Digestion of Municipal Wastewater Biosolids using an Anaerobic Fluidized Bed Bioreactor (AnFBR)

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Graduate Program in Civil and Environmental Engineering

A thesis submitted in partial fulfillment of the requirements for the degree in Master of Engineering Science

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Digestion of Municipal Wastewater Biosolids using an
Anaerobic Fluidized Bed Bioreactor (AnFBR)

(Thesis format: Integrated-Article)

by

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Graduate Program in Engineering Science
Department of Civil and Environmental Engineering

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Engineering and Science

The School of Graduate and Postdoctoral Studies
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London, Ontario, Canada

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Abstract

This research investigated the efficacy of the anaerobic fluidized bed bioreactor (AnFBR) technology in treating municipal wastewater sludges. Primary sludge (PS) and thickened waste activated sludge (TWAS) were studied in two lab-scale AnFBRs using High-density polyethylene (HDPE) as carrier media. PS was investigated at various organic loading rates (OLRs) ranging from 9 to 18 kg chemical oxygen demand (COD)/m³·d corresponding to hydraulic retention times (HRTs) of 2 to 4 days, with maximum COD and volatile suspended solid (VSS) removal efficiency of 70% and 72%, respectively. For TWAS, VSS destruction efficiency varied from 53% at an HRT of 4 days and OLR of 12 kg COD/m³·d to 61% at an HRT of 8 days and an OLR of 6 kg COD/m³·d. The results showed that mesophilic anaerobic fluidized bed bioreactor is highly effective for COD removal and VSS reduction of municipal biosolids compared with conventional anaerobic digestion. Furthermore, the specific bacterial community activity tests showed a significant difference between solid retention times (SRT) based on general VSS and retention times based on the activity of methanogenic, acidogenic, and acetogenic microbes. While SRTs based on VSS measurements in the PS AnFBR were 3.3 days, the activity-based retention times varied from 12.2 to 14.6 days. Similarly, in the TWAS AnFBR, the SRTs based on VSS measurements were 5.0 days, and the activity-based retention times ranged from 8.0 to 9.4 days. These specific microbial activities tests can provide a better understanding of the performance of full-scale digesters, help to determine the rate-limiting process and optimize the operation conditions.
Keywords

Anaerobic fluidized bed bioreactor, primary sludge, TWAS, specific microbial activity test, specific SRTs.
Co-Authorship Statement

Dr. George Nakhla and Dr. Jesse Zhu provided supervision and guidance to the research project. Chapter 3 and 4 have been combined into a single journal paper submitted to Water Research with a manuscript number of WR29700. Drafts for the papers were written initially by Zhenqi Wang. Subsequent modifications were carried out taking the co-authors comments and suggestions. A copy of this paper has been attached in Appendix II.
Acknowledgments

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I greatly appreciate and acknowledge the generous help of Ahmed Eldyasti for his valued guidance and training in the initial part of the project. I also owe special thanks to Mr. J. Wen, Haning Li and my colleagues, who directly or indirectly, offered their help and support during this work.

I also want to thank everyone who supported me throughout the research. I am thankful for their aspiring guidance, invaluably constructive criticism and friendly advice during the project work. I am sincerely grateful to them for sharing their truthful and professional views related to the project.

Thank you,

Zhenqi Wang
Table of Contents

Abstract....................................................................................................................... Error! Bookmark not defined.
Co-Authorship Statement.......................................................................................... iiv
Acknowledgments ..................................................................................................... vvy
List of Tables .......................................................................................................... iix
List of Figures ......................................................................................................... xi
Nomenclature .......................................................................................................... xii
Abbreviation ............................................................................................................ xii

1. Introduction ........................................................................................................ 1
   1.1 Rationale ....................................................................................................... 1
   1.2 Objective .................................................................................................... 2
   1.3 Scope of the Thesis ..................................................................................... 3
   1.4 References .................................................................................................. 4

2. Literature Review .............................................................................................. 5
   2.1 Introduction ................................................................................................. 5
   2.2 Anaerobic Digestion of Biosolids ............................................................... 8
      2.2.1 Anaerobic Suspended Growth Process ................................................ 9
      2.2.2 Anaerobic Attached Growth Process .................................................... 15
   2.3 Anaerobic Fluidized Bed Bioreactor and Biofilm ........................................ 20
      2.3.1 History of Anaerobic Fluidized Bed Bioreactor .................................. 20
      2.3.2 Anaerobic Fluidized Bed Bioreactor Characteristics ........................... 22
      2.3.3 Biofilm Structure ................................................................................ 23
      2.3.4 Biofilm Formation and Detachment ..................................................... 25
   2.4 Bacterial Distribution in Biofilm Reactors .................................................... 27
   2.5 Bacterial Activity Tests ............................................................................... 30
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5.1 Specific Methanogenic Activity (SMA) and Biochemical Methane Potential (BMP) Tests</td>
<td>31</td>
</tr>
<tr>
<td>2.5.2 Specific Acidogenic Activity (SAdA) and Specific Acetogenic Activity (SAtA) Tests</td>
<td>33</td>
</tr>
<tr>
<td>2.6 Synopsis</td>
<td>33</td>
</tr>
<tr>
<td>2.7 References</td>
<td>35</td>
</tr>
<tr>
<td>3. Performance of AnFBR Treating Municipal Wastewater Biosolids</td>
<td>47</td>
</tr>
<tr>
<td>3.1 Introduction and Literature Review</td>
<td>47</td>
</tr>
<tr>
<td>3.2 Materials and Methods</td>
<td>49</td>
</tr>
<tr>
<td>3.2.1 System Description</td>
<td>49</td>
</tr>
<tr>
<td>3.2.2 Commissioning and Start-up</td>
<td>51</td>
</tr>
<tr>
<td>3.2.3 Analytical Methods</td>
<td>53</td>
</tr>
<tr>
<td>3.3 Results and Discussion</td>
<td>55</td>
</tr>
<tr>
<td>3.4 Conclusions</td>
<td>69</td>
</tr>
<tr>
<td>3.5 Reference</td>
<td>69</td>
</tr>
<tr>
<td>4. Activity Tests and Microbial Characterization in the Attached and Suspended Phases</td>
<td>73</td>
</tr>
<tr>
<td>4.1 Introduction and Literature Review</td>
<td>73</td>
</tr>
<tr>
<td>4.2 Materials and Methods</td>
<td>76</td>
</tr>
<tr>
<td>4.2.1 Batch Tests</td>
<td>77</td>
</tr>
<tr>
<td>4.2.2 Analytical Methods</td>
<td>79</td>
</tr>
<tr>
<td>4.3 Results and Discussions</td>
<td>80</td>
</tr>
<tr>
<td>4.3.1 Specific Microbial Activity Tests</td>
<td>80</td>
</tr>
<tr>
<td>4.3.2 Microbial Attachment and Detachment</td>
<td>90</td>
</tr>
<tr>
<td>4.3.3 Active Microbial Characterization</td>
<td>95</td>
</tr>
<tr>
<td>4.4 Conclusions</td>
<td>96</td>
</tr>
<tr>
<td>4.5 References</td>
<td>97</td>
</tr>
<tr>
<td>5. Conclusions and Recommendations</td>
<td>101</td>
</tr>
</tbody>
</table>
5.1 Conclusion ......................................................................................................................... 101
5.2 Limitations of the Current Work.................................................................................. 102
5.3 Recommendations and Future Work............................................................................ 103

Appendix I................................................................................................................................ 104
Appendix II.............................................................................................................................. 106
List of Tables

Chapter 2
Table 2.1 Comparison of aerobic and anaerobic treatment.............................................................. 7
Table 2.2 Comparison of anaerobic suspended growth process .................................................... 13
Table 2.3 Comparison of UASB, anaerobic filter, and AnMBBR.................................................. 19
Table 2.4 Studies and applications of anaerobic fluidized bed bioreactor..................................... 21

Chapter 3
Table 3.1 Performance of conventional anaerobic digester on treating municipal wastewater biosolids at mesophilic condition ................................................................................... 48
Table 3.2 Composition of the synthetic wastewater ...................................................................... 52
Table 3.3 Operation conditions and steady-state performance data of AnFBR fed primary sludge (R1) at S.T.P. .................................................................................................................. 60
Table 3.4 Operation conditions and steady-state performance data of AnFBR fed TWAS (R2) at S.T.P. ........................................................................................................................................... 61

Chapter 4
Table 4.1 Results of microbial activities tests under steady state of phase II for both reactors.... 92
List of Figures

Chapter 2

Figure 2.1 Principles of anaerobic digestion ................................................................. 7
Figure 2.2 Schematic of anaerobic suspended growth reactors ..................................... 10
Figure 2.3 Schematic of anaerobic attached growth reactors .......................................... 16
Figure 2.4 SEM pictures of biofilm .................................................................................. 24

Chapter 3

Figure 3.1 Schematic of the anaerobic fluidized bed bioreactor ...................................... 50
Figure 3.2 HDPE Particles used in reactors .................................................................... 51
Figure 3.3 Hydraulic control panel .................................................................................. 53
Figure 3.4 COD removal efficiency and effluent VSS concentration of reactors during start-up period .............................................................................................................. 56
Figure 3.5 Temporal VSS destruction and TCOD removal of reactors treating PS and TWAS .. 58
Figure 3.6 Temporal methane yields in both reactors treating municipal sludges at S.T.P. .... 65
Figure 3.7 Temporal VFA distributions and concentration in R1 during phase II ................. 66
Figure 3.8 Temporal VFA distributions and concentration in R2 during phase II ................. 67
Figure 3.9 Temporal VFA/ALK ratio of both reactors treating municipal wastewater sludges in phase II .................................................................................................................. 68

Chapter 4

Figure 4.1 Results of 4 rounds SMA tests for PS and TWAS reactors (average±SD) .......... 82
Figure 4.2 Results of 4 rounds SAdA tests for PS and TWAS reactors (average±SD) .......... 83
Figure 4.3 Results of 4 rounds SAtA tests for PS and TWAS reactors (average±SD) .......... 84
Figure 4.4 COD balance of 4 rounds SMA tests of both reactors ..................................... 86
Figure 4.5 Initial and final pH during all activity batch tests ............................................ 89
Figure 4.6 Distribution of active microbial groups in two reactors .................................... 91
# Nomenclature

- $b_s$: First-order detachment rate coefficient
- $C_p$: Particle concentration
- $R_e$: Reynolds number
- $L_f$: Biofilm thickness
- $S_0$: Influent COD concentration
- $S_e$: Effluent COD concentration
- $S_s$: Scum layer COD concentration
- $V_d$: VSS destruction
- $Q_{feeding}$: Flow rate of feeding
- $Q_{scum}$: Accumulating rate of scum layer
- $Q_{effluent}$: Flow rate of effluent
- $W_p$: Weight of total clean particle carriers
- $\sigma$: Shear stress
- $\mu_{max}$: Maximum growth rate
- $\rho_f$: Biofilm VSS concentration
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>TCOD</td>
<td>Total chemical oxygen demand</td>
</tr>
<tr>
<td>sCOD</td>
<td>Soluble chemical oxygen demand</td>
</tr>
<tr>
<td>TSS</td>
<td>Total suspended solid</td>
</tr>
<tr>
<td>VSS</td>
<td>Volatile suspended solid</td>
</tr>
<tr>
<td>BOD&lt;sub&gt;5&lt;/sub&gt;</td>
<td>Biochemical oxygen demand</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile fatty acid</td>
</tr>
<tr>
<td>HDPE</td>
<td>High-density polyethylene</td>
</tr>
<tr>
<td>PS</td>
<td>Primary sludge</td>
</tr>
<tr>
<td>TWAS</td>
<td>Thickened waste active sludge</td>
</tr>
<tr>
<td>SRT</td>
<td>Sludge retention time</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
</tr>
<tr>
<td>AnFBR</td>
<td>Anaerobic fluidized bed reactor</td>
</tr>
<tr>
<td>UASB</td>
<td>Upflow anaerobic sludge blanket</td>
</tr>
<tr>
<td>EGSB</td>
<td>Expanded granular sludge bed</td>
</tr>
<tr>
<td>AnMBBR</td>
<td>Anaerobic moving bed biofilm reactor</td>
</tr>
<tr>
<td>CSTR</td>
<td>Completely stirred tank reactor</td>
</tr>
<tr>
<td>ABR</td>
<td>Anaerobic baffled reactor</td>
</tr>
<tr>
<td>AnSBR</td>
<td>Anaerobic sequencing batch reactor</td>
</tr>
<tr>
<td>AnMBR</td>
<td>Anaerobic membrane bioreactor</td>
</tr>
<tr>
<td>SMA</td>
<td>Specific methanogenic activity</td>
</tr>
<tr>
<td>SAdA</td>
<td>Specific acidogenic activity</td>
</tr>
<tr>
<td>SA&lt;sub&gt;a&lt;/sub&gt;A</td>
<td>Specific acetogenic activity</td>
</tr>
<tr>
<td>SNR</td>
<td>Specific nitrification rate</td>
</tr>
<tr>
<td>SDNR</td>
<td>Specific denitrification rate</td>
</tr>
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</table>
Chapter 1

Introduction

1.1 Rationale

The history of the biological wastewater treatment can be traced back to the end of the 19th century. Since 1896, people started to know that “organisms”, which are also known as activated sludge, can be applied to treat wastewater (Henze, et al., 2008). Within the recent 100 years, the technology of biological wastewater treatment has experienced enormous innovation and the biological principles and kinetics have been gradually revealed by scientists and engineers. The basic principle of the biological wastewater treatment is that the pollutants are absorbed, converted, and digested by the microorganisms as their energy and carbon resources. (Rittmann & McCarty, 2001). Essentially, bioreactors can be divided into two main sub-streams: suspended growth and attached growth (Metcalf & Eddy, 2003).

Anaerobic process, as one of the typical biological treatment, has been widely applied to treating high strength industrial wastewaters due to its capability of sustaining higher volumetric loadings (Heijnen et al., 1988), low nutrient requirements, low biomass yield, and additional biogas (hydrogen, methane) production (Chan, 2009). Although municipal wastewater usually contains low solids concentration (<0.05%), the residues comprising screened solids, grit, primary sludge, and secondary sludge can have an extremely high solids concentration of up to 12%. Unlike the screened solids and grit, the primary sludge and waste active sludge are biodegradable. Therefore, the anaerobic
system turns out to be an ideal bio-process for treating primary sludge (PS) and waste active sludge (WAS) (Metcalf & Eddy, 2003).

Recently, bioparticle technology, as an advanced wastewater treatment technology, started to attract the interest of the researchers due to its advantages compared with the suspended (conventional) wastewater treatment strategy. The wide application of the biofilm technology in the environmental field is attributed to three main reasons (Nicolella, et al., 2000):

• The reactor can be operated at high biomass concentration even without the sludge recirculation;
• Excellent treatment can be achieved even at high hydraulic loading rates due to immobilization of biomass;
• Natural, mixed microbial communities that can operate in synergy can be sustained.

The anaerobic fluidized bed bioreactor (AnFBR) involving biofilm-coated particles has been successfully developed and investigated on digesting municipal and industry biosolids by Nakhla and coworkers (Andalib et al., 2014) to be a potential alternative for conventional anaerobic digesters.

### 1.2 Objective

The thesis has the following goals:
• Investigating the performance of the AnFBR with challenging municipal biosolids e.g., primary sludge (PS) and thickened waste activated sludge (TWAS) at lab-scale.

• Developing a methodology to estimate active biomass SRT, and rationalizing the performance of the AnFBR by evaluating attachment / detachment characteristics.

• Exploring the impact of sonication on the scum layer floating on the top of the reactor

1.3 Scope of the Thesis

This thesis mainly focuses on the anaerobic digestion of acetic acid based synthetic wastewater and municipal wastewater sludges using an anaerobic fluidized bed bioreactor (AnFBR) and exploring the distribution of different specific active microbial groups in the biofilm. Chapter 2 provides a critical literature review on the anaerobic digestion of biosolids, biofilm attachment and detachment, and basic application of anaerobic fluidized bed.

Chapter 3 discusses the operation and performance of the AnFBR in digesting municipal wastewater biosolids. Detailed data of the VSS destruction efficiency, mass balance, biofilm properties, and operational conditions are presented and discussed in this section.
Chapter 4 focuses on the measurements and results of specific microbial activity tests. These tests are novel in the field of examining the distribution of different active bacteria groups in an anaerobic biofilm reactor.

1.4 References


Mogens et al. (2008) Biological Wastewater Treatment Principles, Modelling and Design. IWA Publication


Chapter 2

Literature Review

2.1 Introduction

Anaerobic digestion of biosolids can be divided into four sequential steps (Figure 2.1): hydrolysis (digesting large polymers into small monomers), acidogenesis (converting monomers into volatile fatty acids), acetogenesis (degrading volatile fatty acid into acetic acid, CO2, and H2), and methanogenesis (consuming acetate acid and producing CH4) (Metcalf & Eddy, 2003), carried out by various microbial groups that exist both in suspended phase and attached biofilm phase in biofilm reactors (Switzenbaum, 1983; Heijnen, et al., 1988; Kuba, et al., 1990; Elefsiniotis & Oldham, 1993). During these processes, the complex organic matters are destroyed and the biogas, comprising primarily H2, CH4 and CO2, is generated. Typically, rods (Methanobacterium, Methanobacillus) and spheres (Methanococcus, Methanothrix, and Methanosarcina) are considered as main methanogenic bacteria communities (Metcalf & Eddy, 2003), while phyla Firmicutes and spirochaetales are mostly found during mesophilic acetogenic and acidogenic processes (Lee et al., 2011). Other significant members of these bacterial communities were chloroflexi, Syntrophomonas, Gammaproteobacteria, Actinobacteria, Bacteroidetes and Deferribacteres with Gammaproteobacteria (i.e., Pseudomonas) commonly being representatives of the microbial communities in anaerobic processes of solid substrates (Rincon et al., 2006; McMahon et al., 2001). Anaerobic communities in anaerobic systems are highly dependent on the temperature (Pervin et al., 2013), SRT (Lee et al., 2011), and OLR (Ricon et al., 2008). Pervin et al. (2013) studied the microbial
community composition in mesophilic and thermophilic temperature-phased anaerobic digesters treating activated sludge by applying 16sRNA gene amplicon pyrosequencing and fluorescence in situ hybridization (FISH). They found that Thermotogae sp., Coprothermobacter sp., and Lutispora thermophlia were much more active at temperature higher than 50°C, while Gammaproteobacterium and Thauera sp. contributed most at mesophilic conditions. Ricon et al. (2008) investigated various organic loading rate (OLR) ranging from 0.8 to 11.0 kg COD/m3d in a mesophilic anaerobic completely stirred tank reactor (CSTR) treating olive mill solid residue. Their results showed that the genus Clostridium was representative at a low OLR, while Gammaproteobacteria, Actinobacteria, Bacteroidetes and Deferribacteres were mostly found at high OLRs. Lee et al. (2011) tested the impact of multiple sludge retention times (SRTs) on methanogenesis in anaerobic digestion of thickened mixed sludge. They observed that as the SRT decreased from 20 to 4 days, Chloroflexi and Syntrophomonas declined and two acetogenic genera belonging to the phyla Firmicutes and Spirochaetales increased.

Table 2.1 basically compares the features of anaerobic and aerobic treatment (Yeoh, 1995). As evident from Table 2.1, aerobic systems are more feasible as a secondary treatment facility due to low temperature sensitivity and high effluent quality, while anaerobic process are more practical in treating high biosolids wastewater and pre-treatment because of capability of generating bioenergy and sustaining high loading rates.
Figure 2.1 Principles of anaerobic digestion
(From website: http://www.wtert.eu/default.asp?Menu=13&ShowDok=12, Nov 2014)

Table 2.1 Comparison of aerobic and anaerobic treatment

<table>
<thead>
<tr>
<th>Feature</th>
<th>Aerobic</th>
<th>Anaerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effluent quality</td>
<td>Excellent</td>
<td>Moderate to poor</td>
</tr>
<tr>
<td>Organic loading rate</td>
<td>Moderate</td>
<td>High</td>
</tr>
<tr>
<td>Sludge production</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Nutrient requirement</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Alkalinity requirement</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Temperature sensitivity</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Start-up time</td>
<td>Quick</td>
<td>Slow</td>
</tr>
<tr>
<td>Odor</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Bioenergy and nutrient recovery</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Unlike aerobic systems, anaerobic systems are very temperature sensitive. Generally, mesophilic (20-40°C) and thermophilic (50-60 °C) conditions are utilized in anaerobic processes. The thermophilic processes is believed to be able to provide a higher metabolic rate according to the Arrhenius equation as well as a larger degree of pathogen deactivation, although energy consumption is relatively high compared with mesophilic systems. Actually, Guo et al., (2014) investigated the performance of two lab-scale anaerobic digesters for treating food waste under mesophilic (35 °C) and thermophilic (55 °C) and observed a better performance and richer bacteria species of mesophilic reactor at an OLR of 2.5 kg VS/m³d. However, temperature phased anaerobic digestion processes with a thermophilic acidogenic fermenter and a mesophilic methanogenic fermenter have been shown to enhance the biosolids reduction by 5% and biogas production in acidogenic fermenter by 100% for both food waste (Youn and Shin, 2005) and municipal biosolids digestion (Rubio-Loza and Noyola, 2010).

Under most condition, separating acidification and methanation in two reactors (2-stage) is considered as an optimal design for anaerobic treatment process due to its advantage of high COD reduction capacity, easier pH control, and stable performance (Heijnen et al., 1988).

2.2 Anaerobic Digestion of Biosolids

Anaerobic processes can be simply divided into two main categories: suspended growth and attached growth (biofilm) process. Although the basic metabolic processes are similar for fixed-film and suspended-growth systems, there are still some inherent
differences that provide several advantages and some challenges for the application of attached film processes (Rittmann & McCarty, 2001).

2.1.1 Anaerobic Suspended Growth Process

Anaerobic suspended growth processes, which have been deeply studied and researched, are adopted worldwide as a trusted biological treatment of industry wastewaters as well as a reliable method of digesting biosolids. The typical types of anaerobic suspended growth processes are: completely stirred tank reactor (CSTR), anaerobic baffled bioreactor (ABR), anaerobic sequencing batch reactor (AnSBR), and anaerobic membrane bioreactor (AnMBR) (Shown in Figure 2.2).

A CSTR is a conventional technology contains a reactor equipped with a mixer, which can be applied in single-stage and two-stage digestion. The main difference between these two types of reactor is that fermentation and methanogenesis are separated and acclimated in two stirred tanks by using different retention times. Usually, 2-stage digestion system would need lower total digestion time (Gunaseelan, 1997).
The ABR started to appear in the wastewater field since the early 1980s. The basic function of ABR is similar with upflow anaerobic sludge blanket (UASB), but granulation is not necessary in ABR (Hassan & Dahlan, 2013). In ABR, several vertical or horizontal baffles are placed in series to force the wastewater flow across them. Hence, the solid retention capacity is increased. Therefore, ABR is considered as one of the high...
rate anaerobic bioreactors, and the typical OLR of ABR can reach as high as 40 kg COD/m$^3$d. However, Ayaz et al. (2012) compared the performance of pilot-scale UASB and ABR for treating domestic wastewater at an OLR ranging from 0.4 to 0.7 kg COD/m$^3$d. The result showed the TCOD removal efficiency was 56%-58% in UASB while the ABR only achieved 41%-50% TCOD removal. This indicated that the treatment efficiency of ABR is lower than UASB at the same condition. Furthermore, the application of ABR for treating wastewaters with high suspended organics still remains under research (Hassan & Dahlan, 2013).

The cyclical operation of the ASBR follows four sequential steps: feed, react, settle, and decant. The advantage of ASBR is that it can sustain a higher OLR compared to conventional CSTR due to a high SRT, and a high food-to-microorganism (F/M) ratio at the beginning of the react phase that ensure a high reaction rate and biogas production (Ndegwa et al., 2008). However, the low treatable loading rate (less than 19 kg COD/m$^3$d) is the major disadvantage of this reactor (Shizas and Bagley, 2001).

Anaerobic membrane reactor, as one of advanced technologies, has been further studied within the last two decades. The remarkable advantages, such as low sludge production, low footprint, complete biomass retention, elicited the interest of both the research community and industry (Lin et al., 2013). However, membrane fouling remains the major obstacle limiting the application of AnMBR. The fouling, which is mostly caused by the interaction between the membrane material and the suspended solids, decreases the system productivity, reduce membrane lifespan, and increase energy requirement.
This suggests that AnMBR might not be effective in treating wastewater with high suspended solids.

Table 2.2 summarizes several studies of anaerobic suspended growth system on both industrial waste and municipal wastewater biosolids. Throughout the table, a long HRT is required for these kinds of system to achieve high removal efficiency at a high OLR. Among all of the reactor types, The AnMBR shows remarkably high treatment efficiency. However, this technology also has some disadvantages, such as high capital cost, low packing density (for tubular membranes), and high pumping costs (Lin et al., 2013), which limits the widespread utilization.
Table 2.2 Comparison of anaerobic suspended growth process

<table>
<thead>
<tr>
<th>Reactor type</th>
<th>Substrate type</th>
<th>OLR (kg/m³d)</th>
<th>HRT (h)</th>
<th>COD removal (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CSTR</strong></td>
<td>Cheese processing wastewater</td>
<td>5-14</td>
<td>12-24</td>
<td>20</td>
<td>(Yang et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Cheese whey wastewater</td>
<td>6-47</td>
<td>24-84</td>
<td>-</td>
<td>(Azbar et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Olive pulp wastewater</td>
<td>14-63</td>
<td>7.5-30</td>
<td>-</td>
<td>(Koutrouli et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>Sugar beet wastewater</td>
<td>17</td>
<td>14.2</td>
<td>-</td>
<td>(Hussy et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>Sugary wastewater</td>
<td>10-64</td>
<td>0.5-72</td>
<td>-</td>
<td>(Ueno et al., 1996)</td>
</tr>
<tr>
<td><strong>ABR</strong></td>
<td>Brewery wastewater</td>
<td>5.6</td>
<td>15</td>
<td>92</td>
<td>(Boopathy et al., 1991)</td>
</tr>
<tr>
<td></td>
<td>Soybean wastewater</td>
<td>1.2</td>
<td>39.5</td>
<td>97</td>
<td>(Langenhoff et al., 2000)</td>
</tr>
<tr>
<td></td>
<td>Municipal wastewater</td>
<td>2.62</td>
<td>6</td>
<td>86</td>
<td>(Bodkhe et al., 2009)</td>
</tr>
<tr>
<td><strong>ASBR</strong></td>
<td>Dairy wastewater</td>
<td>2.4-4.7</td>
<td>24</td>
<td>87</td>
<td>(Mohan et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Swine waste</td>
<td>0.4</td>
<td>4-12 (d)</td>
<td>85</td>
<td>(Ndegwa et al., 2013)</td>
</tr>
<tr>
<td><strong>AnMBR</strong></td>
<td>Glucose</td>
<td>1.1</td>
<td>12</td>
<td>99</td>
<td>(Huang et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Cheese whey</td>
<td>19.8</td>
<td>24-96</td>
<td>98.5</td>
<td>(Saddoud et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Brewery</td>
<td>12</td>
<td>140</td>
<td>99</td>
<td>(Torres et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>Olive-mill</td>
<td>0.7</td>
<td>16.7</td>
<td>95</td>
<td>(Stamatelatou et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Municipal wastewater</td>
<td>1</td>
<td>10</td>
<td>88</td>
<td>(Lin et al., 2011)</td>
</tr>
</tbody>
</table>
Recently, anaerobic co-digestion has attracted significant interest due to its ability of enhancing bio-gas production and treatment efficiency by combining two wastewater streams together. Several researches have been conducted to investigate the co-digestion of different substrates over last 15-20 years by simultaneously treating different organic waste streams. Co-digestion has been proven to have a distinct positive effect on methane production rate and methane yields (Kim et al., 2003; Esposito et al., 2012). The main advantages of co-digestion are reported as dilution of toxic compounds, improved nutrients balance and buffering capacity, and synergistic microbial effects (Esposito et al., 2012).

Esposito et al. (2012), who assessed the mesophilic co-digestion of buffalo manure (BM) and organic fraction of the municipal solid water (OFMSW) in biochemical methane potential (BMP) tests, observed that the co-digestion of BM and OFMSW resulted in 12% higher methane production and decreased the possibility of failure for the biological process. Riano et al. (2011) demonstrated promising results for co-digestion of swine manure with winery wastewater, with 81% to 300% improvement in the methane yields at different combinations of substrates at an OLR of 0.85 kg COD/m³d.

Alvarez et al. (2014) reviewed the co-digestion researches within the last three years and pointed out that, within the OLR ranging from 0.85 to 5.50 kg COD/m³d, the treatment efficiency of the co-digester can be improved by 10% to 200% by mixing pig manure or cow manure with other side streams together i.e. distillery wastewater, cheese whey, olive mill waste. Lindorfer et al. (2007) studied the impact of organic loading shock on a
full scale 2-stage mesophilic CSTR co-digesting crops and in Austria and observed that after doubling the organic loading rate from 2.1 to 4.2 kg VS/m$^3$d, the volume related biogas production almost doubled, which indicated that the co-digestion reactor completely accepted the increasing loading rate at an HRT of 75 days.

2.2.2 Anaerobic Attached Growth Process

Although anaerobic digestion has been investigated successfully on both municipal wastes and industrial effluents, i.e. olive oil mill, protein waste (Rintala, et al., 1996; Filidei, et al., 2002; Borja, et al., 2001) the digestion of municipal PS and TWAS is often limited by slow biodegradation rates from slow biomass hydrolysis, and resulting in low solids destruction efficiencies of less than 50% despite long retention times (Metcalf & Eddy, 2003).

In order to optimize the biosolids reduction and capital cost, fixed film reactors turn out to be an alternative method of anaerobic digesting. In these systems, microorganisms grow in a biofilm formed on the surface of a solid support instead of randomly “swimming” in the reactors. The substrates are transport into the biofilm and consumed as the liquid passes the bio-particles (Switzenbaum, 1983). The typical types of anaerobic fixed film bioreactor are: upflow anaerobic sludge blanket (UASB), anaerobic filter, anaerobic fluidized bed, and expanded granular sludge bed (EGSB), which are presented in Figure 2.3.
Figure 2.3 Schematic of anaerobic attached growth reactors
(a) UASB (b) anaerobic filter (c) anaerobic fluidized bed bioreactor (From em-group website http://www.em-group.co.th/Technology_Biogas%20Technology2.html, Nov, 2014)
(d) EGSB (From Nicoletta et al., 2000)
The principle of UASB reactor is similar to the fluidized bed bioreactor; however, the liquid up flow velocity is much smaller in UASB. Research showed that the UASB processes are basically based on the dense granules formed in the reactor (Nicolella et al., 2000). As the raw wastewater enters from the bottom of the reactor, it passes upward through the dense anaerobic sludge layer by the up flow force caused by the influent itself. The dense sludge phase and treated liquid phase are separated at the settler section which maintains the high biomass concentration in the reactor (60-70 kg/m³). This high biomass concentration allows the UASB to sustain a high OLR of 10 to 15 kg COD/m³d with a fairly short HRT of less than 2 days (Nicolella et al., 2000).

Anaerobic filters are widely used as secondary treatment of domestic wastewaters and industrial wastewater to improve solids removal (Francisco et al, 2003). Although anaerobic filters have several advantages, such as high organic removal capacity, short HRT, and ability to withstand load fluctuations, they can only be applied for treating wastewater with a low percentage of suspended solids to prevent the filter from clogging (Eawag et al., 2014). Hence, anaerobic filters are always combined with other treatment.

Expanded granular sludge bed (EGSB) reactors combine both UASB and FBR, which contain granular bioparticle and operate at a slightly higher superficial liquid velocity (5-10 m/hr) (Nicolella et al., 2000). Numbers of EGSBs have been built for treating various types of industrial wastewaters i.e. food, chemical, pharmaceutical with an OLR up to 30 kg COD/m³d (Zoutberg and de Been, 1997). As a family of the UASB, there is no definite difference between UASB and EGSB (Lim and Kim, 2014).
The anaerobic moving bed biofilm reactor (AnMBBR) is a reactor in which polyethylene carrier media are employed and mixed. The large area provided by the support carrier guarantees a high attached biomass concentration, which makes the AnMBBR reliable on sustaining high volumetric loading rate and insensitive towards shock loading. AnMBBR has been successfully investigated on treating vinasse (Sheli et al., 2007), landfill leachate (Chen et al., 2008), and dairy wastewater (Wang et al., 2009) at OLRs ranging from 2 to 30 kg COD/m$^3$d and achieved more than 73% COD removal efficiency.

Table 2.3 illustrates the researches and applications of UASB, anaerobic filter, and AnMBBR. Compared with the anaerobic suspended growth systems, the treatment efficiency of these systems is considerably high at similar HRT and OLR. The details of anaerobic fluidized bed bioreactors are discussed in Section 2.3.
<table>
<thead>
<tr>
<th>Reactor type</th>
<th>Substrate type</th>
<th>OLR (kg COD/m³ d)</th>
<th>HRT (h)</th>
<th>COD removal (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>UASB</td>
<td>Synthetic wastewater</td>
<td>18</td>
<td>17</td>
<td>95</td>
<td>(Kennedy et al., 1989)</td>
</tr>
<tr>
<td></td>
<td>Synthetic wastewater</td>
<td>28</td>
<td>2</td>
<td>90</td>
<td>(Noyola et al., 1988)</td>
</tr>
<tr>
<td></td>
<td>Brewery</td>
<td>14.1</td>
<td>4.9</td>
<td>86</td>
<td>(Switzenbaum, 1983)</td>
</tr>
<tr>
<td></td>
<td>Starch</td>
<td>11</td>
<td>47</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sugar</td>
<td>13.3</td>
<td>24</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alcohol</td>
<td>16</td>
<td>8</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Anaerobic</td>
<td>Domestic sewage</td>
<td>3.1</td>
<td>4</td>
<td>55</td>
<td>(Elmitwalli et al., 2002a)</td>
</tr>
<tr>
<td>filter</td>
<td>Domestic sewage (combined</td>
<td>0.9</td>
<td>12</td>
<td>71</td>
<td>(Elmitwalli et al., 2002b)</td>
</tr>
<tr>
<td></td>
<td>with hybrid reactor)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Municipal wastewater</td>
<td>0.8</td>
<td>12</td>
<td>91</td>
<td>(Bodkhe, 2008)</td>
</tr>
<tr>
<td></td>
<td>Synthetic domestic sewage</td>
<td>1-1.7</td>
<td>10-17</td>
<td>80</td>
<td>(Martin et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>Starch gluten</td>
<td>3.8</td>
<td>22</td>
<td>64</td>
<td>(Switzenbaum, 1983)</td>
</tr>
<tr>
<td></td>
<td>Guar gum</td>
<td>16</td>
<td>24</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>AnMBBR</td>
<td>Vinasses</td>
<td>1.6-29.6 (sCOD)</td>
<td>1.6-6.3 d</td>
<td>81-89</td>
<td>(Sheli et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Landfill leachate</td>
<td>4.1</td>
<td>30</td>
<td>91</td>
<td>(Chen et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Dairy wastewater</td>
<td>2-20</td>
<td>14.5-24</td>
<td>73-86</td>
<td>(Wang et al., 2009)</td>
</tr>
</tbody>
</table>
2.3 Anaerobic Fluidized Bed Bioreactor and Biofilm

2.3.1 History of Anaerobic Fluidized Bed Bioreactor

Compared with conventional bioreactors, fluidized reactors have many advantages, such as enhanced mass and heat transfer rates, stability under shock loadings, achieving high treatment efficiency with low support media, and a uniform distribution within the liquid phase. These features have led to increased productivity and wide application of fluidized bed reactors (Zhu et al., 2000). Anaerobic fluidized bed reactors have been used in the treatment of industrial wastewaters since 1980s e.g. treating food-processing, digesting paper industry wastewater. (Heijnen, et al., 1988), and purifying fermentation wastewater (Holst, et al., 1997). As wastewater travels through the media, the substrate diffuses to the biofilm where it is digested.

Table 2.4 summarizes the application of the anaerobic fluidized bed and performance with different wastewaters. Initially, sand was widely applied as the support media, while other carriers, like zeolite, glass beads, plastic beads, have become more popular, recently, due to energy savings. Most of the literature studies only focused on COD removal instead of solids destruction. These studies superficially displayed and discussed VSS destruction both of industrial wastewaters and municipal wastewaters treatment. Although research has focussed on industrial wastewaters treatment, Nakhla and coworkers explored the potential application of AnFBR for digestion of municipal wastewater biosolids with high VSS concentration.
### Table 2.4 Studies and applications of anaerobic fluidized bed bioreactor

<table>
<thead>
<tr>
<th>Scale</th>
<th>Substrate type</th>
<th>Reactor volume (L)</th>
<th>OLR (kg COD/m³d)</th>
<th>HRT (h)</th>
<th>Carrier media (D in mm)</th>
<th>TSS/VSS reduction (%)</th>
<th>COD removal (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab</td>
<td>Starch</td>
<td>50</td>
<td>18</td>
<td>12</td>
<td>sand (0.6)</td>
<td>N/A</td>
<td>80</td>
<td>(Hichey et al., 1981)</td>
</tr>
<tr>
<td>Lab</td>
<td>Sewage</td>
<td>1</td>
<td>4.5</td>
<td>1</td>
<td>resin (1.0)</td>
<td>N/A</td>
<td>70</td>
<td>(Jewell et al., 1981)</td>
</tr>
<tr>
<td>Lab</td>
<td>Ethanol</td>
<td>50</td>
<td>19</td>
<td>15</td>
<td>sand (0.6)</td>
<td>N/A</td>
<td>85</td>
<td>(Hickey et al., 1981)</td>
</tr>
<tr>
<td>Lab</td>
<td>Whey permeate</td>
<td>0.4</td>
<td>30</td>
<td>2.4</td>
<td>sand (0.24)</td>
<td>N/A</td>
<td>70</td>
<td>(Biver, 1984)</td>
</tr>
<tr>
<td>Lab</td>
<td>Milk waste</td>
<td>4</td>
<td>8</td>
<td>15</td>
<td>sand (0.22)</td>
<td>N/A</td>
<td>90</td>
<td>(Bull et al., 1982)</td>
</tr>
<tr>
<td>Lab</td>
<td>Glucose</td>
<td>1.5</td>
<td>24</td>
<td>0.5</td>
<td>carbon (0.6)</td>
<td>N/A</td>
<td>90</td>
<td>(Chen et al, 1985)</td>
</tr>
<tr>
<td>Pilot</td>
<td>Brewery waste</td>
<td>60</td>
<td>19</td>
<td>15</td>
<td>sand (0.35)</td>
<td>N/A</td>
<td>95</td>
<td>(Hall, 1982)</td>
</tr>
<tr>
<td>Pilot</td>
<td>Soy waste</td>
<td>60</td>
<td>14.5</td>
<td>20</td>
<td>sand (0.5)</td>
<td>N/A</td>
<td>60</td>
<td>(Sutton et al., 1982)</td>
</tr>
<tr>
<td>Pilot</td>
<td>yeast water</td>
<td>310</td>
<td>60</td>
<td>1</td>
<td>sand (0.2)</td>
<td>N/A</td>
<td>90</td>
<td>(Heijnen, 1983)</td>
</tr>
<tr>
<td>Full</td>
<td>Soft-drink</td>
<td>95 m³</td>
<td>9.6</td>
<td>6</td>
<td>sand (0.6)</td>
<td>N/A</td>
<td>77</td>
<td>(Switzenbaum 1983)</td>
</tr>
<tr>
<td>Full</td>
<td>Food canning</td>
<td>120 m³</td>
<td>60</td>
<td>24</td>
<td>biolite (&lt;0.5)</td>
<td>N/A</td>
<td>80</td>
<td>(Holst et al., 1997)</td>
</tr>
<tr>
<td>Lab</td>
<td>Textile wastewater</td>
<td>4</td>
<td>3</td>
<td>24</td>
<td>pumice (0.25-1.44)</td>
<td>N/A</td>
<td>82</td>
<td>(Sen et al, 2003)</td>
</tr>
<tr>
<td>Lab</td>
<td>Trinitrotoluene</td>
<td>1 m³</td>
<td>0.43</td>
<td>3</td>
<td>GAC</td>
<td>N/A</td>
<td>99</td>
<td>(Maloney et al., 2002)</td>
</tr>
<tr>
<td>Lab</td>
<td>Thin stillage</td>
<td>16</td>
<td>29</td>
<td>3.5 d</td>
<td>zeolite (0.5)</td>
<td>78</td>
<td>88</td>
<td>(Andalib et al., 2012)</td>
</tr>
<tr>
<td>Lab</td>
<td>Primary sludge</td>
<td>16</td>
<td>9.5</td>
<td>1.9 d</td>
<td>zeolite (0.5)</td>
<td>82</td>
<td>82</td>
<td>(Andalib et al., 2014)</td>
</tr>
</tbody>
</table>
While traditionally, fluidized bed reactors have been used for low suspended solids streams e.g. treating food-processing, digesting paper industry wastewater, and purifying fermentation wastewater (Heijnen, et al., 1988; Holst, et al., 1997), recently, the mesophilic anaerobic fluidized bed reactor (AnFBR) with zeolite as carrier media (425–610 µm) developed by Nakhla and coworkers, (Andalib et al., 2012), achieved up to 88% TCOD and 78% total suspended solids (TSS) removal at an OLR of 29 kg COD/m³d during the treatment of thin stillage with a TCOD of 130 g/L and TSS of 47 g/L (Andalib et al., 2012). Another AnFBR has been demonstrated for the digestion of primary sludges (Andalib et al., 2014) with a TSS destruction efficiency of 82% at an OLR of 9.5 kg COD/m³d.

2.3.2 Anaerobic Fluidized Bed Bioreactor Characteristics

Various characteristics of the reactor, such as start-up process, inoculation, biofilm formation, and microbial dynamics, are essential to evaluate the performance of anaerobic fluidized bed bioreactors (Saravanane & Murthy, 2000).

During start-up, as the biofilm develops, the thickness of biofilms is highly influenced by the liquid flux rate, Reynolds number, abrasion, and organic loading (Hichey et al., 1991). Synthetic Volatile fatty acid (VFA)-based wastewater i.e. acetate (Hsu and Shiek, 1993), propionate (Heppner et al., 1992), is commonly applied in the start-up period.

Various inoculum sources have been applied as seed for anaerobic fluidized bed treating different wastewater. Municipal secondary anaerobic digester sludges (ADS) was used as
seed for reactors treating thin stillage, municipal primary sludge, and thickened waste active sludges (Andalib et al., 2014), while supernatant from animal manure digesters can be applied for starch-based food processing waste, chemical waste and soft drink bottling waste (Hickey et al., 1991). Ehlinger et al. (1989) investigated the impact of the seed sludge pH on the anaerobic fluidized bed reactor, and claimed that a seed pH ranging from 7 to 8.5 would be optimal.

Hichey et al., (1991) summarised the application of various carriers in anaerobic fluidized bed reactors. At the time of the aforementioned studies, sand was a widely accepted carrier media for treating industrial wastewater, while zeolite and activated carbon were ideal for treating sewage. Recently, plastic media has been proved viable due to its lower density and potential energy saving concern (Eldyasti et al., 2012). Yee et al. (1992) investigated the performance of porous carriers against sands in two identical anaerobic fluidized bed reactors fed acetic acid at an OLR of 6 kg TOC/m³d and observed that the start-up times were reduced by more than 50% in the reactor using porous support carriers.

2.3.3 Biofilm Structure

Biofilms can be generally divided into two zones, the base film and the surface film, both containing a mixture of microorganisms and other particulate material bound together (Grady et al., 1999). The biofilm thickness highly depends on the hydrodynamic characteristics of the system as well as the nature of microorganisms in the biofilm while mass transfer rates are usually limited by the hydrodynamic regime (Characklis &
Marshall, 1990). Figure 2.4 depicts typical biofilm-coated particle observed in anaerobic fluidized bed.

The structure of biofilms is very complex, comprising dead and active bacteria, inert organic and inorganic solids, substrates, and metabolites. Literature confirms that anaerobic biofilms are actually growing in a layered structure with methanogenic bacteria growing at the inner part followed by the acetogenic and acidogenic microbes (Heijnen et al., 1988).

Figure 2. 4 SEM pictures of biofilm
(a) bioparticle on sand; (b) methanogenic biolayer (From Heijnen et al., 1988); (c) attached biofilm in bio-hydrogen AnFBR (From Kuo et al., 2011)
2.3.4 Biofilm Formation and Detachment

In biofilm reactors, the development of the biofilm is determined by the difference between biofilm growth and detachment processes. Biofilm growth mainly relies on the carrier characteristics such as particle size, sphericity, porosity, density, and specific surface area (SSA) (Nicolella, 2000). The detachment of biofilm is usually contributed by abrasion (surface biofilm loss caused by particle collision), erosion (surface biofilm loss caused by shear stress), sloughing (the periodic loss of large biofilm patch) and predator grazing (outer surface biofilm consumed by protozoa) (Nicolella, 2000). During sloughing, a fraction of the biomass is removed down to the substratum but detachment is not effective for the entire surface of the biofilm. Erosion and abrasion, in contrast, are effective for the entire surface of the biofilm.

Chang et al. (1991) derived a model using detachment coefficient ($b_s$) to describe the mechanism of detachment of biofilms in an aerobic liquid-solid fluidized bed. The aforementioned authors studied the impact of liquid shear stress ($\sigma$), biofilm VSS concentration ($\rho_f$), biofilm thickness ($L_f$), biofilm true growth rate ($u$), particle concentration ($C_p$), and the Reynolds number ($R_e$). As a result, they found that the first-order detachment coefficient ($b_s$) was mainly dependent on the shear stress ($\sigma$), particle concentration ($C_p$), and Reynolds number ($R_e$) with negligible impact of density, thickness, and growth rate. The model generated is shown below:

$$b_s = -3.14 + 0.0335 C_p + 19.3 R_e - 3.46 \sigma$$  \hspace{1cm} (2.1)
This equation derived by Chang shows that the $b_s$ is inversely proportional to shear force. In contrast, Rittman (1982) built a model based on smooth aerobic biofilms on unfluidized glass beads saying $b_s$ is proportional to $\sigma^{0.58}$. However, Speitel and DiGiano (1987) observed during their study on paranitrophenol (PNP) in a granular activated carbon (GAC) reactor that the $b_s$ predicted by the Rittmann model underestimated the actual detachment rate, implying that the value of the exponent in Rittmann’s model is greater than 0.58. Liu and Tay (2001) observed a smooth, dense and stable biofilm at a high shear stress in an aerobic rotating disc reactor with a tip velocity of 1.45 m/s treating synthetic wastewater at an OLR of 2.4 kg COD/m³d. Patel et al. (2005) also obtained a relationship between increased shear stress and increased biomass first-order detachment rate coefficients in both aerobic and anoxic columns of a circulating fluidized bed reactor using lava rock (0.6-1.0 mm) as media. Similarly, Reis and Silva (2004), Nakhla et al. (2002) and Turan (2000) also observed that the increase of shear stress will lead to an increase of detachment rate in anaerobic fluidized beds. Similarly, Escudie et al (2011) also confirmed this opinion in his review of anaerobic biofilm reactors.

The total detachment rate of components ($r_d$) can be calculated as the empirical expression below (Stewart, 1993):

$$r_d = b_s \rho_f L_f$$

(2.2)
2.4 Bacterial Distribution in Biofilm Reactors

In most of the suspended growth reactors, it is considered that the liquid phase is completely mixed; therefore the distribution of specific bacteria is even in the whole reactor. In contrast, the distribution of bacteria in attached growth systems is totally different due to the formation of the biofilm on the support media.

Egli et al. (2003) using fluorescent in situ hybridization (FISH) observed that the biofilm layer from a rotating biological contactor biofilm treating high ammonium wastewater, comprised aerobic nitrifiers on the outer layer of the biofilm and anammox bacteria in the inner layer. Vlaeminck (2010), observed that in autotrophic biomass in a granular sludge reactor treating synthetic wastewater at a NLR of 84 g NH$_4^+$-N/m$^3$d, the structure of the biofilm layer from inside to outside was in the following order: anammox bacteria ($u_{\text{max}}=0.1$ d$^{-1}$), nitrite oxidizing bacteria (NOB), and ammonium oxidizing bacteria (AOB) ($u_{\text{max}}=0.14$-1.44 d$^{-1}$). This finding clearly suggests that in the autotrophic biofilm, the slowest growing bacteria grow deep in the biofilm and are thus sheltered from hydrodynamic forces. Fu et al. (2010) explored the biofilm structure in a simultaneous nitrification and denitrification moving bed bioreactor (MBBR) treating synthetic glucose solution at an OLR of 1.2 to 3.6 kg COD/m$^3$d, and observed that the heterotrophic bacteria were in the outer layer.

Mozumder (2014) further studied the impact of substrate concentration on the bacterial distribution in a granular sludge reactor and found that in the absence of organics, the relatively few heterotrophic bacteria grew behind the autotrophic AOB and NOB bacteria.
However, in the presence of organics, the fast growing heterotrophs became the majority on the outer surface of the biofilm. The aforementioned studies of aerobic biofilms clearly demonstrated that in multi-species biofilms, the slow-growing bacteria are present in the inner biofilm layers. Additionally, 16S rDNA/rRNA-targeted oligonucleotide DNA probes were applied for further identifying the microorganism communities in aerobic systems (Mobarry, 1996; Wagner, 1996; Magnusson, 1998; Cheneby, 2000).

The structure of anaerobic biofilms is distinctively different from aerobic mixed-culture biofilms of heterotrophs and nitrifies where the culture interaction and interdependency is not as strong. The various bacterial groups in anaerobic biofilms feed off the products generated by the other cultures and hence it is anticipated that the acidogenesis grow on the outside of the biofilm while the methanogens grow on the inside of the biofilm. Studies of the structure of anaerobic biofilms are limited with most of the studies focusing on the spatial distribution of active organisms along the reactor rather than the distribution inside the biofilm. Bull et al. (1983) observed that methanogens mainly grew attached to the carrier surface while acidifiers tend to appear in the suspended phase when investigating an anaerobic fluidized bed reactor with glucose solution at an HRT of 5 days and OLRs ranging from 6 to 18 kg COD/m$^3$d. Kuba et al. (1990), using zeolite as support media in an anaerobic fluidized bed treating VFAs based synthetic wastewater at an OLR of 4 kg COD/m$^3$d, claimed that not all of the attached biomass were active methanogens.
Hidalgo et al. (2002) carried out specific methanogenic activity (SMA) tests only on the attached biomass in a methanogenic fluidized bed reactor fed with acetic acid and found higher specific methanogenic activity at the top of the fluidized bed than at the bottom. Andalib et al. (2014) observed a much lower detachment rate for methanogens than other biomass, resulting in a methanogenic SRT to overall biomass SRT ratio of 4:1 in an AnFBR reactor treating municipal biosolids. Kuo et al. (2011), using biochemical hydrogen potential (BHP) on attached and suspended biomass from AnFBR treating kitchen wastes mesophilically at an HRT of 7.3 days and OLR of 1.1 kg COD/m$^3$d, determined that the concentration of hydrogen-producing bacteria in suspension is 2.5 times on the carrier media, implying that the acidogenic bacteria grew primarily in suspension. This observation also implied that it might not be suitable to build a single-stage hydrogen production anaerobic fluidized bed since the attached biofilm did not show any advantage against the suspended phase. In contrast, Cresson et al. (2009) applied FISH on the colonized particles obtained from a methanogenic inverse turbulent bed reactor fed with diluted red wine at an OLR of 10.7 kgCOD/m$^3$d, and proved that a relatively homogeneous layered biofilm was generated.

While anaerobic microbial activity in biofilm reactors has been assessed using SMA test, (Kuba et al., 1990; Hidalgo et al., 2002; Andalib et al, 2014), the activity of other anaerobic microbial groups have been scantily used in the literature, presumably due to the common perception that methanogenesis is often the rate limiting anaerobic process. However, this postulation is not valid for solids digestion which is hydrolysis-limited (Alvarez, et al., 2000).
2.5 Bacterial Activity Tests

Generally, sludge activity tests can be conducted in two ways: overall measurement which gives whole information regarding the full degradation process and specific activity measurement which focus on each bacterial group at different degrading stage. Commonly, sludge activity measurements can also be applied to select the inoculum, monitorize the operation, and determine the toxic effect (Soto et al., 1993).

In aerobic systems, specific nitrification tests, specific denitrification tests, and respirometry are widely used to determine the biomass activity and bio-kinetics. Respirometry is a quantifiable way to determining the biological oxygen consumption of a biomass, using well defined experimental conditions. The consumption of oxygen by the biomass enables the bacteria to grow and to remove substrate from influent streams. Applying respirometry techniques to biomass samples from an activated sludge process allows for monitoring, modeling and control of the system. Xu et al. (2006), who ran respirometry on both influent and effluent of the anaerobic reactor, observed that the soluble chemical oxygen demand (sCOD) increased from 40% to 50% of the total COD, and the average maximum heterotrophic growth rate also increased from 1.5 d⁻¹ to 3.5 d⁻¹ in a pilot-scale anaerobic/aerobic system treating tomato-processing wastewater. Chowdury et al., (2011) invented a novel respirometric technique which made it possible to do the respirometry tests directly in a fluidized bed.

The process of respirometry test is very complicated and the entire experiment requires a long reaction and analysis time (5-7 days). However, specific nitrification rate (SNR) test
and specific denitrification rate (SDNR) test are quite straightforward. These batch tests usually can be finished within one day. Practically, specific nitrification tests and specific denitrification tests are helpful to indicate the performance of aerobic/anoxic reactors. Cooper et al. (1990) reported a nitrification rate of 0.09 g NH₄-N/g VSSd in an FBBR treating ammonium-rich (50-100 mg/L) industrial wastewater. Huang et al. (2005) observed a high specific nitrification rate of 0.26-0.47 g NH₄-N/g VSSd in a partial nitrification activated sludge reactor combined with UASB treating pre-settled piggery wastewater at a total kjeldahl nitrogen (TKN) loading rate of 0.64 kg/m³d. Generally, in simultaneous nitrification and denitrification reactors, SDNR is higher than SNR if the substrate concentration is not rate-limiting.

Although the specific activity tests and bio-kinetic tests for aerobic system are well developed, the activity of methanogenic anaerobic cultures is still predominantly based on specific methanogenic activity tests.

2.5.1 Specific Methanogenic Activity (SMA) and Biochemical Methane Potential (BMP) Tests

In anaerobic biodegradation process, methanogenesis is the final stage where the acetate and H₂ are further bio-transformed to CH₄, which is also generally believed to be the rate-limiting step (Alvarez et al., 2002). SMA test is one of the preferred methods to investigate the methanogenic activity profiles of suspended and attached biomass in anaerobic reactors. Researches have shown that SMA is feasible for evaluating the performance of most anaerobic reactor types, such as AnFBR (Araki et al., 1994; Andalib
et al., 2014), AnMBR (Ince et al., 1995; Ho et al., 2010), UASB (Sumino et al., 2007; Mchugh et al., 2004), AnSBR (Banik et al., 1997).

Even at the beginning of the start-up period of a reactor, SMA tests would also be very helpful for investigating the activity of inoculum and estimating the potential organic loading rate (Soto et al., 1993). Usually, acetic acid, as the most readily biodegradable substrate, is used as the substrate in SMA tests. Occasionally, propionic and butyric acids can also be applied as substrates (De Jong, 1986; Field et al., 1988).

Besides SMA tests, Biochemical Methane Potential (BMP) tests are widely accepted for evaluating methane production. However, according to the literature, BMP tests are more likely to determine the characteristic of the various feed than investigate the bio-kinetics of biomass. Several BMP tests were conducted on industry waste and WAS. Labatut et al. (2011) carried out BMP tests on substrates highly rich in lipids using anaerobic digested sludge as inoculum, and observed a 30% higher methane yield if the initial high lipid stream was co-digested with easily-degradable carbohydrates. Alzate et al (2014) studied the anaerobic digestion of lipid-extracted nannochloropsis and observed a 20% higher BMP for the lipid-extracted nannochloropsis than the non-extracted microalgae, and also claimed that the impact of substrate-to-inoculum ratio (0.5 to 1) did not impact the BMP test when the biomass concentration was controlled within 0.5% to 2% by volume.
2.5.2 Specific Acidogenic Activity (SAdA) and Specific Acetogenic Activity (SAtA) Tests

Acidogenesis and acetogenesis refer to the processes which convert monomers into volatile fatty acids and degrade volatile fatty acid into acetic acid, CO$_2$, and H$_2$, respectively. Although acidogenesis and acetogenesis are not usually rate-limiting in most anaerobic systems, the evaluation of these two activities also provides important information about biofilm structure and bio-kinetics (Soto et al., 1933).

However, literature did not show any clear and feasible methods which have been conducted to separately measure specific acetogenic and specific acidogenic bacterial activities. Acetogenic activity is fairly easy to assess by inhibiting methanogenic bacteria and controlling the VFA-based substrate (Nie et al., 2009), while acidogenesis is hard to assess separately since both acetogenesis and acidogenesis are usually happening simultaneously in batch tests because the digested product of acidogenic biomass is the natural substrate for acetogenesis.

2.6 Synopsis

Literature showed that the relationship between shear stress and biomass detachment rate coefficient in aerobic biofilm reactor has been thoroughly studied and understood. However, no research has clearly justified the model of predicting detachment coefficient based on the biomass characteristics, bioparticle features, and reactor hydrodynamics in anaerobic fluidized bed system although anaerobic
fluidized bed reactors have been widely used in treating industrial wastewaters for several decades.

Furthermore, no research has ever clearly distinguished the distribution of the three main active bacterial groups (methanogenic, acetogenic and acidogenic) in a single-stage anaerobic fluidized bed bioreactor treating high solid municipal wastewater sludge. In light of the scarcity of information in the dispersed literature on the structure of anaerobic biofilms, and limited tools for quantification of various microbial groups, the main objectives of this study were to develop a methodology to estimate active biomass SRT, and rationalize the excellent performance of the AnFBR by evaluating attachment/detachment characteristics.

Therefore, the major objective of this research involve operating AnFBRs for digestion of municipal wastewater biosolids, exploring the distribution of three active bacterial groups in biofilm, and comparing the relationship between detachment rate coefficients and shear stress. Specific bacterial activity tests are employed in this research to explore the activity and distribution of bacterial groups in suspended and biofilm phase in anaerobic fluidized bed bioreactors. Additionally, this research derived a new method to separate these two tests from the whole biochemical hydrogen potential test by controlling different pH, substrate and inhibitors, the details of which are explained in Section 4.2.
2.7 References


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Chapter 3

Performance of AnFBR Treating Municipal Wastewater Biosolids

3.1 Introduction and Literature Review

Anaerobic digestion is a preferred and also widely applied treatment process for organic wastes due to its low nutrient requirements, low biomass yield, and biogas (hydrogen, methane) production (Chan, 2009). Anaerobic digestion has been investigated successfully on both municipal wastes, and industrial effluents i.e. olive oil mill, protein waste (Rintala, et al., 1996; Filidei, et al., 2002; Borja, et al., 2001). However, research has been shown that conventional anaerobic digestion processes usually require a hydraulic retention time (HRT) as long as 20 to 40 days in order to get satisfactory removal efficiency (Lee et al., 2011; Alvarez et al., 2014). Additionally, anaerobic digestion of primary sludge (PS) and thickened waste activated sludge (TWAS) is often limited by slow biodegradation rates ensuing from slow biomass hydrolysis, and resulting in low solids destruction efficiencies of less than 50% despite long retention times as shown in Table 3.1. The low destruction efficiencies, combined with low design volumetric volatile solids loadings, would translate not only in large footprint and high capital cost for digesters but also high solids disposal costs.

* Parts of this chapter have been submitted for publication to Water Research (manuscript number WR29700). A full edition of the paper has been attached in Appendix II.
Table 3.1 Performance of conventional anaerobic digester on treating municipal wastewater biosolids at mesophilic condition

<table>
<thead>
<tr>
<th>Scale</th>
<th>Wastewater type</th>
<th>OLR (kg VS/m3d)</th>
<th>HRT (d)</th>
<th>Biosolid reduction (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full</td>
<td>WAS</td>
<td>1.0</td>
<td>21</td>
<td>22</td>
<td>Bolzonella et al., 2005</td>
</tr>
<tr>
<td>Full</td>
<td>WAS</td>
<td>0.8</td>
<td>33</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Full</td>
<td>WAS</td>
<td>1.0</td>
<td>22</td>
<td>15</td>
<td>Lin et al., 1997</td>
</tr>
<tr>
<td>Lab</td>
<td>WAS</td>
<td>0.5-2.4</td>
<td>7.5-20</td>
<td>19-35</td>
<td>Ghyoot &amp; Verstraete, 1997</td>
</tr>
<tr>
<td>Full</td>
<td>PS</td>
<td>0.4-0.8</td>
<td>20</td>
<td>40-45</td>
<td></td>
</tr>
<tr>
<td>Pilot</td>
<td>Combined</td>
<td>1.6-2.6</td>
<td>19-27</td>
<td>41-48</td>
<td>Szikriszt et al., 1988</td>
</tr>
<tr>
<td>Lab</td>
<td>Combined</td>
<td>-</td>
<td>15</td>
<td>32</td>
<td>Tomei et al., 2011</td>
</tr>
<tr>
<td>Lab</td>
<td>Combined</td>
<td>7</td>
<td>15</td>
<td>23</td>
<td>Cecchi et al., 1991</td>
</tr>
<tr>
<td>Full</td>
<td>Combined</td>
<td>1.56</td>
<td>25</td>
<td>50</td>
<td>Lacroix et al., 2014</td>
</tr>
</tbody>
</table>

Compared with conventional bioreactors, fluidized reactors have many advantages, such as enhanced mass and heat transfer rates, stability under shock loadings, high treatment efficiency at high organic loading rates, and a uniform distribution within the liquid phase. These features have led to increased productivity and wide application of fluidized bed reactors (Zhu et al., 2000). Anaerobic fluidized bed reactors have been used in the treatment of low suspended-solids industrial wastewaters since 1980s e.g. food-processing, pulp-and-paper industry wastewater (Heijnen, et al., 1988), and fermentation wastewater (Holst, et al., 1997). As wastewater travels through the media, the substrate diffuses to the biofilm where it is digested. Initially, sand was widely applied as the support media, while other carriers, like zeolite, glass beads, plastic beads, have become more popular, recently, due to energy savings. Most of the literature studies only focused on COD removal instead of solids destruction. These studies superficially displayed and discussed VSS destruction both for industrial wastewaters and municipal wastewaters treatment.
Recently, the mesophilic anaerobic fluidized bed reactor (AnFBR) with zeolite (true density of 2360 kg/m³ and average diameter ranging from 425–610 µm) as carrier media has been successfully developed and investigated by Nakhla and coworkers for the treatment of thin stillage with a total chemical oxygen demand (TCOD) of 130 g/L and total suspended solids (TSS) of 47 g/L (Andalib et al., 2012). The AnFBR also achieve 70% and 56% solids destructions when treating primary sludge (PS) and thickened waste activated sludge (TWAS) at an OLR of 19 kg COD/m³d and 8 kg COD/m³d, respectively (Mustafa et al., 2014).

This research was a further study based on the previous work carried out by Nakhla and coworkers. In this research, lighter HDPE particles were used as carrier media instead of zeolites, which has been proven to save more energy (Eldyasti et al., 2012). Furthermore, more detailed work on the attachment and detachment of various microbial communities was undertaken.

3.2 Materials and Methods

3.2.1 System Description

Two identical lab-scale anaerobic fluidized bed bioreactors (AnFBBRs), demonstrated in Figure 3.1, were investigated for digestion of PS (R1) and TWAS (R2). Each plexiglass reactor contained a 16-liters main anaerobic column (3.6 m height, 8.9 cm long and 5.1 cm width) and a liquid-solid separator from which the digested sludges was separated and circulated to the bottom of the ANFBBR for fluidization. A wet tip gas meter connected to the top of the column was used to measure the biogas flow rate. A mesophilic temperature of 37°C is uniformly maintained throughout the reactor by a water bath (IncuMaxTM WB20C,
USA). A 10-liter container with mixer was used as a feed tank, from which sludges were pumped to the bottom of the column by a peristaltic pump (Masterflex I/P, Masterflex AG, Germany).

![Figure 3.1 Schematic of the anaerobic fluidized bed bioreactor](image)

Approximately 3 kg HDPE media (600 um~850 um) were added into the reactors after compaction, which occupied 22% volume of the 16 L reactor. The HDPE carrier (Figure 3.2)
had a sphericity of 0.9 and a BET surface area of 0.86 m\(^2\)/g, with bulk and true densities of 810 kg/m\(^3\) and 1554 kg/m\(^3\), respectively. The reason for using plastic particles (HDPE) instead of zeolites was due to their potential lower energy consumption (Eldyasti et al., 2012).

![Figure 3. 2 HDPE Particles used in reactors](image)

3.2.2 Commissioning and Start-up

Anaerobic digester sludge (ADS-TSS and VSS concentrations of 25,000 and 18,000 mg/L) from the secondary digester was collected from the St. Mary wastewater treatment plant (Ontario, Canada) and used as the seed for the AnFBRs. After loading with 3 kg of media corresponding to a compacted media volume of 3.5 L, the reactors were filled with 20 L of ADS, fluidized and operated in a batch mode at 100% bed expansion for 7 days to induce microbial attachment at an anaerobic condition provided by initially injecting N\(_2\) gas at the top area. The reactors were then started by feeding synthetic solution containing 10,000 mg COD/L as sodium acetate at a flow rate of 1.8 L/d corresponding to a volumetric OLR of 1.1.
kg COD/m^3^-d based on the 16 L AnFBR working liquid volume. Details of the composition of the synthetic feed are presented in table 3.2 (Andalib et al., 2012). Although the pH of the synthetic feeding was lower than 4 due to a high concentration of acetic acid, the pH in the reactor were maintained at a fairly constant level at around 7.2. The OLR was gradually increased to 18 kg COD/ m^3^-d within 100 days.

The liquid at the top of the reactor was recycled and pumped back to the bottom of the fluidized bed to maintain an upflow velocity at 0.8 cm/s. A hydraulic control panel (Figure 3.3) was built to control the flow rate and obtain accurate measurement of the liquid superficial velocity. A gas release valve was installed at the highest point of the pipe line in the control panel in order to release accumulated gas when necessary. After acclimatization, TWAS and screened PS from the Adelaide wastewater treatment plant (Ontario, Canada) were fed to the AnFBRs. Adelaide WWTP is a single-stage nitrifying wastewater treatment plant operating at an SRT of 3-4 days.

**Table 3.2 Composition of the synthetic wastewater**

<table>
<thead>
<tr>
<th>Feed Comp.</th>
<th>CH₃COOH (mL/LF)</th>
<th>NH₄Cl (g/LF)</th>
<th>K₂HPO₄ (g/LF)</th>
<th>MgSO₄·7H₂O (g/LF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con.</td>
<td>9.5-38</td>
<td>0.93</td>
<td>0.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Feed Comp.</td>
<td>CaCl₂·2H₂O (g/LF)</td>
<td>Yeast (g/LF)</td>
<td>NaHCO₃ (g/LF)</td>
<td>Trace element (mL/LF)</td>
</tr>
<tr>
<td>Con.</td>
<td>0.03</td>
<td>0.03</td>
<td>6.2-24.8</td>
<td>1</td>
</tr>
<tr>
<td>Trace element</td>
<td>FeCl₂·4H₂O</td>
<td>MnCl₂·4H₂O</td>
<td>H₃BO₃</td>
<td>ZnCl₂</td>
</tr>
<tr>
<td>Con. (mg/L)</td>
<td>2000</td>
<td>500</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Trace element</td>
<td>CuCl₂</td>
<td>AlCl₃</td>
<td>CoCl₂·6H₂O</td>
<td>NiCl₂</td>
</tr>
<tr>
<td>Con. (mg/L)</td>
<td>30</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>
3.2.3 Analytical Methods

The influent and effluent samples were constantly collected and analyzed for various water quality parameters such as Total suspended solids (TSS), volatile suspended solids (VSS), Total chemical oxygen demand (TCOD), soluble chemical oxygen demand (sCOD), 5-days biological oxygen demand (BOD₅), Volatile fatty acids (VFA), and alkalinity. Additionally, gas production and gas composition was monitored and recorded daily.

TSS, VSS, BOD₅ were analyzed according to the Standard Methods (APHA, 1992). TCOD, and sCOD, were measured using HACH methods and investigating kits (HACH Odyssey DR/2800) based on potassium dichromate oxidation and spectrophotometric determination. Soluble sample was obtained by applying filter paper with a pore size of 0.45 um. Alkalinity was measured by titration with 0.02 N H₂SO₄ in accordance with the Standard Method No.
2320 (APHA, 1992). VFAs were measured by employing gas chromatograph (Model CP-3800, software version 3.2.6.C, CP-1177 injector, VARIAN). The gas pressures were set as 80 psi for helium, 80 psi for nitrogen, 60 psi for air, and 40 psi for hydrogen, respectively. The gas flow rates were set at 1.5 mL/min, 3.0 mL/min, and 6.0 mL/min for nitrogen, helium, and hydrogen, respectively. The oven and flame ionization detector (FID) temperatures were 250ºC and 300 ºC. The standard curves for analyses determined by gas chromatograph and HACH Odyssey DR/2800 have been attached in Appendix I.

The rate of biogas produced in the anaerobic fluidized bed was measured by a wet tip gas meter (Rebel wet-tip gas meter company, Nashville, TN, USA) connected to the top of anaerobic column. Methane, nitrogen gas, hydrogen gas were determined by injecting 0.6 mL of the biogas composition into a gas chromatograph (Model 310, SRI Instruments, Torrance, CA) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Molesieve 5A, mesh 80/100, 182.88 × 0.3175 cm). The temperatures of the column and the TCD detector were 90 and 105ºC, respectively. Argon was used as carrier gas at a flow rate of 30 mL/min (Andalib, 2012).

In order to measure the biomass attachment, approximately 8-10 g bioparticles were collected from each column and sonicated for 3 h at 30C to detach the biomass from the particle using a Aquasonic sonicator (Model 75HT, ETL Laboratory Investigating Inc., New York). The VSS content of the detached biomass was measured using standard methods (APHA, 1992) and the sonicated particles were weighted after drying at room temperature for 1 d.
3.3 Results and Discussion

During the start-up period, the acetic acid-based synthetic wastewater was fed gradually to the two reactors at OLR ranging from 1.1 kg COD/m³d to 18 kg COD/m³d. R2 was started two months before R1.

As illustrated in Figure 3.4, during the first week, the sCOD removal efficiency in R2 decreased due to the biomass acclimatization. However, starting from the second week, the sCOD removal efficiency started to increase steadily approaching 95% on day 20 at an OLR of 2.2 kg COD/m³d, concomitant with a dramatic decrease in VSS concentrations in liquid (effluent). This observation clearly indicates that the active attached biomass was already developed and growing on the support media.

Since acetic acid is a readily biodegradable substrate (Hsu and Shiek, 1993), the increase in loading rate did not adversely impact the performance of reactor during start-up. The sCOD removal decreased to 80% immediately following loading increases but came back to more than 90% within several days, emphasizing the rapid favourable response of the AnFBR to the dynamic organic loading rates. However, R2 suffered from a pH shock on day 58 when the OLR was increased from 4.5 to 9 kg COD/m³d with pH dropping to 5.0 due to insufficient feed alkalinity, prompting a rapid drop in COD removal 80% to 20% as evidenced by a precipitous decline of COD removal efficiency. After dosing NaOH to bring the pH to 7.2 again, the reactor did not recover for 5 days. The reactor was then drained, reseeded with ADS and started at an OLR of 1.1 kg COD/m³d. This implied that the pH shock might have caused irreversible damage to the AnFBR.
Figure 3.4 COD removal efficiency and effluent VSS concentration of reactors during start-up period
The operation of R1 was quite smooth during the start-up period based on the experience gained from the operation of R2. Both reactors were gradually started up with OLR ranging from 1.1 to 18 kg COD/m³/d gradually. Biogas production was measured every two days and COD balance closure was calculated at more than 95% based on the biogas production and COD consumption during the start-up period.

After successful start-up and commissioning for about 100 days, both reactors were fed with real municipal wastewater biosolids, ie. PS in R1 and TWAS in R2, respectively. Raw PS was initially fed into R1 at an OLR of 9 kg COD/m³/d, however, the large chunks in the raw PS clogged the water distributor and the accumulated pressure caused a pipeline rupture. The reactor was then fed with screened PS, where the large chunks were manually removed through a strainer. Although the reactor still suffered from clogging occasionally, the performance of the PS reactor was still fairly stable. On the other hand, the operation of R2 starting at an initial OLR of 6 kg COD/m³/d was good as the TWAS was more uniform than PS.

Figures 3.5 shows the temporal variations of VSS destruction and the TCOD removal in both reactors during the municipal biosolids run i.e. time 0 corresponds to the initial feeding of PS and TWAS. The TCOD removal efficiency and VSS destruction rate were almost identical for both reactors due to the relatively low sCOD. There were a few fluctuations during the first couple of days for both reactors as the feed was switched from synthetic wastewater to biosolids and the loading rate was changed. However, the VSS reduction efficiency was still increasing during the first 20 days and the reactors achieved a stable performance after 30 days in two investigated phases.
Figure 3.5 Temporal VSS destruction and TCOD removal of reactors treating PS and TWAS
A fairly thick scum layer containing 112 mg/g TSS and 92 mg/g VSS in R1, 136 mg/g TSS and 119 mg/g VSS in R2, was observed floating on the top of the liquid-solid separator in phase II with an accumulation rate of 350 g/d and 270 g/d, respectively. The scum layers were manually removed every 1 or 2 days to ensure the smooth operation of the reactors. Compressed N₂ was bubbled in the reactors for 10 minutes after removing the scum.

Methane yield and VSS destruction efficiency were calculated as follows:

\[
\text{Methane yield} \left( \frac{\text{mL CH}_4}{\text{mg COD}} \right) \text{ (STP)} = \frac{V_{\text{CH}_4}(\text{mL}) \times \frac{273}{273+37}}{S_0 \left( \frac{\text{mg}}{\text{L}} \right) \times Q_{\text{feeding}}(\text{L/d}) - S_e \left( \frac{\text{mg}}{\text{L}} \right) \times Q_{\text{effluent}}(\text{L/d}) - S_s \left( \frac{\text{mg}}{\text{L}} \right) \times Q_{\text{s}}(\text{g/d})}
\]  

(3.1)

\[
\text{VSS destruction} \ (V_d) = 1 - \frac{V_{\text{effluent}} \left( \frac{\text{mg}}{\text{L}} \right) \times Q_{\text{effluent}}(\text{L/d}) + V_{\text{scum}} \left( \text{mg/g} \right) \times Q_{\text{scum}}(\text{g/d})}{V_{\text{feeding}} \left( \text{mg/L} \right) \times Q_{\text{feeding}}(\text{L/d})}
\]  

(3.2)

The steady-state performance data of R1 and R2 are presented in Table 3.3 and 3.4. PS feeding to the AnFBR was started at an OLR of 9 kg COD/m³-d and increased to 18 kg COD/m³-d on the 61st day. The operation of the AnFBR fed with TWAS was finally conducted at an OLR of 12 kg COD/m³-d, and achieved a VSS destruction efficiency of 53% at an HRT of 4 days.
<table>
<thead>
<tr>
<th><strong>Parameter</strong></th>
<th><strong>Start-up</strong></th>
<th><strong>Phase I</strong></th>
<th><strong>Phase II</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of operation (d)</td>
<td>1-60</td>
<td>61-151</td>
<td>151-231</td>
</tr>
<tr>
<td>Feed flow rate (L/d)</td>
<td>1.8-7.2</td>
<td>3.6</td>
<td>7.2</td>
</tr>
<tr>
<td>OLR based on anaerobic reactor (kg COD/m³ d)</td>
<td>1.1-18</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Anaerobic HRT(d)</td>
<td>2.2-8.9</td>
<td>4.4</td>
<td>2.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.2±0.2</td>
<td>7.1±0.4</td>
<td>7.0±0.5</td>
</tr>
<tr>
<td>Attached Biomass (mg/g media)</td>
<td>2.3-5.6</td>
<td>13.6±1.0</td>
<td>24.8±4.1</td>
</tr>
<tr>
<td>Total media (kg)</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

### Feeding characteristics

<table>
<thead>
<tr>
<th><strong>Parameter</strong></th>
<th><strong>Start-up</strong></th>
<th><strong>Phase I</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>TCOD (mg/L)</td>
<td>10,000~40,000</td>
<td>38900±2900</td>
</tr>
<tr>
<td>sCOD (mg/L)</td>
<td></td>
<td>1940±820</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td></td>
<td>30200±3400</td>
</tr>
<tr>
<td>VSS (mg/L)</td>
<td></td>
<td>25700±3100</td>
</tr>
</tbody>
</table>

### Effluent characteristics

<table>
<thead>
<tr>
<th><strong>Parameter</strong></th>
<th><strong>Start-up</strong></th>
<th><strong>Phase I</strong></th>
<th><strong>Phase II</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>TCOD (mg/L)</td>
<td>-</td>
<td>6620±320</td>
<td>8160±740</td>
</tr>
<tr>
<td>sCOD (mg/L)</td>
<td></td>
<td>985±30</td>
<td>1230±200</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>-</td>
<td>4710±950</td>
<td>6090±950</td>
</tr>
<tr>
<td>VSS (mg/L)</td>
<td>-</td>
<td>4060±420</td>
<td>5070±630</td>
</tr>
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</table>

### Scum layer characteristics

<table>
<thead>
<tr>
<th><strong>Parameter</strong></th>
<th><strong>Start-up</strong></th>
<th><strong>Phase I</strong></th>
<th><strong>Phase II</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>TCOD (mg/g)</td>
<td>-</td>
<td>110±40</td>
<td>140±40</td>
</tr>
<tr>
<td>TSS (mg/g)</td>
<td>-</td>
<td>100±20</td>
<td>110±30</td>
</tr>
<tr>
<td>VSS (mg/g)</td>
<td>-</td>
<td>70±25</td>
<td>90±15</td>
</tr>
<tr>
<td>Production rate (g/d)</td>
<td>-</td>
<td>200±35</td>
<td>350±70</td>
</tr>
</tbody>
</table>

### Removal Efficiencies

<table>
<thead>
<tr>
<th><strong>Parameter</strong></th>
<th><strong>Start-up</strong></th>
<th><strong>Phase I</strong></th>
<th><strong>Phase II</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>COD removal eff. (%)</td>
<td>&gt;90%</td>
<td>68</td>
<td>61</td>
</tr>
<tr>
<td>VSS removal eff. (%)</td>
<td>-</td>
<td>69</td>
<td>63</td>
</tr>
</tbody>
</table>

### Methane yields

<table>
<thead>
<tr>
<th><strong>Parameter</strong></th>
<th><strong>Start-up</strong></th>
<th><strong>Phase I</strong></th>
<th><strong>Phase II</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane yield (LCH4/gCOD removed) (STP)</td>
<td>-</td>
<td>0.31</td>
<td>0.28</td>
</tr>
</tbody>
</table>
Table 3.4 Operation conditions and steady-state performance data of AnFBR fed TWAS (R2) at S.T.P.

<table>
<thead>
<tr>
<th>Operating Conditions</th>
<th>Start-up</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
<td><strong>Time of operation (d)</strong></td>
<td><strong>Feed flow rate (L/d)</strong></td>
<td><strong>OLR based on anaerobic reactor (kg COD/m³ d)</strong></td>
</tr>
<tr>
<td></td>
<td>1-120</td>
<td>1.8-7.2</td>
<td>1.1-18</td>
</tr>
<tr>
<td></td>
<td>121-187</td>
<td>2.0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>188-268</td>
<td>4.0</td>
<td>12</td>
</tr>
</tbody>
</table>

| **Feeding characteristics**   |                   |                  |                  |                  |        |                                 |                     |
| TCOD (mg/L)                   | 10,000-40,000     |                  |                  |                  |        |                                 |                     |
| sCOD (mg/L)                   |                   |                  |                  |                  |        |                                 |                     |
| TSS (mg/L)                    |                   |                  |                  |                  |        |                                 |                     |
| VSS (mg/L)                    |                   |                  |                  |                  |        |                                 |                     |

| **Effluent characteristics**  |                   |                  |                  |                  |        |                                 |                     |
| TCOD (mg/L)                   |                   | 10000±900        | 11000±1250       |                  |        |                                 |                     |
| sCOD (mg/L)                   |                   | 1050±450         | 1650±350         |                  |        |                                 |                     |
| TSS (mg/L)                    |                   | 7410±640         | 8100±1050        |                  |        |                                 |                     |
| VSS (mg/L)                    |                   | 6570±600         | 7300±850         |                  |        |                                 |                     |

| **Scum layer characteristics**|                   |                  |                  |                  |        |                                 |                     |
| TCOD (mg/g)                   |                   | 130±10           |                  |                  |        |                                 |                     |
| TSS (mg/g)                    |                   | 90±35            |                  |                  |        |                                 |                     |
| VSS (mg/g)                    |                   | 75±25            |                  |                  |        |                                 |                     |
| Production rate (g/d)         |                   | 170±45           |                  |                  |        |                                 |                     |

| **Removal Efficiencies**      |                   |                  |                  |                  |        |                                 |                     |
| COD removal eff. (%)          | >90%              |                  |                  |                  |        |                                 |                     |
| VSS removal eff. (%)          |                   | 57               |                  |                  |        |                                 |                     |

| **Methane yields**            |                   |                  |                  |                  |        |                                 |                     |
| Methane yield (LCH4/gCOD removed) (STP) |   | 0.33       |                  |                  | 0.31    |                                 |                     |
Where $S_0$ is the influent TCOD concentration, $S_e$ is the effluent TCOD concentration, and $S_s$ is the TCOD concentration in the scum layer. All the values involved and the results are illustrated in Tables 3.2 and 3.3.

The theoretical methane yield at standard temperature and pressure (STP, 0°C and 1 atm) is 0.35 mL/mg COD digested which corresponds to 0.4 mL/mgCOD digested at the operational temperature of 37°C (Metcalf & Eddy, 2003). Thus, it can be used to indicate the COD balance in a closed anaerobic methanogenic system. Figure 3.6 shows the temporal methane yield of both reactors at STP. The average methane yields in R2, of 0.33 and 0.31 for phase I and II, respectively, although slightly low, are within the rational error given the typical 10%-15% accuracy of measuring COD and the 10% accuracy of measuring biogas. The slightly lower yield might have been caused by dissolved methane and opening reactor for scum removal. However, the average methane yield in R1 during phase II was only 0.28 mL/mgCOD digested, which indicated that the COD balance was 20% off. This result might suggest that the COD concentration in the scum layer of R1 is underestimated, which also further infers that the real COD removal and VSS destruction in R1 during phase II might have been lower than 60%. Considering the four methane yields at STP of 0.31 and 0.28 mL/mg COD for phases I and II, respectively in R1, as well as the 0.33 and 0.31 in R2, the overall average methane yield of 0.31 mL/mg COD which is about 12% less than theoretical. Thus, the uncertainty in the reported COD and VSS destruction data is 12% of average. However, given the typical COD mass balance closures of 80% to 90% in anaerobic reactors (Parawira et al.,
2006; Gopala Krishna et al., 2009), the uncertainty of 10% in the performance of AnFBRs in this research is indeed satisfactory.

As shown in Tables 3.2 and 3.3, biofilm attachment increased from 2.3 to 5.6 mg VSS/g particle during the start-up period and further developed to more than 20 mg VSS/g particle at steady-state of phase II. As depicted in Table 4.1, the steady-state SRT based on VSS were 3.3 days and 5 days for PS and TWAS AnFBR, respectively. Although it has been suggested that methanogenic reactors, can be operated stably at SRTs as low as 5 days (Lee et al., 2011), the performance of the two AnFBRs cannot be rationalized by the very low VSS-based SRTs. The details of the specific SRT analysis in this research will be presented in Section 4.3.

Figure 3.7 and 3.8 illustrate the percentage of the total volatile acids and the concentration of the individual VFAs (acetic acid, propionic acid, and butyric acid) in the effluent of R1 and R2, respectively, during phase II. As shown in Figure 3.7 and 3.8, during the operation of R1 and R2 on municipal wastewater biosolids, the fluctuation of VFAs distributions is fairly small. Throughout the operation during phase II, acetic acid and propionic acid accounted for 46% and 43% of the VFA (based on COD), respectively, while butyric acid contributed the remaining 11%. Cruddas et al. (2014), who studied the treatment of domestic wastewater in an anaerobic pond at an OLR of 0.18 kg COD/m³d, observed that 54% of the VFA in effluent was contributed by acetic acid. Similarly, Forster-Carneiro et al. (2008) found that acetic acid usually accounted for 50% of the total volatile acids in the effluent while butyric acid only contributed less than 20% of the
total acid in the effluent of the thermophilic lab-scale batch reactor treating municipal solid waste with the COD concentration ranging from 32 to 41 g/L. Thus, the VFA distribution observed in this research is consistent with selected literature studies. When compared with the sCOD values in Table 3.3 and 3.4, the VFAs (based on equivalent COD) accounted for approximately 80% of the sCOD in the effluent of both reactors. This observation, combined with the fairly constant distribution of VFAs, suggests that the AnFBR achieved stable concentrations of VFAs, and accordingly the technology is not prone to upsets arising from the accumulation of volatile acids.

When operating an anaerobic reactor, VFA (as acetate)--to-alkalinity (as mg CaCO₃) ratio is a widely accepted measurement of anaerobic digestion stability (Chen et al., 2007). Figure 3.9 shows the temporal variation of the VFA (as acetates)-to-alkalinity ratio (α). Generally, VFA-to-alkalinity ratios of less than 0.4 reflect process stability. The α values were initially high in both reactors during phase II due to the increased OLR, but rapidly dropped and were maintained consistently below 0.4 through the steady-state operation.
Figure 3.6 Temporal methane yields in both reactors treating municipal sludges at S.T.P.
Figure 3.7 Temporal VFA distributions and concentration in R1 during phase II
Figure 3.8 Temporal VFA distributions and concentration in R2 during phase II
Figure 3. 9 Temporal VFA/ALK ratio of both reactors treating municipal wastewater sludges in phase II
3.4 Summary and Conclusions

The AnFBR showed an excellent adaptability to shock organic loadings due to the growth of most of the active bacteria on the media and retention of biomass inside the reactor. However, the AnFBR was very sensitive to pH fluctuations.

The performance results showed that mesophilic anaerobic digestion of municipal wastewater biosolids using a fluidized-bed reactor, with HDPE (600 µm~850 µm) as support material, is indeed promising with the reactor much more compact and efficient in removing COD and destroying VSS compared with conventional anaerobic digester. The AnFBR successfully treated the primary sludge at OLR of 18 kg/m$^3$-d and HRT of 2.2 days, achieving COD removal efficiency of 61% and VSS destruction efficiency of 63 % with an uncertainty of 12%. Furthermore, the AnFBR also successfully treated TWAS at OLR of 12 kg/m$^3$-d, achieving COD removal efficiency of 55% and VSS destruction efficiency of 51 % with an uncertainty of about 10%. Thus, the AnFBR can be deemed effective for digester capacity expansion.

3.5 References


Chapter 4*

Activity Tests and Microbial Characterization in the Attached and Suspended Phases

4.1 Introduction and Literature Review

Anaerobic digestion can be divided into four sequential steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Metcalf & Eddy, 2003). Within these four steps, methanogenesis is the final stage where the acetate and H₂ are further bio-transformed to CH₄, which is also generally believed to be the rate-limiting step (Alvarez et al., 2000). The various bacterial groups in anaerobic biofilms feed off the products generated by the other cultures and hence it is anticipated that the acidogenesis grow on the outside of the biofilm while the methanogens grow on the inside of the biofilm. Therefore, the structure of anaerobic biofilms is distinctively different from aerobic mixed-culture biofilms of heterotrophs and nitrifies where the culture interaction and interdependency is not as strong.

In aerobic attached-growth systems, when substrate is not the rate-limiting factor, it is believed that the fast growing heterotrophs become the majority on the outer surface of the biofilm while the slow-growing bacteria are present in the inner biofilm layers. Egli et al. (2003) using fluorescent in situ hybridization (FISH) observed that the biofilm layer

* Parts of this chapter have been submitted for publication to Water Research (manuscript number of WR29700). A full edition of the paper has been attached in Appendix II.
from a rotating biological contactor biofilm treating high ammonium wastewater, comprised aerobic nitrifiers on the outer layer of the biofilm and anammox bacteria in the inner layer. Vlaeminck (2010), observed that the structure of the aerobic biofilm layer from inside to outside was in the following order: anammox bacteria ($u_{\text{max}}=0.1 \text{ d}^{-1}$), nitrite oxidizing bacteria (NOB), and ammonium oxidizing bacteria (AOB) ($u_{\text{max}}=0.14-1.44 \text{ d}^{-1}$) in autotrophic biomass in a granular sludge reactor treating synthetic wastewater at a NLR of 84 g $\text{NH}_4^+\text{-N/m}^3\text{d}$, This finding clearly suggests that in the autotrophic biofilm, the slowest growing bacteria grow deep in the biofilm and are thus sheltered from hydrodynamic forces. Fu et al. (2010) explored the biofilm structure in a simultaneous nitrification and denitrification moving bed bioreactor (MBBR) treating synthetic glucose solution at an OLR of 1.2 to 3.6 kg COD/m$^3$d, and observed that the heterotrophic bacteria were in the outer layer. Mozumder (2014) further studied the impact of substrate concentration on the bacterial distribution in a granular sludge reactor and found that in the absence of organics, the relatively few heterotrophic bacteria grew behind the autotrophic AOB and NOB bacteria. However, in the presence of organics, the fast growing heterotrophs became the majority on the outer surface of the biofilm. The aforementioned studies of aerobic biofilms clearly demonstrated that in multi-species biofilms, the slow-growing bacteria are present in the inner biofilm layers.

Studies of the structure of anaerobic biofilms are limited with most of the studies focusing on the spatial distribution of active organisms along the reactor rather than the distribution inside the biofilm. Bull et al. (1983) observed when investigating an anaerobic fluidized bed reactor with glucose solution at an HRT of 5 days and OLRs
ranging from 6 to 18 kg COD/m^3d that methanogens mainly grow attached to the carrier surface while acidifiers tend to appear in the suspended phase. Kuba et al. (1990), using zeolite as support media in an anaerobic fluidized bed treating VFAs based synthetic wastewater at an OLR of 4 kg COD/m^3d, claimed that not all of the attached biomass were active methanogens.

SMA test is one of the preferred methods to investigate the methanogenic activity profiles of suspended and attached biomass in anaerobic reactors. Researches have shown that SMA is feasible for evaluating the performance of most anaerobic reactor types, such as AnFBR (Araki et al., 1994; Andalib et al., 2014), AnMBR (Ince et al., 1995), UASB (Sumino et al., 2007), AnSBR (Banik et al., 1997).

While anaerobic microbial activity in biofilm reactors has been assessed using the SMA test, (Kuba et al., 1990; Hidalgo et al., 2002; Andalib et al., 2014), the activity of other anaerobic microbial groups have been scantily used in the literature, presumably due to the common perception that methanogenesis is often the rate limiting anaerobic process. Furthermore, studies of the structure of anaerobic biofilms are also limited with most of the studies focusing on the spatial distribution of active organisms along the reactor rather than the distribution inside the biofilm.

In anaerobic biofilm processes, as a result of decoupling the HRT from the SRT and due to the difference in attachment characteristics between biomass and inerts (i.e. nonbiodegradable suspended solids), the performance of the AnFBR cannot be
rationalized based on the widely accepted definition and model of SRT based on VSS. Furthermore, no research has ever clearly distinguished the distribution of the three main active bacterial groups (methanogenic, acetogenic and acidogenic) in a single-stage anaerobic fluidized bed bioreactor treating high solid municipal wastewater sludge. In light of the scarcity of information in the dispersed literature on the structure of anaerobic biofilms, and limited tools for quantification of various microbial groups, the main objectives of this study were to develop a methodology to estimate active biomass SRT, and rationalize the excellent performance of the AnFBR by evaluating attachment/detachment characteristics. In order to investigate the mechanism of the biofilm reactor and obtain the active biomass retention time, a series of batch tests of specific methanogenic activity (SMA), specific acidogenic activity (SAdA), and specific acetogenic activity (SAtA) on both attached biofilm and detached biomass were conducted in this research.

4.2 Materials and Methods

In order to determine the difference of the activities between the suspended phase and biofilm phase, batch tests were carried out to test the specific methanogenic activity (SMA), specific acidogenic activity (SAdA), and specific acetogenic activity (SAtA) of the sonicated supernatant (as attached biomass), and effluent of the reactor (as suspended). Although SMA and biochemical methane potential (BMP) tests have been widely applied to indicate the performance of anaerobic reactors since methane production process is generally believed as the rate-limiting process in anaerobic reactor
(Alvarez, et al., 2000), less attention was paid to the acidification stage comprising both acidogenic and acetogenic processes. Furthermore, the literature did not show any methods which have been applied to separately measure specific acetogenic and specific acidogenic activities. This research established a new method to separate these two tests from the whole biochemical hydrogen potential test by controlling different pH, substrate, and inhibitors.

4.2.1 Batch Tests

After both reactors achieved steady-state in Phase II, four rounds of SMA, SAdA, and SAtA batch tests were conducted in duplicates to investigate the differences in biomass activities between the attached and suspended phases for both reactors. Sonicated supernatant of the bio-particles and the effluent of the anaerobic fluidized reactor were used separately as seed in these tests. The initial substrate-to-biomass (S/X) ratio was set at a constant level of 2.0 g COD/g VSS (Soto et al., 1993; Yoon et al., 2014). The same nutrient solution added during the AnFBR start-up period was also added in the batch test bottles (total liquid volume of 100 mL and headspace volume of 50 mL).

In the SMA tests, acetic acid was used as a substrate to test methane production. A high initial concentration of 5 g/L NaHCO₃ in the bottle was required to maintain stable pH level throughout the entire test, while 200 g/L NaOH was used to control the initial pH at 7.2 in the sample bottle (Andalib et al., 2014).
For the specific acetogenic tests, equal COD of ethanol, propionic acid, butyrate acid, and lactic acid were applied as substrates because propionic and butyric acids are the main degradation products of glucose (Kalyuzhnyi, 1997; Sun et al., 2000), while ethanol and lactic acid are carbohydrates fermentation products (Paramithiotis and Sofou, 2007). Furthermore, a high initial concentration of 5 g/L NaHCO₃ in the bottle was required to maintain stable pH level through the entire batch tests. Horiuchi et al. (2002) investigated the impact of pH control on organic acid production in a glucose fed mesophilic anaerobic reactor and found that there was a butyric acid accumulated at a pH ranging from 5 to 7, while propionate acid tended to accumulate at a pH of 8. In order to get rid of the propionate and butyric acid accumulation in the acetogenic tests, the initial pH was adjusted at 8 in the sample bottle. As the process of fermentation progresses, the pH in the bottle is expected to decrease slightly, and thus, would be suitable for propionate fermentation without butyrate accumulation.

Glucose was employed as the substrate in the specific acidogenic test, while extra acetate acid was also added to provide an acetic acid concentration of 5 g/L in order to inhibit further degradation of propionate to acetic acid (Heijnen, 1988) because that is considered as part of acetogenic process. As Li et al. (2013) reported in their research of VFA distribution during acidogenesis of algal residues, a pH of 6 is optimal for the accumulation of butyric acid in acidogenesis. Therefore, the initial pH for the acidogenesis test was controlled at 6 by adding 182 g/L HCl in order to eradicate further acetogenesis at high acetic acid concentration. An initial concentration of 5 g/L NaHCO₃ was also maintained in the bottle.
The seeds for acidogenic activity tests and acetogenic activity tests were preheated at 90°C for 30 minutes to inhibit the methanogenic bacteria. The initial VSS in the bottles were controlled at approximately 1,500 mg/L and the substrates were dosed at an initial concentration of 3,000 mg/L COD. Two bottles were used as blank which contained the same amount of VSS without any substrate as food. After flushing with nitrogen gas at 5-10 psi for 5 minutes, the sample bottles were then placed in a swirling-action shaker (MaxQ 4000, Incubated and Refrigerated Shaker, Thermo Scientific, CA) and operated at 180 rpm and 37°C.

4.2.2 Analytical Methods

The volume of the gas produced was measured by releasing the bottles headspace pressure using proper glass syringes (Perfektum; Popper & Sons Inc, NY, USA) until gas production ceased (Andalib et al., 2014). The volume of CH₄ and H₂ gas were determined by injecting 0.6 mL of the biogas composition into a gas chromatograph (Model 310, SRI Instruments, Torrance, CA) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Molesieve 5A, mesh 80/100, 182.88 × 0.3175 cm). The initial and final sCOD and were measured using HACH methods and investigating kits (HACH Odyssey DR/2800) for checking the COD balance within the batch tests. Initial and final pHs were measured by using a portable pH meter (OAKTON, pH 11 series). Four rounds of the aforementioned tests were run in duplicate.
4.3 Results and Discussion

4.3.1 Specific Microbial Activity Tests

According to the Monod equation, if substrate concentration is considerable higher than its half saturation constant (K_s), the specific bacteria growth rate obtained can be considered as the maximum rate of bacteria growth. Since the K_s values of the VFAs and glucose are less than 0.2 g/L (Henze and Harremoes, 1983), the initial substrate concentrations in the batch tests were more than 15 times higher than the K_s, and consequently, the specific bacteria growth rate (lag phase and plateau phase are exclusive) obtained in these batch tests represent the maximum growth rate phase.

The specific SRT of each bacterial group can be determined by the biomass specific growth rate in liquid phase and biofilm phase according to the following equation:

\[
SRT = \frac{\text{Rate } 1 \left( \frac{L}{mgd} \right) \times \text{Attachment} \left( \frac{mg}{g} \right) \times W_p (g) + \text{Rate } 2 \left( \frac{L}{mgd} \right) \times VSS_{effluent} \left( \frac{mg}{L} \right) \times V_{reactor} (L)}{\text{Rate } 2 \left( \frac{L}{mgd} \right) \times VSS_{effluent} \left( \frac{mg}{L} \right) \times Q_{feeding} (L/d)}
\]  

(4.1)

Where rates 1 and 2 are reflected by the specific biogas production rate of the attached and suspended biomass, respectively. Attachment is determined by the measurement of biomass attachment of the bioparticle. W_p is the initial weight of clean particles added into the reactor. The VSS in the scum layer was also considered in the VSS_{effluent} here after dividing by the working volume of reactor (16 L).

Figure 4.1 to 4.3 illustrate the results of the 4 rounds various microbial activity tests at 35°C. The specific biogas accumulation rates (biogas generated in sample bottle minus
the biogas generated in blank bottle) in these figures reflect the substrate utilization rate, which corresponds to the specific activity of the targeted microbial group. The specific microbial activities were evaluated by dividing the volume of biogas produced per unit time by the initial weight of VSS in the test bottles. The distribution of active bacteria in the liquid and biofilm can be determined from the product of the specific biogas rates and the total biomass as VSS in the liquid and attached phases.

Given an initial anaerobic environment, a longer lag phase was still observed in SMA batch test bottles (nearly 2 days) while the other two H\textsubscript{2} production batch tests only had less than 10 hours. This observation showed that methanogenic process usually requires longer SRT. Therefore, it is the rate limiting step in the anaerobic fluidized bed.

As shown in Figure 4.1, SMAs were 446 mL\textsubscript{CH\textsubscript{4}}/gVSS d and 350 mL\textsubscript{CH\textsubscript{4}}/gVSS d for the attached biomass treating PS and TWAS, respectively, and 51 mL\textsubscript{CH\textsubscript{4}}/gVSS d and 105 mL\textsubscript{CH\textsubscript{4}}/gVSS d for the suspended phase throughout the SMA tests with standard deviations of ±10% calculated based on the 8 samples comprising duplicates for each of the four rounds. Similarly, higher SAdAs and SATAs were also observed in the attached biomass for both reactors treating PS and TWAS as depicted in Figure 4.2 and 4.3.

As reflected by the slopes in figure 4.2, in the SATAs tests, the substrate utilization rates in the attached phase of both reactors are relatively close. The same aforementioned observation is evident from Figure 4.3. However, the methane generation rate in the attached phase of R1 was almost 30% higher than of R2 as depicted in Figure 4.1. This
suggests that the difference in reactor OLR had a larger impact on methanogens than acidifying biomass.

Figure 4.1 Results of 4 rounds SMA tests for PS and TWAS reactors (average±SD)
Figure 4. 2 Results of 4 rounds SAdA tests for PS and TWAS reactors (average±SD)
Figure 4. 3 Results of 4 rounds SAtA tests for PS and TWAS reactors (average±SD)
Although the differences of microbial activities in attached phase were considerably small between two reactors, the SMAs, SAdAs, and SAtAs in the suspended phase of R2 were almost double the activities observed for R1. This is to be expected given the much higher anaerobic biodegradability of primary sludge relative to TWAS.

The COD balance (Figure 4.4) was calculated as 90%-100% for all test bottles based on initial sCOD, final sCOD, and biogas production as shown below:

\[
\text{COD balance} = \frac{\text{Initial } \text{sCOD}}{\text{Final } \text{sCOD} + \text{V}_{\text{CH}_4,S.T.P}/0.35} \times 100\% \quad \text{(Methanogenic)} \tag{4.2}
\]

\[
\text{COD balance} = \frac{\text{Initial } \text{sCOD}}{\text{Final } \text{sCOD} + \text{V}_{\text{H}_2,S.T.P}/1.41} \times 100\% \quad \text{(Acetogenic and Acidogenic)} \tag{4.3}
\]

As shown in Figure 4.4 (a), almost 90% of the dosed sCOD was finally removed in the SMA test bottles of attached biomass, while more than 80% of the initial sCOD still remained in SAdA and SAtA test bottles. Since it has not been reported that the products and substrates used in each activity test would inhibit the further digestion (except high acetic acid concentration will inhibit the digestion of propionic acid), the methanogenic process was the most effective process of removing COD, while the COD removal during the acidogenic process was negligible compared with the methanogenic process.
Figure 4.4 COD balance of 4 rounds SMA tests of both reactors
Figure 4.4 (b) COD balance of 4 rounds SAtA tests of both reactors
Figure 4.4 (c) COD balance of 4 rounds SAdA tests of both reactors

Figure 4.5 compares the final pH with the initial pH in all batch tests, and the results show that the final pHs were almost stable for all batch tests, which indicates that the sodium bicarbonate buffer dosed was sufficient to maintain a stable pH through the test.
duration. The decrease of pH from 8 to 7.5 in SAtA tests was expected and, actually, preferred to enhance the sequential digestion of butyric and propionic acids to acetic acid.

![Figure 4.5 Initial and final pH during all activity batch tests](Attached)

![Figure 4.5 Initial and final pH during all activity batch tests](Suspended)
4.3.2 Microbial Attachment and Detachment

Figure 4.6 shows the distribution of three aforementioned active microbial groups in the attached and suspended phases in both reactors. Based on the VSS measurement shown in Table 3.3 and 3.4, the total amount of attached biomass in the reactor treating PS was roughly 75 g, while the VSS in the suspended phase was 81 g. However, if the specific bacterial activity was taken into consideration, more than 84% of the active bacteria were attached. Similarly, the attached biomass in the TWAS reactor was 60 g, with a suspended VSS of 117 g with the attached biomass accounting for over 60% of the total active bacteria.

The VSS in the scum layer for the two reactors were approximately 17% (PS) and 25% (TWAS) of the influent VSS in phase II. SMA tests were conducted on samples of the scums and the results showed approximately half the specific microbial activities of the suspended phase in each reactor, suggesting that regular removal of the scum layers would not adversely impact performance. It is apparent that based on microbial activity, the attached biofilm contributed about 90% in reactor 1 (treating primary sludge) and about 60% in reactor 2 (treating TWAS), with the balance contributed by suspended microorganisms. This is attributed to the different microbial attachment/detachment characteristics, as elaborated upon later.

Table 4.1 illustrates the results of the specific bacterial characteristics. The specific activity in the attached phase was 6 to 8 times higher than in the suspended phase in the reactor treating PS for the different bacterial communities. Therefore, the attached phase
was much more active in digesting wastes than the suspended phase even the total mass of VSS were similar for these two parts. The amount of attached VSS in the TWAS reactor was much lower than the VSS in suspended phase. However, the treatment capacity in the attached phase was still higher that the suspended phase due to 3 times higher activity in the attached phase as shown in Table 4.1.

Figure 4.6 Distribution of active microbial groups in two reactors
<table>
<thead>
<tr>
<th></th>
<th>Specific Activity in Suspended Phase (mL CH4 or H2/gVSS.d) (35°C)</th>
<th>Specific Activity in Attached Phase (mL CH4 or H2/gVSS.d)(35°C)</th>
<th>SRT (d)</th>
<th>First Order Detachment Rate Coefficient (d⁻¹)</th>
<th>Expected VSS Reduction Based on Liptak</th>
<th>Actual VSS Reduction in Reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PS</strong> VSS</td>
<td>-</td>
<td>-</td>
<td>3.3</td>
<td>0.69</td>
<td>35%</td>
<td>63%</td>
</tr>
<tr>
<td>Methanogenic Microbe</td>
<td>51±9 (8)</td>
<td>446±47 (8)</td>
<td>14.6±0.62 (8)</td>
<td>0.08</td>
<td>56%</td>
<td></td>
</tr>
<tr>
<td>Acidogenic Microbe</td>
<td>32±10 (8)</td>
<td>191±8 (8)</td>
<td>12.2±0.57 (8)</td>
<td>0.11</td>
<td>54%</td>
<td></td>
</tr>
<tr>
<td>Acetogenic Microbe</td>
<td>61±9 (8)</td>
<td>480±41 (8)</td>
<td>13.2±1.08 (8)</td>
<td>0.09</td>
<td>53%</td>
<td></td>
</tr>
<tr>
<td><strong>TWAS</strong> VSS</td>
<td>-</td>
<td>-</td>
<td>5.2</td>
<td>0.62</td>
<td>41%</td>
<td>51%</td>
</tr>
<tr>
<td>Methanogenic Microbe</td>
<td>105±3 (8)</td>
<td>350±24 (8)</td>
<td>9.4±0.10 (8)</td>
<td>0.19</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>Acidogenic Microbe</td>
<td>63±6(8)</td>
<td>194±12 (8)</td>
<td>8.0±0.43 (8)</td>
<td>0.22</td>
<td>47%</td>
<td></td>
</tr>
<tr>
<td>Acetogenic Microbe</td>
<td>157±11 (8)</td>
<td>480±30 (8)</td>
<td>8.8±0.45 (8)</td>
<td>0.19</td>
<td>49%</td>
<td></td>
</tr>
</tbody>
</table>
Andalib et al. (2012) achieved a biomass attachment of 37.5 mg/g with zeolite as a media, ranging in size from 425 um to 610 um, and a maximum biofilm thickness of 150 um corresponding to a biofilm VSS concentration of 28.6 kg/m3, which translates to estimated biofilm thicknesses in this study of 100 um and 120 um for the reactors treating TWAS and PS, respectively. The average surface area (SSA) based on a bed voidage of 70% and average bio-particle diameter of 945 um was calculated as roughly 1900 m2/m3 according to the following equation (Eldyasti et al., 2012):

\[
SSA = \frac{6}{d_p} (1 - \varepsilon)
\]  
(4.4)

Then, the shear stress was calculated by the following formula (Rittmann, 1982):

\[
\sigma \text{ (dyn/cm}^2) = \frac{100\mu v(1-\varepsilon)^3}{d_p^2(\varepsilon)^3 a(7.46 \times 10^9)}
\]  
(4.5)

Where \( \varepsilon \) is the total bed voidage (70%), \( \mu \) is liquid viscosity (864 g/cm day), \( v \) is the liquid upflow velocities (0.8 cm/s), \( d_p \) is the bioparticle diameter including biofilm (0.0945cm and 0.0925cm for PS and TWAS, respectively), and \( a \) is the specific surface area of biofilm carriers (19 cm2/cm3). The shear stress calculated for two reactors fed with PS and TWAS were 0.36 dyn/cm² and 0.39 dyn/cm², respectively, as compared with 0.2 dyn/cm² for the AnFBR with zeolite (Andalib et al., 2012).

In biofilm reactors, first order detachment rate coefficient (bs) is generally used to describe detachment mechanisms. In fluidized bed bioreactor, bs can be calculated as follow (Patel et al., 2005):

\[
b_s = \frac{\text{Biomass specific maximum rate} \times Q_{\text{effluent}} \times VSS_{\text{effluent}}}{\text{Biomass specific maximum rate} \times M_{\text{media}} \times VSS_{\text{attachment}}} 
\]  
(4.6)
The bs values for different bacterial groups of this research are shown in Table 4.1. It can be observed that the bs of VSS is much larger than other bacterial groups for all cases, which indicates that the active bacteria tend to grow on the media surface while inert bacteria commonly exist in the suspended phase in the AnFBR. The bs/σ ratios for the VSS observed in this study of 1.9 and 1.6 for PS and TWAS, respectively, are comparable to the 1.5 observed in the zeolite AnFBR (Andalib et al., 2012) during the treatment of thin stillage. These results clearly suggest that a high shear force would cause a negative impact on biofilm formation, and system performance, with the detachment.

Nakhla et al. (2002) and Turan (2000) also observed that the increase of shear stress will lead to an increase of detachment rate in anaerobic fluidized bed. Although Rittmann justified that the detachment rate coefficient (bs) is proportional to $\sigma^{0.58}$ according to his model, this empirical model was only based on smooth aerobic biofilms on unfluidized glass beads (Chang et al., 1991). Furthermore, Speitel and DiGiano (1987) observed during their study on paranitrophenol (PNP) in a granular activated carbon (GAC) reactor that the bs predicted by the Rittmann model underestimated the actual detachment rate, implying that the value of the exponent in Rittmann’s model is greater than 0.58. A lower methanogenic microbial detachment coefficient was observed in Andalib’s research using zeolite as carrier media might implies that further research regarding the modification of the plastic support material is required.
4.3.3 Active Microbial Characterization

Liptak empirical equation (Liptak, 1974) is commonly applied to estimate the VSS destruction based on SRT for high-rate digestion system as showed in equation (4.7)

\[ V_d = 13.7 \times \ln(\text{SRT}) + 18.9 \]  

(4.7)

Results of the activity tests showed that the SRT of these three specific bacterial groups were all significantly higher than the SRT calculated directly based on the VSS. As illustrated in Table 4.1, the \( V_d \) calculated based on the specific SRT of methanogenic microbes were much more reliable compared with the general VSS-based SRT.

Literature confirms that biofilms are layered (Heijnen et al., 1988) and mathematical models, such as three dimensions simulation (Noguera, et al., 1999), layered stationary granular model (Tartakovsky, et al., 1996), and hybrid anaerobic reactor model (Saravanan, et al., 2008), were also derived based on this theory. A two tails T-test was conducted comparing each two of the different microbial activity-based SRTs generated for each reactor. Results show that the null hypothesis was rejected at both 90% and 95% confidence level for all of the comparisons, which indicate that the difference of SRTs of each bacterial group were significant. However, the methanogenic bacteria showed the longest SRT among the three active bacterial groups in both reactors. Since the outer surface of the bio-particles are prone to shear forces, the difference of the three SRTs suggests that the methanogenic microbial community, with a slower growth rate, was growing in the inner layer of the biofilm while acidogenic bacteria were growing at the outer layer of the biofilm with the acetogenic microorganism growing in the middle of the biofilm. This phenomenon can also be explained by the substrate gradient. This is
plausible since each microbial group utilizes the degradation products of the one above it in the anaerobic food chain.

Among all these specific activity tests, the lag phase of methanogenic bacteria is the longest. The production of CH₄ usually started on the third day of batch tests while the lag phase of H₂ production is generally a couple of hours. This indicates that the methane production process is the limiting process of AnFBR digesting municipal waste sludges in this research.

### 4.4 Summary and Conclusions

According to the specific methanogenic activity, specific acetogenic activity, and specific acidogenic activity tests conducted in phase II, the activity-based sludge retention times varied from 12.2 to 14.6 days in R1 and 8.0 to 9.4 days in R2, respectively. In R1, more than 84% of the active bacteria were in the biofilm with the remaining 16% in suspended phase, while approximately 60% of active bacteria were in the attached phase in R2.

This research developed a new method of separately measuring the specific acetogenic activity and specific acidogenic activity. Although they are not the rate limiting process in the anaerobic fluidized bed, these measurements are still helpful in determining the biofilm structure in AnFBR. These three microbial activities tests can not only help operators understand and analyse the performance of full-scale digesters better, but also determine the rate-limiting process in the system and then optimize the operational conditions accordingly. For example, if a low SMA is observed in an anaerobic digester,
the operator might need to either reduce the loading rate or increase the sludge retention times.

The SMA and other microbial activities tests showed that the SRT calculation based on general VSS is not accurate enough in predicting and rationalizing the VSS reduction in the anaerobic biofilm systems. The microbial activity tests also indicated that the biofilm was layered with acidogens on the outside, followed by acetogens, and methanogens on the inside.

4.5 References


Chapter 5

Conclusions and Recommendations

5.1 Conclusion

The results of this study demonstrate that mesophilic anaerobic digestion using a fluidized-bed reactor, with HDPE (600 µm~850 µm) as support material, is highly effective for COD removal and VSS destruction of primary and secondary sludge. In phase I, the AnFBR fed with PS achieved a VSS destruction efficiency of 69% and COD removal efficiency of 68% at an OLR of 9 kg COD/m³-d while the other one fed with TWAS showed approximately 57% treatment efficiency at an OLR of 6 kg COD/m³-d. Finally, the AnFBR successfully treated the primary sludge at OLR of 18 kg/m³-d, achieving COD removal efficiency of 61% and VSS destruction efficiency of 63%, and the AnFBR also successfully treated TWAS at OLR of 12 kg/m³-d, achieving COD removal efficiency of 55% and VSS destruction efficiency of 51%. The COD mass on average closed at 88%, implying that the uncertainty in the performance data is only 12%.

The final attached biomass concentration was 25 mg VSS/g HDPE particle, about 50% lower than the approximately 50 mg/g zeolite achieved in zeolite AnFBR by Andalib et al. (2012). Although the attachment capacity of the HDPE is inferior to zeolite, the shear stress in this work at an upflow velocity of 0.8 cm/s is 2 times higher than for zeolite at the upflow velocity of 0.35 cm/s.
The AnFBR showed an excellent adaptability to shock organic loadings due to the growth of most of the active bacteria on the media and retention of biomass inside the reactor. However, the AnFBR was very sensitive to the pH fluctuation, with COD and VSS removal efficiencies dropping from 80% to 20% during the start-up investigating at an OLR of 9 kg COD/m^3d at a pH of 5.

This research developed a new method of separately measuring the SAdA and SAa. Although they are not the rate limiting process in the anaerobic fluidized bed, these measurements are still helpful in determining the biofilm structure in AnFBR.

The SMA and other microbial activities tests showed that the SRT calculation based on general VSS is not accurate in predicting and rationalizing the VSS reduction in the anaerobic biofilm systems. The microbial activity tests also indicated that the biofilm was layered with acidogens on the outside, followed by acetogens, and methanogens on the inside.

### 5.2 Limitations of the Current Work

This research was focused on the biological principle of anaerobic fluidized bed bioreactor instead of the hydrodynamic model in AnFBR. The relationship between bioparticle size, liquid upflow velocity, bed height, and other hydrodynamic elements are not further studied. The SMA, SAa, and SAdA tests were focused on investigating the activity differences between attached biomass and suspended biomass, without optimization of the other test conditions ie. pH, substrate and inhibitor concentrations.
The COD mass balance based on methane yield and the influent and effluent liquid COD during the operation was approximately 88%, which means the actual treatment efficiency might be 10% less. The scum layer appeared on the top of liquid-solid separator also needs more accurate measurement. The reactors need to add more samples ports to investigate the difference of biomass attachment and activities along with the bed height.

### 5.3 Recommendations and Future Work

Since the scum layer was forming at the top of the liquid-solid separator, I would recommend studying the impact of sonication of the scum layer in the future work, which in principle would help to improve the biogas production and stabilize the performance of reactors. Secondly, designing and developing an inverse anaerobic fluidized bed bioreactor would also help to eliminate the problem of scum layer.

Besides that, testing microbial activity distribution along with the bed height would also be necessary for full understanding of the operation of AnFBRs which would facilitate optimization. Furthermore, it is also very interesting and valuable to study and optimize the performance of combining a bio-hydrogen reactor with a methanogenic AnFBR. Last but not least, the co-digestion of PS and TWAS or other organic waste would also be a popular research field in the coming years.
Appendix I

Standard Curves of Lab Equipments

1. Standard Curves of gas chromatograph (Acetic acid, propionic acid, and butyric acid)

   ![Acetic acid plot]
   \[y = 0.00228506x\]
   \[R^2 = 0.78116130\]

   ![Butyric acid plot]
   \[y = 0.00056311x\]
   \[R^2 = 0.99989768\]

   ![Propionic acid plot]
   \[y = 0.00127766x\]
   \[R^2 = 0.70663360\]

2. Standard Curve of HACH Odyssey DR/2800 (COD)
$y = 1.02652941x$

$R^2 = 0.99995591$
Appendix II

Manuscript submitted to Water Research

Microbial Speciation of the Anaerobic Biofilm in a Fluidized Bed Bioreactor

Zhenqi Wang, George Nakhla, Jesse Zhu

ABSTRACT: This paper investigated the efficacy of the anaerobic fluidized bed bioreactor (AnFBR) technology in treating municipal wastewater sludges with a focus on microbial characterization of the biofilm. Primary sludge (PS) and thickened waste activated sludge (TWAS) were studied in two lab-scale AnFBRs using plastic particles as carrier media. PS was tested at various organic loading rates (OLRs) range from 9 to 18 kg COD/m^3·d corresponding to hydraulic retention times (HRTs) from 2 to 4 days, with maximum COD and VSS removal efficiency of 70% and 72%, respectively, while for TWAS, VSS destruction efficiency varied from 53% at an HRT of 4 days and OLR of 12 kg COD/m^3·d to 61% at an HRT of 8 days and an OLR of 6 kg COD/m^3·d. Furthermore, the specific bacterial community activity tests showed a significant difference between solids retention time (SRT) based on general VSS and retention times based on the activity of methanogenic, acidogenic, and acetogenic microbes. While SRTs based on VSS measurements in the PS AnFBR were 3.3 days, the activity-based retention times varied from 12.2 to 14.6 days. Similarly, in the TWAS AnFBR, the SRTs based on VSS measurements were 5.0 days, and the activity-based retention times ranged from 8.0 to 9.4 days.

KEYWORDS: anaerobic biofilm; fluidized bed bioreactor; attachment and detachment; sludge retention time

Abbreviation

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCOD</td>
<td>Total chemical oxygen demand</td>
</tr>
<tr>
<td>sCOD</td>
<td>Soluble chemical oxygen demand</td>
</tr>
<tr>
<td>TSS</td>
<td>Total suspended solid</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>VSS</td>
<td>Volatile suspended solid</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile fatty acids</td>
</tr>
<tr>
<td>HDPE</td>
<td>High-density polyethylene</td>
</tr>
<tr>
<td>PS</td>
<td>Primary sludge</td>
</tr>
<tr>
<td>TWAS</td>
<td>Thickened waste activated sludge</td>
</tr>
<tr>
<td>SRT</td>
<td>Sludge retention time</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
</tr>
<tr>
<td>AnFBR</td>
<td>Anaerobic fluidized bed reactor</td>
</tr>
<tr>
<td>SMA</td>
<td>Specific methanogenic activity</td>
</tr>
<tr>
<td>SAdA</td>
<td>Specific acidogenic activity</td>
</tr>
<tr>
<td>SATaA</td>
<td>Specific acetogenic activity</td>
</tr>
</tbody>
</table>
Highlights:

- Exploring the distribution and activity of three active microbial groups in anaerobic biofilms
- Testing a new methodology to separately measure acetogenic and acidogenic activities
- Comparing the relationship between detachment rate coefficients and shear stress in anaerobic fluidized bed reactors
1. Introduction

Anaerobic digestion is a preferred treatment process for organic wastes due to its low nutrient requirements, low biomass yield, and additional biogas (hydrogen, methane) production (Chan, 2009). Anaerobic digestion has been tested successfully on both municipal wastes, and industrial effluents ie. olive oil mill, protein waste (Rintala, et al., 1996; Filidei, et al., 2002; Borja, et al., 2001). Anaerobic digestion of PS and TWAS is often limited by slow biodegradation rates ensuing from slow biomass hydrolysis, and resulting in low solids destruction efficiencies of less than 50% despite long retention times (Metcalf & Eddy, 2003).

Fluidized bed reactors have been used in various biotechnological applications utilizing low suspended solids streams e.g. treating food-processing, digesting paper industry wastewater, and purifying fermentation wastewater. (Heijnen, et al., 1988; Holst, et al., 1997). The mesophilic anaerobic fluidized bed reactor (AnFBR) with zeolite as carrier media (425–610 µm) developed by Nakhla and coworkers, (Andalib et al., 2012), achieved up to 88% TCOD and 78% TSS removal at an OLR of 29 kg COD/m³d during the treatment of thin stillage with a TCOD of 130 g/L and TSS of 47 g/L (Andalib et al., 2012). The AnFBR has been recently demonstrated for the digestion of primary sludges (Andalib et al., 2014) with a TSS destruction efficiency of 82% at an OLR of 9.5 kg COD/m³d.

In biofilm reactors, the development of the biofilm is determined by the difference between biofilm growth and detachment processes. Biofilm growth mainly relies on the carrier characteristics such as particle size, sphericity, porosity, density, and specific surface area (SSA) (Nicolella, 2000). The detachment of biofilm is usually contributed by abrasion (surface biofilm loss caused by particle collision), erosion (surface biofilm loss caused by shear stress), sloughing (the periodic loss of large biofilm patch) and predator grazing (outer surface biofilm consumed by protozoa) (Nicolella, 2000).

Egli et al. (2003) using fluorescent in situ hybridization (FISH) observed that the biofilm layer from a rotating biological contactor biofilm treating high ammonium wastewater, comprised aerobic nitrifiers
on the outer layer of the biofilm and anammox bacteria in the inner layer. Vlaeminck (2010), observed that in autotrophic biomass in a granular sludge reactor treating synthetic wastewater at a nitrogen loading rate (NLR) of 84 g NH₄⁺-N/m³d, the structure of the biofilm layer from inside to outside was in the following order: anammox bacteria (u_{max}=0.1 \text{ d}^{-1}), nitrite oxidizing bacteria (NOB), and ammonium oxidizing bacteria (AOB) (u_{max}=0.14-1.44 \text{ d}^{-1}). This finding clearly suggests that in the autotrophic biofilm, the slowest growing bacteria grow deep in the biofilm and are thus sheltered from hydrodynamic forces. Fu et al. (2010) explored the biofilm structure in a simultaneous nitrification and denitrification moving bed bioreactor (MBBR) treating synthetic glucose solution at an OLR of 1.2 to 3.6 kg COD/m³d, and observed that the heterotrophic bacteria were in the outer layer.

Mozumder (2014) further studied the impact of substrate concentration on the bacterial distribution in a granular sludge reactor and found that in the absence of organics, the relatively few heterotrophic bacteria grew behind the autotrophic AOB and NOB bacteria. However, in the presence of organics, the fast growing heterotrophs became the majority on the outer surface of the biofilm. The aforementioned studies of aerobic biofilms clearly demonstrated that in multi-species biofilms, the slow-growing bacteria are present in the inner biofilm layers.

The structure of anaerobic biofilms is distinctly different from aerobic mixed-culture biofilms of heterotrophs and nitrifies where the culture interaction and interdependency is not as strong. The various bacterial groups in anaerobic biofilms feed off the products generated by the other cultures and hence it is anticipated that the acidogenesis grow on the outside of the biofilm while the methanogens grow on the inside of the biofilm. Studies of the structure of anaerobic biofilms are limited with most of the studies focusing on the spatial distribution of active organisms along the reactor rather than the distribution inside the biofilm. Bull et al. (1983) observed that methanogens mainly grew attached to the carrier surface while acidifiers tend to appear in the suspended phase when testing an anaerobic fluidized bed reactor with glucose solution at an HRT of 5 days and OLRs ranging from 6 to 18 kgCOD/m³d. Kuba et al. (1990), using zeolite as support media in an anaerobic fluidized bed treating VFAs based synthetic wastewater at an OLR of 4 kgCOD/m³d, claimed that not all of the attached biomass were active.
methanogens. Hidalgo et al. (2002) carried out SMA tests only on the attached biomass in a methanogenic fluidized bed reactor fed with acetic acid and found higher specific methanogenic activity at the top of the fluidized bed than at the bottom. Andalib et al. (2014) observed a much lower detachment rate for methanogens than other biomass, resulting in a methanogenic SRT to overall biomass SRT ratio of 4:1 in an AnFBR reactor treating municipal biosolids. Kuo et al. (2011), using biochemical hydrogen potential (BHP) on attached and suspended biomass from AnFBR treating kitchen wastes mesophilically at an HRT of 7.3 days and OLR of 1.1 kgCOD/m³d, determined that the concentration of hydrogen-producing bacteria in suspension is 2.5 times on the carrier media, implying that the acidogenic bacteria grew primarily in suspension. In contrast, Cresson et al. (2009) applied FISH on the colonized particles obtained from a methanogenic inverse turbulent bed reactor fed with diluted red wine at an OLR of 10.7 kgCOD/m³d, and proved that a relatively homogeneous layered biofilm was generated.

While anaerobic microbial activity in biofilm reactor has been assessed using specific methanogenic activity (SMA) test, (Kuba et al., 1990; Hidalgo et al., 2002; Andalib et al, 2014), the activity of other anaerobic microbial groups have been scantily evaluated in the literature, presumably due to the common perception that methanogenesis is often the rate limiting anaerobic process. However, this postulation is not valid for solids digestion which is hydrolysis-limited (Alvarez, et al., 2000).

In light of the scarcity of information in the dispersed literature on the structure of anaerobic biofilms, and limited tools for quantification of various microbial groups, the main objectives of this study were to develop a methodology to estimate active biomass SRT, and evaluate the attachment / detachment characteristics of the various anaerobic microbial groups.

2. Materials and Methodology

2.1. System Description

Two identical lab-scale ANFBBRs, demonstrated in Figure 1, were tested for digestion of PS and TWAS. Each plexiglass reactor contained a 16-liters main anaerobic column (3.6 m height, 8.9 cm long and 5.1 cm width) and a liquid-solid separator from which the digested sludges was
separated and circulated to the bottom of the ANFBBR for fluidization. A wet tip gas meter connected to the top of the column was used to measure the biogas flow rate. A mesophilic temperature of 37°C is uniformly maintained throughout the reactor by a water bath (IncuMaxTM WB20C, USA). A 10-liter container with mixer was used as a feed tank, from which sludges were pumped to the bottom of the column by a peristaltic pump (Masterflex I/P, Masterflex AG, Germany). Approximately 3 kg HDPE media (600 um~850 um) were added into the reactors after compaction, which occupied 22% volume of the 16 L reactor. The HDPE carrier had a sphericity of 0.9 and a BET surface area of 0.86 m²/g, with bulk and true densities of 810 kg/m³ and 1554 kg/m³, respectively. The reason for using plastic particles (HDPE) instead of zeolites was due to their potential lower energy consumption (Eldyasti et al., 2012).

2.2. Commissioning and Start-up

Anaerobic digester sludge (ADS-TSS and VSS concentrations of 25,000 and 18,000 mg/L) from the secondary digester was collected from the St. Mary wastewater treatment plant (Ontario, Canada) and used as the inoculation for the AnFBRs. After loading with 3 kg of media corresponding to a compacted media volume of 3.5 L, the reactors were filled with 20 L of ADS, fluidized and operated in a batch mode at 100% bed expansion for 7 days to induce microbial attachment. The reactors were then started by feeding synthetic solution containing 10,000 mg COD/L as sodium acetate at a flow rate of 1.8 L/d corresponding to a volumetric OLR of 1.1 kg COD/m³-d based on the 16 L AnFBR working liquid volume. The OLR was gradually increased to 18 kg COD/ m³-d within 100 days. Details of the composition of the synthetic feed are presented elsewhere (Andalib et al., 2012).

The liquid at the top of the reactor was recycled and pumped back to the bottom of the fluidized bed to maintain an up flow velocity at 0.8 cm/s as an energy saving concern. After acclimatization period, PS and TWAS from the Adelaide wastewater treatment plant (Ontario, Canada) were fed to the AnFBRs. Adelaide WWTP is a single-stage nitrifying wastewater treatment plant with a SRT of 3-4 days. The
operating conditions for the two AnFBRs over the course of the study are presented in Tables 1. The influent and effluent were collected and analyzed for various water quality parameters such as TSS, VSS, TCOD, SCOD, VFA, and alkalinity. Additionally, gas production and gas composition was monitored and recorded daily. The analytical techniques for the aforementioned parameters are detailed elsewhere (Andalib et al., 2012). Attached biomass concentrations (biosolids) were measured using APHA Standard Method No. 2540G (APHA, 1998).

2.3. Specific Methanogenic Activity (SMA), Specific Acidogenic Activity (SAdA), and Specific Acetogenic Activity (SAtA) Batch Tests

Anaerobic digestion can be divided into four sequential steps: hydrolysis (digesting large polymers into small monomers), acidogenesis (converting monomers into volatile fatty acids), acetogenesis (degrading volatile fatty acid into acetic acid, CO₂, and H₂), and methanogenesis (consuming acetate acid and producing CH₄) (Metcalf & Eddy, 2003), carried out by various microbial groups that exist both in suspended phase and attached biofilm phase in biofilm reactors (Switzenbaum, 1983; Heijnen, et al., 1988; Kuba, et al., 1990; Elefsiniotis & Oldham, 1993). In anaerobic biofilm processes, as a result of decoupling the HRT from the SRT and due to the difference in attachment characteristics between biomass and inerts (ie. nonbiodegradable suspended solids), the performance of the AnFBR cannot be rationalized based on the widely accepted definition and model of SRT based on VSS. In order to investigate the mechanism of the biofilm reactor and obtain the active biomass retention time, a series of batch tests of SMA, SAdA, and SAtA on both attached biofilm and detached biomass were conducted in this research.

Sonicated supernatant of the bioparticles and the effluent of the anaerobic fluidized reactor were used separately as seed in these tests. The initial substrate-to-biomass (S/X) ratio was set at a constant level of 2.0 g COD/g VSS. The SMA test details can be found in elsewhere (Andalib, et al., 2014). For the specific acetogenic tests, equal COD of ethanol, propionic acid, butyrate acid, and lactic acid were applied as substrates because propionic and butyraic acids are the main degradation products of glucose (Kalyuzhnyi, 1997) i.e. carbohydrates as well as short and medium chain fatty acids i.e. valeric acid,
hexanoic acid, produced during lipids degradation (Sun et al., 2000), while ethanol and lactic acid are carbohydrates fermentation products (Paramithiotis and Sofou, 2007). Furthermore, a high initial concentration of 5 g/L NaHCO$_3$ in the bottle was required to maintain stable pH level through the entire batch tests. Horiuchi et al. (2002) tested the impact of pH control on organic acid production in a glucose fed mesophilic anaerobic reactor and found that butyric acid accumulated at a pH ranging from 5 to 7, while propionate acid tended to accumulate at a pH of 8. In order to avoid the propionate and butyric acid accumulation in the acetogenic tests, the initial pH in the sample bottle was adjusted to 8. As the process of fermentation progresses, the pH in the bottle is expected to decrease slightly, and thus, would be suitable for propionate fermentation without butyrate accumulation.

Glucose was employed as the substrate in the specific acidogenic test, while extra acetic acid was also added to at a concentration of 5 g/L in order to inhibit further degradation of propionate to acetate as this reaction is considered part of acetogenesis (Heijnen, 1988). As Li et al. (2013) reported in their research of VFA distribution during acidogenesis of algal residues, a pH of 6 is optimal for the accumulation of butyric acid in acidogenesis. Therefore, the initial pH for the acidogenesis test was controlled at 6 by adding 5N HCl in order to eliminate further acetogenesis. An initial concentration of 5 g/L NaHCO$_3$ was also maintained in the bottle.

The seeds for the acidogenic activity tests and acetogenic activity tests were preheated at 90ºC for 30 minutes to inhibit the methanogenic bacteria. The initial VSS in the bottles were controlled at approximately 1,500 mg/L and the substrates were dosed at an initial concentration of 3,000 mg/L COD. Two bottles were used as blank which contained the same amount of VSS without any substrate. After flushing with nitrogen gas at 5-10 psi for 5 minutes, the sample bottles were then placed in a swirling-action shaker (MaxQ 4000, Incubated and Refrigerated Shaker, Thermo Scientific, CA) and operated at 180 rpm and 37ºC.

3. Results and Discussion

3.1. Performance of the AnFBRs Digestion of Primary Sludge and TWAS
The steady-state performance data of the AnFBR treating PS and TWAS is presented in Table 1. PS feeding to the AnFBR was started at an OLR of 9 kg COD/m³-d and increased to 18 kg COD/m³-d after 90 days. The PS was screened before being fed to the reactors in order to remove all large chunks which might clog the reactor. The operation of the AnFBR fed with TWAS was finally conducted at an OLR of 12 kg COD/m³-d, and achieved a VSS destruction efficiency of 53% at a HRT of 4 days.

A fairly thick scum layer, containing 112 mg/g TSS and 92 mg/g VSS in PS, 136 mg/g TSS and 119 mg/g VSS in TWAS, was observed floating on the top of the liquid-solid separator with an accumulation rate of 350 g/d and 270 g/d, respectively. Based on the VSS measurement showed in Table 1, the total amount of attached biomass in the reactor treating PS was roughly 75 g, while the VSS in the suspended phase was 81 g. However, if the specific bacterial activity was taken into consideration, more than 84% of the active bacteria were attached. Similarly, the attached biomass in the TWAS reactor was 60 g, with a suspended VSS of 117 g with the attached biomass accounting for over 60% of the total active bacteria.

The VSS in the scum layer for the two reactors were approximately 17% (PS) and 25% (TWAS) of the influent VSS. SMA tests were conducted on samples of the scums and the results showed approximately half the specific microbial activities of the suspended phase in each reactor, suggesting that regular removal of the scum layers would not adversely impact performance. Thus, scum layers were manually removed every 1 or 2 days to ensure the smooth operation of the reactors.

Methane yield and VSS destruction efficiency were calculated as follows:

\[
\text{Methane yield} = \frac{v_{CH_4}}{S_0 \left(\frac{mg}{L}\right) \times Q_{\text{feeding}} \left(\frac{L}{d}\right) - S_e \left(\frac{mg}{L}\right) \times Q_{\text{effluent}} \left(\frac{L}{d}\right) - S_s \left(\frac{mg}{g}\right) \times Q_s \left(\frac{g}{d}\right)}
\]

(1)

\[
V_{\text{sS destruction}} = 1 - \frac{v_{\text{effluent}} \left(\frac{mg}{L}\right) \times Q_{\text{effluent}} \left(\frac{L}{d}\right) + V_{\text{sS scum}} \left(\frac{mg}{g}\right) \times Q_{\text{scum}} \left(\frac{g}{d}\right)}{V_{\text{sS feeding}} \left(\frac{mg}{L}\right) \times Q_{\text{feeding}} \left(\frac{L}{d}\right)}
\]

(2)

Where \(S_0\) is the influent TCOD concentration, \(S_e\) is the effluent TCOD concentration, and \(S_s\) is the TCOD concentration in the scum layer. All the values involved and the results are illustrated in Table 1.
Figures 2a and 2b show the temporal variation of VSS destruction and the TCOD removal in both reactors. There were a few fluctuations during the first couple of days for both reactors as the feed was switched from synthetic wastewater to biosolids. However, the treatment efficiency was still increasing during the first 20 days and the reactor achieved a stable treatment performance after 30 days.

As shown in Table 1, biofilm attachment increased from 2.3 to 5.6 mg VSS/g particle during the start-up period and further developed to more than 20 mg VSS/g particle at steady-state. As depicted in Table 2, the steady-state SRT based on VSS were 3.3 days and 5 days for PS and TWAS AnFBR, respectively. Although it has been suggested that methanogenic reactors, can be operated stably at SRTs as low as 5 days (Lee et al., 2011), the performance of the two AnFBRs cannot be rationalized by the very low VSS-based SRTs.

The VFA (as acetate)-to-alkalinity ratio ($\alpha$) (Figure 2C) shows that after 10 days of start-up, $\alpha$ values consistently below 0.4 through the whole operation. Among the VFAs, acetic acid and propionic acid accounted for 46% and 43% of the VFA (based on COD), respectively, while butyric acid contributed the remaining 11%. Cruddas et al. (2014), who studied the treatment of domestic wastewater in an anaerobic pond at an OLR of 0.18 kg COD/m$^3$/d, observed that 54% of the VFA in effluent was contributed by acetic acid. Similarly, Forster-Carneiro et al. (2008) found that acetic acid usually accounted for 50% of the total volatile acids in the effluent while butyric acid only contributed less than 20% of the total acid in the effluent of the thermophilic lab-scale batch reactor treating municipal solid waste with the COD concentration ranging from 32 to 41 g/L. Thus, the VFA distribution observed in this research is consistent with selected literature studies.

3.2 Specific Microbial Activity Tests

Four rounds of specific microbial activity tests were conducted after both reactors reached steady-state. The initial VSS in the bottles were controlled at approximately 1,500 mg/L and the substrates were dosed at an initial concentration of 3,000 mg/L COD. The results of these tests are
illustrated in Figure 3. The maximum specific biogas production rate in each test reflected the growth rate of the correlated bacteria.

Since the $K_s$ values of the VFAs and glucose are less than 0.2 g/L (Henze and Harremoes, 1983), the initial substrate concentrations in the batch tests were more than 15 times higher than the $K_s$, and consequently, the specific bacteria growth rate (lag phase and plateau phase are exclusive) obtained in these batch tests represent the maximum growth rate phase (Figure 3). The specific biogas accumulation rates in these figures reflect the substrate utilizing rate, which corresponding to the specific activity of the targeted microbial group. The difference between the biogas accumulation rates of the suspended and attached phases indicates the distribution of active bacteria in the liquid and biofilm. The specific microbial activities were evaluated by dividing the volume of biogas produced per unit time by the initial weight of VSS in the test bottles.

As shown in Figure 3 (a), SMAs were 446 mL$_{CH_4}$/gVSS d and 350 mL$_{CH_4}$/gVSS d for the attached biomass treating PS and TWAS, respectively, and 51 mL$_{CH_4}$/gVSS d and 105 mL$_{CH_4}$/gVSS d for the suspended phase throughout the SMA tests with standard deviations of ±10%. Similarly, higher SAdAs and SAtAs were also observed in the attached biomass for both reactors treating PS and TWAS as depicted in Figure 3 (b) and (c). These results indicate that most of the active bacteria existed in the attached biofilm rather than suspended in the liquid phase.

The specific SRT of different microbial groups were calculated by using the formula below:

$$SRT = \frac{\text{Biomass specific maximum rate 1\text{-Attachment (mg/g)} \cdot \text{Weight}_{\text{particle}}\cdot \text{g} + \text{Biomass maximum rate 2\text{-VSS}_{\text{effluent}}\cdot (mg/L) \cdot V_{\text{reactor}}\cdot L}}}{\text{Biomass maximum rate 2\text{-VSS}_{\text{effluent}}\cdot (mg/L) \cdot Q_{\text{feeding}}\cdot (L/d)}}$$

(3)

As discussed before, the biomass specific maximum rates 1 and 2 are reflected by the specific biogas production rate of the attached and suspended biomass respectively. The VSS in the scum layer was also considered in the VSS$_{\text{effluent}}$ here after dividing by the working volume of reactor (16 L).
Liptak empirical equation (Liptak, 1974) is commonly applied to estimate the VSS destruction based on SRT for high-rate digestion system as showed in equation (4)

\[ V_d = 13.7 \times \ln(\text{SRT}) + 18.9 \]  

(4)

Results of the activity tests showed that the SRT of these three specific bacterial groups were all significantly higher than the SRT calculated directly based on the VSS. As illustrated in Table 2, the \( V_d \) calculated based on the specific SRT of methanogenic microbes were much more reliable compared with the general VSS-based SRT.

Literature confirms that biofilms are layered (Heijn en et al., 1988) and mathematical models, such as three dimensional simulation (Noguera, et al., 1999), layered stationary granular model (Tartakovsky, et al., 1996), and hybrid anaerobic reactor model (Saravanan, et al., 2008), were also derived based on this theory. A two tails T-test was conducted comparing each two of the different microbial activity-based SRTs generated for each reactor. Results show that the null hypothesis was rejected at both 90% and 95% confidence level for all of the comparisons, which indicate that the difference of SRTs of each bacterial group were significant. However, the methanogenic bacteria showed the longest SRT among the three active bacterial groups in both reactors. Since the outer surface of the bio-particles are prone to shear forces, the difference of the three SRTs suggests that the methanogenic microbial community, with a slower growth rate, was growing in the inner layer of the biofilm while acidogenic bacteria were growing at the outer layer of the biofilm with the acetogenic microorganism growing in the middle of the biofilm. This phenomenon can also be explained by the substrate gradient. This is plausible since each microbial group utilizes the degradation products of the one above it in the anaerobic food chain.

3.3. Microbial Attachment and Detachment

Table 2 illustrates the results of the specific bacterial characteristics. The specific activity in the attached phase was 6 to 8 times higher than in the suspended phase in the reactor treating PS for the different bacterial communities. Therefore, the attached phase was much more active in digesting wastes than the suspended phase even the total mass of VSS were similar for these two parts. The amount of
attached VSS in the TWAS reactor was much lower than the VSS in suspended phase. However, the treatment capacity in the attached phase was still higher that the suspended phase due to the 3 times higher activity in the attached phase.

Andalib et al. (2012) achieved a biomass attachment of 37.5 mg/g with zeolite as a media, ranging in size from 425 um to 610 um, and a maximum biofilm thickness of 150 um corresponding to a biofilm VSS concentration of 28.6 kg/m³, which translates to estimated biofilm thicknesses in this study of 100 um and 120 um for the reactors treating TWAS and PS, respectively. The average surface area (SSA) based on a bed voidage of 70% and average bio-particle diameter of 945 um was calculated as roughly 1900 m²/m³ according to the following equation (Eldyasti et al.,2012):

\[ \text{SSA} = \frac{6}{d_p} (1 - \varepsilon) \]  

(5)

Then, the shear stress was calculated by the following formula (Rittmann, 1982):

\[ \sigma \ (\text{dyn/cm}^2) = \frac{100\mu v(1-\varepsilon)^3}{d_p^3(\varepsilon^3a(7.46\times10^9)(5)} \] 

(6)

Where \( \varepsilon \) is the total bed voidage (70%), \( \mu \) is liquid viscosity (864 g/cm day), \( v \) is the liquid upflow velocities (0.8 cm/s), \( d_p \) is the bioparticle diameter including biofilm (0.0945cm and 0.0925 for PS and TWAS,respectively), and \( a \) is the specific surface area of biofilm carriers (19 cm²/cm³). The shear stress calculated for two reactors fed with PS and TWAS were 0.36 dyn/cm² and 0.39 dyn/cm², respectively, as compared with 0.2 dyn/cm² for the AnFBR with zeolite (Andalib et al., 2012).

In biofilm reactors, first order detachment rate coefficient (\( b_s \)) is generally used to describe detachment mechanisms. In fluidized bed bioreactor, \( b_s \) can be calculated as follow (Patel, et al., 2005):

\[ b_s = \frac{\text{Biomass specific maximum rate}_2 \times \text{Q}_{\text{effluent}} \times VSS_{\text{effluent}}}{\text{Biomass specific maximum rate}_1 \times M_{\text{media}} \times VSS_{\text{attachment}}} \]  

(6)

The \( b_s \) for different bacterial groups of this research as well as Andalib’s study (2012) are shown in Table 2. It can be observed that the \( b_s \) of VSS is much larger than other bacterial groups for all cases, which indicates that the active bacteria tend to grow on the media surface while inert bacteria commonly
exist in the suspended phase in the AnFBR. The $b_\sigma$ ratios for the VSS observed in this study of 1.9 and 1.6 for PS and TWAS, respectively, are comparable to the 1.5 observed in the zeolite AnFBR (Andalib et al., 2012) during the treatment of thin stillage. These results clearly suggest that a high shear force would cause a negative impact on biofilm formation, and system performance, with the detachment. Nakhla et al. (2002) and Turan (2000) also observed that the increase of shear stress will lead to an increase of detachment rate in anaerobic fluidized bed. Although Rittmann justified that the detachment rate coefficient ($b_\sigma$) is proportional to $\sigma^{0.58}$ according to his model, this empirical model was only based on smooth aerobic biofilms on unfluidized glass beads (Chang et al., 1991). Furthermore, Speitel and DiGiano (1987) observed during their study on paranitrophenol (PNP) in a granular activated carbon (GAC) reactor that the $b_\sigma$ predicted by the Rittmann model underestimated the actual detachment rate, implying that the value of the exponent in Rittmann’s model is greater than 0.58.

4. Conclusions

The results of this study demonstrate that mesophilic anaerobic digestion using a fluidized-bed reactor, with HDPE (600 um~850 um) as support material, is highly effective for COD removal and VSS destruction of primary and secondary sludge. The AnFBR successfully treated the primary sludge at OLR of 18 kg/m³-d, achieving COD removal efficiency of 61% and VSS destruction efficiency of 63%, and the AnFBR also successfully treated TWAS at OLR of 12 kg/m³-d, achieving COD removal efficiency of 55% and VSS destruction efficiency of 51%.

The SMA and other activities tests showed that the SRT calculation based on general VSS is not accurate in predicting and rationalizing the VSS reduction in the anaerobic biofilm systems. The microbial activity tests also indicated that the biofilm was layered with acidogens on the outside, followed by acetogens, and methanogens on the inside.
ACKNOWLEDGMENT

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REFERENCES


Figure Captions:

**Figure 1.** Schematic of the anaerobic fluidized bed bioreactor (Adapted from Andalib, 2014)
Figure 2. (a) VSS destruction and TCOD removal (PS)
(b) VSS destruction and TCOD removal (TWAS)
(c) VFA/ALK ratio
Figure 3. (a) SMA tests for PS and TWAS reactors (average±SD)

(b) SAdA tests for PS and TWAS reactors (average±SD)

(c) SAtA tests for PS and TWAS reactors (average±SD)
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Start-up</th>
<th>PS</th>
<th>TWAS</th>
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<tbody>
<tr>
<td>Time of operation (d)</td>
<td>120</td>
<td>90</td>
<td>90</td>
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<td>Feed flow rate (L/d)</td>
<td>1.8-7.2</td>
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<td>2.2-8.9</td>
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<td>Attached Biomass (mg/g media)</td>
<td>2.3-5.6</td>
<td>24.8±4.1</td>
<td>20.1±3.6</td>
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<td>Total media (kg)</td>
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### Feeding characteristics

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<tr>
<td>TCOD (mg/L)</td>
<td>10,000~40,000</td>
<td>38921±2897</td>
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<td>sCOD (mg/L)</td>
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<td>1942±822</td>
<td>3410±1042</td>
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<td>TSS (mg/L)</td>
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<td>34671±5185</td>
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<td>VSS (mg/L)</td>
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### Effluent characteristics

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<tr>
<td>TCOD (mg/L)</td>
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<td>8165±741</td>
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<td>sCOD (mg/L)</td>
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<td>VSS (mg/L)</td>
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<td>5068±633</td>
<td>7317±842</td>
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### Scum layer characteristics

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<tr>
<td>TCOD (mg/g)</td>
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<td>142±37</td>
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<td>TSS (mg/g)</td>
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<td>112±28</td>
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<td>VSS (mg/g)</td>
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<td>92±15</td>
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<td>Production rate (g/d)</td>
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### Removal Efficiencies

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<td>COD removal eff. (%)</td>
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<td>61</td>
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<tr>
<td>VSS removal eff. (%)</td>
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<td>63</td>
<td>51</td>
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### Methane yields

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<th>TWAS</th>
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<tbody>
<tr>
<td>Methane yield (LCH4/gCOD removed)</td>
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<td>0.32</td>
<td>0.35</td>
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Table 2. Results of microbial activities tests under steady state for both reactors

<table>
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<tr>
<th></th>
<th>Specific Activity in Suspended Phase (mL CH₄ or H₂/gVSS.d)</th>
<th>Specific Activity in Attached Phase (mL CH₄ or H₂/gVSS.d)</th>
<th>SRT (d)</th>
<th>First Order Detachment Rate Coefficient (d⁻¹)</th>
<th>Expected VSS Reduction Based on Liptak</th>
<th>Actual VSS Reduction in Reactors</th>
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<tr>
<td>Methanogenic Microbe</td>
<td>51±9 (8)</td>
<td>433±74 (8)</td>
<td>3.3</td>
<td>14.6±0.62 (8)</td>
<td>0.69</td>
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<td>Acidogenic Microbe</td>
<td>32±10 (8)</td>
<td>191±8 (8)</td>
<td>1.22±0.57 (8)</td>
<td>0.11</td>
<td>54%</td>
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<tr>
<td>Acetogenic Microbe</td>
<td>61±9 (8)</td>
<td>456±41 (8)</td>
<td>13.2±1.08 (8)</td>
<td>0.09</td>
<td>53%</td>
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<tr>
<td>TWAS</td>
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<tr>
<td>Methanogenic Microbe</td>
<td>105±3 (8)</td>
<td>350±4 (8)</td>
<td>9.4±0.10 (8)</td>
<td>0.19</td>
<td>50%</td>
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<td>Acidogenic Microbe</td>
<td>63±6 (8)</td>
<td>179±12 (8)</td>
<td>8.0±0.43 (8)</td>
<td>0.22</td>
<td>47%</td>
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<td>Acetogenic Microbe</td>
<td>157±11 (8)</td>
<td>501±30 (8)</td>
<td>8.8±0.45 (8)</td>
<td>0.19</td>
<td>49%</td>
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<tr>
<td>(Andalib, 2012) Methanogenic Microbe</td>
<td>-</td>
<td>-</td>
<td></td>
<td>0.30</td>
<td>-</td>
<td>-</td>
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