuel 169, 7–14. https://doi.org/10.1016/j.fuel.2015.11.091

X. S. Jia, H.H.P.F. and H.F., 1996. Surface charge and extracellular polymer of sludge in the anaerobic degradation process. Water Sci. Technol. 34, 309–316.

Xiao, B., Liu, C., Liu, J., Guo, X., 2015. Evaluation of the microbial cell structure damages in alkaline pretreatment of waste activated sludge. Bioresour. Technol. 196, 109–115. https://doi.org/10.1016/j.biortech.2015.07.056

Xiao, B., Qin, Y., Zhang, W., Wu, J., Qiang, H., Liu, J., Li, Y.Y., 2018. Temperature-phased anaerobic digestion of food waste: A comparison with single-stage digestions based on performance and energy balance. Bioresour. Technol. 249, 826–834. https://doi.org/10.1016/j.biortech.2017.10.084

Xiao, Q., Yan, H., Wei, Y., Wang, Y., Zeng, F., Zheng, X., 2012. Optimization of H2O2 dosage in microwave-H 2O2 process for sludge pretreatment with uniform design method. J. Environ. Sci. (China) 24, 2060–2067. https://doi.org/10.1016/S1001-0742(11)60998-4

Xie, B., Gu, J., Lu, J., 2010. Surface properties of bacteria from activated sludge in relation to bioflocculation. J. Environ. Sci. 22, 1840–1845.

Xu, G., Chen, S., Shi, J., Wang, S., Zhu, G., 2010. Combination treatment of ultrasound and ozone for improving solubilization and anaerobic biodegradability of waste activated sludge. J. Hazard. Mater. 180, 340–346. https://doi.org/10.1016/j.jhazmat.2010.04.036

Xu, J., Yuan, H., Lin, J., Yuan, wenxiang, 2014. Evaluation of thermal, thermal-alkaline, alkaline and electrochemical pretreatments on sludge to enhance anaerobic biogas production. J. Taiwan Inst. Chem. Eng. 45, 2531–2536. https://doi.org/10.1016/j.jtice.2014.05.029

Xu, X.R., Li, X.Z., 2010. Degradation of azo dye Orange G in aqueous solutions by persulfate with ferrous ion. Sep. Purif. Technol. 72, 105–111. https://doi.org/10.1016/j.seppur.2010.01.012

Yang, Q., Luo, K., Li, X. ming, Wang, D. bo, Zheng, W., Zeng, G. ming, Liu, J. jin, 2010. Enhanced efficiency of biological excess sludge hydrolysis under anaerobic digestion by additional enzymes. Bioresour. Technol. 101, 2924–2930. https://doi.org/10.1016/j.biortech.2009.11.012

Yang, Q., Yi, J., Luo, K., Jing, X., Li, X., Liu, Y., Zeng, G., 2013. Improving disintegration and acidification of waste activated sludge by combined alkaline and microwave pretreatment. Process Saf. Environ. Prot. 91, 521–526. https://doi.org/10.1016/j.psep.2012.12.003

Yang, Y., Chen, Q., Guo, J., Hu, Z., 2015. Kinetics and methane gas yields of selected C1 to C5 organic acids in anaerobic digestion. Water Res. 87, 112–118. https://doi.org/10.1016/j.watres.2015.09.012

Yeneneh, A.M., 2014. Study on Performance Enhancement of Anaerobic Digestion of Municipal Sewage Sludge. Curtin University.

Yeneneh, A.M., Hong, E., Sen, T.K., Kayaalp, A., Ang, H.M., 2016. Effects of Temperature, Polymer Dose, and Solid Concentration on the Rheological Characteristics and Dewaterability of Digested Sludge of Wastewater Treatment Plant (WWTP). Water. Air. Soil Pollut. 227. https://doi.org/10.1007/s11270-016-2820-4

Yeneneh, A.M., Kayaalp, A., Sen, T.K., Ang, H.M., 2015a. Effect of Combined Microwave-Ultrasonic Pretreatment of Real Mixed Sludge on the Enhancement of Anaerobic Digester Performance. Water. Air. Soil Pollut. 226. https://doi.org/10.1007/s11270-015-2586-0

Yeneneh, A.M., Kayaalp, A., Sen, T.K., Ang, H.M., 2015b. Effect of microwave and combined microwave-ultrasonic pretreatment on anaerobic digestion of mixed real sludge. J. Environ. Chem. Eng. 3, 2514–2521. https://doi.org/10.1016/j.jece.2015.09.003

Yeneneh, A.M., Sen, T.K., Ang, H.M., Kayaalp, A., 2017a. Optimisation of Microwave, Ultrasonic and Combined Microwave-Ultrasonic Pretreatment Conditions for Enhanced Anaerobic Digestion. Water. Air. Soil Pollut. 228. https://doi.org/10.1007/s11270-016-3197-0

Yeneneh, A.M., Sen, T.K., Kayaalp, A., Ang, H.M., 2017b. Effect of primary sludge to waste activated sludge mixing ratio on anaerobic digester performance. Desalin. Water Treat. 71, 79–84. https://doi.org/10.5004/dwt.2017.20483

Yeom, I.T., Lee, K.R., Ahn, K.H., Lee, S.H., 2002. Effects of ozone treatment on the biodegradability of sludge from municipal wastewater treatment plants. Water Sci. Technol. 46, 421–5.

Yi, W.G., Lo, K. V., Mavinic, D.S., 2014. Effects of microwave, ultrasonic and enzymatic treatment on chemical and physical properties of waste-activated sludge. J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng. 49, 203–209. https://doi.org/10.1080/10934529.2013.838880

Yin, G.Q., Liao, P.H., Lo, K. V., 2008. Sewage sludge treatment using microwave-enhanced advanced oxidation process. J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng. 43, 191–201. https://doi.org/10.1080/10934520701781590

Yu, G.H., He, P.J., Shao, L.M., He, P.P., 2008. Stratification structure of sludge flocs with implications to dewaterability. Environ. Sci. Technol. 42, 7944–7949.

https://doi.org/10.1021/es8016717

Yu, H.Q.M., Tay, J.H., Fang, H.H.P., 2001. The roles of calcium in sludge granulation during UASB reactor start-up. Water res 35, 1052–1060.

Yu, Q., Lei, H., Yu, G., Feng, X., Li, Z., Wu, Z., 2009. Influence of microwave irradiation on sludge dewaterability. Chem. Eng. J. 155, 88–93. https://doi.org/10.1016/j.cej.2009.07.010

Yu, S., Zhang, G., Li, J., Zhao, Z., Kang, X., 2013. Effect of endogenous hydrolytic enzymes pretreatment on the anaerobic digestion of sludge. Bioresour. Technol. 146, 758–761. https://doi.org/10.1016/j.biortech.2013.07.087

Yu, T., Deng, Y., Liu, H., Yang, C., Wu, B., Zeng, G., Lu, L., Nishimura, F., 2017. Effect of alkaline microwaving pretreatment on anaerobic digestion and biogas production of swine manure /631/326/2522 /631/326/171/1878 article. Sci. Rep. 7, 1–8. https://doi.org/10.1038/s41598-017-01706-3

Yu, Y., Chan, W.I., Liao, P.H., Lo, K. V., 2010. Disinfection and solubilization of sewage sludge using the microwave enhanced advanced oxidation process. J. Hazard. Mater. 181, 1143–1147. https://doi.org/10.1016/j.jhazmat.2010.05.134

Zahedi, S., Icaran, P., Yuan, Z., Pijuan, M., 2016. Assessment of free nitrous acid pre-treatment on a mixture of primary sludge and waste activated sludge: Effect of exposure time and concentration. Bioresour. Technol. 216, 870–875. https://doi.org/10.1016/j.biortech.2016.06.038

Zhang, A., Wang, J., Li, Y., 2015. Performance of calcium peroxide for removal of endocrine-disrupting compounds in waste activated sludge and promotion of sludge solubilization. Water Res. 71, 125–139. https://doi.org/10.1016/j.watres.2015.01.005

Zhang, J., Liu, J., Wang, Y., Yu, D., Sui, Q., Wang, R., Chen, M., Tong, J., Wei, Y., 2017. Profiles and drivers of antibiotic resistance genes distribution in one-stage and two-stage sludge anaerobic digestion based on microwave-H2O2 pretreatment. Bioresour. Technol. 241, 573–581. https://doi.org/10.1016/j.biortech.2017.05.157

Zhang, J., Yang, H., Li, W., Wen, Y., Fu, X., Chang, J., 2018. Variations of sludge characteristics during the advanced anaerobic digestion process and the dewaterability of the treated sludge conditioning with PFS , PDMDAAC and synthesized PFS-PDMDAAC. Water Sci. Technol.

78, 1189–1198. https://doi.org/10.2166/wst.2018.387

Zhang, J., Yang, K., Wang, H., Zheng, L., Ma, F., Lv, B., Zhang, J., Yang, K., Wang, H., Zheng, L., Ma, F., Lv, B., 2016. Impact of microwave treatment on dewaterability of sludge during Fenton oxidation. Desalin. Water Treat. 57, 14424–14432. https://doi.org/10.1080/19443994.2015.1065765

Zhang, L., Gao, R., Naka, A., Hendrickx, T.L.G., Rijnaarts, H.H.M., Zeeman, G., 2016. Hydrolysis rate constants at 10–25 °C can be more than doubled by a short anaerobic pre-hydrolysis at 35 °C. Water Res. 104, 283–291. https://doi.org/10.1016/j.watres.2016.07.038

Zhang, L., Loh, K.C., Zhang, J., Mao, L., Tong, Y.W., Wang, C.H., Dai, Y., 2019. Three-stage anaerobic co-digestion of food waste and waste activated sludge: Identifying bacterial and methanogenic archaeal communities and their correlations with performance parameters. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2019.121333

Zhang, P., Shen, Y., Guo, J.-S., Li, C., Wang, H., Chen, Y.-P., Yan, P., Yang, J.-X., Fang, F., 2015. Extracellular protein analysis of activated sludge and their functions in wastewater treatment plant by shotgun proteomics. Sci. Rep. 5. https://doi.org/10.1038/srep12041

Zhang, Q.-Q., Jin, R.-C., 2015. The Application of Low-Intensity Ultrasound Irradiation in Biological Wastewater Treatment: A Review. Crit. Rev. Environ. Sci. Technol. 45, 2728–2761. https://doi.org/10.1080/10643389.2015.1046772

Zhang, Q., Hu, J., Lee, D.J., 2016. Biogas from anaerobic digestion processes: Research updates. Renew. Energy 98, 108–119. https://doi.org/10.1016/j.renene.2016.02.029

Zhang, S., Zhang, P., Zhang, G., Fan, J., Zhang, Y., 2012. Enhancement of anaerobic sludge digestion by high-pressure homogenization. Bioresour. Technol. 118, 496–501. https://doi.org/10.1016/j.biortech.2012.05.089

Zhang, S., Zhang, R., 2014. Effects of Microwave Pretreatment of Apple Raw Material on the Nutrients and Antioxidant Activities of Apple Juice 2014, 1–10.

Zhang, T., Wang, Q., Ye, L., Batstone, D., Yuan, Z., 2015. Combined free nitrous acid and hydrogen peroxide pre-treatment of waste activated sludge enhances methane production via organic molecule breakdown. Sci. Rep. https://doi.org/10.1038/srep16631

Zhang, W., Peng, S., Xiao, P., He, J., Yang, P., Xu, S., Wang, D., 2015a. Understanding the evolution of stratified extracellular polymeric substances in full-scale activated sludges in relation to dewaterability. RSC Adv. 5, 1282–1294. https://doi.org/10.1039/c4ra13379j

Zhang, W., Yang, P., Xiao, P., Xu, S., Liu, Y., Liu, F., Wang, D., 2015b. Dynamic variation in physicochemical properties of activated sludge floc from different WWTPs and its influence on sludge dewaterability and settleability. Colloids Surfaces A Physicochem. Eng. Asp. 467, 124–134. https://doi.org/10.1016/j.colsurfa.2014.11.027

Zhang, W., Yang, P., Yang, X., Chen, Z., Wang, D., 2015c. Insights into the respective role of acidification and oxidation for enhancing anaerobic digested sludge dewatering performance with Fenton process. Bioresour. Technol. 181, 247–253. https://doi.org/10.1016/j.biortech.2015.01.003

Zhang, X., Bishop, P.L., 2003. Biodegradability of biofilm extracellular polymeric substances. Chemosphere 50, 63–69. https://doi.org/10.1016/S0045-6535(02)00319-3

Zhang, X., Wang, Y., Li, G., Qu, J., 2006. Oxidative decomposition of azo dye C.I. Acid Orange 7 (AO7) under microwave electrodeless lamp irradiation in the presence of H2O2. J. Hazard. Mater. 134, 183–189. https://doi.org/10.1016/j.jhazmat.2005.10.046

Zhang, Y., Zhang, P., Ma, B., Wu, H., Zhang, S., Xu, X., 2012. Sewage sludge disintegration by high-pressure homogenization: A sludge disintegration model. J. Environ. Sci. 24, 814–820. https://doi.org/10.1016/S1001-0742(11)60834-6

Zhao, H., Kalivendi, S., Zhang, H., Joseph, J., Nithipatikom, K., Vásquez-Vivar, J., Kalyanaraman, B., 2003. Superoxide reacts with hydroethidine but forms a fluorescent product that is distinctly different from ethidium: Potential implications in intracellular fluorescence detection of superoxide. Free Radic. Biol. Med. 34, 1359–1368. https://doi.org/10.1016/S0891-5849(03)00142-4

Zhen, G., Wang, J., Lu, X., Su, L., Zhu, X., Zhou, T., Zhao, Y., 2019. Effective gel-like floc matrix destruction and water seepage for enhancing waste activated sludge dewaterability under hybrid microwave-initiated Fe(II)-persulfate oxidation process. Chemosphere 221, 141–153. https://doi.org/10.1016/j.chemosphere.2019.01.037

Zhou, A., Liu, W., Varrone, C., Wang, Y., Wang, A., Yue, X., 2015. Evaluation of surfactants on waste activated sludge fermentation by pyrosequencing analysis. Bioresour. Technol. 192, 835–840. https://doi.org/10.1016/j.biortech.2015.06.017

Zhou, B.W., Shin, S.G., Hwang, K., Ahn, J.H., Hwang, S., 2010. Effect of microwave irradiation on cellular disintegration of Gram positive and negative cells. Appl. Microbiol. Biotechnol. 87, 765–770. https://doi.org/10.1007/s00253-010-2574-7

Zhou, J., Mavinic, D.S., Kelly, H.G., Ramey, W.D., 2002. Effects of temperatures and extracellular proteins on dewaterability of thermophilically digested biosolids. J. Environ. Eng. Sci. 1, 409–415. https://doi.org/10.1139/S02-029

Zhou, J., Zheng, G., Zhang, X., Zhou, L., 2014. Influences of extracellular polymeric substances on the dewaterability of sewage sludge during bioleaching. PLoS One 9. https://doi.org/10.1371/journal.pone.0102688

Zhou, X., 2015. Improving dewaterability of waste activated sludge through advanced oxidization process. The University of Queensland.

Zhou, X., Jiang, G., Wang, Q., Yuan, Z., 2015. Role of indigenous iron in improving sludge dewaterability through peroxidation. Sci. Rep. 5, 1–6. https://doi.org/10.1038/srep07516

Zhou, Y., Takaoka, M., Wang, W., Liu, X., Oshita, K., 2013. Effect of thermal hydrolysis pre-treatment on anaerobic digestion of municipal biowaste: A pilot scale study in China. J. Biosci. Bioeng. 116, 101–105. https://doi.org/10.1016/j.jbiosc.2013.01.014

Zhuang, M., Ding, C., Zhu, A., Tian, Y., 2014. Ratiometric Fluorescence Probe for Monitoring Hydroxyl Radical in Live Cells Based on Gold Nanoclusters. Anal. Chem. 86, 1829–1836. https://doi.org/10.1021/ac403810g

Zinatizadeh, A.A.L., Mohamed, A.R., Mashitah, M.D., Abdullah, A.Z., Isa, M.H., 2007. Optimization of pre-treated palm oil mill effluent digestion in an up-flow anaerobic sludge fixed film bioreactor: A comparative study. Biochem. Eng. J. 35, 226–237. https://doi.org/10.1016/j.bej.2007.01.018

Zonta, Ž., Alves, M.M., Flotats, X., Palatsi, J., 2013. Modelling inhibitory effects of long chain fatty acids in the anaerobic digestion process. Water Res. 47, 1369–1380. https://doi.org/10.1016/j.watres.2012.12.007

Zorba, G.T., Sanin, F.D., 2013. Disintegration of sludge by sonication and improvement of methane production rates in batch anaerobic digesters. Clean - Soil, Air, Water 41, 396–402. https://doi.org/10.1002/clen.201100391

APPENDIX

This section includes a list of attribution tables attested by the co-authors with respect to their contribution to the published work of the thesis. The publications have been listed in chronological order with the co-author's acknowledgement of his/her contribution.

Publication 1:

Ambrose, H.W., Philip, L., Sen, T.K., Suraishkumar, G.K., 2019. The Effect of Combined Microwave and Hydrogen Peroxide Pretreatment on Sludge Characteristics and Oxidation Status of Waste Activated Sludge, in: Lefèbvre, B. (Ed.), The Activated Sludge Process: Methods and Recent Developments. Nova Science Publishers, pp. 105–141.

Table A 1: Attribution table for publication 1.

Author	Conception and design	Acquisition of data and method	Data condition and manipulation	Analyses and statistical method	Interpretation and discussion	Final approval
Herald Wilson Ambrose	✓	✓	✓	✓	✓	
I acknowledge that these represent my contribution to the above research output Signed:						
Ligy Philip	✓	✓				✓
I acknowledge that these represent my contribution to the above research output Signed:						
Tushar Kanti Sen	✓	✓				✓
I acknowledge that these represent my contribution to the above research output Signed:						
G. K. Suraishkumar	✓				✓	✓
I acknowledge that these represent my contribution to the above research output Signed:						

Publication 2:

Ambrose, H.W., Chin, C.T.L., Hong, E., Philip, L., Suraishkumar, G.K., Sen, T.K., Khiadani, M., 2020. Effect of hybrid (microwave-H2O2) feed sludge pretreatment on single and two-stage anaerobic digestion efficiency of real mixed sewage sludge. Process Saf. Environ. Prot. 136, 194–202. https://doi.org/10.1016/j.psep.2020.01.032

Table A 2: Attribution table for publication 2.

Author	Conception and design	Acquisition of data and method	Data condition and manipulation	Analyses and statistical method	Interpretation and discussion	Final approval
Herald Wilson Ambrose	✓	✓	✓	✓	✓	
I acknowledge that these represent my contribution to the above research output Signed: Herald						
Calvin Tse-Liang Chin		✓	✓			
I acknowledge that these represent my contribution to the above research output Signed: Calvin						
Eugene Hong		✓				
I acknowledge that these represent my contribution to the above research output Signed:						
Ligy Philip						✓
I acknowledge that these represent my contribution to the above research output Signed: Ligy Philip						
G. K. Suraishkumar	✓				✓	✓
I acknowledge that these represent my contribution to the above research output Signed:						
Tushar Kanti Sen	✓					✓
I acknowledge that these represent my contribution to the above research output Signed:						
Mehdi Khiadani						✓
I acknowledge that these represent my contribution to the above research output Signed:						

Publication 3:

Ambrose, H.W., Philip, L., Suraishkumar, G.K., Karthikaichamy, A., Sen, T.K., 2020. Anaerobic co-digestion of activated sludge and fruit and vegetable waste: Evaluation of mixing ratio and impact of hybrid (microwave and hydrogen peroxide) sludge pre- treatment on two-stage digester stability and biogas yield. J. Water Process Eng. 37. https://doi.org/10.1016/j.jwpe.2020.101498

Table A 3: Attribution table for publication 3.

<table>
<tr><th>Author</th><th>Conception and design</th><th>Acquisition of data and method</th><th>Data condition and manipulation</th><th>Analyses and statistical method</th><th>Interpretation and discussion</th><th>Final approval</th></tr>
<tr><td>Herald Wilson Ambrose</td><td>✓</td><td>✓</td><td>✓</td><td>✓</td><td>✓</td><td></td></tr>
<tr><td colspan="7">I acknowledge that these represent my contribution to the above research output
Signed: Herald</td></tr>
<tr><td>Ligy Philip</td><td></td><td></td><td></td><td></td><td></td><td>✓</td></tr>
<tr><td colspan="7">I acknowledge that these represent my contribution to the above research output
Signed: Ligy Philip</td></tr>
<tr><td>G. K. Suraishkumar</td><td>✓</td><td></td><td></td><td></td><td>✓</td><td>✓</td></tr>
<tr><td colspan="7">I acknowledge that these represent my contribution to the above research output
Signed:</td></tr>
<tr><td>Anbarasu Karthikaichamy</td><td></td><td></td><td></td><td>✓</td><td></td><td></td></tr>
<tr><td colspan="7">I acknowledge that these represent my contribution to the above research output
Signed:</td></tr>
<tr><td>Tushar Kanti Sen</td><td>✓</td><td></td><td></td><td></td><td></td><td>✓</td></tr>
<tr><td colspan="7">I acknowledge that these represent my contribution to the above research output
Signed:</td></tr>
</table>