Optimization of Anaerobic Digestion of Sewage Sludge Using Thermophilic Anaerobic Pre-Treatment

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Optimization of anaerobic digestion of sewage sludge using thermophilic anaerobic pre-treatment

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Contents

Contents................................................................................................................1

Title page..............................................................................................................4

Preface................................................................................................................5

Acknowledgement..............................................................................................6

Summary (English)..................................................................................................7

1. Introduction.........................................................................................................9

2. Issues on sewage sludge..................................................................................12
   2.1 Generation and composition.......................................................................12
   2.2 Environmental impact...............................................................................13
   2.3 Treatment and disposal methods.............................................................14

3. Principles in anaerobic digestion of sewage sludge ......................................16
   3.1 The 4-step anaerobic digestion model.......................................................16
      3.1.1 Hydrolysis.........................................................................................16
      3.1.2 Acidogenesis....................................................................................17
      3.1.3 Acetogenesis....................................................................................17
      3.1.4 Methanogenesis..............................................................................18
   3.2 Factors affecting anaerobic digestion process..........................................19
      3.2.1 Specific characteristics of sewage sludge.......................................20
      3.2.2 Temperature....................................................................................20
      3.2.3 pH....................................................................................................22
      3.2.4 Macro- and micronutrients..............................................................22
      3.2.5 Inhibition..........................................................................................22
      3.2.6 Toxicity.............................................................................................24
      3.2.7 Retention time..................................................................................24
      3.2.8 Agitation...........................................................................................24
      3.2.9 Feeding strategy..............................................................................25
   3.3 Optimization of anaerobic digestion for sewage sludge treatment...........25

4. Thermophilic anaerobic pre-treatment..........................................................28
   4.1 Summary of the pre-treatment methods....................................................28
   4.2 Rationale for conducting pre-treatment under thermophilic anaerobic
      conditions.....................................................................................................30
      4.2.1 Exploitation of the anaerobic thermopiles for hydrolysis and
         acidogenesis..............................................................................................30
      4.2.2 Simultaneous achievement of thermal pre-treatment effects............31
      4.2.3 Simultaneous achievement of pasteurization..................................32
      4.2.4 Easy for operation...........................................................................32
      4.2.5 Internal-supplied energy for heating...............................................32
   4.3 Determination of optimal temperature and retention time........................33
      4.3.1 Rationale for determination of optimal temperature and retention
         time...........................................................................................................33
      4.3.2 Matrix of temperature and retention time combinations..................33
      4.3.3 Experiments....................................................................................34
      4.3.4 The optimal combination of temperature and retention time...........35
4.4 Differentiation of the effects on the hydrolysis of the organic particulates…37
4.4.1 The different characteristics of primary sludge and waste activated sludge…………………………………………………………………………………………37
4.4.2 Experiments……………………………………………………………………37
4.4.3 Differentiation of the biological and thermal effects………………………37

5. Two-step anaerobic digestion……………………………………………………...41
5.1 Theoretical background of two-phase AD process…………………………...42
5.2 Comparison of the two-phase process with the single-phase process………42
5.2.1 Operation at normal RT……………………………………………………..42
5.2.2 Operation at reduced RT……………………………………………………..43
5.2.3 Perturbation tests……………………………………………………………..45
5.3 Summary of the two phase process…………………………………………47

6. Conclusion..................................................................................................49

References........................................................................................................51

Papers:

Paper-I: Thermophilic anaerobic pre-treatment for hydrolysis and hygienization of sewage sludge
Paper-II: Comparison of the two-phase anaerobic digestion (73°C/55°C) the single-phase anaerobic digestion (55°C) in treating sewage sludge
Paper-III: Biological and thermal effects of thermophilic anaerobic pre-treatment on the hydrolysis of organic solids in sewage sludge
Paper-IV: Thermal pre-treatment of primary sludge and secondary sludge at 70°C prior to anaerobic digestion
Paper-V: Effect of hyper-thermophilic pre-treatment on thermophilic anaerobic digestion of primary sludge

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April, 2006
PREFACE

This thesis presents the results from the work done for my Ph.D study at BioScience and Technology, BioCentrum-DTU, the Technical University of Denmark during the period from February 1, 2003 to April 30, 2006 with Professor Birgitte Kier Ahring as supervisor.

The thesis is organized in two parts. Part one includes the literature survey of the sewage sludge problems, the theoretical background of anaerobic digestion, the methods for pre-treatment, and the lab work results of the studies on the effects and mechanisms of thermophilic anaerobic pre-treatment, comparison of the two-phase process with the single-phase process, and the considerations of the start-up, operation as well as the economic assessment of the two-phase process. Part two contains five papers submitted to international journals and one oral presentation at an international conference. The papers and the presentation are:


**Paper II:** Lu, J. and Ahring, B.K. (2006) Comparison of the two-phase anaerobic digestion (73°C/55°C) with the single-phase anaerobic digestion (55°C) in treating sewage sludge. Manuscript submitted to *Water Science and Technology*.


- 5 -
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I would like to express my thankfulness to my former colleagues in Fushun EPA in China, my family and my Danish and Chinese friends for their support and encouragement during this study.

The project was financed by DTU. Besides, Daloon Fonden and the Sino-Danish Scientific and Technological Cooperation Committee also provided stipend for my stay in Denmark during this study.

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SUMMARY

The purpose of my Ph.D study is to develop an innovative and cost-efficient method for optimal biogas production and reuse of sewage sludge. Topics including thermophilic anaerobic pre-treatment, enhancement of hydrolysis of organic particulates, pathogen reduction effect, and efficiency and stability of two-phase anaerobic digestion are therefore focused on.

After the optimal temperature and retention time for the pre-treatment had been determined by manipulating continuous stirred tank reactors running at temperatures in the range of 55-80°C for the retention times in the range of 0.5-3.0 days (Paper I), a two-phase anaerobic process with a pre-treatment phase under optimal conditions before the methane phase was tested in terms of reactor organic reduction efficiency, pathogen reduction effect and process stability by using a single-phase process as control (Paper II). To further investigate the pre-treatment mechanisms, a study on distinguishing different effect mechanisms was carried out (Paper III). In addition, in order to identify the microbial activities, energy balance for the two-phase reactor system, two sets of two-phase process (70°C/55°C) were studied, in which primary and waste activate sludge was used as substrate, respectively (Paper IV and Paper V).

It was identified that pre-treatment under thermophilic anaerobic conditions, with the optimal combination of temperature and retention time of 73°C and 2 days, simultaneous enhancement of hydrolysis of the organic particulates in the sludge, high degree of acidification of the hydrolysis products and achievement of satisfactory pathogen reduction effect were obtained. The enhanced hydrolysis and acidification were contributed by thermal and biological effects as the major mechanisms for both primary sludge and waste activated sludge. For waste activated sludge, an additional mechanism, microbial cell lysis, was also involved.

Thermophilic anaerobic pre-treatment running at the identified optimal condition, i.e., at the temperature of 73°C for a retention time of 2 days, was employed as the acid-phase of the two-phase anaerobic digestion system. By running the two-phase CSTR process (73°C/55°C) with a parallel single-phase CSTR process (55°C) as control, it
was verified that the two-phase process could keep not only the satisfactory pathogen reduction effect that the single-phase process could not achieve, but also possessed superiorities over the single-phase process such as increased efficiency in converting waste organic material into biogas and enhanced process stability due to the effect of pre-treatment. Microbial activities of the two-phase process were higher than those of the single-phase process.

It was concluded from this study that thermophilic anaerobic pre-treatment can be used to optimize anaerobic digestion process for sewage sludge treatment. The optimized two-phase process is high-rate, efficient and cost-effective, and possesses the capability to eliminate the pathogens. The significance of implementing the optimized two-phase anaerobic digestion process lies in the following aspects:

1. The environmental problem caused by sewage sludge, which is a global one and getting more and more severe, can be solved in a sustainable way;
2. The energy saved in the organic material of the sewage sludge can be extracted in the form of biogas, which is CO$_2$ neutral and renewable, and can be used to produce electricity and heat;
3. The thorough elimination of pathogens makes it possible to recycle the plant nutrients and inert organic material in the digested effluent back to the farmland as fertilizer and soil conditioner without any fear of spreading of epidemic disease;
4. The heat needed to keep the process temperature can be obtained by burning the biogas produced by the process itself, so there is no dependency on the external energy supply.
1. INTRODUCTION

The development of wastewater treatment technology together with the implementation of stringent environmental legislation has successfully protected the aquatic system from pollution in many of the countries of the world. However, sewage sludge, as the by-product of the wastewater treatment plant, is also generated at the same time. Unlike the other kinds of waste, the generation of which can be reduced by clean-production technology, sewage sludge is not evitable and its generation will, on the contrary, increase along with the increase of wastewater discharge and treatment rate (Wang, 1997). Sewage sludge is now becoming a worldwide environmental problem because of its increasing production and its high contents of organic waste and pathogens, as well as xenobiotics and heavy metals. If not being treated or disposed properly, this dangerous waste may cause the environment and human as well as animal health exposed to tremendous threat (Ahring, 2003).

Anaerobic digestion is a biological process that can degrade waste organic material by the concerted action of a wide range of microorganisms in the absence of oxygen. The process consists of a complex series of reactions that convert a wide array of polymeric substances such as carbohydrates, proteins, and lipids, having carbon atoms at various oxidation and/or reduction states, to one-carbon molecules in its most oxidized state ($\text{CO}_2$) and its most reduced state ($\text{CH}_4$). In a variety of anaerobic environments, such as the intestinal tract of animals, marine and fresh water sediment, paddy fields, sewage sludge, water logged soils, and in the region of volcanic hot springs and deep-sea hydrothermal vents, naturally exists this process (Westermann, 1996; Madigan, et al., 2003).

Anaerobic digestion has been manipulated by man for many years to treat sewage sludge (Hamzawi, et al., 1998). Before anaerobic digestion, the organic material in the sludge also automatically decay due to the biological activities of the extensive existence of microorganisms in the sludge, producing offensive, odorous and reduced end products such as fatty acids, mercaptans and amines. After anaerobic digestion,
the digestate consists of an odor free residue with appearance similar to peat. Methane produced by the anaerobic digestion process is a clean, CO$_2$ neutral and renewable energy that can be used to produce heat and electricity. Further more, anaerobic digestion seems to be the only cost-effective method that makes it possible for sewage sludge to use farmland as a safe and permanent outlet destination with positive effect, i.e., the digestate, which has retained plant nutrients such as N and P, can be recycled as fertilizer and soil conditioner back to the farmland and thus keeps these natural nutrients recycled within a closed loop ecosystem, and remain or improve the soil structure of the farmland. The unique features of anaerobic digestion have made it superior to any other methods such as landfill, incineration, aerobic treatment and etc., which have ever been and are still being used for the treatment and disposal of sewage sludge in some parts of the world.

However, the advantages of anaerobic digestion in the treatment of sewage have not been brought into full play. This process is still far from optimization. When conducted at mesophilic temperatures, the process retention time normally has to be set as long as 30 days. When conducted at thermophilic temperatures, although the retention time can be reduced to 15 days, special care for operation has to be taken for the stability of the process. No matter under which temperature conditions, only around 50% to 60% of the organic material can be degraded, leaving a large potential to increase the biogas production. Besides, even though it is has been widely believed that anaerobic digestion process is of the capability of reducing the pathogens (Bendixen, 1999; Nielsen & Petersen, 2000), the properties of the digested effluent is still hardly meeting the stringent requirement by legislations in relation to land application of sewage sludge in agriculture (Vesilind, 2000).

In recent years, due to the directive of minimization of landfill and calling for reuse and recycle of the waste by the new waste management policies (EU, 2000), and the eagerness for extraction energy from waste including sewage sludge to ease up the dependence on energy from fossil fuels (Chynoweth, et al., 2001), tremendous research has been carried to optimize the anaerobic digestion process. Better understanding of the basic mechanisms occurring in the anaerobic process, conducting the process at thermophilic temperatures, application of different kinds of methods for
pre-treatment, development in process monitoring and control, and phase separation have contributed to the improvement of anaerobic digestion process from different aspects. However, simultaneous achievement of both biogas increase and digestate pasteurization has never been obtained by using a cost-effective and single effort.

This study is to optimize the anaerobic digestion process using thermophilic anaerobic pre-treatment. It is hoped that the pre-treatment could enhance the hydrolysis of the organic particulates in the sludge so as to increase the efficiency of the organic material conversion to methane, and at the same time, pathogens in the digested sludge can be totally eliminated during this pre-treatment step so as to the digested effluent can meet the requirement on reuse of the digested sludge in agriculture.
2. ISSUES ON SEWAGE SLUDGE

2.1 Generation and composition

Sewage sludge is an unwanted and inevitable by-product from wastewater treatment plants, the purpose of which is to clarify wastewater. Sewage sludge is generated by sedimentation both before and after the bio-treatment process, named as primary sludge and waste activated sludge (or secondary sludge), respectively. In some wastewater treatment plants, tertiary sludge may be produced as well due to the tertiary or polishing treatment of the biologically treated wastewater. However, the quantity of this kind sludge is relatively small and the organic fraction in the solids is slight, so this fraction is often ignored.

Primary sludge is of a non-homogeneous nature because of some rather coarse constituents in it. After thickening in the primary settling tank, the solids content is about 5-10%, of which about 70% consists of organic matter. Wastewater normally contains thousands of different organics, so the composition of primary sludge is very complex. Secondary sludge consists of waste activated sludge from the aeration tanks or humus from the trickling filters, both of which are composed of microorganisms and other life forms, which are withdrawn or flushed out of the system. In addition, small proportions of adsorbed suspended solids and colloids derived from the wastewater are included. By normal thickening and/or dewatering technologies, the solids concentration in secondary sludge is about 1-6%, in which the organic fraction is also around 70%. In general, primary sludge and secondary sludge account for 60-80% and 20-40% of the total sewage sludge based on the volatile solids (VS) contents, respectively, and the percentage depends not only on the technique of the wastewater treatment process, but also depends on the sources of the wastewater. (Mudrack & Kunst, 1986; Henze, et al., 2000).

The production of sewage sludge is huge and has been a worldwide problem. In 2000, the sewage sludge production in Denmark and in the EU member states was about 160,000 and 8,500,000 tons of dry weight, respectively (Magoarou, 2000; Jensen and Jepsen, 2005). To the global perspective, especially in the developing courtiers, along with the increase in population, development in urbanization and the implementation
of the stringent legislation and regulation related to wastewater and sewage sludge, there will be more and more wastewater to be treated and thus more and more sewage sludge to be generated and treated (Hamer, et al., 1985).

2.2 Environmental impact
Wastewater treated in the wastewater treatment plant consists of domestic sewage, industrial effluents and storm-water runoff from roads and other paved areas, and therefore, sewage sludge contains not only waste organic material, but also pathogenic microorganisms, as well as trace amount of pollutants such as xenobiotics and heavy metals. Sewage sludge is a good substrate for bacteria and therefore putrefies very rapidly (Mudrack & Knust, 1986), causing offensive emission, growth of flies or pathogens, as well as contamination of soil and aquatic system. The pathogens that are concentrated into the sewage sludge include viruses, bacteria, protozoans and larger parasites such as human roundworms, tapeworms and liver flukes. These pathogens are capable of independent existence. They will multiply under suitable conditions. Under conditions unsuitable for growth, they are not necessarily lethal. Some of them may further evolve their structures such as cysts or spores as a means of survival in adverse conditions. Without proper treatment, they may spread diseases. Heavy metals such as cadmium, mercury, zinc, etc., are toxic. They may enter the sewage sludge via industrial discharges, and also in some cases from galvanized water pipes. Xenobiotics such as PCBs, herbicides, insecticides and etc, are chemical substances that are foreign to the biological system and may accumulate in human body through food chain. So, if sewage sludge is not properly treated or disposed, it can make the environment and the health of human and animals exposed to a tremendous threat (Hänel, 1988; Mudrack & Kunst, 1986; Carrington, 2001; Ahring, 2003).

2.3 Treatment and disposal methods
Many methods have been developed and used in the treatment and/or disposal of sewage sludge, but the operation of the treatment or disposal is usually expensive and/or easy to contaminate the environment. For most of the wastewater treatment plants, the treatment and disposal of sewage sludge accounts for 50% or more of the total capital and operation cost (Benefield & Randall, 1985). So the processing and disposal of sewage sludge are increasingly being the topics of environmental,
financial and technological concerns (Woodard & Wukasch, 1994). The following are the methods that can be referred to from literatures up to today:

- Spreading on wild land
- Discarding into the ocean
- Direct application on farmland
- Landfill
- Incineration
- Aerobic composting
- Anaerobic digestion
- Injection into old oil wells

Among these methods, spreading on wild land, discarding into the ocean and direct application on farmland are the most primitive disposal methods. Due to the obvious contamination to the environment, these methods have been in practice forbidden in many of the countries and regions. Landfill only postpones the problem since the leakages can contaminate the ground water and are subjected to advanced treatment in most European countries involving high purification cost. Further more, expenditure for development of landfill areas is getting higher due to the decrease of space in highly populated countries. So, disposal of sewage sludge by landfill is to be phased out, even though 35-40% of the sludge in Europe is still deposited in landfill today (Ødegaard et al., 2002). Incineration seems to be a good method because, after dewatering and burning, there is almost nothing left compared to the original huge sludge volume. However, it costs energy for dewatering and normally needs input of external fuel. Also, the disposal of ash and the treatment of exhaust gas are expensive (Ahring, 2003). Aerobic composting cannot recover the energy of the biomass in the sludge, and may cause odor problem. Injection into old oil wells is not applicable for countries and regions where there exists no oil field.

So, it has been generally accepted that, among all the treatment and disposal methods that have been used up to now, anaerobic digestion is universal and sustainable. This is because anaerobic digestion can stabilize the organic materials, reduce the number of pathogens (Bendixen, 1999; Nielsen and Petersen, 2000), degrade xenobiotics (Hartmann and Ahring, 2003), recover CO\textsubscript{2} neutral energy in the form of CH\textsubscript{4}, as well
as make it possible to recycle the plant nutrients in it back to the farmland. And therefore, anaerobic digestion complies with today’s sludge management policies, i.e., to reduce the waste stream to landfill and to recycle the organic material and the plant nutrients back to the agricultural soil (EU, 2000; Ahring, 2003). So, anaerobic digestion of sewage sludge is worth of further studying.
3. PRINCIPLES IN ANAEROBIC DIGESTION OF SEWAGE SLUDGE

3.1 The 4-step anaerobic digestion model

Anaerobic digestion is a multiple bio-process, of which four main steps can be identified (Ghost 1975; Kasper and Wuhrmann, 1978; Gujer and Zehnder, 1982; Madigan, et al., 2003), namely hydrolysis, acidogenesis, acetogenesis and methanogenesis, involving six major distinct processes (Figure 3-1).

Figure 3-1. Proposed reaction scheme for the anaerobic digestion of sewage sludge. Adapted from Gujer and Zehnder (1983). Percentages indicate substrate flow in the form of COD or CH₄ equivalents. Only the net flow of substrates through external pools is indicated. Numbers in the circles identify different process.

3.1.1 Hydrolysis

In this step, complex organic polymers are hydrolyzed into smaller units such as sugars, long-chain fatty acids and amino acids. This is carried out by different groups of obligate or facultative fermentative bacteria through excreting extracellular enzymes (Kaseng et al., 1992). The proteolytic bacteria produce proteases that catalyze the hydrolysis of proteins into amino acids (Figure 3-1, 1A); the cellolytic and xylanolytic bacteria produce cellulases and/or xylanases that degrade cellulose and xylan (both are carbohydrates) to glucose and xylose, respectively (Figure 3-1, 1B); and the lipolytic bacteria produce lipases that degrade lipids to glycerol and long chain fatty acids (Figure 3-1, 1C).
3.1.2 Acidogenesis

The dissolved sugars, long-chain fatty acids and amino acids produced by hydrolysis are used in this step either by fermentative bacteria (Figure 3-1, 2) or by anaerobic oxidizers (Figure 3-1, 3) (Gujer and Zehnder, 1983), forming acetate and other short-chain fatty acids, alcohols, hydrogen and carbon dioxide. Acidogenesis is a robust and often the fastest step in the whole anaerobic digestion process. When protons are used as electron acceptor with concurrent hydrogen production, the oxidation of substrate by fermentative bacteria provides the largest amount of energy. In a well-operated anaerobic reactor, about 70-80% of the hydrolysis products will be transformed directly to methanogenic substrates i.e., hydrogen, carbon dioxide and acetate, with the remaining 20-30% transformed into other intermediate products, such as volatile fatty acids (VFAs) and alcohols (Gujer and Zehnder, 1983; Schink, 1997; Ahring, 2003).

However, when the produced hydrogen cannot be consumed simultaneously by the hydrogen-utilizing methanogens, the increasing concentration of hydrogen is inhibitory to the hydrogen production. In this case, the fermentative bacteria will switch metabolism so that they can still obtain some energy, and then about 50-70% of the hydrolysis products will be transformed into the intermediate products (Bryant, 1979; Klass, 1984; Ahring, 2003).

The intermediate products produced in acidogenesis step cannot be utilized by the methanogens, and must be further degraded in the acetogenesis step by acetogens.

3.1.3 Acetogenesis

Since the intermediate products must be further oxidized to acetate, H₂ and CO₂ before they are used by methanogens, the acetogenesis step is crucial for the successful production of biogas (Figure 3-1, 4). In contrast to the fermentative bacteria, the acetogens are obligately symbiotic bacteria (McInemey, et al., 1980; Boone & Bryant, 1980; Westermann, 1996). Under standard conditions, these oxidation processes are endothermic, i.e., energy demanding and cannot grow when the H₂ partial pressure is high. As shown in Figure 3-2, only when H₂ partial pressure is lower than a certain level, the oxidation of propionate and butyrate can be possible. Also in contrast to the fermentative bacteria, acetogens cannot switch their metabolic
pathway but reduce of $H^+$ to $H_2$. Therefore, the proceeding of acetogenesis is relying on the presence of hydrogen-utilizing methanogens to remove $H_2$.

Table 3-2. Free energy change of acetogenesis and methanogenesis from $H_2$ as a function of the $p_{H_2}$. Data derived from Archer (1983). Calculation based on standard values for free energies at pH 7, 25°C, 34 mM $HCO_3^-$, 1mM VFAs and $p_{CH_4}$ 0.7 atm.

3.1.4 Methanogenesis

The final step of the anaerobic digestion is called methanogenesis that mineralizes the fermentative products to methane. This step is carried out by two main groups of methanogens: the aceticlastic methanogens, which degrade acetate, belonging to the genera *Methanosarcina* and *Methanosaera* (Figure 3-1, 5), and the hydrogen-utilizing methanogens (Figure 3-1, 6), of which an array of genera exist. Methanogens belong to *Archae*, a unique group of microorganisms, phylogenetically different from the main group of prokaryotic microorganisms (Madigan, et al., 2003). It is estimated that, under stabilized conditions, about 70% of methane is produced by the acetate-utilizing methanogens and 30% by the hydrogen consuming methanogens (Smith et al., 1980; Klass, 1984).

Methanogenesis is regarded as the motive force of the whole anaerobic degradation. In contrast to some of the acetogens, methanogenesis is an energy producing process under standard conditions (Figure 3-2). Only if the presence of the hydrogen-utilizing methanogens keeping the partial pressure low, can the acetogens perform a catabolic oxidation which would not be energy yielding if the hydrogen-consuming bacteria had
This biological phenomenon is called ‘interspecies hydrogen transfer’. As it can be seen from data shown in Table 3-1, due to the consumption of H\textsubscript{2} and acetate by the methanogens, the oxidation of propionate and butyrate is exothermic and possible under standard conditions.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>( \Delta G^\circ )(kJ/reaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetogenesis</td>
<td></td>
</tr>
<tr>
<td>(1) ( CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2 )</td>
<td>+ 116.4</td>
</tr>
<tr>
<td>(2) ( CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + HCO_3^- + H^+ + 2H_2 )</td>
<td>+ 88.2</td>
</tr>
<tr>
<td>Methanogenesis</td>
<td></td>
</tr>
<tr>
<td>(3) ( 4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O )</td>
<td>- 175.5</td>
</tr>
<tr>
<td>(4) ( CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- )</td>
<td>- 31.0</td>
</tr>
<tr>
<td>Syntrophic conversion</td>
<td></td>
</tr>
<tr>
<td>(5) ( CH_3CH_2COO^- + \frac{1}{2} H_2O \rightarrow \frac{1}{2} HCO_3^- + \frac{1}{2} H^+ + \frac{1}{2} CH_4 )</td>
<td>- 46.2</td>
</tr>
<tr>
<td>(6) ( CH_3CH_2CH_2COO^- + \frac{1}{2} H_2O \rightarrow \frac{1}{2} HCO_3^- + \frac{1}{2} H^+ + \frac{1}{2} CH_4 )</td>
<td>- 61.6</td>
</tr>
</tbody>
</table>

Values for Gibbs free energy (\( \Delta G^\circ \)) are given under standard conditions, i.e., 1M, 1atm, pH=7.0, T=25°C. Values of acetogenesis and methanogenesis are adapted from Westermann (1996).

Also, it has to be noticed that, from Figure 3-2 and Equation 3 in Table 3-1, when H\textsubscript{2} partial pressure is lowered, the energy yield of H\textsubscript{2}-utilizing methanogens will be reduced. This implies that there is a certain narrow H\textsubscript{2} partial pressure range where oxidation of fatty acids with H\textsubscript{2} as a product, and methanogenesis with H\textsubscript{2} as a substrate is possible (Dolfing, 1988; Westermann, 1996).

Although the main products of the anaerobic process are carbon dioxide and methane, minor quantities of nitrogen, hydrogen, ammonia and hydrogen sulfide (usually less than 1% of the total gas volume) are also generated (McInerney et al., 1980). The mixture of the gaseous products is termed as biogas.

### 3.2 Factors affecting anaerobic digestion process

As mentioned above, anaerobic digestion is a very complicated process depending upon the synergy interactions between the various groups of microorganisms involved. A fine balance between these microbial communities is necessary for
successful digestion giving the largest conversion rate of organic material to methane. When anaerobic process is used for the digestion of sewage sludge, it might be affected by the specific characteristics of the sewage sludge to be treated, environmental factors such as temperature, pH, presence of inhibitory or toxic substances, etc., and operational factors such as hydraulic and solid retention time, mixing and feeding strategies and so on.

3.2.1 Specific characteristics of sewage sludge

The specific characteristics of sewage sludge include the type of sludge (primary sludge, secondary sludge or the mixture of these two types of sludge), composition of the complex polymeric organics (percentage of carbohydrates, proteins and lipids), solid concentration and size distribution. Normally, variations in composition and in size distribution of primary sludge are bigger than that of secondary sludge. When sewage sludge with different specific characteristics is used as feedstock, the reactor performance may change, especially when CSTR type reactor is used (Chyi & Dague, 1994; Ferreiro & Soto, 2003; Palmowski and Muller, 2003). The degradation rate of secondary sludge is only one-half of that of primary sludge (Ghosh, 1995). Also, for the secondary sludge, the degradation rate is affected by the sludge age. For example, by using the same anaerobic digestion process, the degradation rate is only 14% for the feed with a sludge age of 30 days, and 31% for the feed with a sludge age of 5 days (Gossett, J. & Belsere, 1982). When anaerobic digestion is studied at lab scale, especially when small volume of reactor is used, the specific characteristics of the sludge to be used should be identified. Meanwhile, as the organics in the sludge may decay even at 4°C, proper methods to store the sludge should be used so that the variations of reactor performance caused by the influent fluctuation can be avoided (paper I and II).

3.2.2 Temperature

Temperature is one of the most important environmental parameters for anaerobic digestion. Biologically speaking, temperature determines if a certain kind of microorganism can survive or grow in the reactor and if they are living there with their highest activities. Practically speaking, higher temperature means high
consumption of energy. So, the choice of temperature and control of the level in question are of crucial significance for anaerobic digestion. At different temperature ranges, the microbial consortia are different. Anaerobic digestion can be carried out by anaerobic psychrophiles, mesophiles, thermophiles and extreme thermophiles, the optimum working temperature ranges of which are 20-25°C, 30-37°C, 50-55°C and above 65°C, respectively [Madigan et al. 2000]. A constant temperature is very important for a microbial consortium because once it has adapted to a certain temperature, it can tolerate a very small changes in temperature. Especially an increase of the temperature just above the optimum can soon lead to a drastic decrease of the growth rate of the microbes. Figure 3-3 shows the relations between temperature and growth rate for different bacteria consortia.

![Figure 3-3. Relationship between bacterial growth rate and temperature](Madigan et al. 2003)

When anaerobic digestion is carried out at thermophilic temperatures, many advantages such as higher conversion rate, better pathogen reduction effect, and shorter retention time are observed than when it is carried out at mesophilic temperature. But it is normally performed at temperatures close to the upper limit of some of the organisms involved in the process. This is why the process is especially sensitive to temperature fluctuations (Ahring, et al., 2001). Normally, the temperatures are kept below 55-60°C, which corresponds to the upper temperature limit of the thermophilic strains of *Methanosarcina* (Zinder et al. 1984; Sorensen 1996).
3.2.3 pH

Each group of microorganisms has their optimum pH range. Apart from the influence on the growth of microorganisms, pH can affect other factors such as dissociation of compounds (ammonia, sulfide, organic acids, and etc.) of great importance for the whole process of anaerobic digestion. pH in anaerobic digesters is mainly controlled by the bicarbonate buffer system (Rozzi, 1991; Pretoius, 1994). Therefore, pH in biogas plants depends on the partial pressure of CO₂ and the concentration of alkaline and acid components in the liquid phase. Experimental results from many studies shows that when temperature and retention time have been determined, the pH in a process fed with identical sludge will be stabilized at a certain value that benefits the dominant microorganisms (Eastman & Ferguson, 1981; Miron, et al., 2000). In wastewater treatment plant, the pH value in the anaerobic digestion reactor is normally in the range of 7.0-7.5, which is beneficial to the growth of methanogens.

3.2.4 Macro- and micronutrients

Macronutrients are the elements that the cellular material of the anaerobic microorganisms comprises, including hydrogen, nitrogen, oxygen, carbon, sulfur, phosphorus, potassium, calcium, magnesium and iron. Normally, anaerobic microorganisms require these elements presented with a concentration around 10⁻⁴ M. In addition to the micronutrients, a number of other elements, such as Ni and Co must be present in small amount, i.e. below 10⁻⁴ M. This is because that these elements are important for the growth of anaerobic organisms. For example, Ni is necessary for activating factor F₄₃₀, which is a co-factor involved in methanogenesis. But it can be inhibitory for fermentative as well as methanogens if it is present in high concentration. For anaerobic treatment of mixed waste, such as sewage sludge, it is often assumed that the necessary nutrients are available and in non-limiting amounts. However, at treatment of single waste or wastewater fraction, the degradation can be limited by the availability of nutrients. There are examples that supplementation of Ni and Co stimulates anaerobic process (Speece, 1983; Frostell, 1985).

3.2.5 Inhibition

3.2.5.1 Inhibition by ammonia
Ammonia (NH$_3$/NH$_4$) can be toxic to anaerobic digestion and the active component responsible for ammonia inhibition is the unionized form of ammonia, i.e. free NH$_3$. The free NH$_3$ concentration can be calculated by the following equilibrium equation:

$$[\text{NH}_3] = \frac{[\text{I-NH}_3]}{(1+[H^+]/k_A)} \quad (\text{Eq. 3-1})$$

where $[\text{NH}_3]$, $[\text{I-NH}_3]$ and $[H^+]$, are the free ammonia, total ammonia and proton concentration, respectively, and $k_A$ is the dissociation constant, which increases with temperature. So, ammonia inhibition is higher under high temperatures and high pH values. Among the microorganisms involved in the anaerobic process, methanogens are especially sensitive to ammonia inhibition. Thus, when an anaerobic process is inhibited by ammonia, the concentration of VFAs will increase and this will lead to a decrease of pH. The decrease of pH will partly counteract the effect of ammonia due to a decrease in the free ammonia concentration. This phenomenon is called “inhibited steady state”.

3.2.5.2 Inhibition by nitrogen oxides

It is reported that hydrogen-utilizing methanogens can be inhibited by nitrogen oxides, such as nitrate, nitrite and nitric oxide; both are present in natural marine sediments and in man-made environments with whole-cell of *Methanobacterium thermoautotrophicum* and *Methanobacterium formicicum* suspensions. And experimental results suggested that the inhibitory effect was not due to redox change or substrate competition, but due to the inhibition of the activity of some component of the methanogenic enzyme complex itself (Balderston & Payne, 1976). Hydrogen-utilizing methanogenesis is one of the major pathways in anaerobic digestion process, especially when anaerobic digestion is conducted at thermophilic temperatures, and acetate-utilizing methanogenesis is inhibited by elevated temperature, acetate oxidation followed by hydrogen-utilizing methanogenesis is the only way to convert acetate (Ahring, 2003). So, sewage sludge from a wastewater treatment plant without denitrification process may bring the danger of posing inhibition to the anaerobic
3.2.5.3 Inhibition by shock loading of substrates

A sudden increased load of proteins will result in formation of ammonia, so substrate may cause inhibition as well. Besides proteins, a sudden addition of lipids to the reactor can cause inhibition of the anaerobic process, since the hydrolytic, acidogenic and methanogenic bacteria can be inhibited by accumulation of long chain fatty acids produced during hydrolysis of lipids. Generally, it takes long period for the reactor to adapt to the overload of this kind of substrates. Another case of substrate inhibition is the shock overload of easily degradable substrates, which can be quickly hydrolyzed and fermented by the acidogens resulting in a sudden decrease in pH of the reactor and finally inhibit the methanogens.

3.2.6 Toxicity

Besides ammonia and nitrate/nitrite, heavy metals, such as Zn, Cu and Cd can be toxic to acidogenic bacteria [Ahiring & Westermann, 1983; Zinder et al. 1984]. However, many of these elements and compounds can be tolerated in relatively high concentration due to absorption in inert material contained in the reactor.

3.2.7 Retention time

For the CSTR reactors, which are the most prevailingly used types of reactors, hydraulic and solid retention time is the same. Retention time is an important operational parameter that is easy to operate and control. Tremendous efforts has been put into the research of the effect of retention time on anaerobic digestion (Bouzas, et al., 2002; Elefsiniotis & Oldham, 1994 a, b; Perot, et al., 1988; Zhang & Noike, 1994). Biologically, only those whose doubling time are shorter than the retention time can be kept in the reactor, so retention time is one of the best parameter to be manipulated for separating and enriching different groups of the microbes involved in the anaerobic process. Also, retention time determines the time that
substrates can be attacked by the enzymes in the reactor. In practice, longer HRT means bigger working volume of reactor and higher investment and operation costs.

### 3.2.8 Agitation strategy

It is normally believed that agitation is necessary to help the diffusion of substrate and increase their contacts with the microbes, especially when raw sludge is intermittently fed into the reactor. Agitation strategy can affect anaerobic digestion of sewage sludge and optimum agitation strategy should be found (Perot, et al., 1988). However, Banister & Pretorius (1998) found that, when using primary sludge for VFA production, vigorous mixing was not helpful. Also, a study carried out by Stroot, et al. (2001) using mixture wastes of organic fraction of municipal solid waste, primary sludge and secondary sludge show that continuous mixing was not necessary for good performance of the anaerobic process and was inhibitory at higher loading rates. In addition, it was also found that mixing levels might be used as an operational tool to stabilize unstable anaerobic reactor.

### 3.2.9 Feeding strategy

Practically, anaerobic reactors treating sewage sludge in wastewater treatment plants are fed semi-continuously instead of continuously. For example, in Lundtofte Wastewater Treatment Plant, the frequency of feeding of raw sludge to the reactor is once per hour. Feeding frequency determines the ratio of food to microbe (F/M) when the retention time and the working volume have been fixed. Normally, the ratio can be satisfied so that there is no negative effect on the stability and on the performance of the anaerobic reactors. However, when hygienic property of the digestate is considered, the minimum guaranteed retention time (MGRT) of the sludge in the reactor should be taken into consideration (Farrell, et al., 1988). Then, the F/M ratio and MGRT should be compromised by the adjustment of feeding frequency.

### 3.3 Optimization of anaerobic digestion for sewage sludge treatment

Even though anaerobic digestion is a good biological process and has a long history and a worldwide application in treating sewage sludge, this biotechnology is still far from being optimized. The organic material in sewage sludge is in the form of
particulates, and this makes hydrolysis of these particulates the obstacle of the whole anaerobic digestion (Ghosh, 1975; Eastman & Fergusson, 1981; Li & Noike, 1992). Meanwhile, some of the microbial groups involved are slowly growing and sensitive to changes in operating conditions and to variations of influent sludge composition and concentration, which are caused by the inhomogeneity of the nature of the sludge. These usually causes instability of the reactor performance (Huysman et al., 1983; Gijzen et al., 1988; Rozzi and DiPinto, 1994), leading to the decrease in biogas production. Consequently, only around 50% of organic material in sewage is degraded and converted to biogas by conventional anaerobic digestion process. Figure 3-4 shows an example from our lab experiment.

![Figure 3-4](image)

Figure 3-4. Schematic diagram of the conversion rate in each step and the final COD distribution of mixed sewage sludge after anaerobic digestion in a single-phase reactor (55°C, 15 days).

As it can be seen in Figure 3-4 (data adapted from our lab experiment), when the raw sludge, a mixture of primary sludge and secondary sludge with a solid concentration of 34.6g/l, was digested in the conventional single-phase thermophilic anaerobic reactor running at 55°C for a retention time of 15 days, the remaining solid concentration in the effluent was 20.5g/l. No accumulation of d-COD or VFA was found. The conversion rate of organic material from the form of particulates to hydrolysis products, measured as dissolved COD is only about half of the rate from hydrolysis products to acidogenesis products, measured as VFA, or the rate from acidogenesis products to methanogenesis products, measured as CH₄. So, there exists a large potential to optimize the process for exploitation of energy from sewage sludge. The interest in increasing the conversion of the organic material is further linked to
the reduction in the final amount of solid, which has to be disposed after the treatment (Ahring, 2003).

The other aspect to optimize the anaerobic digestion process is to make it possible to recycle the digestate, the effluent from anaerobic digestion process, back to the farmland. The digestate consists of an odor free residue with appearance similar to peat. The plant nutrients such as N, P and etc. are retained in the digestate after anaerobic digestion. Although the application of the digestate to farmland is the best final disposal option and also encouraged by the EU directives (EU, 2000), commitment to the relevant criteria can seldom be held by the conventional anaerobic digestion process. Besides xenobiotics and heavy metals, the concentrations of which in sewage sludge can be reduced by control at source (Jensen & Jepsen, 2005), pathogens are normally the most critical parameter limited by the criteria and the agriculture sector concerns very much about the risk of spreading disease. Studies have shown that anaerobic digestion can kill pathogens, especially when it is conducted at thermophilic temperatures (Bendixen 1999; Nielsen and Partersen, 2000). It will be significant if it is possible for sewage sludge to use farmland as a safe and permanent outlet destination with only positive effect, i.e., the digestate can be recycled as fertilizer and soil conditioner back to the farmland so as to keep these natural nutrients within a closed loop system, and remain or improve the soil structure of the farmland.

So, efforts must be made to enhance the hydrolysis and improve the process stability for biogas production and to eliminate the pathogens.
4. THERMOPHILIC ANAEROBIC PRE-TREATMENT

4.1 Summary of the pre-treatment methods

The pre-treatment methods that are most frequently cited in literature are summarized in Table 4-1.

<table>
<thead>
<tr>
<th>Method</th>
<th>Sludge</th>
<th>Improvements</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stirred ball-mill (22Wh/l)</td>
<td>WAS</td>
<td>23% hydrolysis, 14% increase in VS removal</td>
<td>Müller, 2004</td>
</tr>
<tr>
<td>Jetting-smashing (50bar, 5 times)</td>
<td>WAS</td>
<td>15% increase in VS removal</td>
<td>Choi et al., 1997</td>
</tr>
<tr>
<td>Ultrasound (40Hz, 375W/l, 1h)</td>
<td>WAS</td>
<td>20% in VS removal</td>
<td>Kim et al., 2003</td>
</tr>
<tr>
<td>Electron beam (0.5-1.0 kGy)</td>
<td>WAS</td>
<td>30-52% increase in hydrolysis and app. 90% in VFA, RT can be reduced from 20 days to 10 days when digestion in CSTR</td>
<td>Shin and Kang, 2003</td>
</tr>
<tr>
<td>Chemical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaOH (40 meq/l, 24 h)</td>
<td>WAS</td>
<td>36.3% hydrolysis of total COD</td>
<td>Chiu et al., 1997</td>
</tr>
<tr>
<td>Ozone (0.05g/g TS)</td>
<td>WAS</td>
<td>37% hydrolysis, 28% increase in VS removal</td>
<td>Goel et al., 2003</td>
</tr>
<tr>
<td>Thermal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low temperature</td>
<td>WAS</td>
<td>30-50% increase in biogas production</td>
<td>Hiraoka et al, 1984</td>
</tr>
<tr>
<td>High temperature</td>
<td>WAS</td>
<td>60% increase in VS removal, and 100% increase in biogas production</td>
<td>Li and Noke, 1992</td>
</tr>
<tr>
<td>Combined (Mech.+ chem., ther.+ chem.)</td>
<td>WAS</td>
<td>89% hydrolysis</td>
<td>Chiu et al., 1997</td>
</tr>
<tr>
<td>Ultrasound + NaOH (40meq/l, 375W, 24 h)</td>
<td>WAS</td>
<td>55% hydrolysis, 58% increase in CH₄ production</td>
<td>Haug et al., 1978</td>
</tr>
<tr>
<td>175°C+NaOH (30meq/l, 121°C, 1h)</td>
<td>WAS</td>
<td>7% increase in CH₄ production</td>
<td>Wang et al, 1999</td>
</tr>
<tr>
<td>Freezing and thawing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermophilic aerobic (70°C, 5 h)</td>
<td>WAS</td>
<td>30% VS removal, 150% increase in biogas production</td>
<td>Hasegawa et al., 2000</td>
</tr>
<tr>
<td>Micro-aeration (37°C, 4d)</td>
<td>PS</td>
<td>60% increase in hydrolysis</td>
<td>Johansen and Bakke, 2005</td>
</tr>
<tr>
<td>Anaerobic (25°C, 3d)</td>
<td>PS</td>
<td>13% increase in hydrolysis, 50% increase in VFA</td>
<td>Miron et al., 2000</td>
</tr>
<tr>
<td>Anaerobic (37°C, 3d)</td>
<td>PS</td>
<td>17% increase in hydrolysis, 64% increase in VFA</td>
<td>Eastman and Ferguson, 1981</td>
</tr>
<tr>
<td>Anaerobic (49.8°C, 3.1d)</td>
<td>WAS</td>
<td>20% increase in hydrolysis, 80% increase in VFA</td>
<td>Ghosh et al., 1995</td>
</tr>
</tbody>
</table>

Different methods have different mechanisms. Theoretically, mechanical disintegration provides energy necessary for the disruption of organic particulates or microbial cells by pressure, transactional or rotational energy, creating tensions on the surface of the particles or cells. Stirred ball mill disintegrates particles by crushing. High-pressure homogeniser disintegrates particles by cavitation effects due to sudden pressure release. Ultrasonic homogeniser leads to sludge disintegration by vibration. Mechanical jet technique has the similar principle as dissolved air flotation, by which
sludge stream is pressurized to 5-50 bars, the pressure is subsequently released across a nozzle and the sludge stream impinges on a splash plate. Electron beam uses electrical pulses to induce shock waves in solid and liquid media. When the power supply is switched on, the voltage across the electrodes is about several 10 kV. Eventually, it comes to a ‘breakthrough’ between the electrodes and thus breaks the organic materials. The disintegration of the organic particles will increase the surface area of the particles for the accessibility of the enzymes in the reactor. The disruption of cells will release the intercellular content that is more soluble.

Because the essence of hydrolysis is the decomposition of organic polymers involving the splitting of bond and the addition of the hydrogen cation and the hydroxide anion of water, addition of acids or alkalis can increase the concentration of these ions and thus increase the hydrolysis reaction rate. The aim of ozone pre-treatment is a partial oxidation and a hydrolysis of the organic matter. A complete oxidation is avoided and in stead larger molecules are cracked into smaller ones and hardly degradable compounds are transferred into more easily degradable ones.

Thermal pre-treatment is normally used to treatment waste activated sludge in the temperature range from 60-180°C. In this temperature range, the cell walls can be destroyed and this makes the proteins accessible for biological degradation. Because mechanical, chemical and thermal methods belong to different mechanisms, so these methods can be combined and different effects from each method can be obtained simultaneously.

By freezing and thawing activated sludge, the size of the floc structure will irreversibly reduced and the sludge will be more compact. This method is not commonly used, although it might be of potential application in the cold weather regions.

In biological pre-treatment, the rate of hydrolysis is a function of the source of hydrolytic enzymes, and thus the activity and population of the microbial population
in the reactor. Studies on the direct addition of enzymes, either by addition of complex enzymes or by addition of a mixture of carbohydrolases, peptidases and lipases, confirmed the enhancement of hydrolysis.

As it can be seen from Table 4-1, all of the pre-treatment methods have positive effect on the enhancement of hydrolysis and thus can improve the anaerobic digestion process. But we have to notice that due to the difference in energy input, dosage of chemical reagent, treatment time and the type of sludge with different solid concentrations used in different studies, the effects on hydrolysis enhancement, volatile solid removal and biogas production are not comparable in terms of treatment cost versus treatment efficiency. In general, mechanical, chemical, thermal and freezing and thawing methods need short treatment time, from minutes to hours. Compared with these pre-treatment methods, biological treatment methods need longer treatment time, from hours to days.

Normally, it is regarded that the input of energy for mechanical pre-treatment is lower than for thermal pre-treatment, but it needs investment of mechanical installations and costs for maintenance operation. Thermal pre-treatment uses heat energy, which is cheap and normally can be obtained from the burning of biogas on the treatment site. Since thermal pre-treatment at higher temperatures normally produces inhibitory substances to the anaerobic digestion process, temperatures lower than 100°C is normally used to pre-treat waste activated sludge. Chemical methods by addition of alkali and acids might be expensive and have to introduce foreign ions to the digestion system. Among the chemical methods, addition of ozone is the best, because it does not introduce foreign ions and only consume electricity for generating ozone. A significant advantage of biological anaerobic methods is the relatively higher production of volatile fatty acids than the other pre-treatment methods.

4.2. Rationale for conducting pre-treatment under thermophilic anaerobic conditions
4.2.1 Exploitation of the anaerobic thermophiles for hydrolysis and acidogenesis
Thermophilic anaerobic bacteria widely exist in natural and man-made environments, such as hot spring and food processing industry (Kristjansson and Stetter, 1992), and
can be sassily enriched. For some thermophilic anaerobic bacteria, they can not only hydrolyze the large spectrum of organic polymers such as cellulose, hemicellulose, proteins, pectin, and etc., but also carry out the fermentation of starch, pullulan, glucose and xylan to acids and etc. (Wiegel, 1992). These thermophiles have growth temperatures range from 20°C to 90°C, and most of them have the optimal temperature in the range from 60°C to 78°C. Table 4-2 lists some of the anaerobic thermophiles. Because their optimum temperature is far away from the normal temperature range, application of these microbes for waste treatment is seldom considered. Their roles in hydrolysis and further acidogenesis of waster organic material have not been brought into full play. If the hydrolysis and acidogenesis activities can be high enough, the pre-treatment step can be incorporated into the two-phase anaerobic digestion system severing as the acid-phase.

Table 4-2. Example thermophilic anaerobes

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate</th>
<th>$T_{\text{min}}$ (°C)</th>
<th>$T_{\text{opt}}$ (°C)</th>
<th>$T_{\text{max}}$ (°C)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dictyoglomus turgidus</td>
<td>Cellulose</td>
<td>50</td>
<td>72</td>
<td>80</td>
<td>5.2-9.0</td>
</tr>
<tr>
<td>Thermoanaerobacter cellulololysis</td>
<td>Cellulose</td>
<td>37</td>
<td>75</td>
<td>80</td>
<td>5.4-8.9</td>
</tr>
<tr>
<td>Caldocellum saccharolyticum</td>
<td>Cellulose</td>
<td>----</td>
<td>68</td>
<td>75</td>
<td>5.0-7.3</td>
</tr>
<tr>
<td>C. thermocellum</td>
<td>Xylan</td>
<td>----</td>
<td>60</td>
<td>68</td>
<td>5.7</td>
</tr>
<tr>
<td>Clostridium fervidus</td>
<td>Xylan</td>
<td>37</td>
<td>68</td>
<td>78</td>
<td>5.5-9.0</td>
</tr>
<tr>
<td>C. thermosulfurogenes</td>
<td>Starch</td>
<td>----</td>
<td>60</td>
<td>75</td>
<td>6.0</td>
</tr>
<tr>
<td>Dictyoglomus turgidus</td>
<td>Pectin</td>
<td>50</td>
<td>72</td>
<td>86</td>
<td>5.2-9.0</td>
</tr>
<tr>
<td>D. thermophilum</td>
<td>Pectin</td>
<td>----</td>
<td>78</td>
<td>80</td>
<td>7.0</td>
</tr>
<tr>
<td>Clostridium thermodolacticu</td>
<td>Pectin</td>
<td>45</td>
<td>60</td>
<td>70</td>
<td>6.4-7.8</td>
</tr>
<tr>
<td>Thermobacteroides proteolyticus</td>
<td>Protein</td>
<td>35</td>
<td>63</td>
<td>75</td>
<td>5.0-8.5</td>
</tr>
<tr>
<td>T. leptospartum</td>
<td>Protein</td>
<td>----</td>
<td>60</td>
<td>71</td>
<td>7.5</td>
</tr>
<tr>
<td>Clostridium thermodolacticu</td>
<td>Glucose</td>
<td>50</td>
<td>65</td>
<td>75</td>
<td>7.2</td>
</tr>
<tr>
<td>C. fervidus</td>
<td>Pentose</td>
<td>----</td>
<td>68</td>
<td>80</td>
<td>7.3</td>
</tr>
</tbody>
</table>


4.2.2 Simultaneous achievement of thermal pre-treatment effects

Because thermal pre-treatment and biological pre-treatment belong to different mechanisms, in the temperature range of 60 °C to 78 °C, the thermal effects on hydrolysis improvement can be obtained simultaneously, as it has been reported that when waste activated sludge is thermally pre-treated at 60°C to 80°C, both of the organic matter destruction rate and methane generation rate can be increased

4.2.3 Simultaneous achievement of pasteurization
In particular, it is expected that, when pre-treatment is conducted at thermophilic temperatures, pasteurization of the digestate will be obtained simultaneously. Pasteurization means the process, treatment, or combination thereof, that can reduce the most resistant microorganism(s) of public health significant to a level that is not likely to present a public health risk under normal conditions of distribution and storage (USA National Advisory Committee on Microbiological Criteria for Foods). Normally, it needs 7 mins at 70°C, 30 mins at 65°C, 2 hours at 60°C, 15 hours at 55°C and 3 days at 50°C (Strauch, 1991 and 1998). Studies using simple media under defined condition have shown that most of the pathogenic bacteria can be inactivated at temperatures in excess of 70°C over a relatively short period of time or lower temperatures over longer time periods (Carrington, 2001). It was expected that satisfactory pathogen reduction effect in the complex media, sewage sludge, could also be obtained, because pathogen inactivation is in principle due to heat and the holding time.

4.2.4 Easy for operation
This pre-treatment process can be easy for operation. Since the process temperature is lower than 100°C, it is not necessary to make the pre-treatment reactor as a pressure vessel. The odor problem can be eliminated since there is no need for aeration, and thus it can be conducted in a closed system. If the sludge has been pasteurized by this pre-treatment step, it will be convenient for the next digestion step because of no fear of pathogenic contamination.

4.2.5 Internal-supplied energy for heating
This pre-treatment process can be economically feasible. In many wastewater treatment plants, for example, the Lundtofte Wastewater treatment plant in Denmark, the heat from CHP (combined heat and power) unit burning the biogas produced from the sewage sludge treatment process is surplus after supplying to the local heating system. Heat needed for elevating the sludge to thermophilic temperature can be, at least partially, provided by the heat from the excess heat. Especially when heat
exchanger is installed, large percent of the heat can be recovered (Zupancic and Ros, 2003), so this pre-treatment method is not highly dependent on external energy or chemical, as the other pre-treatment methods have to demand.

4.3 Determination of optimal temperature and retention time

4.3.1 Rationale for determination of optimal temperature and retention time

Temperature and retention time are important environmental and operation parameters. In the studies regarding two-phase anaerobic digestion of different wastes including manure, agricultural waste and sludge, different temperature and retention time combinations, such as 68°C/3days, 60-70°C/4.3days, 60°C/3hours, 55°C/4hours, have been used for the pre-treatment or acid phase operation (Nielsen, et al., 2003; Scherer, et al., 2000; Roberts, et al., 1999). However, the main purposes of the pre-treatment used in these studies were to increase organic waste degradation and thus to generate more biogas. The optimization of temperature and retention time has never been considered, or considered without taking pasteurization into consideration.

As we have discussed previously, temperature and retention time are directly related to the microbiology, determination of reactor size and operational cost of the anaerobic process, so optimum combination of temperature and retention time should be found, i.e., under the condition of which, values of temperature and retention time are kept as low as possible whereas relatively high rates of hydrolysis, acidogenesis and satisfied pasteurization are obtained at the same time.

4.3.2 Matrix of temperature and retention time combinations

Temperature and retention time are dynamically related to each other with respect to the effects of hydrolysis, acidogenesis and pasteurization. For example, when a strain of a certain kind of fermentative bacteria are not growing at their optimum temperature, by extending the fermentation time, the same amount of fermentative products can also be obtained as when they are growing at the optimum temperature for a relatively shorter period of fermentation time. It is the same for pasteurization. The outcome of 2 hours at 60°C is the same as 15 hours at 55°C (Carrington, 2001). So, there exists a matrix of temperature and retention time combinations that regulates the effects of thermophilic anaerobic pre-treatment. The following matrix of combinations was carried out in our study:
\[
\begin{align*}
&\{t_1, r_1, t_2, r_2, t_3, r_3, t_4, r_4, t_5, r_5, t_6, r_6\} \\
&\{t_1, r_1, t_2, r_2, t_3, r_3, t_4, r_4, t_5, r_5, t_6, r_6\} \\
&\{t_1, r_1, t_2, r_2, t_3, r_3, t_4, r_4, t_5, r_5, t_6, r_6\} \\
&\{t_1, r_1, t_2, r_2, t_3, r_3, t_4, r_4, t_5, r_5, t_6, r_6\} \\
&\{t_1, r_1, t_2, r_2, t_3, r_3, t_4, r_4, t_5, r_5, t_6, r_6\} \\
\end{align*}
\]

(Equation 4-1)

Where \(t_1, t_2, t_3, t_4, t_5, t_6\) equals to 55\(^\circ\)C, 60 \(^\circ\)C, 65 \(^\circ\)C, 70 \(^\circ\)C, 75 \(^\circ\)C and 80 \(^\circ\)C, respectively, and \(r_1, r_2, r_3, r_4, r_5, r_6\) equals to 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 days, respectively. The selected temperature and retention time spans are considered to include most of the optimum growth temperatures and the doubling times of the thermophilic bacteria.

4.3.3 Experiments

In the experiments carried out by Hiraoka, et al. (1984) and Wang, et al. (1997) for studying the effect of thermal pre-treatment, biological effects on the hydrolysis were ignored, so there was no need of inoculation. The inoculum used in our experiments was taken from a continuously stirred tank reactor running at 55 \(^\circ\)C for a RT of 15 days, with feeding of sewage sludge as substrate for more than one year. It was assumed that thermophilic bacteria that could be enriched and active in participating the hydrolysis and acidogenesis of the organics in the sludge were included in the inoculum or could be obtained from the influent sludge.

After significant biological activities were observed in all of the simplified reactors, the cultivated inoculum were transferred into the corresponding reactors which were installed with gas and liquid sampling, agitation, and the influent feeding, effluent wasting, biogas counting apparatuses, which were automatically controlled by computer system installed with software called Lab-view. The reactors running at respective temperatures were started with RT of 3.0 days, and then step-wisely shifted to 2.5, 2.0, 1.5, 1.0 and 0.5 days, respectively. For each of the retention times, the operation period lasted for at least 3 times of the retention time to make sure that the
steady stage of the reactor at the retention in question was reached. At the steady stage of each of the retention times, reactor performance was monitored in terms of pH, hydrolysis production measured as soluble COD, acidogenesis production measured as VFA, and methanogenesis production for which CH\(_4\) and H\(_2\) production were measured.

To evaluate pathogen reduction effect (PRE) of each of the reactor runs, attention was paid to the temperature and minimum guaranteed retention time (MGRT), to both of which PRE is only related in this circumstance. MGRT is determined by feeding interval, and therefore, feeding frequency is of great importance for the PRE of a reactor running at a certain temperature (Huyard, et al., 2000). Theoretically, for better PRE, the feeding frequency should be kept as low as possible. However, in practice, the ratio of food to microorganism (F/M) should be taken into consideration as well. When RT is fixed, reduction of feeding frequency will lead to the increase of F/M value, resulting in the breakdown of the biological system in the reactor. On the other hand, feeding frequency is also related to the frequency of switch-on and switch-off of the influent feeding and effluent wasting pumps and the size of influent and effluent storage tanks. In this study, PRE was examined when feeding frequency was set at once per hour for all RTs tested.

4.3.4 The optimal combination of temperature and retention time
As we have discussed, the purpose of conducting pre-treatment under thermophilic anaerobic conditions was to obtain both enhanced hydrolysis and satisfactory pathogen reduction so that sewage sludge can be better stabilized, more biogas produced and the digested sludge recycled to the farmland without fear of epidemic diseases. And therefore, the choice of temperature and RT should guarantee the total elimination of pathogens and high concentration of s-COD. In addition, since the present of VFA in the influent can increase the microbial activity and the thus improve the performance of the next step AD reactor (Zhang and Noike, 1991; Skiadas et al., 2005; Lu and Ahring, 2006), higher VFA concentration in the pre-treated sludge is preferred. Besides, since methanogenesis consumes VFA, it should be avoided. So, the hierarchy adopted here in determining the suitable temperature and RT for thermophilic anaerobic pre-treatment follows the order of a thorough
elimination of pathogens, a higher s-COD concentration, a higher VFA concentration and finally a low s-COD loss to the gas phase.

Since experimental results show that the highest values for s-COD concentration, VFA concentration and pathogen reduction effect and lowest value for s-COD loss were not obtained at the same temperature and RT combination, only a range of the most suitable temperature and RT for thermophilic anaerobic pre-treatment was actually able to be determined from this study. We found that for pathogen reduction, only when temperature is higher than 70°C and RT longer than 2 day, or then temperature higher than 75°C and RT longer than 0.5 days, can the indicator organism be totally eliminated. Relatively higher d-COD concentration (not lower than 85% of the highest value) can be obtained at temperatures of 65°C, 70°C 75°C and 80°C when RT in the range of 2.5, 1.5, 1.0 and 0.5 to 3.0 days, respectively. Relatively high VFA concentration (not lower than 85% of the highest value) can be obtained in the temperature range of 60°C, 65°C and 70°C when RT was set in the range of 2.5, 2.5 and 2.0 to 3.0 days, respectively. To limit biogas production (lower than 10% of the total s-COD produced), at 55°C, 60°C and 65°C, RT should be shorter than 0.5, 2.0 and 2.5 day, respectively. Figure 4-1 shows the compromised boundary for choosing temperature and RT to conduct a satisfactory pre-treatment. By using linear equation it can be calculated that, if temperature and RT is chosen within the boundary, s-COD concentration will be in the range of 12.1 to 14.1g/l, corresponding that 29.0% to 33.8% of the solids can be hydrolyzed in terms of COD, and 47.1% to 70.2% of the hydrolysis products are in the form of VFA.

Figure 4-1 The optimal temperature and RT boundary for thermophilic anaerobic treatment
In practice, the choice should keep temperature and RT values as low as possible so as to reduce the operation cost and to reduce the reactor volume, for example, setting temperature at 73°C and RT for 2.0 days, so that operation energy (heat) and reactor volume can be kept as low and small as possible.

4.4 Differentiation of the effects on the hydrolysis the organic particulates
To investigate further the mechanisms involved in the hydrolysis of organic particulates in primary and waste activated sludge thermophilic anaerobic pre-treatment, a study on distinguishing different effect mechanisms was carried out (see Paper III).

4.4.1 The different characteristics of primary sludge and waste activated sludge
The sewage sludge used in the study thermophilic anaerobic pre-treatment was the mixture of primary sludge and waste activated sludge accounting for about 71% and 29% of the total VS, respectively. Normally, these two types of sludge in wastewater treatment plant are anaerobically treated together. For the wastewater treatment plants where only primary sludge is treated at mesophilic temperatures, and waste activated sludge is not anaerobically treated, with the prevailing shift of operation at thermophilic temperatures, enough space will be provided for the treatment of secondary sludge (Nielsen & Petersen, 2000). Although primary sludge and waste activated sludge are digested together in practice and also in this study the mixture of these two types of sludge was used, it has to be kept in mind that the main contents in these two types of sludge are very different. The particulate constituents in primary sludge are food scraps, excrement, paper and cellulose (nappy liners, etc.) that normally present in raw sewage. Waste activated sludge is mainly composed of microorganisms and other life forms with small proportions of adsorbed suspended solids and colloids derived form the sewage (Mudrack & Knust, 1986). So, in the study of the mechanisms of thermophilic anaerobic pre-treatment involved in hydrolysis and acidogenesis, these two types of sludge were separated. As it can be seen from Table 4-3, the characteristics of the primary sludge and waste activated sludge, especially the chemical composition in terms of proteins, lipids and carbohydrates are quite different.

4.4.2 Experiments
Two series of continuous experiments were carried out. In Series I, three completely stirred tank reactors running at 60, 70 and 80°C, respectively, were fed with primary sludge as feedstock. In Series II, another three completely stirred tank reactors running also at 60, 70 and 80°C, respectively, were fed with waste activated sludge as feedstock. The RT for all of the six reactors was set at 2.0 days. When reactors approached their steady stages, three representative samples were taken at reasonable intervals and used for analysis of pH, VFA, d-COD, VSS, lipids, proteins. Content of carbohydrates was obtained by subtracting lipids and proteins from VSS. Biogas production for each reactor runs was counted, and the content of methane and hydrogen in the biogas was also monitored.

Table 4-3 Characteristics of the primary sludge and waste activated sludge

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Primary sludge</th>
<th>Waste activated sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Components in solid form</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSS</td>
<td>g/l</td>
<td>24.54 ± 0.22</td>
<td>17.06 ± 0.70</td>
</tr>
<tr>
<td>VSS</td>
<td>g/l</td>
<td>17.39 ± 0.17</td>
<td>9.61 ± 0.35</td>
</tr>
<tr>
<td>Lipids(^{b})</td>
<td>g/l</td>
<td>3.85 ± 0.27</td>
<td>0.44 ± 0.10</td>
</tr>
<tr>
<td>Proteins(^{b})</td>
<td>g/l</td>
<td>3.15 ± 0.06</td>
<td>5.27 ± 0.12</td>
</tr>
<tr>
<td>Carbohydrates(^{c})</td>
<td>g/l</td>
<td>10.39 ± 0.21</td>
<td>1.46 ± 0.14</td>
</tr>
<tr>
<td>COD</td>
<td>g/l</td>
<td>27.91 ± 1.25</td>
<td>14.09 ± 0.86</td>
</tr>
<tr>
<td>Components in soluble form</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COD</td>
<td>g/l</td>
<td>1.02 ± 0.03</td>
<td>0.64 ± 0.04</td>
</tr>
<tr>
<td>VFA</td>
<td>g/l</td>
<td>0.55 ± 0.01</td>
<td>0.23 ± 0.01</td>
</tr>
</tbody>
</table>

a. Values are average of three measurements with standard deviation; b. Measurements carried out after samples were centrifuged and the solid fraction was washed twice and re-suspended to the original volume; c. Value obtained by calculation according to the assumption that VSS is composed of lipids, proteins and carbohydrates; d. The sum of acetate, propionate, n-butyrate and n-valerate.

The inoculum used for the batch experiment was taken from the above-mentioned reactors during their steady stages. The initial substrate concentration (S\(_o\)) and the initial biomass (X\(_o\)) concentration have a significant effect on the biological reaction rate (Moreno, et al., 1999). The higher the S\(_o\)/X\(_o\) ratio is, the higher the reaction rate will be, and vice versa (German, 2002). In this study, the initial ratio of substrate to microbial biomass, i.e., S\(_o\)/X\(_o\) was set at 3:1. This is because the ratios higher or lower than this resulted in either unobvious biological effect or the biological evolution was too fast to follow. To differentiate the biological effect and the thermal effect, interferences from the microbial activities in the raw sludge and the biomass in the inocula sludge had to be eliminated. This was done by using 3 groups of controls, i.e.,
the inocula, the raw sludge and the raw sludge treated by NaN₃ that inhibited the microbial activities in the raw sludge (Slanetz & Bartley, 1957). To avoid drawbacks that batch experiments usually encountered such as adherence of organic solid on the vial wall, impossibility of organic solids to be sampled together with the liquid by syringe with small-sized needles, and etc., the ‘multiple flask’ method (Sanders, 2002) was followed in this study. The sampled vials were quenched in a –20°C freezer and analyzed in the end of the experiment.

4.4.3 Differentiation of the biological and thermal effects
As shown in Figure 4-1, experiment results confirmed that both biological and thermal effects contribute to the hydrolysis of organic particulates. For waste activated sludge, the additional mechanism, thermal lysis of microbial cell, was also proved.

For the hydrolysis of different types of organic compounds, i.e. lipids, proteins and carbohydrates, their dependency on thermal effect and biological effect were different. The hydrolysis of lipids and proteins was more dependent on biological activity than the hydrolysis of carbohydrates, while the hydrolysis of carbohydrates was more dependent on thermal effect than lipids and proteins. For both of primary sludge and waste activated sludge, at 60°C and 70°C, i.e., at the temperatures of which microbial activity was high, larger percent of lipids and proteins were hydrolyzed than at 80°C. For primary sludge, at 80°C, the biological effect is negligible, and high percent of carbohydrates are hydrolyzed by thermal effect. For waste activated sludge, at 80°C, thermal lysis of microbial cell was the dominant mechanism resulting in the high degradation rate of proteins released from the microbial cells.

In differentiating the contributions to hydrolysis from biological effect and thermal effect, batch experiment for a period of 72 hours shown that, for the pre-treatment of primary sludge, an increase of 49.5% and 48.32% of hydrolysis product could be obtained due to biological activity at 60°C and 70°C, respectively. At 60°C, 33.1% of the hydrolysate was caused by biological activity and the rest, 69.9%, was caused by thermal effect. At 70°C, 32.6% was caused by biological activity, and 67.4% is caused by thermal effect.
For the pre-treatment of waste activated sludge, an increase of 50.7% and 46.0% of hydrolysis product could be obtained due to biological activity at 60°C and 70°C, respectively. At 60°C, 33.6% of the hydrolysate was caused by biological activity and the rest, 66.4%, was caused by thermal effect. At 70°C, 31.5% was caused by biological activity, and 68.5% was caused by thermal effect.

At 80°C, for the pre-treatment of both primary sludge and waste activated sludge, almost all of the hydrolysis is caused by thermal effect. Biological effect is negligible. The week biological effect might be due to the failure in enriching the microbes that can grow at this high temperature or might be the microbes were enriched, but with low activity at this high temperature. This temperature was recommended as the upper limit for thermophilic anaerobic pre-treatment for sewage sludge.
5. TOW-PHASE ANAEROBIC DIGESTION

5.1 Theoretical background of two-phase AD process

Conventionally, AD is conducted in a single-phase reactor that must be operated at conditions conducive to the growth of all the microorganisms involved in the whole process if the waste organic material should be stabilized. However, the physiology, nutrient needs, growth kinetics and sensitivity to environmental conditions of hydrolytic-fermentative bacteria and methanogens are different, so it is not possible to select a single set of reactor operating conditions that can maximize the growth of both groups of the microorganisms (Demirel & Yenigün, 2002). For example, acidogenic bacteria are limited to a pH interval from approximately 5.2-6.5 and a minimum doubling time of 2.0 days, while methanogens often have an optimum pH of 7.5-8.5 and a minimum doubling time longer than 3.6 days (Solera, et al, 2002). So, conditions that are favorable to the growth of the hydrolytic-fermentative bacteria such as short hydraulic retention time, acidic pH and increased temperature, are inhibitory to the methanogens. Consequently, single-phase reactors are usually operated at relatively long HRTs, neutral or slightly basic pH and temperatures just below the optimal temperature (methanogenic activities will be drastically dropped when temperature is higher the optimum (Ahring, 2003; Madigan, et al., 2003)). But even by doing so, single-phase AD process is still subjected to instability due to changes of temperatures and inadvertent organic, hydraulic or toxic overloads caused by short-term variations in waste flows or characteristics (Henry, et al., 1987). Studies carried out on the stability of biogas plants in Denmark also showed that cases with high fluctuation of VFA level could usually be linked to specific events such as temperature instability or abrupt changes in substrate composition. (Angelidaki, et al., 2005).

To optimize AD process, two-phase system, in which the whole AD process is artificially separated into acid-phase and methane-phase, has been studied since 1970s (Pohland & Ghosh, 1971). The most obvious advantage of the two-phase process is the possible selection and enrichment for hydrolytic-fermentative bacteria and methanogens in each reactor by independent control of the reactor operating conditions. Thus, the acid phase can be optimized for hydrolytic-fermentative bacteria and the methane phase for methanogens. And therefore, higher microbial population...
levels (Zhang & Noike, 1990) and increased activities (Skiadas, et al., 2005) can be achieved.

In this study, the thermophilic anaerobic pre-treatment was engrafted into the two-phase AD process. The pre-treatment reactor was run at 73°C for a RT of 2 days so that the compromised enhancements on hydrolysis, acidogenesis and pathogen reduction effect could be kept, as has been discussed in the last chapter. For the methane reactor, the operation temperature was set at thermophilic mode because it has been testified that thermophilic AD has many advantages over the mesophilic AD (Ahring, 1994, 2003, Ahring et al., 2002). The optimum temperature for the aceticlastic methanogens is around 60°C, above which the activity of the methanogens will drastically drop. So, for security reason, the temperature for thermophilic AD in practice is usually set at 55°C, just below the optimum temperature (Ahring, 2003). Due to the enhanced stabilization rate at thermophilic temperatures, RT for thermophilic AD is normally set at 15 days, which is half of what the mesophilic AD needed (Hamer, et al., 1985; Nielsen & Petersen, 2000).

In order to investigate the advantages of the two-phase process over the single-phase process, a single-phase process under the conditions of 55°C and RT equal to the total RT of the two-phase process was run in parallel with the two-phase process.

5.2 Comparison of the two-phase process with the single-phase process

5.2.1 Operation at normal RT

Comparable performance of the two methane reactors, before one of them used in the two-phase process was connected to the acid-phase reactor, was obtained by using the same start-up strategy described by Ahring (2003) and by exchanging the biomass between these two methane reactors. After the pre-treatment reactor had been strategically connected with the second-phase methane reactor, the superiority of the two-phase process over the single-phase process was displayed. As it had been expected, the two-phase process could exert the satisfactory pathogen reduction effect, while 350 of Faecal Streptococci, the microorganism indicator used in this study, was monitored in one milliliter of the effluent of the single-phase process. The VS reduction rate and CH₄ production rate of the two-phase process was 4.48% and
11.66% higher than those of the single-phase process, respectively. The reactor performance of the single-phase process and the two-phase process is shown in Table 5-1.

Table 5-1. Comparison of the operation and performance of the single- and two-phase process

<table>
<thead>
<tr>
<th>System</th>
<th>Single-phase</th>
<th>Two-phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactor</td>
<td>R1</td>
<td>R2-1</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>55</td>
<td>73</td>
</tr>
<tr>
<td>RT (day)</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>pH</td>
<td>7.66&lt;sup&gt;a&lt;/sup&gt; (0.07&lt;sup&gt;b&lt;/sup&gt;, 15&lt;sup&gt;c&lt;/sup&gt;)</td>
<td>5.68 (0.06, 15)</td>
</tr>
<tr>
<td>VFA (mg-COD/l)</td>
<td>664 (121, 15)</td>
<td>7972 (174, 15)</td>
</tr>
<tr>
<td>FS (CFU/ml)</td>
<td>350 (28, 5)</td>
<td>0</td>
</tr>
<tr>
<td>CH&lt;sub&gt;4&lt;/sub&gt; in biogas (%)</td>
<td>61.6 (2.1, 10)</td>
<td>3.3 (0.6, 15)</td>
</tr>
<tr>
<td>Methane production (ml/d)</td>
<td>2333 (205, 15)</td>
<td>68 (15, 15)</td>
</tr>
<tr>
<td>VS removal rate (%)</td>
<td>60.49 (2.4, 5)</td>
<td>Not measured</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean value; <sup>b</sup> Standard deviation; <sup>c</sup> Number of measurements.

5.2.2 Operation at reduced RT

As illustrated in Table 5-1 and in Figure 5-1, the VFA concentration in the effluent of the two-phase process was much lower than that of the single-phase process. The former was only 32% of the latter, indicating that the two-phase process could convert VFA to a more complete level. When observing the methane production during one circle of feeding to the methane reactors, it was found that the methane production rate in the beginning period was much faster than in the latter period for the two-phase process in comparison with the single-phase process.
As it can be seen in Figure 5-2, it only took about 3.2 hours for the two-phase process to produce the same amount of CH$_4$ as what the single-phase process produced in 6 hours. From these phenomena, it can be deduced that the acetogenic and the methanogenic activities in the methane reactor of the two-phase process were higher than those in reactor of the single-phase process, and the enhanced activities in the two-phase process had been far from being brought into full play. Also, it was demonstrated that the methane potential of the effluent of two-phase process was very low. Around 66.42% of the total COD in the influent sludge had been removed by the two-phase process for a total RT of 17 days. Batch experiment showed that it was difficult to further increase the removal rate, even by alkali-thermal treatment, as it is shown in Figure 5-3. So, effort was exerted to test the possibility to reduce the RT of the methane reactor of the two-phase process to 13 days, 11 days, 9 days, 7 days and 5 days with the RT of the pre-treatment reactor being constant at 2 days. For comparison reasons, the RT of the reactor in the single-phase process was correspondingly set at 15 days, 13 days, 11 days, 9 days and 7 days, respectively.

As it is shown in Figure 5-4, when running at each of the above-mentioned corresponding RTs, the VS reduction rate of the two-phase process was always higher than that of the single-phase process. Even when the two-phase process was run at the total RT of 9 days, i.e., 2 days for the pre-treatment reactor and 7 days for the methane reactor, its VS degradation rate was still as high as 60.61%. This result is still
comparable to that of the single-phase process when running at RT of 15 days, which is 60.54%.

![Figure 5-4. Methane production and VS reduction of the single-phase and the two-phase process running at different RTs (The methane production and VS reduction in the pre-treatment reactor of the two-step process are ignored).](image)

### 5.2.3 Perturbation tests

From Figure 5-1, it can be noticed that the variation of biogas production and VFA concentration of the two-phase process was smaller than that of the single-phase process, indicating that the two-phase process was more stable than the single-phase process. To test the process stability, sodium nitrate was added in the influent sludge to a concentration of 0.1 M to inhibit the methanogens (Balderston & Payne, 1976) when both of the single-phase process and the two-phase process were run for RT of 9 days (for the two-phase process, RT for R2-1 and R2-2 was set at 2 days and 7 days, respectively). It can be seen from Figure 5-5a that the dosage of NaNO₃ caused severe instability to the single-phase reactor. This was indicated by the sudden drop of CH₄ production and increase of VFA accumulation. This instability lasted for about 20 days. Stability the two-phase process also suffered negative effect.

Reduced production of VFA in R2-1 and slight VFA accumulation and CH₄ drop in R2-2 were also noticed. However, this slight instability in the two-phase lasted only for about 5 days. In the single-phase process, methanogens were directly exposed to the toxic substance added, so they were immediately inhibited.
The consequence of the inhibition might cause the accumulation of VFA and drop of pH, so the condition for methanogens might become even worse. This is why the instability lasted for a longer period. In the two-phase process, however, the toxic substance first contacted with the biomass in the pre-treatment reactor. The acidogens are known to be robust and in the pre-treatment reactor de-nitrification reaction might be carried out as shown in Equation 5-1.

\[ C_{x}H_{y}O_{z} + 4NO_3^- \rightarrow 6CO_2 + 5H_2O + 2N_2 + \text{energy} \quad (\text{Eq. 5-1}) \]

So, the inhibition of nitrate was eased off before it would have affected the methanogens in the methane reactor. Due to the high methanogenic activity in the methane reactor, the accumulated VFA was quickly turned over. So, no further damage to the process stability was caused.

In the AD system, a precious balance between the hydrolyzing-fermenting bacteria and the methanogens are needed (Ahring, 2003). A sudden inadvertent overloading of
easily degradable substrate may cause a sudden increase of VFA and results in the break down of the stable stage of the AD process (Westermann, 2003). In this study, when glucose was added to double the organic loading rate of these two processes, different results were found, as shown in Figure 5b. For the single-phase process, CH$_4$ production increased in the beginning and then decreased. This can be explained by the fact that accumulation of VFA led to the drop of pH and then caused inhibition on the methanogens. On the contrary, the two-phase process could turn over almost all of the added glucose producing more CH$_4$. No severe disturbance was observed. This again illustrated the enhanced methanogenic activity due to phase separation.

5.3 Summary of two-phase anaerobic digestion process

From this study, it can be concluded that two-phase process is superior to the conventional single-phase process.

The effluent from two-phase process is hygienically satisfied and could be used as fertilizer and soil conditioner in the farmland without any fear of spreading diseases, while the effluent from the single-phase process has to undergo special sanitation treatment before application on farmland or has to go into other final disposal streams such as landfill, incineration, and etc that might be costly.

The two-phase process is a high rate process. If RT is set for the same period, more VS can be degraded and more biogas can be produced by the two-phase process than the single-phase process. To achieve the comparable methane production rate and VS reduction rate, the two-phase process needed shorter RT than the single-phase system. When RT of the two-phase process was set for 9 days, of which 2 days was used for the pre-treatment reactor and 7 days for the methane reactor, the VS reduction rate was as high as 60.61%, which was still comparable to that of the single-phase process when RT was set at 15 days.

Process stability can be greatly enhanced by phase separation. Perturbation test using pulse dosage of sodium nitrate and glucose demonstrated that two-phase process could buffer the shock overload of inhibitory substance and easily degradable substrate, while the single-phase was broken down by the fluctuations coming from the influent.
Even though the two-phase process may cost more energy and capital in construction and operation of whole AD system, considering the smaller volume of reactor needed due to the reduction of RT, the valuable application of its effluent on farmland and the elimination of the cost on effluent disposal, the two-phase process is still attractive.
6. CONCLUSIONS

In this study, it was confirmed that enhancement of hydrolysis of the organic particulates in the sludge, high degree of acidification of the hydrolysis products and achievement of satisfactory pathogen reduction effect were obtained by pre-treatment under thermophilic anaerobic conditions simultaneously.

Thermophilic anaerobic pre-treatment running at the identified optimal condition, i.e., at the temperature of 73°C for a retention time of 2 days, was employed as the acid-phase of the two-phase anaerobic digestion system. By running a two-phase CSTR process (73°C/55°C) with a parallel single-phase CSTR process (55°C) as control, it was verified that the two-phase process could keep not only the satisfactory pathogen reduction effect that the single-phase process couldn’t achieve, but also possessed superiorities over the single-phase process such as increased efficiency in converting waste organic material into biogas and enhanced process stability due to the effect of pre-treatment. Microbial activities of the two-phase process were higher than those of the single-phase process. To help the implementation of the two-phase process in to practical application for sewage sludge treatment, it was suggested that proper start-up and operation strategies should be used.

It was concluded from this study that thermophilic anaerobic pre-treatment can be used to optimize anaerobic digestion process for sewage treatment. The optimized two-phase process is high-rate, efficient and cost-effective, and possesses the capability to eliminate the pathogens. The significance of implementing the optimized two-phase process lies in the following aspects:

5. The environmental problem caused by sewage sludge, which is a global one and getting more and more severe, can be solved in a sustainable way;
6. The energy saved in the organic material of the sewage sludge can be extracted in the form of biogas, which is CO₂ neutral and renewable, and can be used to produce electricity and heat;
7. The thorough elimination of pathogens makes it possible to recycle the plant nutrients and inert organic material in the digested effluent back to the farmland as fertilizer and soil conditioner without fear of spreading of epidemic disease;

8. The heat needed to keep the process temperature can be obtained by burning the biogas produced by the process itself, so there is no dependency on the external energy supply.

For future studies, it is suggested that identification of the composition of the microbes in both the pre-treatment reactor and the methane reactor should be focused. Besides temperature, RT and feeding frequency, other factors such as organic solid concentration, reactor agitation and start-up strategy should be further studied as well.


