Wet and Dry Anaerobic Digestion of Biowaste and of Co-substrates

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Abstract

The increase of the population and the economic development throughout the world has also brought about an unexpected increase of the energy demand and of the amount of solid wastes. Energy is the basis of social advancement and economic development. Human comfortable life is depending on advancing science and technology. The presently fast advancing lifestyle in developed and developing countries is particularly associated with an increased energy demand. The social and economic development has also brought a problem of municipal solid waste generation and management. Treatment of municipal solid waste by anaerobic digestion can solve the environmental problems caused by this organic solid waste and also supply biogas as renewable energy for a sustainable development. Biowaste can be processed into a suspension, containing a high proportion of biodegraable substances by addition of process water and the suspension treated in wet anaerobic digestion (total solid content: TS <15%). Alternatively biowaste can directly be treated by dry anaerobic digestion (TS >15%). In this study the improvement of wet anaerobic digestion by addition of co-substrates and the effect of moisture on dry anaerobic digestion were investigated.

In order to find out appropriate co-substrates for improvement of wet anaerobic digestion of biowaste, characteristics and biogas production potential of some potential substrates, as well as the performance of co-digesters treating biowaste with different sorts and different amounts of co-substrates were examined. Sewage sludges, old bread, yoghurt and food waste were examined as potential co-substrates in batch and continuous assays. Only old bread and food waste had a higher biogas production potential as biowaste in batch assay. In continuous assays addition of activated sludge from a sewage treatment plant had no positive impact on anaerobic digestion of biowaste. More biogas was

produced during co-digestion of biowaste with old bread than with other organic waste substrates.

From bread as the best potential co-substrate, two sorts, wheat and rye bread, were used for improvement of biogas production in anaerobic digesters treating biowaste. Before addition of bread into anaerobic biowaste digesters, acidification behavior and buffer capacities of wheat bread suspension, rye bread suspension and biowaste suspension were examined. Acidification of wheat bread (WBS), rye bread (RBS) and fresh biowaste suspensions (FBS) led respectively to lactate + acetate, lactate + acetate + n-butyrate, and acetate + propionate + n-butyrate. The buffer capacity of RBS was twice higher than that of WBS. The addition of old bread into anaerobic digesters treating biowaste not only linearly increased biogas production but also improved the gas production rates. At the shortest HRT of 6.2 days in full-scale biowaste digestion reactors, co-digestion with old bread could be operated safely at a very high organic loading rate (OLR) of up to 22 $kg \cdot m^{-3} \cdot d^{-1}$.

Propionate as the most delicate intermediate during anaerobic digestion was added to anaerobic biowaste digesters. To determine its maximal possible degradation rates during anaerobic digestion, a reactor was fed Monday to Friday with an OLR of 12/14 kg $COD_{biowaste} \cdot m^{-3} \cdot d^{-1}$ plus propionate up to a final OLR of 18 kg $COD \cdot m^{-3} \cdot d^{-1}$. No feed was supplied on weekends as it was the case in full-scale. To maintain permanently high propionate oxidizing activity (POA) a basic OLR of 3 $kg \cdot COD_{proionate} \cdot m^{-3} \cdot d^{-1}$ all week + 11 $kg \cdot COD_{biowaste} \cdot m^{-3} \cdot d^{-1}$ from Monday to Friday was supplied. Finally a reactor was operated with an OLR of 12 $kg \cdot COD_{biowaste} \cdot m^{-3} \cdot d^{-1}$ from Monday to Friday and 5 kg $COD_{propionate} \cdot m^{-3} \cdot d^{-1}$ from Friday night to Monday morning to maintain a constant gas production for permanent operation of a gas engine. Propionate degradation rates (PDRs) were determined for biowaste, biowaste + propionate and for solely propionate feeding. Decreasing PDRs during starvation were analysed. The POA was higher after propionate supply than after biowaste

feeding and decreased faster during starvation of a propionate fed than of a biowaste fed inoculum.

Biogas production from biowaste with 20%, 25% and 30 % dry matter (DM) by "box-type dry anaerobic digestion" was investigated for incubation at 20, 37 and 55 ^{o}C. Dry anaerobic digestion (DAD) with 20 % DM-containing biowaste was possible at 20 – 55 ^{o}C, whereas with 25% DM-containing biowaste successful digestion was only possible at 37^{o}C and 55^{o}C. No or only little biogas was produced in reactors with 30 % DM at 20, 37 or 55 ^{o}C. The methane production rate in the DAD reactor with 20 % DM content was almost the same at 37^{o}C or 55^{o}C, whereas the DAD reactor with 25 % DM content had a higher methane production rate at 55^{o}C.

Zusammenfassung

Das stetige Wachstum der Weltbevölkerung und der Weltwirtschaft resultiert in einem exponentiell ansteigenden Energiebedarf sowie einer immer größer werdenden Menge an Abfall. Für sozialen Fortschritt und Wirtschaftswachstum ist die Verfügbarkeit von Energie unabdingbar. Der derzeit in Industrie- und Schwellenländern ständig ansteigende Lebensstandard ist mit einem steigenden Energiebedarf gekoppelt. Der zunehmende Wohlstand in Industriestaaten, Schwellen- und Entwicklungsländern führt weltweit zu hohen Mengen an organischen Abfällen. Diese neigen bei der Ablagerung zu Faulung und müssen vorher stabilisiert werden. Zur Stabilisierung gibt es zwei grundlegend verschiedene Möglichkeiten: die aerobe Kompostierung und die anaerobe Vergärung. Für die Kompostierung ist sowohl separat gesammelter Biomüll in Industriestaaten als auch organischer Marktabfall in Entwicklungsstaaten (verfügbare Hauptmenge organischen Abfalls) zu nass, sodass am besten eine Nassvergärung zur Faulung und Stabilisierung (für weitere Nutzung der Gärungsrückstände als organischer Dünger) durchgeführt werden sollte. Dabei wird ein Großteil des Energiegehalts freigesetzt. Das gebildete Biogas muss genutzt werden, da Biogas ein klimarelevantes Gas ist und nicht in die Atmosphäre gelangen darf. Für die Vergärung stehen je nach Feuchtegehalt zwei Prozessarten zur Verfügung, die Naßvergärung (Trockensubstanzgehalt: TS < 15 %) und die Trockenvergärung (TS >>15%), die beide in unterschiedlichen Temperaturbereichen - psychrophil, mesophil oder thermophil - durchgeführt werden können.

In dieser Arbeit werden Gärversuche mit separat gesammelten Biomüll nach Sortierung durch Trockenvergärung und nach Aufbereitung durch Zerfaserung in einem Hydropulper in einer Naßvergärung in verschiedenen Temperaturbereichen entweder alleine als Mono-vergärungen oder nach Zusatz von verschiedenen Co-Substraten (Faulschlamm, Belebtschlamm, Suspensionen

von Essensresten, abgelaufene Joghurtchargen, Altbrot) als Co-Fermentationen untersucht. Dazu werden Abbaugrad und Biogaspotential aller möglichen Co-Substrate und die zwischenzeitlich ausgeschiedenen Fettsäuren bestimmt, um deren Verhalten bei Zusatz zu Biomüllreaktoren abschätzen zu können. Die Batch-Ansätze haben gezeigt, dass das Gasbildungspotential von Biomüll bei 330 $ml \cdot g^{-1}$COD lag. Das Gasbildungspotential von altem Brot (356 $ml \cdot g^{-1}$COD) und von Speiseresten (392 $ml \cdot g^{-1}$COD) war höher als das von Biomüll. In kontinuierlichen Gäransätzen hatte der Zusatz von Belebtschlamm keinen positiven Effekt auf die anaerobe Vergärung von Biomüll. Durch die Mischung von Biomüll mit altem Brot oder Speiseresten kann die Gasproduktion im Vergleich zur Biomüll-Monovergärung dauerhaft von 0.24 $L \cdot g^{-1}$ COD d^{-1} auf Werte von höher als 0.3 $L \cdot g^{-1}$ COD d^{-1} gesteigert werden.

Um das Brot, das sich als das am besten geeignete Co-Substrat herausstellte, weiter zu charakterisieren, wurden Weizen- (WBS) und Roggenbrot-suspensionen (RBS), bevor diese mit der Biomüll-Suspension gemischt wurden, hinsichtlich ihrer Pufferkapazität und ihrer Versäuerung untersucht. Die Versäuerung von WBS hatte die Bildung von Laktat und Acetat, die Versäuerung von RBS die Bildung von Laktat, Acetat und n-Butyrat zur Folge. In der Biomüllsuspension wurde zusätzlich zu Acetat und n-Butyrat auch Propionat gefunden. Im Vergleich zu WBS war die Pufferkapazität von RBS doppelt so hoch. Die Co-Vergärung von altem Brot und Biomüll führte, verglichen mit einer Biomüll-Monovergärung, zu einer höheren Biogas-produktion und zu verbesserten Gasbildungsraten. Bei WBS- und RBS-Zusatz von bis zu 50 % der OLR wurde die Gasproduktion um 90% bzw. 130% verbessert. Um die maximal mögliche OLR für Co-Vergärungen mit altem Brot zu bestimmen wurden zwei Co-fermentationen, eine mit WBS und die andere mit RBS bei festgesetzter HRT kontinuierlich betrieben. Bei einer für die Praxis kurzen hydraulischen Verweilzeit (HRT) von 6,2 Tagen kann eine Co-

Vergärung mit Brot mit einer sehr hohen organischen Raumbelastung (OLR) von bis zu 22 $kg \cdot m^{-3} \cdot d^{-1}$ durchgeführt werden.

Die Anreicherung von Propionat stellt eine kritische Phase während der anaeroben Vergärung dar. Um die maximal mögliche Propionat-Abbaurate während der anaeroben Vergärung zu bestimmen, wurde ein Reaktor von Montag bis Freitag mit einer OLR von 12/14 kg $CSB_{Biomüll} \cdot m^{-3} \cdot d^{-1}$ und mit Propionat-Zusatz bis zu einer OLR_{gesamt} von 18 kg $CSB \cdot m^{-3} \cdot d^{-1}$ beschickt. Im Gegensatz zu den meisten großtechnisch betriebenen Methanreaktoren wurde aber wie in Karlsruhe am Wochenende kein Biomüll und Propionat zugegeben. Um permanent eine hohe Propionat-oxidierende Aktivität (POA) in der Suspension des Methanreaktors aufrecht zu erhalten, wurde über die gesamte Woche eine OLR von 3 kg $CSB_{Propionat} \cdot m^{-3} \cdot d^{-1}$ eingestellt und von Montag bis Freitag zusätzlich 11 kg $CSB_{Biomüll} \cdot m^{-3} \cdot d^{-1}$ zugegeben. Außerdem wurde ein Reaktor von Montag bis Freitag mit einer OLR von 12 kg $CSB_{Biomüll} \cdot m^{-3} \cdot d^{-1}$ (nicht komplett abbaubar) und von Freitag Nacht bis Montag Morgen mit einer OLR von 5 kg $CSB_{Propionat} \cdot m^{-3} \cdot d^{-1}$ (komplett abbaubar) betrieben mit dem Ziel, eine konstante Gasproduktion über 7 Tage aufrecht zu erhalten, um einen permanenten Betrieb des Stromgenerators zu ermöglichen. In allen Ansätzen von Biomüll ± Propionat wurden die Propionat-Abbauraten (PDR) für Zugabe von Biomüll alleine, Biomüll plus Propionat und Propionat alleine bestimmt, wobei sinkende PDR in Hungerphasen detektiert wurden. Die POA war nach der Zudosierung von Propionat höher als nach der Zugabe von Biomüll und sank in der Hungerphase nach Propionat-Zugabe schneller als nach der Zugabe von Biomüll.

Für die Bestimmung der Gasproduktion von Biomüll mit 20 %, 25 % und 30 % Trockensubstanzgehalt (DM) wurden sogenannte „Garagenfermenter“ bzw. Box-Fermenter nachsimuliert und dabei verschiedene Temperaturbereiche (Inkubation bei 20, 37 und 55 °C) getestet. Während eine anaerobe Vergärung des 20 % DM-haltigen Biomülls zwischen 20 und 55 °C möglich war, konnte

Biomüll mit 25 % DM nur bei 37 °C und 55 °C vergärt werden. In den Reaktoren, die mit 30 % DM-haltigen Biomüll beschickt wurden, wurde unabhängig von der Temperatur (20, 37 oder 55 °C), keine oder nur eine sehr geringe Gasproduktion beobachtet. Die Methanproduktionsrate war in den Ansätzen mit 20 % DM-haltigen Biomüll von der Temperatur unabhängig. In den Ansätzen mit 25 % DM-haltigem Biomüll war die Methan-Produktionsrate bei 55 °C höher als bei 37 °C. Die Vergärungseffizienz der Biomüll-Trockenvergärung mit 20% DM war in den drei Temperaturbereichen, die der Biomülltrockenvergärung mit 25% DM bei 37^{o}C und 55^{o}C (mesophiler und thermophiler Temperaturbereich) etwa gleich gut wie bei der Nassvergärung.

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AD : Anaerobic digestion

COD : Chemical oxygen demand

CSTR : Continuously stirred tank reactor

DAD : Dry anaerobic digestion

DM : Dry matter

FBS : Fresh biowaste suspension

HRT : Hydraulic retention time

MSW : Municipal solid waste

OFMSW : Organic fraction of municipal solid waste

OLR : Organic loading rate

PDR : Propionate degradation rate

POA : Propionate oxidizing activity

RBS : Rye bread suspension

TS : Total solid

VFA : Volatile fatty acid

VS : Volatile solids

WAD : Wet anaerobic digestion

WBS : Wheat bread suspension

Chapter 1

Introduction

1.1 Worldwide environmental problems

The total population of the world has tremendously increased over the past two decades and is expected to double in the middle of the 21st century (Anon, 1995).The increase of the population and the economic development has also brought about an unexpected increase of the amount of solid waste and of the energy demand.

All activities around human life depend on energy availability. In other words, energy is the foundation of social advancement and economic development. Human comfortable life is depending on advancing science and technology and today advancing lifestyle is particularly associated with an increased energy demand. In 2013, global primary energy consumption increased by 2.3%, an acceleration over that of 2012 by 1.8% (BP, 2014).

The International Energy Agency has reported that the demand on energy will increase during this century by a factor of two or three (IEA 2006). Until today, about 88% of the world's primary energy consumption is still supplied from fossil fuels (oil, gas, and coal) (Weiland, 2010). For each of the fossil fuels, global consumption increased much more rapidly than production. The fossil fuels resources are, however, not endless to exploit. There is considerable evidence that excessive use of environmental resources has a significant negatively impact on human future. How to achieve a bright and reasonable energy future for human development with minimal environmental impacts must be taken into consideration. More use of renewable energy resources and development of energy technologies for a better conversion of wastes into renewable energy is one possibility to reduce/solve the energy shortage. Since the 1970th, there has been a worldwide attention of renewable resources and

numerous research attempts have been undertaken to invent new technologies or improve existing technologies for higher efficiency. It has been proven that the energy conversion systems based on renewable energy have several beneficial impacts on environmental, economic and political issues of the world (McGowan, 1990). In general renewable energy technologies produce useful energy by converting natural resources with energy recovery. Table 1.1 shows the renewable energy technologies of different natural sources and their maturity. Until today, several renewable energy sources e.g. hydropower, wind and biomass have successfully replaced part of fossil fuels. Solar energy technologies, particularly photovoltaic (PV) systems have progressed rapidly during the past two decades. Now some new technologies e.g., bio fuel generation and ocean thermal energy utilization are considered to have great future potential for energy supply (Dincer, 2000).

The social and economic development, not only in highly industrialized countries but also in developing and emerging countries has brought another problem: Masses of municipal solid waste (MSW) generation. MSW management is a major challenge for local governments in both, urban and rural areas throughout the world. An integrated solid waste management (ISWM) system, which consists of reducing, reusing, recycling (3-R principle) and disposal of non-recyclable waste material, is considered to be the most optimized waste management system. Some advanced industrial countries such as USA, Germany, Japan and Sweden have achieved significant success in solid waste management. There have been some revolutionary changes in the strategies of solid waste management in those advanced industrial countries during the 1960-2006. For solid waste management most attention has been focused on reduction and on recycling procedures. The proportion of solid wastes for disposal on sanitary landfills has been greatly reduced (Yuan et al., 2006).

Table 1.1 Maturity of renewable technologies (Dincer, 2000)

Proven capability	Transition phase	Future potential
Hydropower	Wind	Advanced Turbines
		Geothermal
Geothermal	Geothermal	Hot dry rock
Hydrothermal	Hydrothermal	Geo pressure
		Magma
Biomass	Bio fuels	Bio fuels
Direct combustion	Ethanol from corn	Methane
Gasification	Municipal waste	
	Active solar	
Passive solar	Buildings	Solar Thermal
Buildings	Process heat	Advanced electricity
	Solar Thermal	High-temperature processes
	Thermal/gas hybrid	
Photo voltaic	Photo voltaic	Photo voltaic
Small remote	Remote power	Utility power
Specialty products	Diesel hybrids	
		Ocean Thermal

Compared with highly developed industrial countries, the rapidly growing cities in developing countries still have a long way to go for proper solid waste management with respect to reusing and recycling, management strategies and waste treatment technologies. A serious and growing problem of solid wastes is especially coming up in China. China has a rapid economic development since it started reforms and an open–to-the–outside-world policy in 1978. The population keeps growing quickly, urbanization and industrialization proceed rapidly and, although those three phenomena have brought a steady improvement of living standards in China, one of the negative consequences is more municipal solid waste generation and heavy environmental pollution. The solid waste pollution has meanwhile raised vast public concern.

1.2 Current situation of municipal solid waste management in China

China has the largest population of the world, about 1.37 billion people in 2010 and China has experienced rapid urbanization in the past 20 years. The number of municipalities and the urban population has remarkably increased. The urban population and better economic conditions are the two most important factors contributing to the quantity of municipal solid waste. The annual increase rate is 8-10% from 1985 to 1995 and 3-5% after 1995 (Wang and Nie, 2001). MSW were constantly increasing , reaching 170.81 million tons by 2012. This amount accounted for 29% of the worlds annual MSW generation (Dong et al., 2001). The annual generation of MSW in China is expected to reach 172 and 200 million tons by 2013 and 2020(Zhou et al., 2014; Cheng and Hu, 2010).

China has gone through a rapid economic development and the GDP increased steadily in the past 20 years. But with growing GDP and the improvement of living standards, the quantity of MSW generation has increased dramatically, as shown in Figure 1.1. The MSW generation can be expressed by population development and the GDP yields using equation 1 (Wang and Nie, 2001):

Equation 1: $G_{MSW} = 0.225286P + 0.049732E_{GDP} + 2640.2355$

Where G is MSW generation, P is population and E is the GDP. It can be predicted that with the present population growth and economic development, the MSW generation will continue to rapidly increase in China.

Like most developing countries, China has still not unfolded the potentials of anaerobic digestion as a technology for the treatment of waste and wastewater to solve environmental and energy problems. Before 1990 there were very few environmentally sound waste management facilities in China. The percentage of environmentally sound treatment of solid waste was only 0.5 – 2.5%. Since 1991 China began to pay more attention to improve this situation. From 1990 to 1998 the percentage of treatment and dispoal of MSW has increased from 2.32% to 58.5%. Nowadays there are three major methods for the MSW

treatment: landfills, incineration and composting (Tchobanoglous et al., 1993; Daskalopoulos et al., 1997; Wang and Nie 2001, 2001). At present 79% of MSW are disposed in sanitary landfills, 19% by composting or recycling and only 2% by incineration (Zhang et al., 2010).

The treatment and disposal waste is dependent on its characteristics. Municipal solid waste in many cities in China has a high moisture content and a low heating value, that is why the incineration of waste in China is not yet regarded as the best or most appropriate technology.

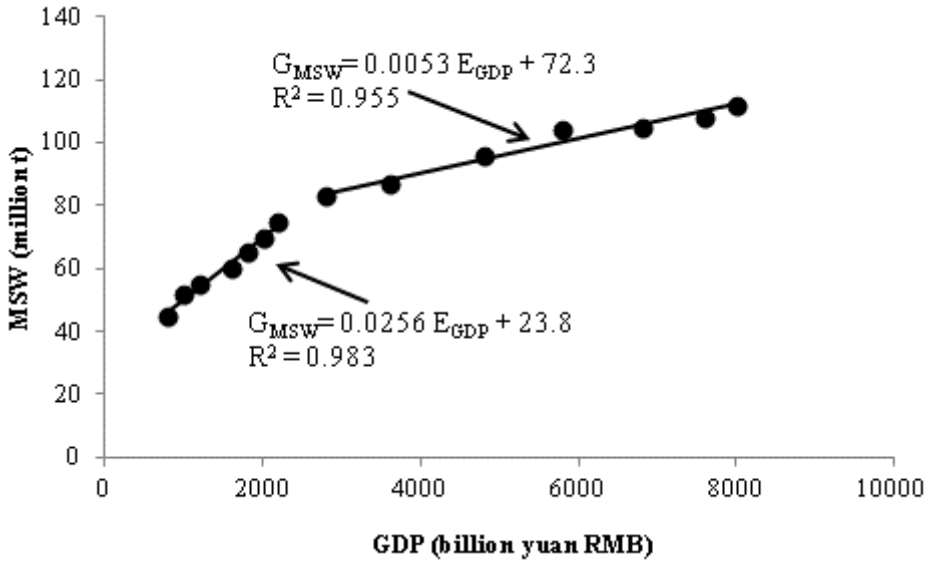

Figure 1.1 Correlation between MSW generation and GDP in China (Wang and Nie, 2001)

1.3 Treatment of solid waste by anaerobic digestion (AD)

By now solid waste disposal on sanitary landfills is still the predominantly used way because of financial reasons (it is cheap) and its simplicity. However, the drawback of land filling is obvious: First of all, landfills have a negative environmental impact, since landfills of MSW significantly contribute to greenhouse gas emission, which might lead to epidemic diseases and climate changes. Secondly, sanitary land filling prevents recovering the resources and recycle energy. Modern MSW landfill sites, which are appropriate for waste disposal, are very limited (Weiss, 1974; Lema et al., 1988; Christensen, 2012).

As alternatives, composting and anaerobic digestion are ways of achieving the main trends of today´s waste management policy: Reduce and reuse a main stream of waste to recover energy and resources. Composting is a simple and inexpensive process, but it needs large areas, emits uncontrolled leachate and is a net energy consumer (Braber, 1995; Domingo and Nadal 2009; Walker et al., 2009).

Compared to composting anaerobic digestion has several merits (Mata-Alvarez et al., 2000; Lens and Verstraete, 2001; Hartmann and Ahring, 2006). AD is an efficient and feasible process to solve diversified waste problems. It requires less area than composting procedures and emits less bad odour and green house gases. More important, AD is a net energy producer. Furthermore, the digestate of an anaerobic digester may serve as fertilizers or soil conditioners. Disposal of the dried digestate in a landfill could reduce landfill gas emission and organic leachate contamination.

1.4 Theoretical background and literature review

1.4.1 Anaerobic digestion

Anaerobic digestion is a sequence of biochemical processes in which microorganisms break down solid or soluble biodegradable material in the absence of oxygen to finally biogas. The whole process consists of a series of biochemical reactions (Stronach et al., 1986; Ahring, 2003). Initial substrates for bacteria in anaerobic digestion include carbohydrates, lipids and proteins, as well as more resistant cellulose, hemi cellulose and eventually lignin. Especially the fraction of wet organic wastes from municipal source-sorted collection has a very complex composition. Carbohydrates are macro-molecules that contain many monomers of sugars and are either homo polymers or hetero polymers. The monomers of carbohydrates are known as mono saccharides that contain four to seven carbon units. When numerous mono saccharides are assembled together, poly saccharides are formed. The common formula for carbohydrates

is (CH2O)x. Mono saccharides are water soluble and can easily enter bacterial cells by diffusion through the cell wall or active uptake through the cytoplasmic membrane, whereas poly saccharides must be hydrolyzed before they can be transported across the cell wall and the cytoplasmic membrane. Lipids in biowaste that is fed into anaerobic digesters include solid and emulged fats and oils. The backbone of lipids is glycerol, which binds up to three long-chain, saturated or unsaturated fatty acids by ester bonds. Complex liquid or solid fats or oily substances are hydrolyzed to small and monomer molecules in the anaerobic digester fluid and further degraded to organic acids and to finally biogas in a sequence of reactions. Proteins are also complex macro molecules with a high molecular weight. They consist of long chains of 22 amino acids that contain an amino group (-NH2) at the α-carbon atom and a carboxyl group (-COOH). Complex proteins with high molecular weight are formed by peptide bonds between amino acids and cannot be transported cross the bacterial cell membrane. After hydrolysis of peptide bonds by proteases released soluble oligopeptides (≤ 6-7 amino acids) or amino acids from protein degradation can enter the bacteria cell (Geradi, 2003; Gallert and Winter, 2005). The overall conversion during anaerobic digestion includes many single reactions in different bacteria of the anaerobic consortium for the degradation of organic material to methane, carbon dioxide, ammonia and hydrogen sulfide, which can be described by applying the generic formula of Buswell (Equation 2; Buswell and Mueller, 1952):

Equation 2: $C_cH_hN_nS_s + 1/4\ (4c - h - 2o + 3n + 2s)\ H_2O \rightarrow$
$1/8\ (4c - h + 2o + 3n + 2s)\ CO_2 + 1/8\ (4c + h - 2o - 3n - 2s)\ CH_4 + nNH_3 + sH_2S$

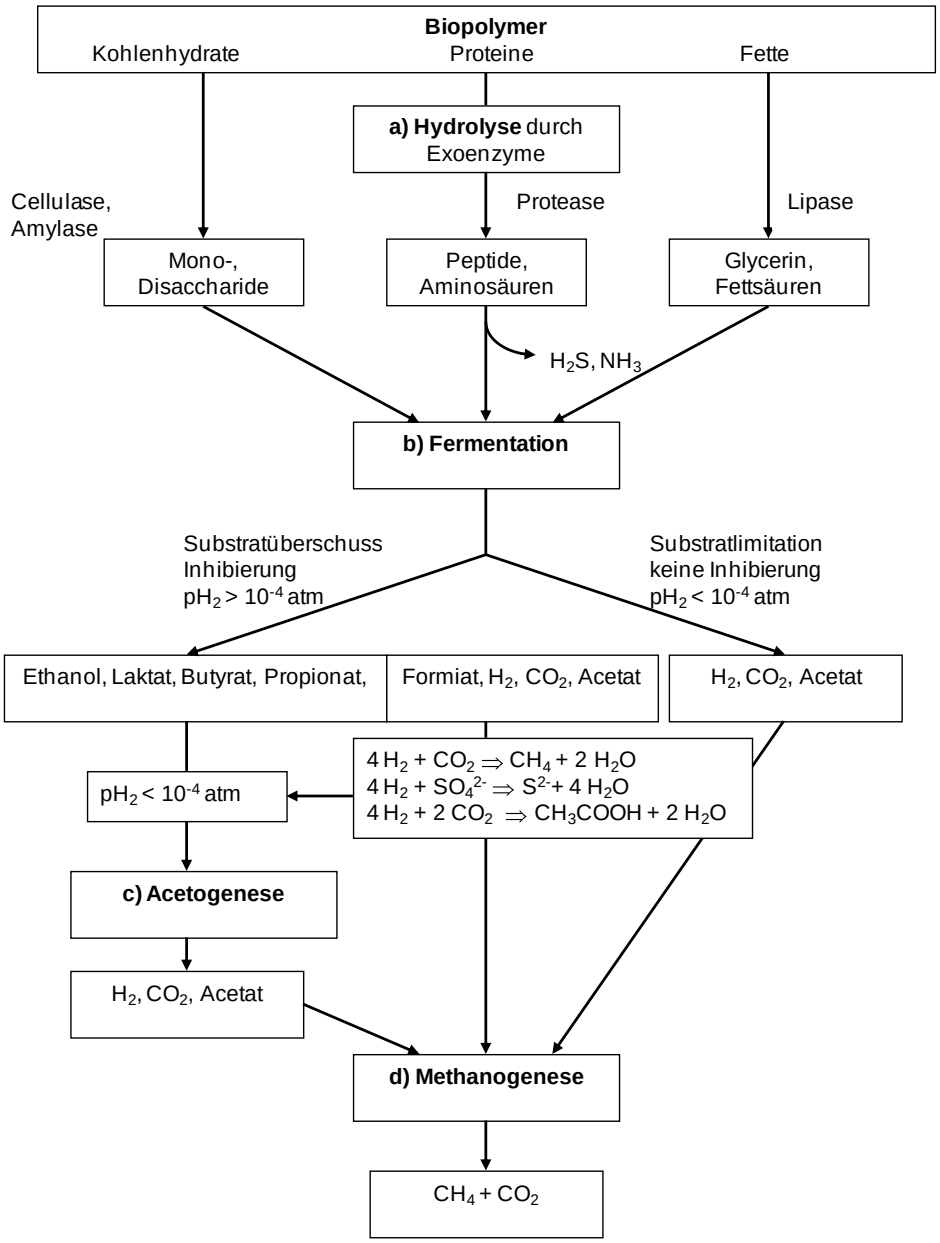

Figure 1.2 Schematic representation of anaerobic digestion (Gallert und Winter 2015).
a) Hydrolyzing bacteria, b) acidogenic (fermenting) bacteria, c) acetogenic (obligately acetate and H_2 forming) bacteria and d) methanogenic bacteria (hydrogenotrophic and acetotrophic methanogens)

The anaerobic digestion process that ends with the production of biogas, can be divided into different stages. Depending on the substrates three or four degradation stages a) - d) (Fig. 1.2) are considered: If fibres (e.g. cellulose) or globuli-forming (e.g. starch) substrates must be degraded an extra-cellular hydrolysis stage a) by cellulases, amylases, proteases and lipases must precede

acidogenesis, acetogenesis and methanognesis. Acidogenesis (b), acetogenesis (c) and methanogenesis (d) are the three stages for biogas formation from soluble substances. Many different consortia of microorganisms with different functions in the overall degradation process are needed for the anaerobic digestion process. The products of biochemical reactions and the three or four stages a) - d) in the anaerobic digestion process are schematically illustrated in Figure 1.2 (Gallert und Winter 2015).

STAGE 1: HYDROLYSIS of BIOPOLYMERS

At the beginning of the anaerobic digestion complex insoluble compounds must undergo hydrolysis, so that the substrates get water-soluble and can be transported cross the cytoplasmic membrane of bacteria. Polymeric substances such as carbohydrates, fats and proteins consist of many monomeric molecules, assembled together by unique chemical bonds under release of H_2O. Hydrolytic bacteria are capable of breaking those chemical bonds to release monomeric products. In this step, carbohydrates, lipids and proteins are respectively converted to soluble sugars, long-chain fatty acids, alcohols or glycerol and soluble peptides or amino acids. Since the hydrolysis stage is very slow (the surface for binding extracellular enzymes is small compared to the volume of particles or fibres) and energy- consuming, it is very often considered as the rate-limiting step for the anaerobic digestion process (McCarty and Mosey, 1991; Veeken et al., 2000; Gallert and Winter 2005).

STAGE 2 and 3: VOLATILE FATTY ACID- AND ALCOHOL-FORMING b) and ACETATE-FORMING STAGE c)

The volatile fatty acid (VFA)-forming stage can be divided into b) acidogenesis and c) acetogenesis. In stage b) soluble monomers forming molecules stemming from hydrolysis of polymer or being present already in wastewater are degraded by a large diversity of facultative anaerobes and anaerobes through many fermentative processes. The degradation of these monomers results in the

production of CO_2, H_2, alcohols, organic acids, some organic-nitrogen compounds and organic sulfur compounds. Major acids and alcohols production from fermentation processes in stage b) during anaerobic digestion are presented in Table 1.2.

Table 1.2 Organic compounds produced during anaerobic digestion

Name	Formula
Acetate	CH_3COOH
Butanol	$CH_3(CH_2)_2CH_2OH$
Butyrate	$CH_3(CH_2)_2CH_2COOH$
Capric acid	$CH_3(CH_2)_4COOH$
Formate	$HCOOH$
Ethanol	CH_3CH_2OH
Lactate	$CH_3CHOHCOOH$
Methanol	CH_3OH
Propanol	$CH_3CH_2CH_2OH$
Propionate	CH_3CH_2COOH
Succinate	$HOOCCH_2CH_2COOH$

Acetate is the most important of the VFAs, and is the principal organic acid used as a substrate by methane-forming bacteria. All other fatty acids and alcohols must be converted to acetate, CO_2 and H_2 during acetogenesis (stage c) and only then acetate, CO_2 and H_2 can be converted carbon dioxide and methane by d) methanogenic bacteria. Some alcohols, organic acids and organic-nitrogen compounds such as acetate, formate, methanol and methylamines can be used directly as substrates by methane-forming bacteria and subatances such as ethanol, butyrate and propionate can be used after they are degraded in an energy-consuming process to acetate (acetogenesis). Acetogenesis (stage c) occurs in the VFA-forming stage, in which some low molecular weight volatile fatty acids are degraded to acetate by obligate hydrogen-forming acetogenic bacteria. A balanced anaerobic digestion process

demands that the products from the microorganisms responsible for hydrolyzing and fermenting the substrates to hydrogen and acetate are simultaneously used by the methane-forming bacteria (Gerardi, 2003; Gallert and Winter 2005).

STAGE 4 – METHANOGENIC STAGE

In the final stage d), methane is formed by methane producing bacteria. Methane is formed mostly from acetate, CO_2 and H_2 but also from same organic compounds other than acetate. There are three principal groups of methane-forming bacteria.

Group 1: Hydrogenotrophic methanogens

The hydrogenotrophic methanogens use hydrogen and CO_2 and form methane (Equation 3). During this conversion the hydrogenotrophic methanogens maintain a low partial hydrogen pressure in the anaerobic digester that is necessary for acetogenic bacteria.

Equation 3: $CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$

Group 2: Acetotrophic methanogens

Acetate is converted to methane and CO_2 by the acetotrophic methane bacteria (Equation 4). The hydrogenotrophic methanogens can then convert the CO_2 produced from acetate to methane, if surplus hydrogen is available from other sources. Some hydrogenotrophic methanogens can also use CO to produce methane (Equation 5).

Equation 4: $CH_3COOH \rightarrow CH_4 + CO_2$

Equation 5: $4CO + 2H_2O \rightarrow CH_4 + 3CO_2$

The acetotrophic methanogens generate methane more slowly than the hydrogenotrophic methanogens and are sensitive against the accumulation of

hydrogen. A low partial hydrogen pressure is favourable for acetotrophic methanogens. High hydrogen partial pressure reduces not only the acetate but also the methane production.

Group 3: Methylotrophic methanogens

The methylotrophic methanogens produce methane directly from methyl groups of e.g. methnaol or methylamines (Equations 6, 7).

Equation 6: $4\ CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$

Equation 7: $4\ (CH_3)_3\text{-}N + 6\ H_2O \rightarrow 9CH_4 + 3CO_2 + 4NH_3$

Each methane-forming bacterium has a specific substrate or group of substrates that can be degraded (Table 1.3) and the use of different substrates by methane-forming bacteria results also in different energy gains (Gerardi, 2003; Metcalf and Eddy, 2003).

Table 1.3 Selection of some species of methane forming bacteria and their substrates

Species	Substrates
Methanobacterium formicium	Carbon dioxide, formate, hydrogen
Methanobacterium thermoantotrophicum	Carbon dioxide, hydrogen, carbon monoxide
Methanococcus frisius	Hydrogen, methanol, methylamine
Methanococcus mazei	Acetate, methanol, methylamine
Methanosarcina barkeri	Carbon dioxide, hydrogen, acetate, methanol, methylamine

1.4.2 Operational conditions of anaerobic digestion

1.4.2.1 Start-up

Start-up of anaerobic digestion (AD) for solid waste treatment is a sensible process and may require a relatively long time. Often the first feedstock that provides the substrates for anaerobic digestion also provides the bacteria needed for degradation of these compounds and the methane production. The major

problems during the start-up phase are the slow growth of anaerobic bacteria of the acetogenic and methanogenic stage and the acidification of the reactor content by VFA-forming bacteria that grow faster. During the early days of a start-up phase no or only very little methane is produced and volatile fatty acids may be accumulating by production from fast-growing acidogenic bacteria. High-rate anaerobic digestion for solid waste treatment depends on syntrophic interaction of fatty acid degrading acetogens with acetate and H_2/CO_2-utilizing methanogens to avoid accumulation of VFAs. If the accumulation of VFAs leads to acidification of the anaerobic digester, pH declines and causes a failure of AD. Monitoring and control of pH and fatty acids concentration during the start-up period are essential. The digesters pH should be maintained within the optimum level of 6.8 – 7.2. Except for pH, factors such as inoculum quality, organic loading rate, temperature and biodegradable substrates influence the duration of the start-up phase. Anaerobic digester start-up should proceed constantly and the time to archive a steady-state of anaerobic reactions should be as short as possible. Far less than 1 month is required to archive a stable operation, that is reflected by the biogas production and a stable volatile acid-to-alkalinity ratio (Gallert et al., 2008). Difficulties during the anaerobic digester start-up may be overcome by inoculating the digester with digested sludge. The steady-state conditions for increasing the OLR could be archived in less than 3 weeks by using digested sludge for start-up (Nayono et al., 2010).

1.4.2.2 Hydraulic retention time (HRT) and organic loading rate (OLR)

The HRT is the average time that a certain substrate or sludge is in the anaerobic digester. The generation time is the time required for bacteria to double in numbers. Most of the slow-growing methane-forming bacteria have relatively long generation times compared with aerobic bacteria, facultative anaerobic bacteria and strict anaerobic volatile fatty acids forming bacteria (Zehnder, 1988). Due to the long generation time of methane-forming bacteria, typical retention times for anaerobic digester operation with continuous mixing

used to be more than 10 days, in sewage treatment 20 - 40 days. The very long HRT for sewage treatment was also due to hygienic reasons. If the HRT was too short than 10 days, significant washout of methane-forming bacteria may lead to failure of AD (Gerardi, 2003). The HRT regulates the conversion of volatile solids to gaseous products in anaerobic digesters. The final disposition of the digested sludge and the rate of methane production depend on the design of the reactor and on the HRT. HRT is one of the most important operational condition that influence the performance of an anaerobic digestion system. The optimum HRT of an anaerobic digester can vary depending on factors like the type of waste, configuration of the digester and on the microorganisms involved in the process. Long HRTs are advantageous for degradation of not immediately or not easily degradable organic matter. However, a shortening of the HRT may lead to an increase in biogas production rate and volumetric methane productivity (Nges and Liu, 2010). Shorter retention times may be used under thermophilic conditions. Fdez.-Güelfo et al. (2012) observed that 15 days was the optimum HRT for the dry anaerobic digestion of the organic fraction of municipal solid waste at thermophilic temperature.

The organic loading rate (OLR) is the amount of organic matter that is loaded into a certain volume of a digester during a certain times, normally given as kg COD m^3 d^{-1}. The maximal OLR at still maximal conversion efficiency describes the highest biological conversion capacity of anaerobic digestion wastewater or sludge under consideration. The value of OLR could be related to the HRT. With the same feedstock, the higher HRT the lower OLR is. Dry anaerobic digestion may tolerate higher OLR than wet anaerobic digestion, but is much slower.

1.4.2.3 Temperature

Temperature plays an important role for anaerobic digestion. Maintenance of optimum digester temperature is essential for anaerobic digestion. Fluctuation

of temperature, even only over a few degrees, could affect almost all biological activity, including the inhibition of some anaerobic bacteria, especially acetogenic and methane-forming bacteria. Additionally, temperature not only has an effect on the activity of the microorganisms but also could influence other important factors such as gas transfer (Metcalf and Eddy, 2003). Temperature influences methane-forming bacteria and volatile fatty acid-forming bacteria, but its effect on hydrolysis of particulate matter is only little. Hydrolytic bacteria are less sensitive to temperature changes than methane-forming bacteria and acid-forming bacteria. The effect of temperature is based on its impact on enzymatic activity or reactions. Increasing the temperature results in more enzymatic activity. Therefore, retention times will be decreased with increasing temperatures.

Anaerobic digestion can take place at psychrophilic temperature at about 20^{o}C, but it is commonly operated at two temperature ranges: at mesophilic temperature around 35^{o}C and at thermophilic temperature range from 50^{o}C to 60^{o}C. Mesophilic bacteria are active in a wider temperature ranges than thermophilic bacteria and can tolerate greater changes in the environmental parameters (Figure 1.3). Anaerobic digestion in municipal wastewater treatment plants is mostly operated at mesophilic temperature (Gerardi, 2003). Compared with anaerobic digestion at mesophilic temperature, the biodegradability and methane yield were greater at thermophilic temperature (Cecchi et al., 1991; Jha et al., 2013). During the start-up period of a dry anaerobic digestion system, better performance can be archived at thermophilic temperature (Lu et al., 2007). The anaerobic digester can well perform with shorter retention time, when the temperature is gradually increased from mesophilic temperature to themophilic (Juanga, 2005; Amani et al., 2011). However, the energy requirement for maintaining the digester at thermophilic temperature is much higher than in a mesophilic process. Thermophilic bacteria are very sensitive to even some small temperature changes and thermophilic anaerobic digestion is

less stable due to more heat input. Therefore, most of the anaerobic digesters are currently operated at mesophilic temperatures, even though its retention time is little longer and methane yield may be a litle lower.

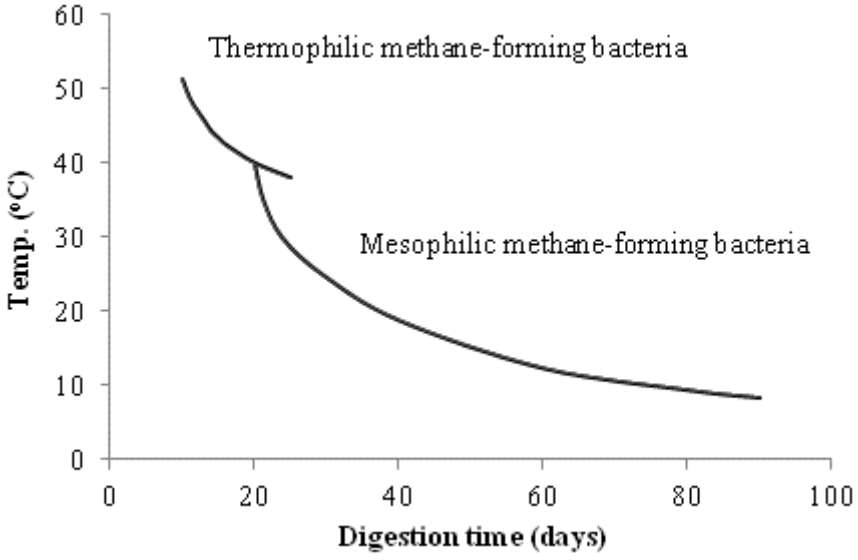

Figure 1.3 Active temperature range for methane-producing bacteria.

1.4.2.4 pH values for anaerobic digestion

The pH value has a great effect on enzymatic activity and digester performance in anaerobic digestion systems. Enzymatic activity of acid-forming bacteria may be not inhibited above pH 5.0, whereas enzymatic activity of methane-forming bacteria cannot occur below pH 6.2. Most anaerobic bacteria including methane-forming bacteria can perform in a pH range of 6.5 to 7.5, but optimally in the range of 6.8 to 7.2. A bad digestion performance or failure of an anaerobic digestion process may occur, if the pH drops to less than 6.1 or increases to more than 8.3 (Lay et al., 1997). The production of volatile acids may cause a decreasing pH in digesters, but during balanced digestion the volatile acids are consumed by methanogens and alkalinity as well as ammonia (from protein degradation) is generated, so that the pH of the digester should be stable. Decreasing of the pH below the normal range is an important indicator of failure of an anaerobic digester, that can be caused by:

- failure of conversion VFAs to methane by acetogenic bacteria and methanogens due to a low buffer capacity causing a too low pH;
- introduction of an organic acids containing substrate to the anaerobic digester;
- inhibition of acetogenic and methanogenic activity by toxic substances.

In order to avoid failure of anaerobic digestion due to decreasing pH, some chemicals can be used to adjust the pH of anaerobic digesters (Table 1.4) to maintain a high bicarbonate alkalinity. Sodium bicarbonate and potassium bicarbonate may be the best choice to adjust the pH, because these compounds have minimal adverse impacts on acid-forming bacteria and methanogens require a high alcalinity for optimal methane production (Gerardi, 2003).

Table 1.4 Chemicals generally used for pH adjustment (Gerardi, 2003)

Chemical	Formula	Buffering cation
Sodium bicarbonate	$NaHCO_3$	Na^+
Potassium bicarbonate	$KHCO_3$	K^+
Sodium carbonate	Na_2CO_3	Na^+
Potassium carbonate	K_2CO_3	K^+
Calcium carbonate	$CaCO_3$	Ca^{2+}
Calcium hydroxide	$Ca(OH)_2$	Ca^{2+}
Sodium nitrate	$NaNO_3$	Na^+

The toxicity of some inhibitors is also dependent on pH value. For example, ammonia and hydrogen sulfide have an inhibitory effect only in their non-ionized forms (Lay et al., 1997), and the proportion of their non-ionized to ionized forms depends on the pH value. Therefore, ammonia is toxic above pH 7, while hydrogen sulfide is toxic when pH is below 7 (Ward et al., 2008).

1.4.2.5 Volatile fatty acids (VFAs)

VFAs are important intermediates produced during anaerobic digestion in the acidogenic phase. Under balanced conditions, the rate of production of the intermediates is matched by the consumption rate. However toxins in the feed,

high dry matter or high OLR close to overloading and temperature fluctuations can lead to an imbalance of the process, that is caused by accumulation of VFAs, especially of propionate, acetate and butyrate. Propinate is one of the most important intermediates in anaerobic digestion. Degradation of propionate is considered as the rate-limiting step in the whole anaerobic digestion process (Vavilin et al.2003). Propionate oxidation under methanogenic conditions requires a hydrogen partial pressure of <6.5 Pa in a narrow thermodynamically defined window. Propionate is accumulating more easily than acetate and butyrate due to its low conversion rate (Shin et al., 2010). It is well accepted that propionate or the ratio between propionate and other VFAs may serve as indicators of process imbalances. There is no obvious biogas production decrease at propionate concentrations of 2750 $mg \cdot L^{-1}$ (Pullammanappallil et al., 2001). Hill et al. (1987) suggested that a propionate/acetate ratio higher than 1.4 indicated the failure of anaerobic digestion.

1.4.2.6 Feedstock

A feasible anaerobic digestion for solid waste treatment is determined by the biogas production potential and biodegradability of different solid wastes. The biogas production potential and biodegradability of substrates mainly depends on the amount of the carbohydrates, proteins and lipids (Hartmann and Ahring, 2006). Since the methane yield from lipids is higher than from other substances, wastes containing more lipids are regarded as attractive substrate for biogas production. However, a low hydrolysis rate constant may be obtained, when the organic waste contains excessive lipids (Neves et al., 2008). The content of lignocellulosic compounds of organic wastes has a significant influence on their degradability. Due to the presence of lignin the hydrolyse of cellulose and hemi cellulose is considered as the rate limiting step in anaerobic digestion (Adney et al., 1991). The nutrient ratio C:N dependents also on the composition of waste. For a balanced nutrition, the C/N ratio should range between 20 - 30 : 1 in

substrates where the carbon constitutes the energy source for microorganisms and nitrogen serves as a critical nutrient for microbial growth (Kayhanian and Hardy, 1994; Jha et al., 2011). Water content in the substrates is essential not only for the activities of the anaerobic bacteria but also for the gas transfer. Anaerobic microbial consortia for biogas production from organic matter require a water activity of >0.91 for high-rate hydrolysis of polymer, acidogenesis of monomers, acetogenesis of fatty acids and methanogensis of acetate and of CO_2/H_2 (Rockland and Beuchal, 1987).

1.4.2.7 Mixing

Mixing can improve the anaerobic digestion process by distribution of microorganisms and substrates throughout the digester and also enhances heat transfer. Mixing creates a homogeneous condition in anaerobic digesters and ensures smooth transfer of organic substrates to microorganisms. Mixing can not only provide sufficient contact between the microorganisms and incoming substrates, but also ensure the contact between acetate-forming bacteria and methane-forming bacteria (the metabolic activities of those bacteria require a close spatial contact between them). Mixing can also prevent stratification and reduce the build-up of scum in the anaerobic digester. Furthermore, mixing also promotes release of gaseous products such as methane, CO_2, H_2, H_2S, NH_3 etc. from the anaerobic reactor. The function of mixing during anaerobic digestion is described in Table 1.5.

Mixing methods may be grouped into intermittent and continuous mixing modes. Intermittent mixing has been proved successful and efficient in anaerobic digestion of livestock waste (Mills, 1979; Smith et al., 1979). In some cases intermittent mixing improved methane production in comparison with continuous mixing (Kaparaju et al., 2008). Stroot et al. (2001) and Ben-Hasson et al. (1985) also observed that continuous mixing is disadvantageous for high solids anaerobic digestion. Minimal mixing or unmixed digestion allowed better

performance of anaerobic digestion with higher gas production in comparison with continuously mixed digestion. However, Hashimoto (1982) reported that more biogas was produced during anaerobic digestion of beef cattle wastes with continuous mixing than under intermittent mixing or under non-mixing conditions. Mixing can be accomplished through different methods: mechanical agitation, gas recirculation from the gas head at the bottom of the digester and hydraulic mixing by recirculation of digesting sludge with a pump are the most applied procedures (Karim et al., 2005; Appels et al., 2008). Gas recirculation is operated with a gas pump and a draft tube arrangement. Gas is collected at the top of the reactor and re-injected into the reactor through or at the bottom. For sludge recirculation, digesting sludge is withdrawn below the top of reactor by a pump (below an eventually present scum layer to avoid clogging of the pipes) and re-injected through the bottom of the reactor. Mechanical agitation is maintained by the use of an axial-flow-impeller, and the content of the digester is mixed through more or less intensive rotation of the impeller.

Table 1.5 Advantages of mixing during anaerobic digestion (Gerardi, 2003)

Eliminating or reducing scum build-up
Eliminating thermal stratification or localized pockets of depressed temperature
Maintaining of the digester content´s chemical and physical uniformity
Rapid dispersion of metabolic products
Minimizing toxicity
Prevent deposition of grit

1.4.2.8 Inhibitor

A variety of inhibitory substances can cause upset and failure of anaerobic digestion processes, if more than a threshold amount is present in the digester content. Inhibitory substances can be components of feedstock or may be high concentrations of intermediate products generated during anaerobic digestion.

Inhibitors have significant influence on microbiological mechanisms or bacterial growth and destroy the balance between different groups of microorganisms in anaerobic digestion processes. Inhibition is commonly indicated by a decrease of methane production, increase in volatile acid concentration and decrease of the pH value. Inhibitors with a high toxic potential for anaerobic digestion are diverse and numerous: the most commonly reported are ammonia, hydrogen sulfide, and heavy metals, but may also be biocides or antibiotics produced by fungi in rotten input material.

Ammonia

Ammonia is transferred into an anaerobic digester by pre-digested material or produced during anaerobic degradation of the nitrogenous matter such as proteins and amino acids. Inorganic ammonia nitrogen exists in two forms, ammonium ions ($NH4^{+}$) and free ammonia (FA, NH_3). Ammonium ions are the main N-nutrient source for bacteria in anaerobic digestion, whereas free ammonia is considered as the main cause of inhibition. The amount of ammonium ions and the amount of free ammonia in an anaerobic digester depend on the pH value. The amount of free ammonia increases with increasing pH above 7. Mechanisms for ammonia inhibition are a change of the intracellular pH, increase of maintenance energy requirement and inhibition of specific enzyme activities (Wittmann et al., 1995). Methanogens are more sensitive towards ammonia than the other types of anaerobic microorganisms (Kayhanian, 1999). Inhibitory concentrations of ammonia vary widely because of different pH values, temperature and acclimation. Inhibiting ammonia concentrations for mesophilic anaerobic digestion range from 2.8 $g \cdot kg^{-1}$ to 8 $g \cdot kg^{-1}$ and from 2.5 $g \cdot kg^{-1}$ to 4 $g \cdot kg^{-1}$ for thermophilic processes (Poggi-Varaldo et al., 1997; Angelidaki and Ahring, 1993).

Hydrogen sulfide

Municipal wastewater and solid wastes generally contain sulfate, which is relatively non-inhibitory to anaerobic microorganisms. During anaerobic

digestion sulfate is reduced to hydrogen sulfide by sulfate reducing bacteria (SRB). The dissolved hydrogen sulfide gas causes toxicity. The inhibition caused by sulfate reduction is a two stage inhibition. In the first stage, the competition for common organic and inorganic substrates from SRB suppresses methane production (Harada et al., 1994). The second stage of inhibition is the toxicity of sulfide to anaerobic bacteria (Chen et al., 2008). Hydrogen sulfide is one of the most toxic compounds to anaerobic digestion system. Sulfide toxicity can be diminished by dilution of the feed stream and arrangement of a sulfide removal step (Chen et al., 2008), e.g. by stripping or heavy metal precipitation.

Heavy metals

Heavy metals such as cobalt, copper, iron, nickel and zinc are elements that are present in complex substrates for anaerobic digestion. Some heavy metal ions at trace concentration are essential elements for enzymatic activity of anaerobic bacteria. However, because heavy metals are not required in high concentration, excessive concentrations may cause toxicity in anaerobic digestion. Heavy metals exert toxicity by inactivating enzymatic system, when they bind to the thiol groups of amino acids in enzymes or replace naturally occurring metals in prosthetic groups of enzymes (Vallee and Ulner, 1972; Sanchez et al., 1996).

1.4.3 Process technology for anaerobic digestion of organic solid wastes

From the moisture content of different substrates, two main types of anaerobic digestion processes can be distinguished for organic solid waste treatment, generally referred to as wet and dry anaerobic digestion. Those two basic process types can be arranged as a single phase digestion, where the complete process is accomplished in one reactor, or as two phase digestion, where two reactors are used in series. The reactors can be operated in a batch mode or in continuous mode. The operating temperature can be set in the range for psychrophilic (ambient temperature), mesophilic (37^{o}C) or thermophilic (55^{o}C)

reaction conditions. The above process technologies are possible in a wide range of combination (Figure 1.4).

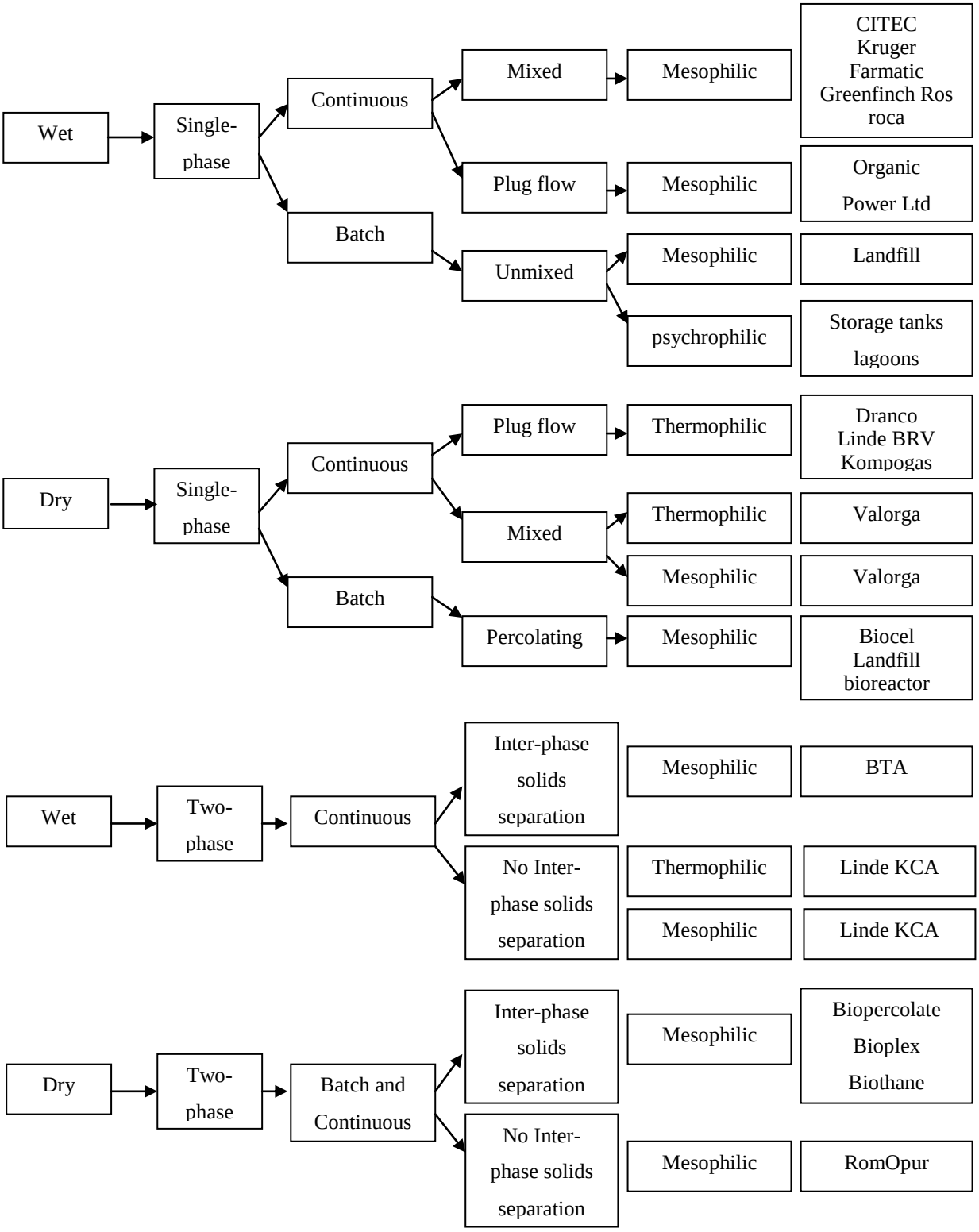

Figure 1.4 Different process technologies for anaerobic digestion of organic solid waste (Banks and Stentiford, 2007).

Wet and dry anaerobic digestion:

In wet anaerobic digestion processes the total solid content of the feed substrate is approximately 10 – 15% dry matter (DM), whereas in dry anaerobic digestion processes the total solid content of the feed substrate is kept between 25 – 40% DM (Lissens et al., 2001). Prior to feeding substrates into the wet anaerobic digester, the feedstock is conditioned to the appropriate DM content by adding process water as required. Continuously stirred tank reactors (CSTR) or wet single-pass digesters are generally used in wet anaerobic digestion processes. These can be characterised as ideally mixed when a mechanical stirring or hydraulic (liquid recycling) or pneumatic mixing (biogas injection) is installed. In dry AD processes, a complete mixing of the contents is almost impossible. Currently such reactors are commercially operated as "garage reactors" with periodical rearrangement in batch mode or as plug flow digesters in continuous mode. Those reactors are not considered as completely mixed; the incoming substrates have not sufficient contact with the microbial population, thus some pre-treatment is necessary to ensure that the active inoculum is present in each 'plug' of feedstock.

In the dry systems there is no or minimal requirement for recirculation of water or the use of fresh water. If no process water is added this reduces the need for dewatering equipment for the residues. Details of mass balances in wet and dry AD processes are shown in Figure 1.5 and Figure 1.6. The dry AD process use less fresh water than the wet AD process, and the wastewater produced in dry AD process is only a quarter of that in wet AD process (Luning et al., 2003).

Both wet and dry anaerobic digestion processes can be applied for organic solid waste treatment and have own respective advantages and disadvantages. The wet AD process have commonly an ideal mixing that ensures the necessary contact between the substrates and microorganisms, and addition of process water "dilutes" inhibitory substances. Compared with wet AD processes, the dry anaerobic treatment offer some advantages such as lower energy requirement

for heating and mixing, less process water addition, reduced nutrient losses during storage and distribution of residues. Energetically it has a more effective performance as it requires less pre-treatment and can be operated with higher organic loading rates. Furthermore, this process produces less leachate and the digested residues can be easy further treated by composting or directly used as an organic fertilizer, if free of heavy metals or other toxicants (Mata-Alvarez, 2003; Jha et al., 2011).

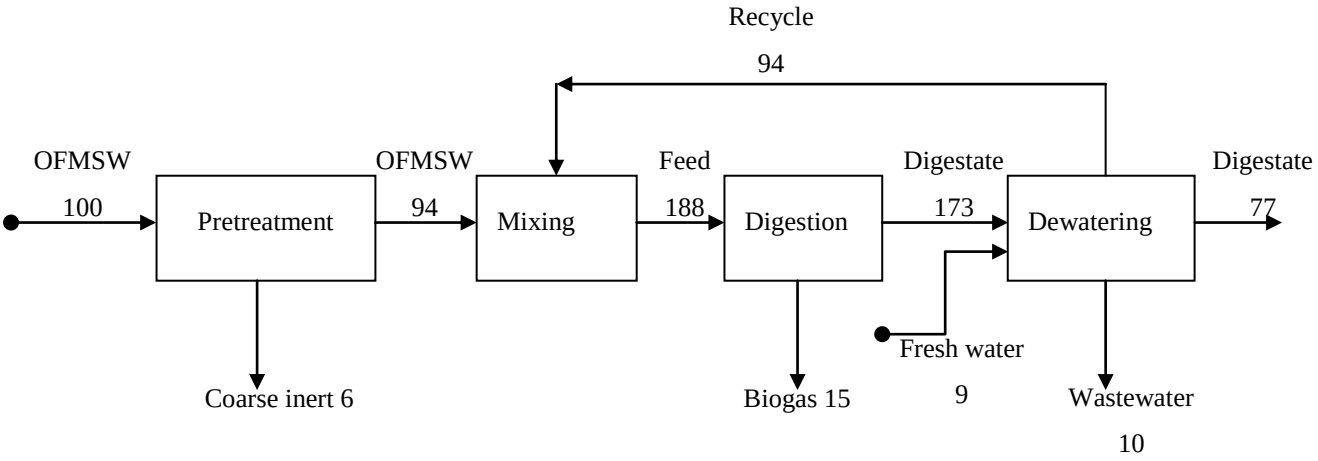

Figure 1.5 Mass balance of dry anaerobic digestion (Luning et al., 2003).

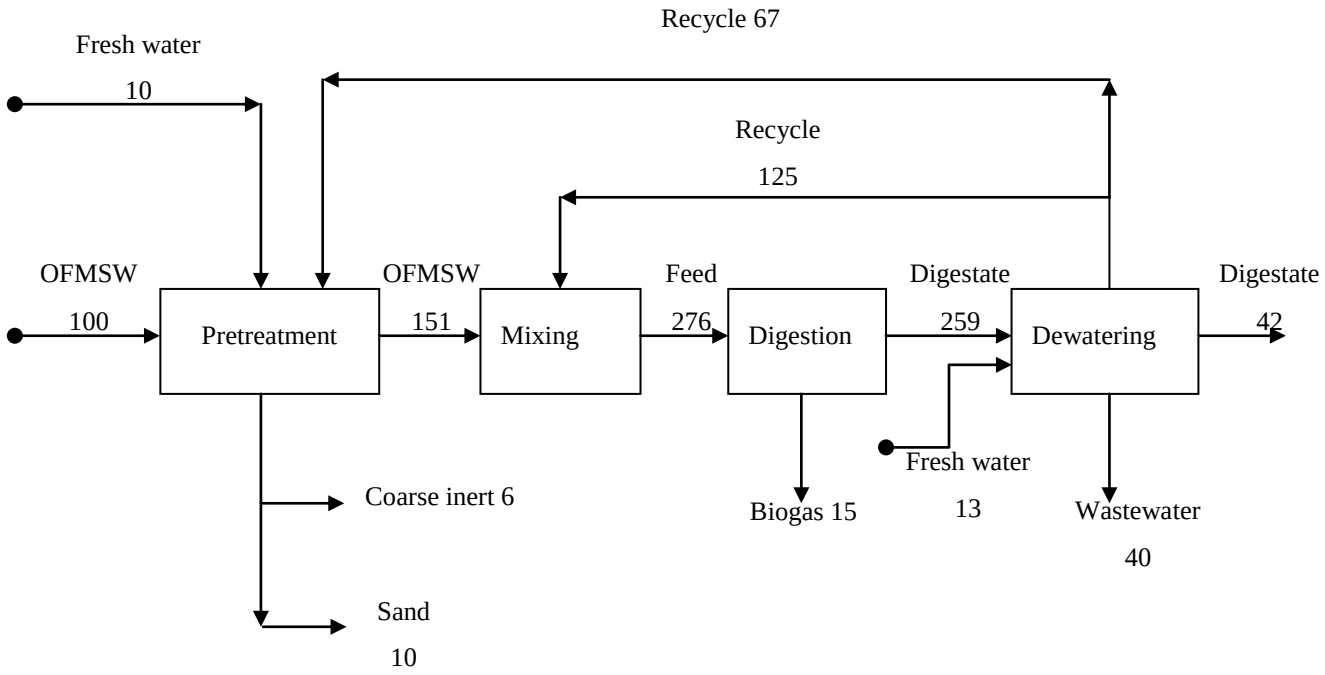

Figure 1.6 Mass balance of wet anaerobic digestion (Luning et al., 2003).

In the dry anaerobic digestion the moisture content plays an important role. The majority of the reports on dry anaerobic digestion deal with stirred tank reactors and substrates that contained up to 25 % dry matters (Bolzonella et al., 2006; Cecchi et al., 1991; Mata-Alvarez et al., 1993; Pavan et al., 2000). Only Abbassi-Guendouz et al. (2012) reported a successful DAD of biowaste with a DM content of 30% in half of their experimental reactors. Model equations for batch assays revealed that mass transfer was strongly limited in the DAD reactors with more than 30% DM content. Anaerobic microbial consortia for biogas production from organic matter require an aequous environment with a water activity of >0.91 (e.g. Rockland and Beuchal, 1987) for high-rate hydrolysis of polymers, acidogenesis of monomers, acetogenesis of fatty acids and methanogensis of acetate and of CO_2/H_2. At the high dry matter content of non-moistened solid waste there may not be enough bio available water for an optimal DAD.

Batch and continuous mode anaerobic digestion

Batch and continuous mode are the two main feeding modes used in anaerobic digestion. In a continuous feeding mode the feedstock is continuously pumped into a digester and the same amount of digested residue is withdrawn from the digester. In a batch feeding mode the feedstock is added once per day into a digester and the added substrates is fermented for the time until the next batch is added. If feedstock is added several times per day batchwise a fed-batch system or semi-continuous system is maintained.

A batch dry digestion mode is considered as an economical and inexpensive process. This process requires less pre-treatment of wastes and no sophisticated mixing equipment. No process water is added to the reactor´s feedstock for DAD in a batch mode. If any moistening is performed, the leachate is sprayed on top of the reactor onto the digesting material to improve water activity and biogas production. Due to the lower investment costs for DAD in comparison to wet anaerobic digestion (WAD), batch systems are attractive for developing

countries. In praxi, the "Biocel process" is based on batch DAD system. A Biocel plant can treat up to 50000 t biowaste per year. Fresh biowaste, which contains 30 – 40 % dry matter and the inoculum is mixed and loaded into the digester. The digester is closed with gas-tight doors. The temperature maintains itself at 35 – 40 ^{o}C and leachate is re-circulated into the reactors (Brummeler, 2000).

Continuous digestion systems can be divided into single stage and multi-stage processes. The digester operations consist of feeding and withdrawal, mixing, heating and gas collection. A single stage system consists of one reactor, in which all biochemical reactions occur. The different microbial groups involved in anaerobic digestion for biogas production have different growth rates and tolerance of fluctuations in operational conditions. This can cause an imbalance between the volatile fatty acid production rate and methane production rate. Therefore, the single stage system is more sensitive for disturbances than multi-stage systems. The multi-stage system consists of at least two reactors. In most multi-stage systems volatile fatty acid production takes place in one reactor and acetogenesis, as well as methanogenesis occur in the second reactor. The multi-stage system can optimise the conditions for each phase by providing separate reactors. In praxi, single stage systems are preferred because they have less investment costs and require simple technical support. For example the Dranco, Kompogas and Linde BRV processes are all single stage DAD systems. The single stage WAD systems are commonly referred to as continuous stirred tank reactors (CSTR). Examples of multi-stage DAD systems are the Portagester-manufactured Bioplex and the Dutch Biothane process (Banks and Stentiford, 2007).

1.4.4 Anaerobic digestion process improvement through co-digestion

In recent years significant effort has been dedicated to find methods of improving the digester performance. An interesting and innovative option is co-digestion. Co-digestion is the simultaneous digestion of a homogeneous mixture of two or more substrates. A positive synergism in the digester medium can improve gas production and process stability. Co-digestion offers some economical and technological advantages, such as:

a) Improvement of nutrient balance. The optimal nutrient ratio of C:N:P should be 300:5:1. Co-digestion with some appropriate substrates may provide a better nutrient balance of nutrient-deficient substrates. It is also capable of maintaining a proper mixture of minerals. b) Optimisation of rheological qualities. Substrate with poor fluidity, aggregating or bulking materials can be more efficiently handled after homogenisation with some more dilute liquid substrates. The materials with high concentrations of disturbing or inhibiting components can also benefit by dilution with other co-substrates.

c) Steady biogas yield throughout seasons. The seasonal fluctuation of a main substrate can be compensated by some other co-substrates in order to obtain a continuous gas yield.

d) Cooperation between agriculture and industry. Some agricultural products such as crops and animal manure have demonstrated good biogas potentials and have become a popular co-substrate in waste or wastewater treatment plants (Braun, 2002; Mata-Alvarez et al., 2014).

A variety of substrates have been reported as co-substrates for anaerobic digestion of MSW. Co-substrates should easily be bio degradable and bring in a high gas production potential, contain macro and micro nutrients to improve the characteristics of the digester medium, but not contain toxic substances. They must be available in terms of quantity and price. Co-digestion of solid waste

with food waste (Nayono et al., 2010), paper waste (Kim and Oh, 2011; Lin et al., 2012), distillery grains (Wang et al., 2012), press water from composting processes (Nayono et al., 2010) or animal manure (Zhang et al., 2011) as co-substrates has been reported. Nayono et al. (2010) demonstrated that press water from the organic fraction of domestic waste and food waste from restaurants can serve as co-substrates for biowaste digestion to improved biogas production. By feeding those co-substrates up to OLRs of 20 kg $COD \cdot m^{-3} \cdot d^{-1}$ gas production increased linearly with the OLR. Kim and Oh (2011) examined co-digestion of biowaste with paper waste in a continuously run dry digester. The biogas production increased as HRT decreased until 40 d. In a study of Wang et al. (2012) the co-digestion of distillery grains (DG) and biowaste (MSW) with four different ratios was examined. Compared to the mono-digestion, biogas production was improved 25-75% with all four DG/MSW ratios due to synergistic effects. Zhang et al. (2011) stated that co-digestion of biowaste with piggery wastewater improved the biogas yield due to the supply of missing trace elements by the co-substrates.

Chapter 2

Goal and objectives

The advantages of co-digestion for organic waste treatment have been known for a long time. Co-digestion is established especially in agricultural biogas plants to improve biogas yield and to stabilize the wastes for use as an organic fertilizer. Until today a variety of substrates has also been examined as co-substrates for anaerobic digestion of municipal solid waste (MSW) to improve biogas yield and process stability. There are still many co-substrate candidates for use in municipal wet or dry organic waste digestion such as cantine residues, overdue bread from bakeries or overlayed yogurt from dairy factories. These substrates are highly concentrated, easily accessible, readily bio degradable and may have great biogas production potential, but are barely reported be used as co-substrates in anaerobic digesters for treatment of MSW. Aim of this study was thus to determine the effect of co-substrate addition for anaerobic digestion of MSW following objectives such as:

- Examination of the biogas production potential of different co-substrates during anaerobic digestion of these biowaste fractions together with MSW
- Elucidation of the maximal permanent or peak loading rate for stable biowaste co-digestion with MSW when OLR was stepwise increased with the co-substrates until failure and possibilities for re-establishment of balanced digestion
- Analysis of maximal propionate degradation rates when the OLR was stepwise increased with co-substrates and by propionic acid addition

As an innovative waste-recycling method to treat high-solid-content waste, dry anaerobic digestion (DAD) has been gaining more attention for practical application in MSW treatment, although the scientific basis is not yet

established sufficiently. In praxi DAD was chosen by farmers as a low-tech digestion possibility of agricultural wastes and initially was performed garage or box fermenters. To improve digestion speed and efficiency digesters were developed that more and more resemble stirred tank reactors, but with much less moisture. Most literature reports focused on stirred tank-type reactors and almost no information is available about DAD performance in box fermenters. Another aim of this study was thus to describe DAD of biowastes in box fermenters by investigating the following objectives:

- Evaluation of the biogas production and VFAs accumulation during dry anaerobic digestion of biowaste with different dry matter content - Effect of water activity on anaerobic digestion
- Influence of the temperature changes in a range from ambient ($20^{o}C$) to mesophilic ($37^{o}C$) and thermophilic ($55^{o}C$) temperature on DAD

Chapter 3

Materials and Methods

3.1 Substrates

3.1.1 Biowaste

The biowaste and biowaste suspension used in this study was the source-sorted organic fraction of municipal solid wastes (OFMSW), that was collected in the City of Karlsruhe and treated in the biowaste treatment plant of Karlsruhe. Operation of the full-scale biowaste treatment plant is the basic background of this study. The separated biowaste fraction in households of the City of Karlsruhe was collected with rotating drum trucks and unloaded into the deep bunker of the WTP for full-scale anaerobic digestion (Gallert et al., 2003, Nayono et al., 2009). Source-sorted OFMSW is transported from the deep bunker by conveyer bands to a drum mill, where it is shredded. From there it passes an electro magnet for metal removal and is transported via another conveyer band to the BTA/MAT hydropulper (Phillip Müller, Stuttgart). In the hydropulper 6 m^3 of the crushed biowaste are suspended in 12 m^3 of process water (supernatant of centrifuged digester effluent + rain water), that makes the moisture content of biowaste suspension more than 90% in order to perform a wet anaerobic digestion. The heavy mineralic fraction (stones, forks, cans, ceramics, etc) are removed from the hydropulper grit bottom and the floating light fraction (mostly plastics) are withdrawn from a scum layer at the top of the hydropulper. During interim storage fine sand is separated through two hydro cyclones. The impurity-free biowaste suspension from interim storage is then loaded into the anaerobic digester every 2-3 hours. The obtained biowaste suspension (composition see Table 4.1) is digested in a cylindrical tank reactor (CSTR) with gas injection to avoid sedimentation.

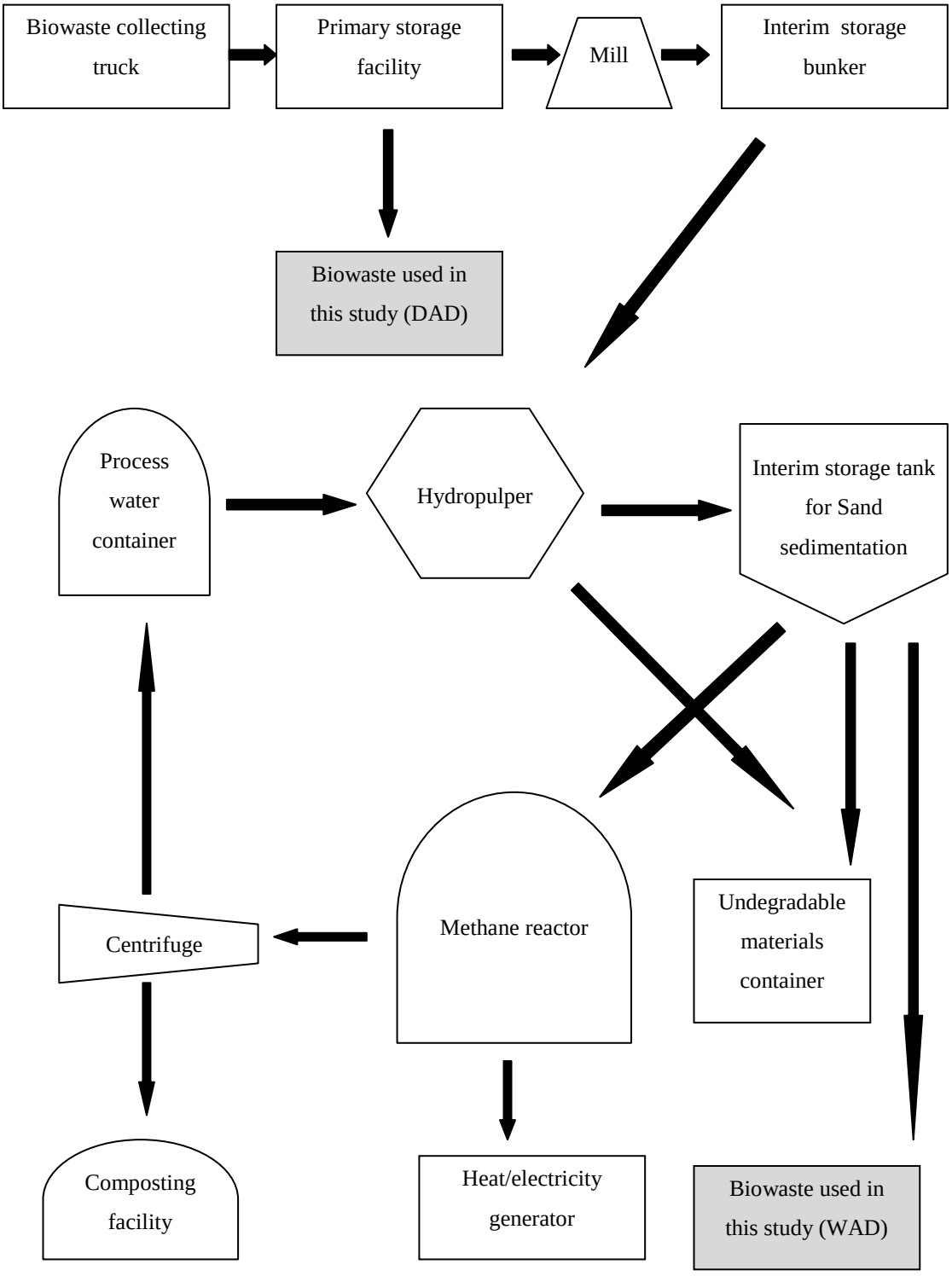

Figure 3.1 Schematic representation of biowaste treatment in Karlsruhe, German.

The digester had a total volume of 1350 m^3 and a maximum working volume of 1120 m^3 and was operated at 35 ± 2°C. Up to 72 tons source-sorted OFMSW are processed and digested per day. Digested suspension must be treated and separated into process water and residues through centrifugation. Figure 3.1 depicts all processes involved in the biowaste treatment plant Karlsruhe.

For DAD-experiments in the laboratory collected biowaste from the deep bunker was manually sorted before crushing in cutter mill (ZG Raiffeissen, Karlsruhe) to 1 cm length. The shredded biowaste with a DM content of about 30% was applied for the laboratory DAD experiment.

The biowaste suspension samples for WAD experiments were collected from the interim storage tank, after heavy metal and plastics removal in the hydropulper and sand removal in the interim storage tank, before uploading into the methane reactor. The collected samples were further sieved again in the laboratory and stored in a refrigerator until use.

Anaerobic digestion for solid waste treatment requires a relatively long time for start-up, a condition attributed to the slow growth of anaerobic bacteria. In order to accelerate the start-up period of the laboratory experiments, effluent from methane reactor of the biowaste treatment plant Karlsruhe was used as a source of anaerobic sludge inocula for WAD experiments. Solid residues of digested biowaste, taken from the extrusion pipe of the sludge centrifuge were taken as a source of microorganisms for DAD experiments.

3.1.2 Co-substrates

Sewage sludge, old bread, yoghurt and food waste were used in this study as co-substrates for anaerobic digestion of biowaste.

Sewage sludge was collected from the wastewater treatment plant Berghausen, in which the wastewater from community of Berghausen is purified by a combination of mechanical and biological treatment processes. The flow scheme of this wastewater treatment plant is depicted in Figure 3.2. During the

mechanical stage part of the mineral ingredients and floating materials are removed by sand sedimentation and fat flotation. In the subsequent primary clarifier the fuzzy and granular ingredients are eliminated by sedimentation under reduced flow speed. After passing the primary clarifier the wastewater flows into the biological treatment stage, which consists of a circular tank and two rectangular tanks. In the circular tank nitrate is converted to elemental nitrogen under anoxic condition by pre-denitrification. In the both rectangular tanks carbon elimination by activated sludge treatment and nitrification is established under aeration. The organic carbon compounds are degraded to carbon dioxide, water and ammonia and the ammonia is converted to nitrate via nitrite. From the nitrification tank the nitrified wastewater is returned to the denitrification tank by internal recycling. At the end of the biological treatment stages wastewater and activated sludge are separated in a sediment tank. The primary sludge and the activated sludge were respectively taken from the primary clarifier and the aeration tank and stored in refrigerator until it is used.

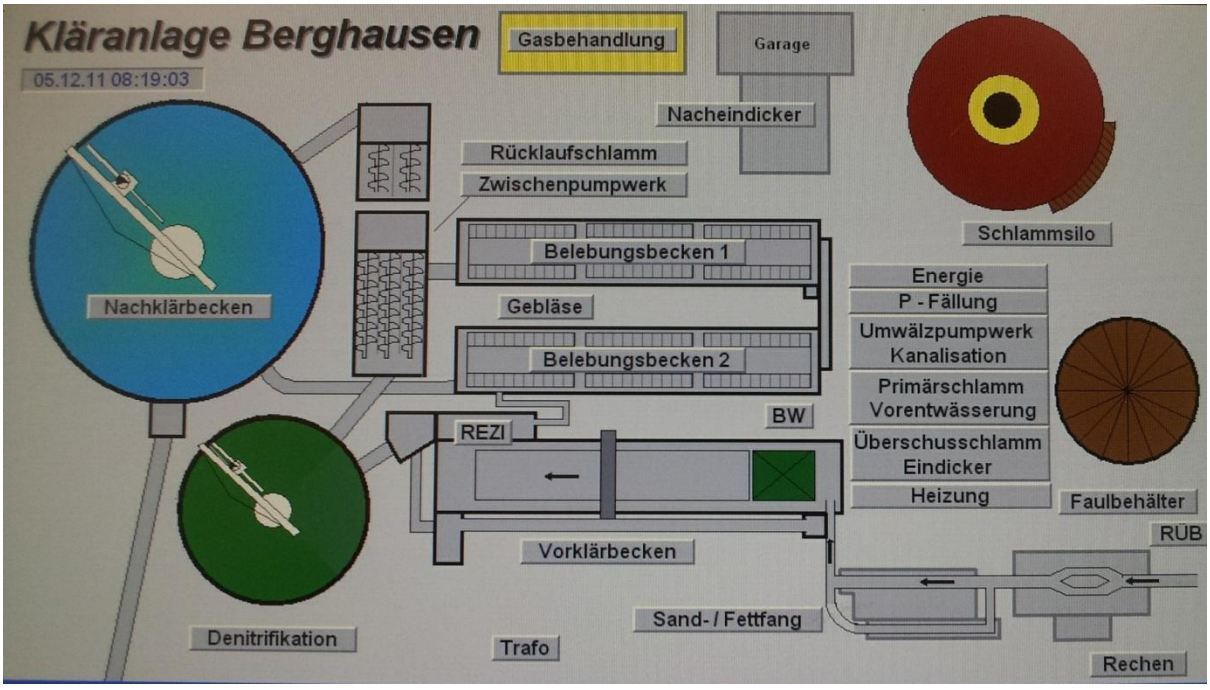

Figure 3.2 Schematic representation of the wastewater treatment plant Berghausen.

In the City Karlsruhe some industrial bakeries collect overlayed bread from the bakery shops and bring it to the biowaste treatment plant of Karlsruhe. It is mashed together with biowaste in a hydropulper for defibering and in a wet biowaste digestion plant. The overlayed bread used in this study was sorted into

wheat bread (flour from wheat grains, bake with yeasts) and rye bread (flour from rye grains, baked with sour dough) in the biowaste treatment plant. The two sorts of bread were frozen and stored in freezer until use. The yogurt for this study was a low-fat joghurt with 1.5% fat content, bought from a supermarket. The food waste used in this study was collected from the university canteen of KIT, grinded with an Ultra-Turrax T50 (Janke & Kunkel GmbH, German) to particles of less than 3mm, the suspension was filled in portion of 1 L into plastic bottles and frozen until use.

3.2 Laboratory-scale digester set-up

3.2.1 Serum bottle reactors

The serum bottle reactors had a working volume of maximally 100 mL. The temperature was maintained at 37^{o}C in an incubator, a shake platform was installed in the incubators to maintain mixing of the suspensions. Reactors were closed with rubber stoppers and sampling as well as feeding were done by injection through the rubber stopper (Figure 3.3). These little "reactors" were used for determination of VFA degradation rates.

3.2.2 Schott bottle reactor

The Schott bottle reactors (Schott, Mainz, Germany) had a different size, the most commonly used had working volume of 1 L and 3.5 L. A constant temperature of reactor of 37^{o}C was achieved by a warm water cover – thermostated water was pumped through plastic tubes surrounding the reactor (Figure 3.3). The suspension in reactor was homogenously mixed with a magnetic stirrer. Biogas from the reactor was measured with Milli-Gas Counters (Ritter model MGC-1 V30) and analyzed with a Blue Sense Model BACCom 12 CB methane/CO_2 gas detector and registered by a computer unit (System Blue Sense Gas GmbH, D-45099 Herten, Germany). This type of reactor was

applied for DAD experiments and also for experiments to determine the biogas production potential of substrates.

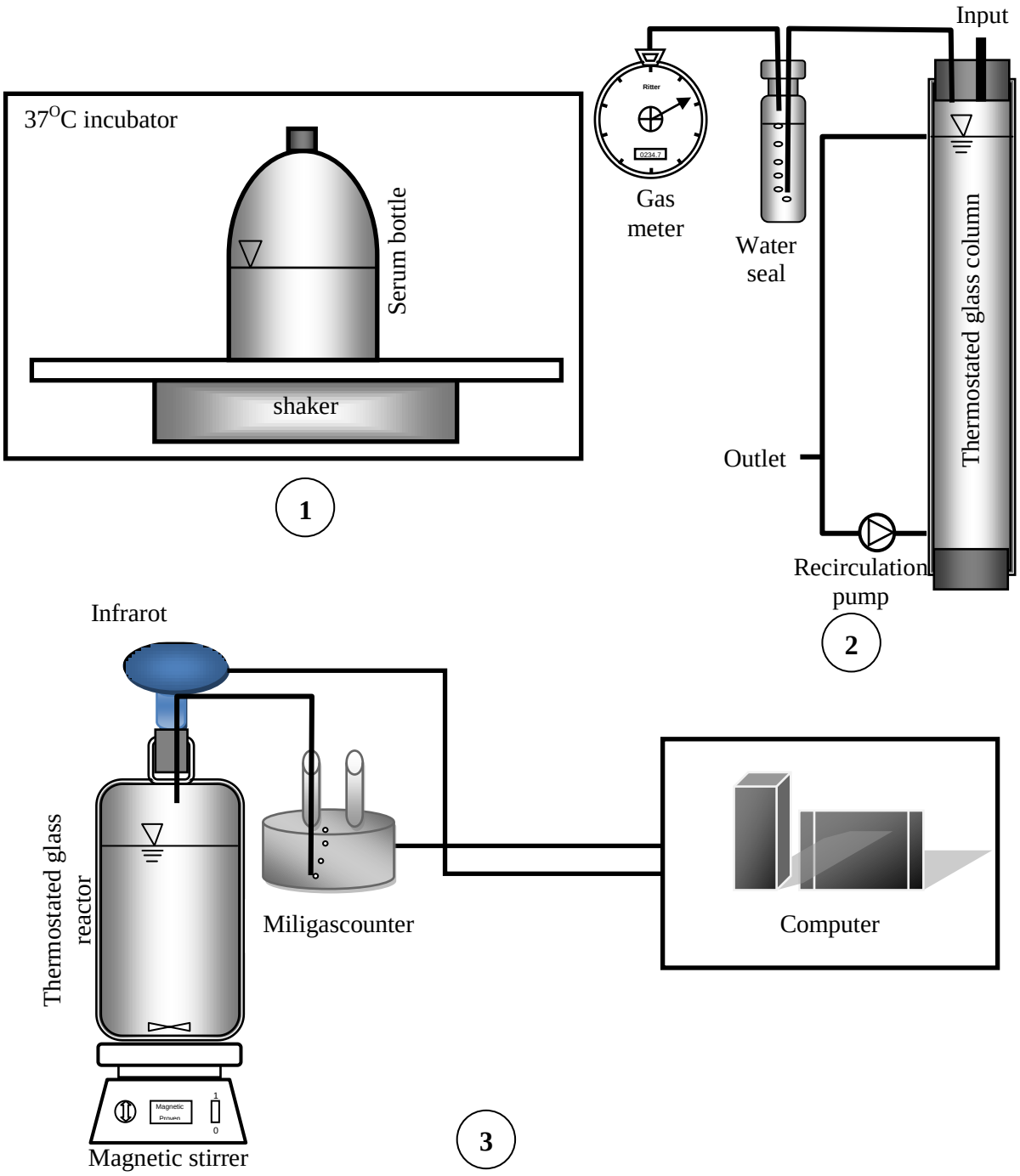

Figure 3.3 Schematic representation of reactors used in this study. 1. Serum bottle reactor; 2. Glass column reactor; 3. Schott glass reactor

3.2.3 Glass column reactor

The laboratory-scale semi-continuous reactors simulated the full-scale anaerobic digestion reactor in practice. The reactors consisted of a glass column with a liquid working volume of 8 L (inner diameter 0.1 m, total height 1.50). Top and bottom of the reactors were sealed with rubber stoppers. The thermostated reactors maintained a temperature at 37 ^{o}C though that warm water cover. In order to maintain a homogeneous mixing of suspensions, suspension from the top of the reactor was circulated to the bottom of the reactor by a peristaltic pump (Whatson Marlow, Germany). Inlet and Outlet was respectively installed on the top and bottom of the reactors, substrates were fed manually through the inlet pipe after effluent was taken from the outlet. Biogas production from the reactor was measured by a wet gas meter (Figure 3.3). This type of reactor was employed for WAD experiment for biowaste co-digestion with different substrates.

3.3 Experimental design

3.3.1 Batch assays for determination of the biogas production potential of substrates

Biogas productivity from biowaste and other different substrates was determined in batch assays using Schott bottle reactors (Figure 3.4). In the batch assay 20 ml of fresh biowaste suspension containing 1.6 g COD, or 10-30 ml of different co-substrates containing 2-3 g COD, was digested with 1 L digested sludge from the methane reactor of the biowaste treatment plant of Karlsruhe as a source of active anaerobic methanogenic consortia.

3.3.2 Dry anaerobic digestion (DAD) of biowaste with different moisture content

DAD experiment for biowaste with different moisture content was accomplished in batch assays using 3.5 L glass reactor (Figure 3.5).

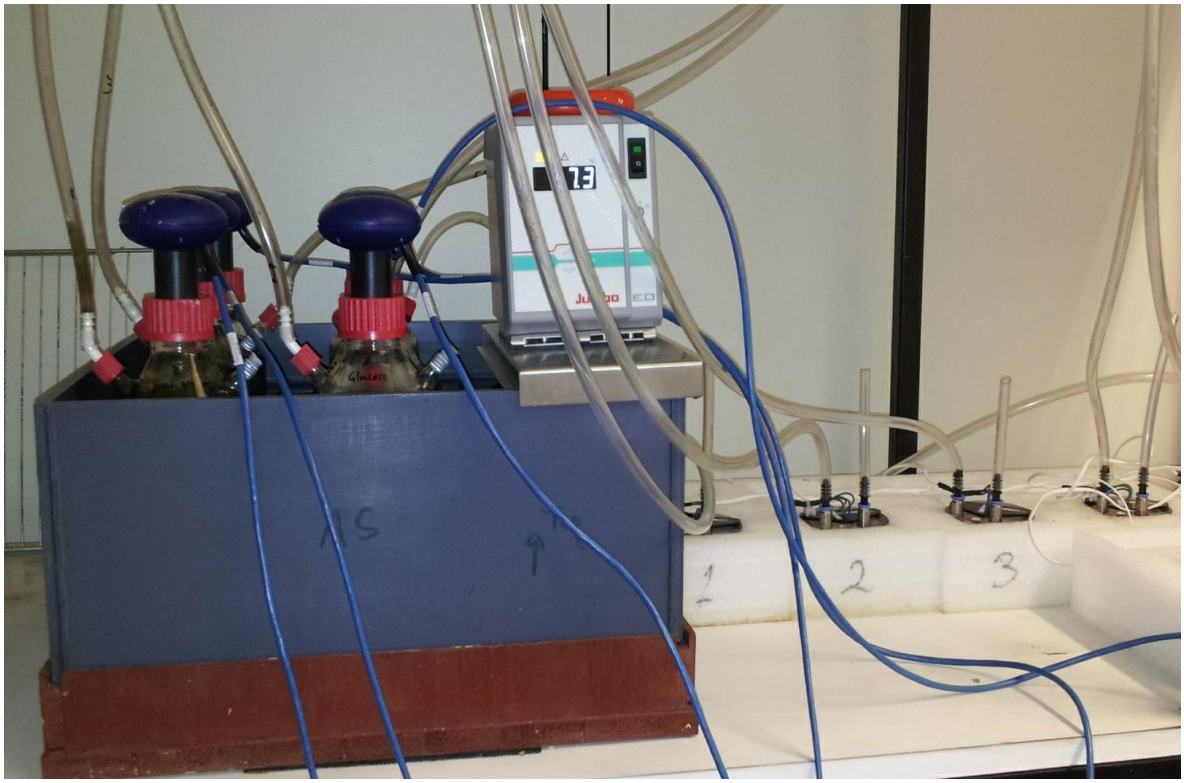

Figure 3.4 Reactors set-up for determination of the biogas production potential of biowaste and co-substrates.

Portions of 10 kg of fresh biowaste and of 10 kg solid residues of digested biowaste suspension were mixed thoroughly. Little water was added to obtain a DM content of 30%. In order to obtain biowaste fraction with 25% and 20% DM content, the above mixture was accordingly diluted with water. Parallel DAD reactors were filled with 2 kg of above prepared biowaste fractions that contains respectively 30%, 25% and 20% DM. One reactor was only fed with 2 kg solid residues that contained 25% DM and was incubated as a control. Reactors were initially flushed with nitrogen, closed with rubber stoppers and incubated at room temperature (22^{o}C). Subsequently the incubation temperature was raised to 37±0.5^{o}C and 55±0.5^{o}C, that will be in detail described in Figure 4.16 – 4.18). Re-feeding cycles of the reactors were also operated at 37±0.5^{o}C and 55±0.5^{o}C. After feeding and re-feeding the reactors, the pH was immediately adjusted with 5M NaOH to above 8. Then, due to acidification, the

pH had to be corrected after 5, 10 and 30 days to maintain conditions for harmless methanogenesis. Aqueous leachate which accumulated at the bottom of the reactors after 3-4 days was regularly remixed into the solid fraction by manual shaking of the reactors. For measuring the pH and volatile fatty acid concentrations 1 ml of leachate was withdrawn from the bottom of the reactor through a valve.

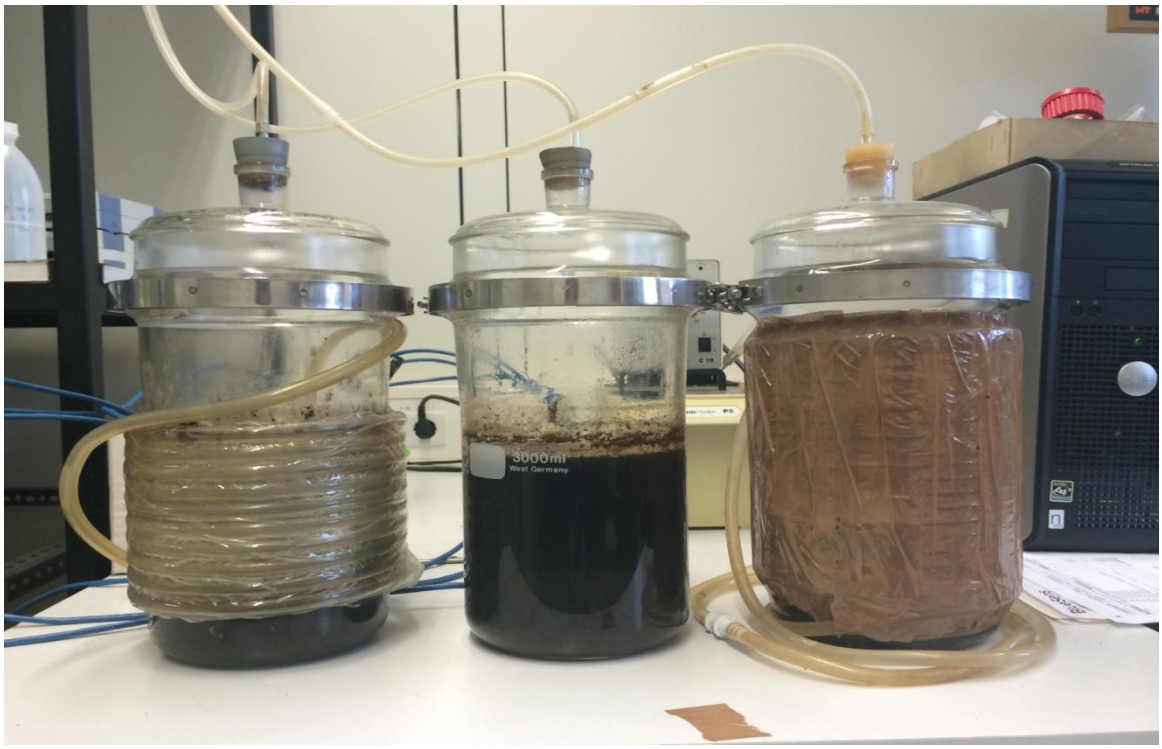

Figure 3.5 Batch assays for dry anaerobic digestion of biowaste with different moisture content.

3.3.3 Batch acidification experiments

Batch acidification experiments were operated with wheat bread, rye bread and biowaste suspensions. 900 ml of each suspension were mixed with 100 ml digested biowaste suspension in a Schott-glass reactor. Reactors were initially flushed with nitrogen and then incubated at 37^{o}C for 90 hours on a shaking platter to avoid sedimentation. Representative samples were withdrawn every 24 hours to determine the VFA concentration and pH.

3.3.4 Co-digestion of biowaste and bread suspension

For biowaste co-digestion with wheat and rye bread, glass column reactors with 10 L volume, 8 L working volume were used. Initially the reactors were fed twice a day only with biowaste at a HRT of 8 days, equal to an OLR of 14.3 kg COD $m^{-3}d^{-1}$. From Monday to Friday at 8:30 a.m. and 5.00 p.m. half a liter of digested biowaste suspension was pumped out of each reactor and replaced by fresh biowaste suspension. After reaching the steady state, in addition to biowaste suspension, increasing amounts of 0.1 – 0.5 L d^{-1} of wheat bread suspension (WBR 1) or rye bread suspension (RBR 2) were added as feed stock into reactors. Wheat bread and rye bread suspension as co-substrates were added to biowaste suspension every morning and evening. The suspensions were mixed thoroughly before feeding. In order to examine the influence of addition of bread as co-substrate on WAD of biowaste and to determine the difference between wheat and rye bread as co-substrates, biogas production, methane content, COD, TS, VS, pH and VFA of the effluent were measured before feeding.

3.3.5 Co-digestion of biowaste with propionic acid

Four cylindrical glass reactors with a total volume of 10 L and a working volume of 8 L, wrapped with silicon tubing for warm water circulation from a thermostat to maintain 37 °C, were fed from Monday to Friday at 8 a.m. and 6 p.m. with fresh biowaste suspension, replacing 600 ml of digested biowaste. No feed was added on Saturday and Sunday as in the full-scale plant of Karlsruhe. The laboratory digesters were run at 12 or 14 kg COD m^{-3} d^{-1} organic loading rate (OLR) with biowaste batch 1 or batch 2, respectively as a “basic load”. Reactor 1 was run as a control for 50 days at an OLR of 12 kg COD m^{-3} d^{-1} (biowaste batch 1). In reactor 2 the OLR was also 12 kg COD m^{-3} d^{-1}, maintained with biowaste suspension batch1 until day 55 and then 14 kg COD m^{-3} d^{-1}, maintained with biowaste batch 2. The OLR with biowaste was increased

stepwise by addition of respective amounts of propionic acid (addition of 0.7 g propionic acid per L equals an OLR increase of 1 kg COD m^{-3} d^{-1}) to maximally 18 kg m^{-3} d^{-1}. During glass repairs (days 75 – 85) the reactor content was stored under anaerobic conditions but not fed. Reactor 3 was run with a basic OLR of 3 kg COD m^{-3} d^{-1}, maintained by propionic acid addition all week to keep the propionate-oxidizing bacteria (POB) active. Biowaste suspension was available from Monday to Friday and was added to give an additional OLR of 11 kg COD m^{-3} d^{-1}. Reactor 4 was fed with biowaste suspension from Monday to Friday at an OLR of 11 kg COD m^{-3} d^{-1} and with propionic acid from Friday night to Monday morning at an OLR of 5 kg COD m^{-3} d^{-1}. With this feeding regime the same daily gas production as with biowaste, necessary for continuous operation of a generator, was obtained.

Figure 3.6 Batch assays for determination of VFA degradation to biogas

3.3.6 VFA degradation kinetics

Serum bottle reactors were used to determine the fatty acid degradation rates (Figure 3.6). 40 ml of homogenized digester samples were transferred into

serum bottles that were plugged with rubber stoppers and gassed with nitrogen at a gassing station. Assays were incubated for one day to degrade residual acetate and propionate. To start experiments for rate determination 50 mmol L^{-1} acetate or propionate, respectively, was injected into single serum bottles with a syringe and duplicate assays were incubated at 37^{o}C on a shaker. During incubation sample were withdrawn with a syringe and the concentration of acetate, propionate and n-butyrate was determined by gas chromatography. Mean degradation rates were calculated for logarithmic or linear degradation phases of the respective acid in two parallel assays.

3.4 Analytical methods

3.4.1 Chemical oxygen demand (COD)

The chemical oxygen demand represents the oxygen equivalent of the organic matter content of a sample that can be full oxidized to carbon dioxide with strong oxidizing agents under acidic conditions. In this study the COD was determined according to the method of Wolf and Nordmann (1977). This method can oxidize organic matter at 95-100% of the theoretical value. Potassium dichromate ($K_2Cr_2O_7$) was used as the oxidizing agent in a solution of sulfuric acid (H_2SO_4) and phosphoric acid (H_3PO_4) (3:1). The catalyst was silver sulfate (Ag_2SO_4). The sample was centrifuged and 1 ml of diluted supernatant was mixed thoroughly with 1.5 ml COD reagent containing the oxidizing agent. After incubation of the sample in a thermo bloc (Thermo, Bielefeld) at 150^{o}C for 2 hours, the absorbance of the built green color of released Cr^{3+} ions due to $K_2Cr_2O_7$ reduction was determined with a Spectrophotometer at 615 nm (Ultrospec II spectrophotometer- Biochrom Ltd., Cambridge). The concentration of unknown samples was calculated by comparison with a standard curve prepared with potassium hydrogen phthalate (C8H5KO4) (0-1400 mg L^{-1} COD).

3.4.2 Volatile fatty acid (VFA)

The concentration of volatile fatty acid was determined by gas chromatography (United Technologies PACKARD model 437A) with a flame ionization detector (FID) using a Teflon column packed with Chromosorb 101 (Germany). Mixture of hydrogen (30 mL. min^{-1}) and synthetic air (300 mL min^{-1}) were used as burning gas. Nitrogen (30 ml min^{-1}) was used to serve the gas chromatograph as carrier gas at an oven temperature of 180^{o}C and an injector and detector temperature of 210^{o}C. The Teflon column packed with Chromosorb 101 served for separation of fatty acids. The samples were centrifuged for 10 min at 15.000 rpm. The clear supernatant was diluted with 4% H_3PO_4. One ml diluted sample was injected into the injection port of the GC with a 10 µl syringe (Hamilton, USA). A standard of mixed volatile fatty acid, i.e. acetate, propionate, iso- and n-butyrate and iso- and n-valerate (5M each), was injected as reference before analysis of the samples. The calculation of VFA was based on peak area comparison between the tested samples and a standard sample.

3.4.3 Biogas composition

The composition of biogas from continuous reactors was measured with a gas chromatograph (Chrompack CP 9001). The gas chromatograph was equipped with a micro volume thermal conductivity detector (TCD) and a capillary column CarboPlot ®007 WLD FS (with 0.53 mm of inner diameter and 27.5 m of length) packed with Poropack N (Sigma, Germany). Nitrogen was the carrier gas at a flow rate of 30 ml min^{-1} with the following temperature setting: column 110^{o}C, detector 220^{o}C and injector 250^{o}C respectively. The filament temperature of TCD was set automatically approximately 100^{o}C above the detector block temperature. The pressure at the control panel for both analysis and reference regulators was 160 kPa. 0.1 ml gas samples from reactors were injected into the gas chromatograph with a 0.5 ml Pressure-Lok® syringe (VICI precision sampling Corp., baton Rouge, Louisiana). A biogas standard

consisting of 60% methane and 40% carbon dioxide was injected every time first as the reference concentration for the GC calibration.

The composition of biogas from batch assays was determined by the BlueSens gas sensor (BlueSens gas sensor GmbH, Herten, Germany). A gas senor consists of a senor head and a measuring adapter. The sensor head contains an IR-radiation source, two beam detectors and the evaluation electronics. The light beam was reflected in the measuring adapter and there it was weakened by the analyte. The attenuate light was measured with the detector, and the concentration of CH_4 and CO_2 was evaluated using the electronics.

3.4.4 Biogas production

Daily biogas production of continuous reactors was measured with a wet gas meter (Ritter, Bochum-Langendreer, Germany), which was based on the principle of water displacement. In the batch assays, the MilliGascounter® was employed for measuring of the biogas production. The measured gas passed through a micro capillary from bottom into the gas counter, which is filled with a barrier fluid. A balance pedal in the fluid was tipped by the rising gas bubbles. The volume measurement of gas is achieved by counting the tipping.

3.4.5 D- and L-lactic acid

D- and L-lactic acid was analysed by using an enzymatic test kit of Boehringer Mannheim (R-Biopharm AG, Darmstadt, Germany). In the presence of D-lactate dehydrogenase (D-LDH), D-lactic acid is oxidized to pyruvate by nicotinamide-adenine dinucleotide (NAD). The oxidation of L-lactic acid requires the presence of the enzyme L-lactate dehydrogenase (L-LDH). The equilibrium of these reactions lies on the side of lactate. By trapping pyruvate in a subsequent reaction catalyzed by the enzyme glutamate-pyruvate transaminase (GPT) in the presence of L-glutamate, the equilibrium can be shifted towards pyruvate and NADH. The amount of NADH formed in the reactions is

stoichiometric to the amount of D- and L-lactic acid. The increase in NADH is determined by means of its light absorbance at 340 nm.

The test reagent:

1. Bottle 1 with approx. 30 ml solution, consisting of glycine hydrazine puffer, pH 10.0, and L-glutamic acid
2. Bottle 2 with NAD, lyophilizate
3. Bottle 3 with glutamate-pyruvate transaminase suspension
4. Bottle 4 with D-lactate dehydrogenase solution
5. Bottle 4 with L-lactate dehydrogenase solution

Procedure:

Pipette into cuvettes	Blank	Sample
Solution 1	1 ml	1 ml
Solution 2	0.2 ml	0.2 ml
Suspension 3	0.02 ml	0.02 ml
Sample	-	0.1 ml
water	1 ml	0.9 ml
Mix, and read absorbances of solutions (A1). Start reaction by addtion of:		
Solution 4	0.02 ml	0.02 ml
Mix, after 30 min read absorbances of of blank and sample (A2) Add:		
Solution 5	0.02 ml	0.02 ml
Mix, after 30 min read absorbances of blank and sample (A3)		

The absorbance differences (A2-A1) was determined for blank and sample. The absorbance difference of the blank was subtracted from the absorbance difference of the sample to obtain $\Delta A_{\text{D-Lactic acid}}$.

The absorbance differences (A3-A2) were determined for blank and samples. The absorbance difference of the blank was subtracted from the absorbance difference of the sample to obtain $\Delta A_{\text{L-Lactic acid}}$.

The calculation was as follows:

$$C = V \cdot MW \cdot \Delta A / (\varepsilon \cdot d \cdot v \cdot 1000)$$

V= final volume (ml)

v = sample volume (ml)

MW = molecular weight of the substance ($g \cdot mol^{-1}$)

d = light path (cm)

ε = extinction coefficient of NADH at 340 nm (6.3 $L \cdot mmol^{-1} \cdot cm^{-1}$)

Ammonia nitrogen (NH_4-N)

Ammonia was determined according to DEV (1983) procedure E5 (DIN 38406).

The two reagents used for analysis of ammonia were:

Reagent A; 13 g sodiumsalicylate, 13 g Tri-Natriumcitrate-Dihydrat and 0.097 g 2-Nitroprussidnatrium-Dihydrat were dissolved in 500 ml de-ionized H_2O. Reagent B; 1.6 g NaOH and 0.1 g Dichlorocyanuric acid-Na-Dihydrat were dissolved in 50 ml deionized H_2O. Ammonia ions react at a pH value of about 2.6 with hypochlorite and sodium salicylate in the presence of sodium pentacyanonitrosylferrate as a catalyst to a blue-coloured product which could be measured in a spectrophotometer at 655 nm. For analysis, 0.125 ml reagents A and 0.125 ml reagent B were added into 1 ml sample, and then measured after 1–3 hours incubation at room temperature.

3.4.6 Total Kjeldahl nitrogen (TKN)

Total Kjeldahl nitrogen (TKN) is the sum concentration of both, organic nitrogen and ammonia nitrogen. TKN was determined by using standard methods (APHA, 1989) with residue after distillation of NH_4^+-N. The organic nitrogen was converted to ammonia, then the total ammonia was distilled into an acid absorbing solution and determined by titration method. Sulphuric acid

was employed as the oxidizing agent. The oxidation proceeded rapidly at temperatures slightly above the boiling point of sulphuric acid (340oC). When the organic nitrogen has been released as ammonia nitrogen, it was determined by using preceding distillation. Ammonia was distilled into a solution of boric acid and determined by titration with H_2SO_4 with a indicator.

3.4.7 Total solids and volatile solids

The total solids content (TS) and volatile solids content (VS) of the sample were determined according to Standard Methods of wastewater analysis (DIN 38409, DEV,1983). To determine the TS, a defined volume or weight (V) of homogenised samples was placed in ceramic vessel (m_a) and kept in a hot air oven (Memmert, Germany) at 105^{o}C for 24 hours for evaporation of moisture until a constant weight (m_b). For determining VS, the samples after TS determination were oxidized in a Muffle Furnace (Heraeus instruments, Germany) at 550^{o}C for 2 hours (m_c) and the minerals content of the sludge sample was subtracted from the total solid content.

$$TS = (M_b - M_a)/V \cdot 1000$$

$$VS = (M_b - M_c)/V \cdot 1000$$

M_a = weight of empty vessel

M_b = weight of vessel and dried sample

M_c = weight of vessel and ash sample

V = volume of sample

3.4.8 Acid capacity for a pH 4.3 (KS4.3)

$K_{S4.3}$ is a value to describe the buffering capacity of a solution against acidification. The acidity was determined according to DIN 38409-7 (DEV, 1983). 200 ml of bread suspension, biowaste suspension and digested biowaste

suspension (V_p) were titrated with 0.1 M HCl until the pH value reached 4.3. $K_{S4.3}$ was calculated from the amount of 0.1 M HCl (V_a) required to reach pH 4.3 according to the following formula:

$$K = V_a \cdot C \cdot 1000 / V_p$$

V_a = volume of HCl

C = concentration of HCl

V_p = volume of sample

3.5 Basic calculations:

3.5.1 Hydraulic retention time (HRT)

Hydraulic retention time (HRT) is the average residence time of the waste suspension in the reactor. It is calculated by comparing the working volume of the reactor and the effluent volume.

$$HRT = V/Q$$

V = liquid volume of reactor

Q = is daily effluent

3.5.2 Organic loading rate (OLR)

The organic loading rate (OLR) is the amount of organic matter, that is loaded to one volumetric unit of reactor per time unit. The OLR is calculated by using the following equation:

$$OLR = O_c \cdot Q / V$$

O_c = organic matter concentration of substrate (represented as COD or VS concentration)

Q = feeding rate

V = liquid volume of reactor

Chapter 4

Results and Discussion

4.1 Potential of different organic materials as co-substrates for anaerobic digestion

As for other renewable energies the biogas demand has rapidly increased in recent years. Anaerobic digestion of municipal solid waste not only solves the problem of an appropriate treatment of putrescent wastes but also provides biogas for electricity generation in combined heat and power units. However, anaerobic digestion of single substrates (mono-digestion) could not always archive an optimal efficiency due to substrates properties. By addition of co-substrates (co-digestion) the drawback could be partly compensated. Co-substrates must consist of easily degradable organic matter and have a great biogas production potential. In addition to this they must be available in terms of quantity and price.

In this part of the study, sewage sludge respectively from the primary clarifier, the secondary clarifier after the aeration tank, some waste from our daily life (bread and yoghurt) and food waste collected from restaurants, hospitals and university canteens were evaluated as co-substrate candidates. The biogas production potential of biowaste and of each of the co-substrates was determined in batch assays. The suitability of every substrate as co-substrates for co-digestion with biowaste was examined in continuously operated reactors during a short time period.

4.1.1 Characteristics of the main substrate and of co-substrates

Characteristics of the main substrate “biowaste” and of different co-substrates used in this study, such as activated sludge and primary sludge from sewage treatment plants, overlayed bread, yoghurt and other “food wastes”) are described in Table 4.1. The biowaste suspension used for experiments in this

part was collected from the full-scale anaerobic digester in Karlsruhe, where the suspension in digested in a wet anaerobic digestion system. During the pre-treatment of biowaste for anaerobic digestion, one portion of fresh biowaste was suspended with 2 portions of process water for hydropulping. The dry matter (total solids, TS) values of the biowaste suspension after hydropulping ranged from 5 – 6 %. Activated sludge and primary sewage sludge from the wastewater treatment plant Karlsruhe contained respectively 2 % and 4 % TS. Since the original activated sludge contained only 2% TS and had a relatively low COD value (21 $g \cdot L^{-1}$) for anaerobic digestion, the activated sludge was concentrated to a TS of 5% by removal of a part of the supernatant after sedimentation of the sludge flocs. Food waste was mashed, had a maximal TS content of 10%, and was suitable for wet anaerobic digestion as such. White and dark bread mash was prepared with tap water to contain 5 % TS. Yoghurt contained 4% TS, which was in the same range as the TS of biowaste.

It can be seen from Table 4.1 that bread, yoghurt and food waste had a very high content of organic matter. The volatile solids (VS, organic matter) content of those three substrates were all above 90% of the TS content. Concerning the COD, the value for food waste was much higher than that of other co-substrates and that of biowaste. The bread mash and yoghurt had a similar COD as biowaste, whereas the COD of activated sewage sludge and of primary sewage sludge was only little more than half of that of biowaste. The total nitrogen content of yoghurt was twice higher than that of biowaste, so the COD:N ratio of yoghurt was much lower as that of biowaste. Due to the high COD value and low nitrogen content food waste had the highest COD:N ratio. The COD:N ratio of biowaste, bread mash and primary sludge was about half as high and the COD:N ratio of activated sludge and that of yoghurt was even lower. As has been discussed in the previous chapter, a C:N ratio within a range of 25-30 is considered to provide optimal growth conditions for the bacteria during anaerobic digestion. Concerning the optimal C:N ratio, primary sludge and

bread mash seemed to be appropriate co-substrates. The C:N ratio is, however, not the only criterion to judge about the suitability of a co-substrate for anaerobic digestion. A high volatile fatty acid content as in biowaste or of only acetate as in biowaste, primary sewage sludge and food waste, generated by acidification prior to anaerobic digestion points towards rapid anaerobic digestion. The biogas production potential in batch assays and its performance during application in continuous reactor operation must also be examined

Table 4.1 Main characteristics of biowaste and of co-substrates

	Biowaste	Activated sludge	Primary sludge	Bread mash	Yoghurt	Food waste
Total solids (%)	4.6 -5.9	1.8 -4.8	3.5-4.3	5.1	3.8	9.8
Volatile solids (%)	3.3 -4.5	1.2 -3.2	2.3 -2.6	4.9	3.6	9.0
Organic fraction (%)	72 -76	67	66	96	95	92
COD_{total} ($g \cdot L^{-1}$)	80 -105	21 -46	39 -48	78	86	198
Electrical conductivity ($ms \cdot cm^{-1}$)	12	4.0	2.2	4.45	6.25	8.5
pH	4.5	6.4	5.4	6.3	4.5	4.5
Total Kjeldahl nitrogen (TKN, $g \cdot L^{-1}$)	2.3	1.9	1.5	2.5	4.6	3.3
NH_4^+-Nitrogen ($g \cdot L^{-1}$)	0.48	0.14	0.18	0	0.38	0.12
C/N ratio	35 -46	11 -24	26 -32	32	19	60
Acetic acid ($g \cdot L^{-1}$)	3.29	2.2	5.76	0.1	0	5.8
Propionic acid ($g \cdot L^{-1}$)	3.64	0.74	0.77	0	0	1.1
Butyric acid ($g \cdot L^{-1}$)	3.16	0.44	0	0	0	0

4.1.2 Biogas production potential of biowaste and of the used co-substrates

The biogas production potential of a substrate is dependent on its composition, on the content of carbohydrates, proteins and lipids, as well as on the amount of cellulose, hemi cellulose or lignin and on the extent to which degradation is possible at the provided retention time (Nayono et al. 2008). The biogas

production of biowaste suspensions during incubations in batch assays was described in Figure 4.1. In the batch assay 20 ml of fresh biowaste suspension, containing 1.6 g COD, was digested with 1 L digested sludge from the methane reactor of the biowaste treatment plant of Karlsruhe as a source of active anaerobic methanogenic consortia. Figure 4.1 shows that the biogas production increased linearly during the first 20 hours with the highest biogas production rate at around 18.8 $ml \cdot g^{-1}COD \cdot h^{-1}$.

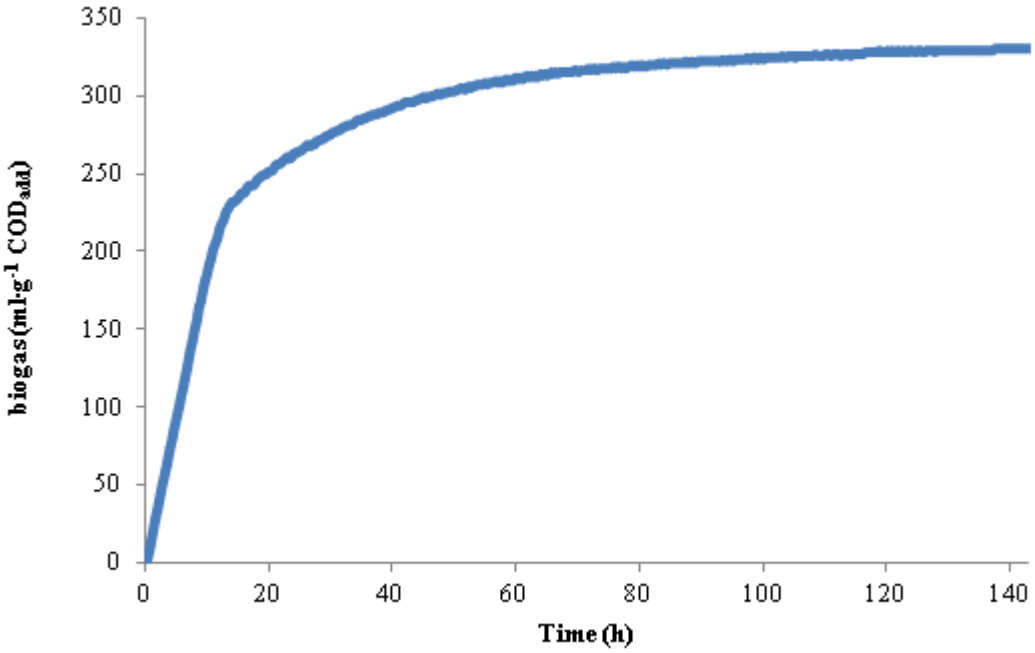

Figure 4.1 Biogas production of biowaste.
(Batch assays of biowaste were incubated on a rotator shaker at 110 rpm and at 37 ^{o}C).

After 80 hours biogas production ceased almost completely and in the following days only 3% more biogas was released from the reactor. The maximum biogas production potential of biowaste was 330 $ml \cdot g^{-1}COD$. The average methane concentration of the biogas from biowaste in batch assay was 70% and the biodegradability of biowaste by anaerobic digestion was 44%.

This result corresponded to other reports about biogas productivity from biowaste. Nayono et al. (2008) has reported that the maximum biogas production potential from biowaste in batch assay was 0.39 $m^3 \cdot kg^{-1}$COD and 0.59 $m^3 \cdot kg^{-1}VS_{added}$. The highest biogas production rate of 14.5 $ml \cdot g^{-1}COD \cdot h^{-1}$ was obtained during the first 48 hours, where still many readily biodegradable substances were available. The methane content of biogas from biowaste digestion was 62% (Nayono et al. 2008).

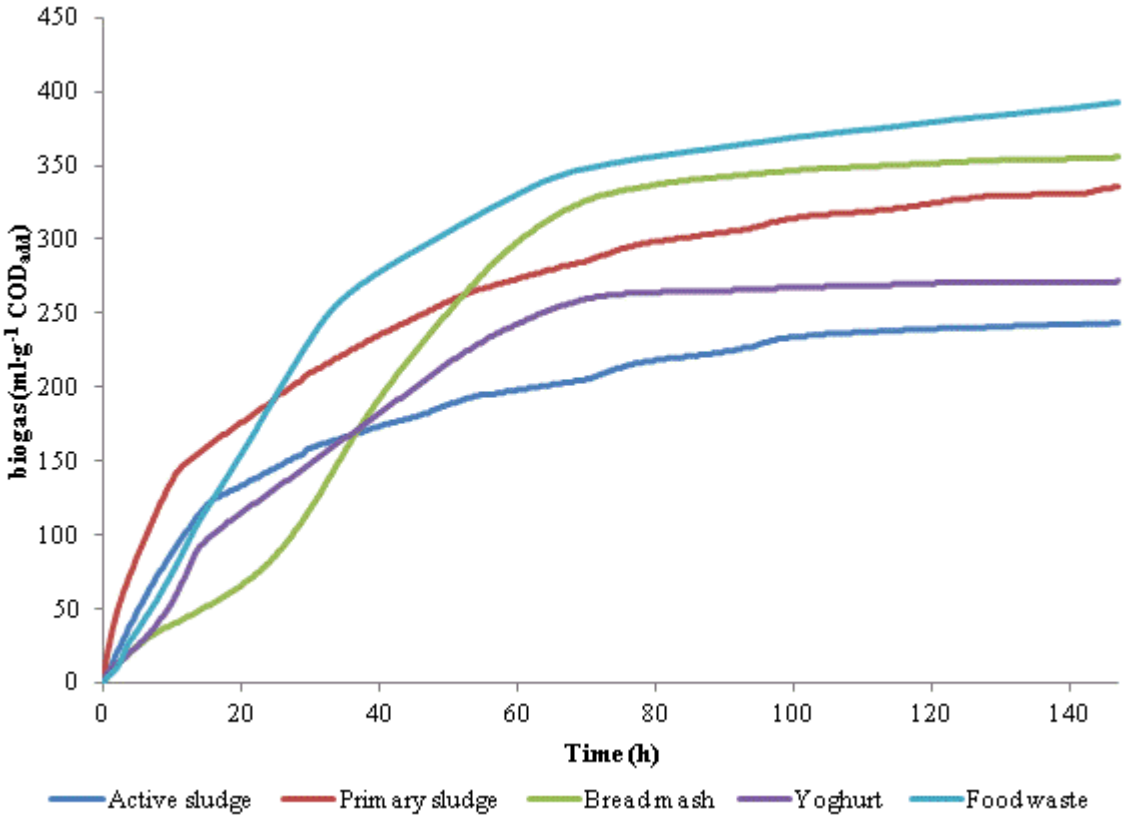

Figure 4.2 Biogas production potential of co-substrates in batch assay.
(Conditions as for Fig. 4.1)

The course of biogas production of different substrates with time in batch assays is documented in Figure 4.2. Compared to the biogas production from biowaste (Fig. 4.1), the gas production from all other co-substrates (Fig. 4.2) was much slower during the first 20 hours of digestion. In the biowaste assay 250 ml biogas per g COD were generated during the first 20 hours, whereas the biogas

production from co-substrates during the first 20 hours ranged from 50 to maximally 200 ml. This may be attributed to the adaption time of anaerobic bacteria required for digestion of "new" substrates. Maximum biogas production potentials and maximum gas production rates are summarized in Table 4.2.

Table 4.2 Maximum biogas production of different co-substrates (Conditions as for Fig. 4.1, 4.2)

	Activated sludge	Primary sewage sludge	Bread mash	Yoghurt	Food waste
Biogas production potential ($ml \cdot g^{-1}COD$)	244	336	356	273	392
Maximum gas production rate ($ml \cdot g^{-1}COD \cdot h^{-1}$)	9.3	14.2	7.7	9.6	7.7
Methane concentration (%)	54	55	68	68	70

During feeding of only foodwaste, about 50 ml more biogas per gram COD were released than with biowaste feeding. This may have been caused by the higher fat content of foodwaste. Biogas production from triglycerides as the main component of fats can reach up to 1.434 $L \cdot kg^{-1}$, but that from carbohydrates and protein can theoretically only reach 0.746 $L \cdot kg^{-1}$. The biogas production potential of bread and primary sewage sludge was similar as that of biowaste, but the degradation processes of those two substrates were totally different. During digestion of primary sewage sludge, more than 50% of the biogas was released within the first 20 hours and the gas production rate sharply decreased subsequently. During digestion of bread mash from overlayed bread types, biogas formation in the first 20 hours was extremely slow. The highest biogas production rate was obtained within the following 24 hours with 7.7 $ml \cdot g^{-1}$ $COD \cdot h^{-1}$. After 80 hours the biogas production had almost ceased completely. As has been analysed in the previous section, the C:N ratio of bread and primary sewage sludge were similar as those of biowaste. The mentioned

two co-substrates contained a low amount of fat, which resulted in a similar biogas production potential from bread, primary sewage sludge and biowaste. The biogas production from yoghurt and activated sludge was respectively 273 and 244 $ml \cdot g^{-1}$ COD, which was much lower than the biogas production potential of biowaste.

4.1.3 Anaerobic digestion of biowaste as the main substrate

Ten L glass column reactors with a working volume of 8 L, fed with the main substrate biowaste were operated in semi-continuous mode at 8 days HRT. The biowaste was fed twice a day in the morning at 8.30 a.m. and in the evening at 5.00 p.m., and there was no feeding during the weekends as in the full-scale biowaste treatment plant. During semi-continuous digestion, biogas production, COD elimination, the methane content of the biogas and the VFA accumulation were measured to determine the performance of anaerobic digestion of source-sorted biowaste.

Figure 4.3 presents COD elimination, daily biogas production and the methane content of the biogas during mono-digestion of biowaste in one month for steady state conditions. The COD of the biowaste suspension used in this experiment was 80 $g \cdot L^{-1}$ and the loading corresponded to an OLR of 10 kg $COD \cdot m^{-3} \cdot d^{-1}$. At this feeding schedule and for this OLR, which both represent a simulation of the full-scale operation of the biowaste digester of the city of Karlsruhe, a steady state was reached 15–20 days after start. When the biowaste suspension in the reactor was not fed with fresh suspension on Saturdays and Sundays biogas production rapidly decreased from average 22 $L \cdot d^{-1}$ obtained from Tuesday to Friday to less than 5 $L \cdot d^{-1}$ from Friday night until Monday morning. After resuming biowaste feeding on Mondays, biogas generation was much lower than on Tuesday until Friday. One week after start-up, the COD removal has reached 64% and in following days it ranged from 60% to 67%. The average methane content of the produced biogas was 70%.

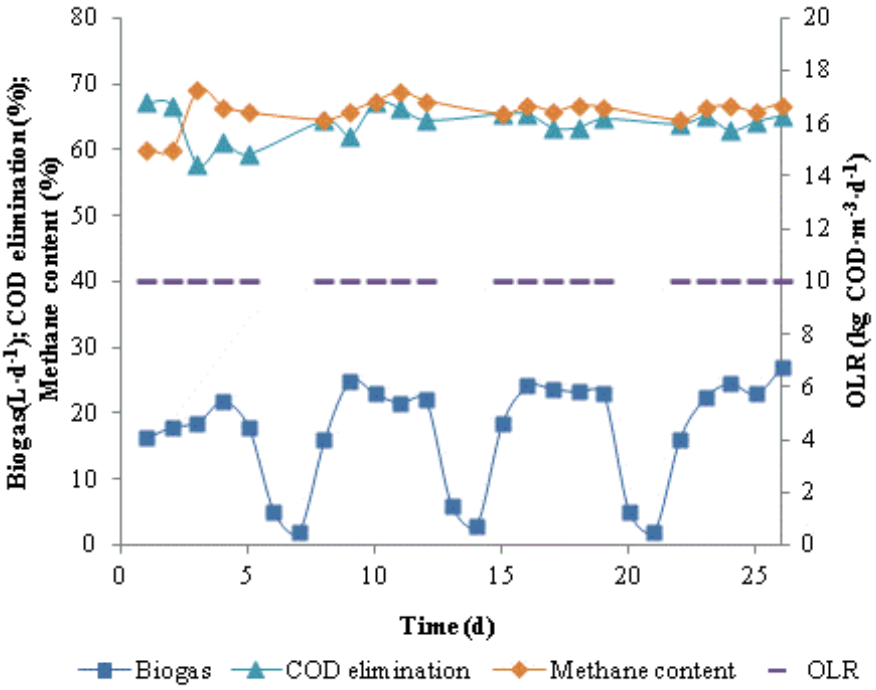

Figure 4.3 Performance of mono-digestion of biowaste suspension during steady state with an OLR of 10 kg·m^{-3}·d^{-1} under "in praxi" conditions.

Figure 4.4 presents pH values and volatile fatty acid concentrations during the start-up period of the biowaste digester. During the whole time, no n- or i-butyric acid and n- and i-valeric acid was detected. A low concentration of acetic acid was detected in the first week after start-up, which was completely degraded in the second week. Propionic acid accumulated in the first two weeks after start during the 5 days with biowaste feeding, but was completely degraded during weekends, when no biowaste was fed. The maximum concentration of acetic acid and propionic acid was, respectively, 0.14 and 0.57 g·L^{-1}. Gallert et al. (2003) have reported the performance of a laboratory biowaste digester with increasing OLR and decreasing HRT. The biogas production and degradation of organic material were linearly increasing with the OLR from 4.3 to 19 kg COD·m^{-3}·d^{-1}. However, the COD removal efficiency decreased with increasing OLR. The average biogas production of the anaerobic biowaste digester at an OLR of 10 kg COD·m^{-3}·d^{-1} (HRT = 8.5 days) was 27

$L \cdot d^{-1}$. VFAs were accumulating after each reduction of the HRT and the VFAs accumulation disappeared in the following week at the same HRT. A high and longer-lasting VFAs accumulation was observed, when the HRT was reduced to 5.7 d, indicating a metabolic limitation of the bacterial population in the reactor.

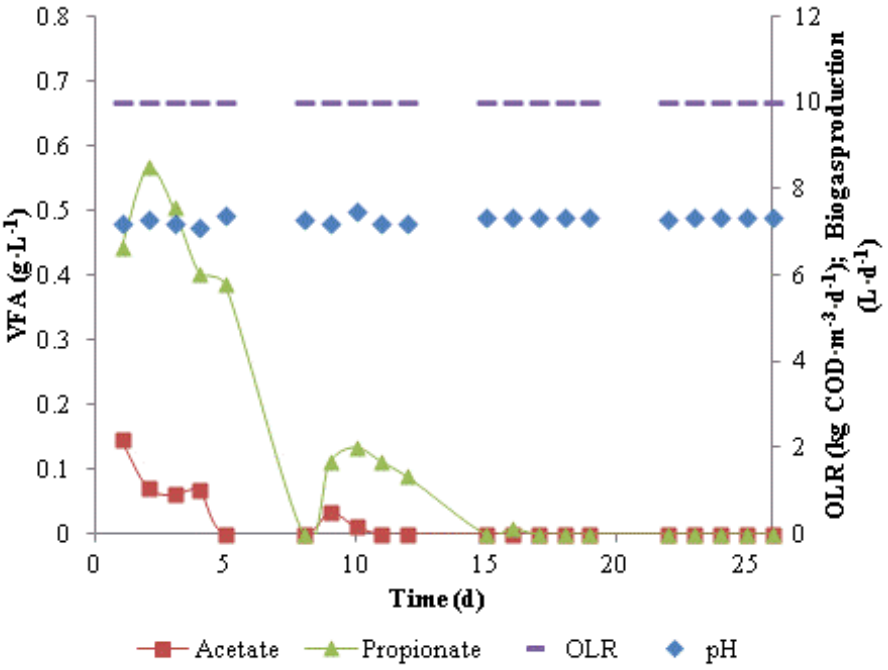

Figure 4.4 pH and VFA levels during mono-digestion of biowaste.
(Feeding mode as in full-scale)

4.1.4 Co-digestion of biowaste with different co-substrates in continuous mode

The same type of glass column reactor as for the mono-digestion of biowaste was used in this experiment. The reactor was fed with biowaste and different co-substrates at a constant HRT of 8 days. Instead of feeding daily 1L biowaste as in the mono-digestion, the reactor was daily fed with 0.5 L biowaste plus 0.5 L co-substrate. Each anaerobic co-digester ran for a month, biogas production and the methane content of biogas, as well as the COD of effluent were daily measured to evaluate the reactor performance. The average values of the whole process were calculated and summarized in Table 4.3.

Table 4.3 Performance of anaerobic co-digestion of biowaste with different substrates

	Biowaste + activated sewage sludge	Biowaste + primary sewage sludge	Biowaste + bread	Biowaste + yoghurt	Biowaste + food waste
HRT (d)	8	8	8	8	8
OLR (kg COD·m^{-3}·d^{-1})	5.2 - 6.2	6 - 7.6	7.6 -7.9	10 - 10.5	6.9 - 7.5
COD Elimination (%)	60	61	69	60	67
Biogas production ($L·d^{-1}$)	10.9	15.2	29.6	25.7	22.3
Methane content (%)	69	70	60	62	68
Biogas production rate ($L·g^{-1}$ COD·d^{-1})	0.19	0.26	0.35	0.30	0.32

Due to the varying concentration of the substrates the ORL at 8 d HRT varied from 5.2 – 10.5 kg COD·m^{-3}·d^{-1}, whereas the COD elimination varied only from 60 – 69 %. The COD elimination during co-digestion of biowaste with bread and food waste was higher than during mono-digestion of biowaste. The methane content was lower in the biogas of the two substrates that tended to acidify (bread and yoghurt: 60-61 %) and higher in the biogas of co-substrates that had a high buffer capacity (primary sludge, activated sludge and food wastes: 68-70 %). A lower methane content may be the consequence of a reduction of alkalinity in the digester liquid due to slight pH-shifts towards acidic pH by higher fatty acid concentrations in early phases of anaerobic digestion. The daily biogas production from co-digestion of biowaste with sewage sludge was less than from mono-digestion of biowaste, whereas the other three co-digesters produced more biogas than the mono-digester. When the reactors were fed with different OLR, it is difficult to compare the gas

productivity from each co-digester. Therefore, biogas production per unit organic loading was calculated to evaluate the impact of addition of different co-substrates on biogas production in a biowaste co-digester. The biogas production rate during mono-digestion of biowaste was 0.24 $L \cdot g^{-1}$ $COD \cdot d^{-1}$. From Table 4.3 it can be seen that addition of bread, yoghurt and food waste as co-substrates for anaerobic digestion of biowaste improved the biogas productivity. The biogas productivity during co-digestion of bread with biowaste was 0.35 $L \cdot g^{-1}$ $COD \cdot d^{-1}$, which was 50% higher than during mono-digestion of biowaste. It seemed that bread may be the best co-substrate in anaerobic digestion of biowaste for improvement or initiation of rapid additional biogas production.

Several different organic materials have been reported as co-substrates for anaerobic digestion of municipal solid waste. Hartmann et al. (2003) reported that co-digestion with sewage sludge could improve the characteristics of municipal solid waste for digestion, leading to a better C/N ratio and increasing the moisture content. Mixtures of municipal solid wastes and sewage sludge within the range of 80:20 on TS basis brought an optimal AD performance. Some pure organic substrates such as cellulose, peptone and oil were evaluted for co-digestion with biowaste. The results suggested that FOG wastes (fat, oil, grease containing wastes) were the most suitable co-substrates for anaerobic digestion treating biowaste (Ponsa et al., 2011). Furthermore, co-digestion of FOG with biowaste has been carried out in two full-scale anaerobic digesters and it has been demonstrated that a rate of 10-30% FOG by volume of feedstock increased biogas production by 30-80% (Baily, 2007; Muller et al., 2010). Nayono et al. (2010) utilized foodwaste from restaurants or hosptial canteens as co-substrate to improve biogas production during anaerobic digestion of biowaste. A maximal net biogas production improvement of 31.9% was achieved during co-digestion of biowaste with the addition of 15% foodwaste by volume. In the present study old bread showed a great biogas production

potential and improved the performance of continuous anaerobic biowaste digestion. Highly concentrated suspensions of old bread or bakery goods seem to be an ideal co-substrates for anaerobic co-digestion with biowaste. However, until today co-digestion with old bread or bakery goods was barely reported, although McDonalds, Dunkin Donuts and other food chains throw away tons of surplus food every day. Anaerobic co-digestion of biowaste with old bread was particularly investigated in this study.

4.2 Anaerobic co-digestion of biowaste with bread for improvement of biogas production (Modified from Li et al., 2015 in press)

It has been demonstrated that using co-substrates is an effective way to improve biogas production by anaerobic digestion within a short time. An eligible co-substrate must satisfy the following conditions: It must be easily available in sufficient quantities, either free of costs or at a reasonable price. In Europe and North America wheat bread (white bread) or rye bread (dark bread) as the most important basic food is baked daily and sold fresh in bakeries on the same day or at latest on the next day. After one day bread in most bakeries is no longer sold or even sellable as human food and is considered a waste. Co-substrates for anaerobic digestion should easily be biodegradable and bring a high gas production potential. As has been analysed in the previous part, bread has very high organic matter content above 90%, was easily degradable by anaerobic bacteria and its biogas production potential was little higher than that of biowaste. It has been reported that the melanoids of dark bread, which are formed during baking of rye bread, seem to serve as a carbon source for anaerobic bacteria, accelerating e.g. growth of bifidobacteria (Borrelli and Fogliano, 2005). Acetic and lactic acid would be the main fermentation products, and these organic acids can be degraded to biogas. Furthermore if free of molds, bread doesn’t contain any dangerous or poisonous substances, which may inhibit anaerobic digestion. For that reasons old bread is considered as an

ideal co-substrate in biogas plants for energy production. Currently in Karlsruhe and several other cities in Germany, bread factories collect overlayed bread from their bakery shops every 2nd day and bring it to the municipal biowaste digestion plant for co-digestion with the wet organic fraction of source-sorted municipal waste.

In previous experiments the anaerobic co-digestion of biowaste with bread has been examined in a continuous reactor for a short time period. In this part of my study, the suitability of wheat bread and rye bread as co-substrates for anaerobic digestion of biowaste was examined during an extended time span. The OLR was increased by addition of increasing amounts of co-substrates and the performance of anaerobic digestion was described by biogas production, COD elimination and VFA accumulation and degradation. The maximum OLR and optimum ratio between the main substrate and the co-substrates was also determined by addition of increasing amount of co-substrates until failure.

4.2.1 Characteristics of biowaste and co-substrates

The main characteristics of the biowaste and all possible co-substrates are presented in Table 4.4. The biowaste suspension used in this experiment was collected from the biowaste treatment plant of Karlsruhe. Due to the long duration of this experiment, three portions were taken from the biowaste treatment plant at different time, therefore the COD and solids content of the biowaste suspension slightly fluctuated. In order to operate the continuous reactors at variable organic loading rates (OLR) without a significant change of the hydraulic retention time (HRT) in co-fermentations, bread suspensions were prepared to contain about 3 times as many total solids (TS) or volatile solids (VS) than the technically prepared biowaste suspension for full-scale digestion. On average, the COD of bread suspensions was about 2 – 3 times higher than that of the biowaste suspension. Concerning the total nitrogen content, the bread suspension contained also about twice as many nitrogen compounds than the

biowaste suspension, so a similar C: N ratio of bread and biowaste suspension could be inferred. The organic fraction of bread was almost 100%, whereas the organic fraction of biowaste was about 70%-80%, which was clearly less than that of bread. Both bread suspensions contained only little volatile fatty acids and lactic acid, whereas there was a high concentration of acetic acid and some propionic acid as well as some n-butyric acid in biowaste suspensions. Fresh rye bread suspensions did not contain more lactic acid than wheat bread and biowaste suspensions, although rye bread was prepared with sour dough (containing hetero- and homofermentative lactic acid bacteria) and wheat bread with baker´s yeast (alcoholic fermentation) as propellants (Li et al., 2014 in press).

Table 4.4 Composition of fresh biowaste (FBS), wheat bread (WBS) and rye bread RBS) suspensions (Reproduced from Li et al., 2014 in press)

Parameter	FBS	WBS	RBS
COD_{total} ($g \cdot L^{-1}$)	82.3 - 114.3	142.1 - 222.1	203.0 - 208.3
Total solids (%)	6.0 - 6.1	13.9 - 18.4	19.5 - 20.6
Volatile solids (%)	4.1 - 5.1	13.4 - 17.9	18.6 - 19.7
Organic fraction (%)	69 – 85	97	96
Total Kjeldahl nitrogen (TKN, $g \cdot L^{-}$)	2.0 - 2.3	4.3	4.5
NH_4^+-Nitrogen ($g \cdot L^{-1}$)	0.5	0	0
Electrical conductivity ($mS \cdot cm^{-1}$)	12	4.5	4.7
pH	5.1	6.3	6.5
Acetic acid ($g \cdot L^{-1}$)	2.1	0.3	0.7
Propionic acid ($g \cdot L^{-1}$)	1.3	0	0
Butyric acid ($g \cdot L^{-1}$)	1.3	0	0
Lactic acid ($g \cdot L^{-1}$)	0.4	0.4	0.5

Formation of volatile fatty acids and the buffer capacity are important factors in biowaste digesters, if co-fermentation with overlayed food material is

considered for improvement of biogas productivity. For this reason VFA spectra and pH changes were determined during acidification of wheat (WBS) or rye bread suspensions (RBS) as possible co-substrates for biogas production from fresh biowaste suspension (FBS).

4.2.2 Acidification and buffer capacities of wheat bread, rye bread and biowaste suspensions (reproduced from Li et al., 2015 in press)

Figure 4.5 represents the course of VFA and pH during acidification of wheat bread suspension (WBS), rye bread suspension (RBS) and fresh biowaste suspension (FBS) in closed Schott-bottle reactors at 37°C. During acidification for 40 – 65 h only around 2 $g \cdot L^{-1}$ acetate accumulated in WBS (Fig. 4.5a), whereas 5 $g \cdot L^{-1}$ acetate plus 2 $g \cdot L^{-1}$ n-butyrate, but no propionate accumulated in RBS (Fig. 4.5b) and 3 $g \cdot L^{-1}$ acetate, 1.5 $g \cdot L^{-1}$ propionate and 2 $g \cdot L^{-1}$ n-butyrate accumulated in FBS (Fig. 4.5c). In WBS and RBS the pH dropped to 3.7, (Fig. 4.5a, 4.5b) mainly caused by 6.8 $g \cdot L^{-1}$ D/L-lactate that was formed in addition to the above mentioned volatile fatty acids (VFA) in both bread suspensions after 40 h. The lactate was not converted to propionate as in sourdough cultures (Zhang et al., 2010), or by other lactate-producing bacteria, even after a prolonged incubation. Propionate would prevent spoilage of bread suspensions during storage by antimicrobial agents-producing fungi. No lactate was found in the FBS and, although a 2-fold increase of acetate and propionate concentrations and a 5-fold increase of the n-butyrate concentration were observed during acidification, the pH increased from 5.1 to 5.5 (Fig. 4.5c). The slight pH increase during VFA formation was presumably caused by ammonia, that was released during proteolysis of proteins from FBS and deamination of amino acids (Gallert and Winter, 2005). The buffering capacity, determined as $K_{S4.3}$ (in brackets initial → final pH), was 7.8 $mmol \cdot L^{-1}$ for WBS (pH 6.3 → 4.3) and 14.4 $mmol \cdot L^{-1}$ for RBS (pH 6.5 → 4.3), and this explains why for

approximately the same pH decrease the VFA content of acidified RBS could be much higher than the VFA content of WBS for the same lactic acid content.

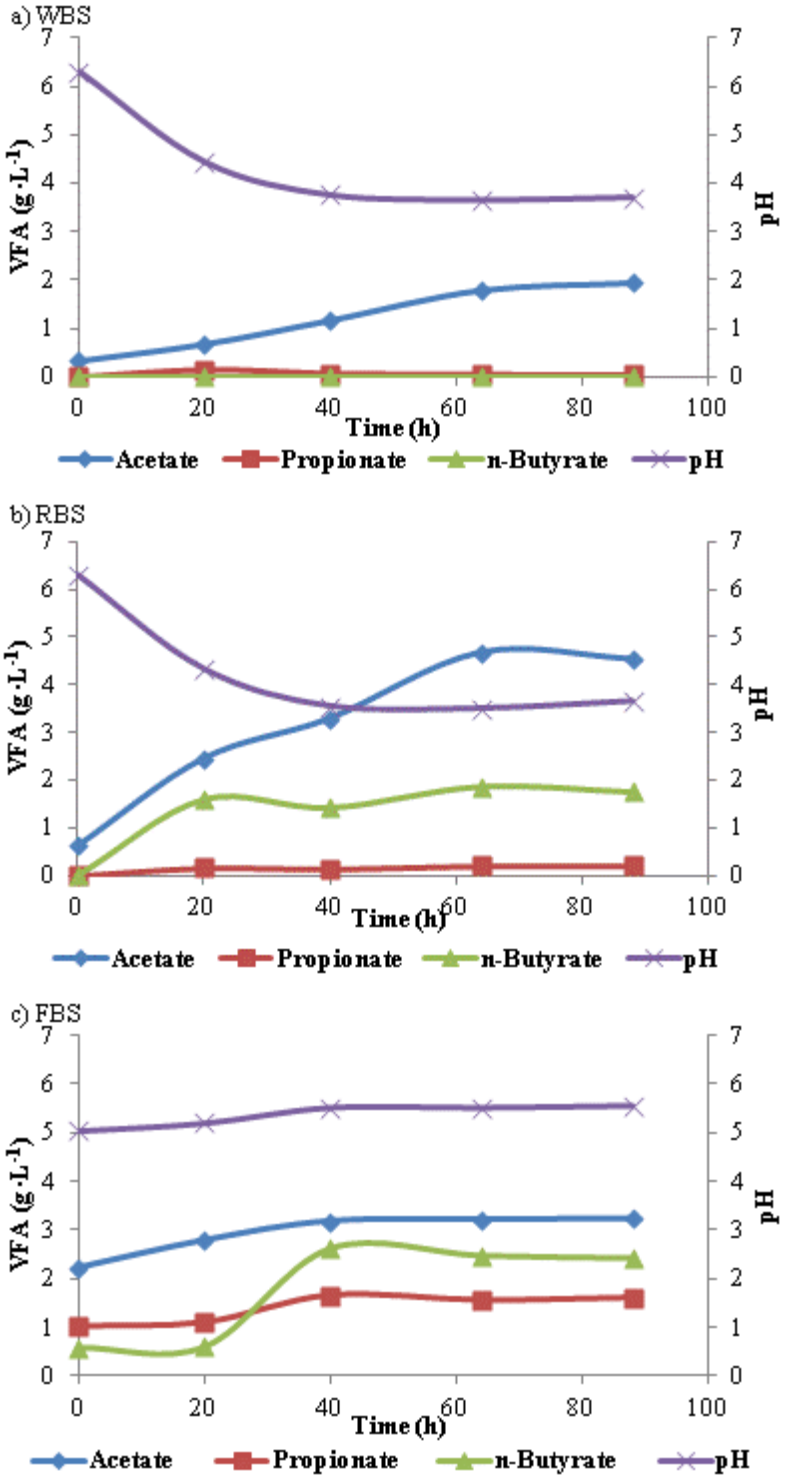

Figure 4.5 Acetate, propionate and n-butyrate formation during acidification of wheat bread (WBS) (a), rye bread (RBS) (b) and fresh biowaste suspensions (FBS) (c). (Taken from Li et al. 2014)

FBS, already significantly acidified at delivery (pH = 5.1), had a much higher $K_{S4.3}$ of 46.8 mmol·L^{-1} which increased more than 3-fold to 152.4 mmol·L^{-1} in digested biowaste (reactor effluent) at a pH of 7.3. The low buffer capacity of wheat and rye bread suspensions would lead to a dropping pH during acidification, which could be compensated by co-digestion with well buffering biowaste (Gallert et al., 1998).

Since acetic acid is a weak acid, and an accumulation rate of only 30 mg·L^{-1}·h^{-1} in WBS (and in FBS) or 70 mg·L^{-1}·h^{-1} in RBS (estimated from Fig. 4.6) was observed, which was not enough to cause the strong decrease of the pH in both assays, the high amounts of strong lactic acid (6.8 g·L^{-1}) must have been responsible for the pH drop. In FBS lactic acid was not formed above the initially present 0.4 g·L^{-1} (Table 4.6). During acidification acetate increased in all assays, whereas propionate and especially n-butyrate concentrations were only increasing in the assays with RBS and FBS.

4.2.3 Performance of anaerobic co-digestion of fresh biowaste with white bread and with rye bread suspensions

For co-digestion experiments two reactors were started in parallel with digester effluent of the biowaste digestion plant of Karlsruhe. A steady state organic loading rate (OLR) of 14.3 kg·m^{-3}·d^{-1} was maintained by feeding the 2 reactors with FBS twice per day (as in practice) for 25 days before co-fermentation of FBS + WBS in reactor 1 or FBS + RBS in reactor 2 was started. An OLR of 14 kg·m^{-3}·d^{-1} by continuing the FBS-feeding was maintained further on in both reactors while increasing amounts of WBS or RBS were added to stepwise increase the OLR. The variation of HRTs and the corresponding increase of OLRs during the co-digestion process are represented in Figure 4.6.

1 L fresh biowaste suspension was daily fed into the reactor during the whole experiment. The increase of OLR was initially carried out by addition of 50 ml WBS or RBS into FBS-fed reactors 1 and 2. After two weeks the addition of

WBS and RBS was increased to 200 ml per day. The maximum addition of 500 ml per day was reached after 120 days. By addition of 500 ml of either WBS or RBS, the reactors had a problem with converting fatty acids to biogas and the suspensions acidified after a short time. Addition of 400 ml bread suspension was considered as maximum, with a still positive impact on anaerobic digestion of biowaste. The OLR was increased stepwise by addition of WBS to a final OLR of 24.2 $kg \cdot m^{-3} \cdot d^{-1}$ during co-digestion with WBS and to 24.9 during co-digestion with RBS. The addition of co-substrates caused a reduction of the HRT from 8 days to 5.3 days.

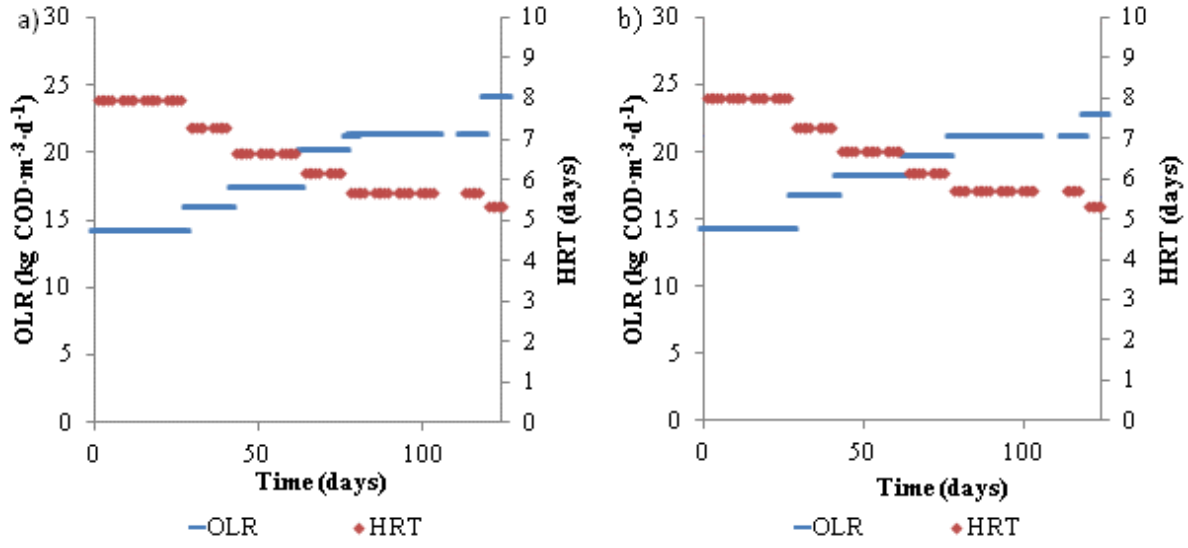

Figure 4.6 Variation of OLR and HRT by addition of co-substrates WBS (a) or RBS (b) into reactors 1 or 2, respectively for co-digestion with FBW.

The variation of daily biogas production, the methane content of produced biogas, as well as TS, VS and the ammonia content in effluent of the co-digestion of FBW with WBS and RBS at increasing OLR was respectively depicted in Figure 4.7a and 4.7b. Due to no feeding on weekends, the biogas production was drastically decreasing from Friday to Monday. The biogas production increased with stepwise increments of the bread suspensions until an addition of 400 ml WBS or RBS per day. When 500 ml WBS or RBS was

added together with the daily biowaste feeding, the biogas production in the co-digesters almost ceased, indicating overloading.

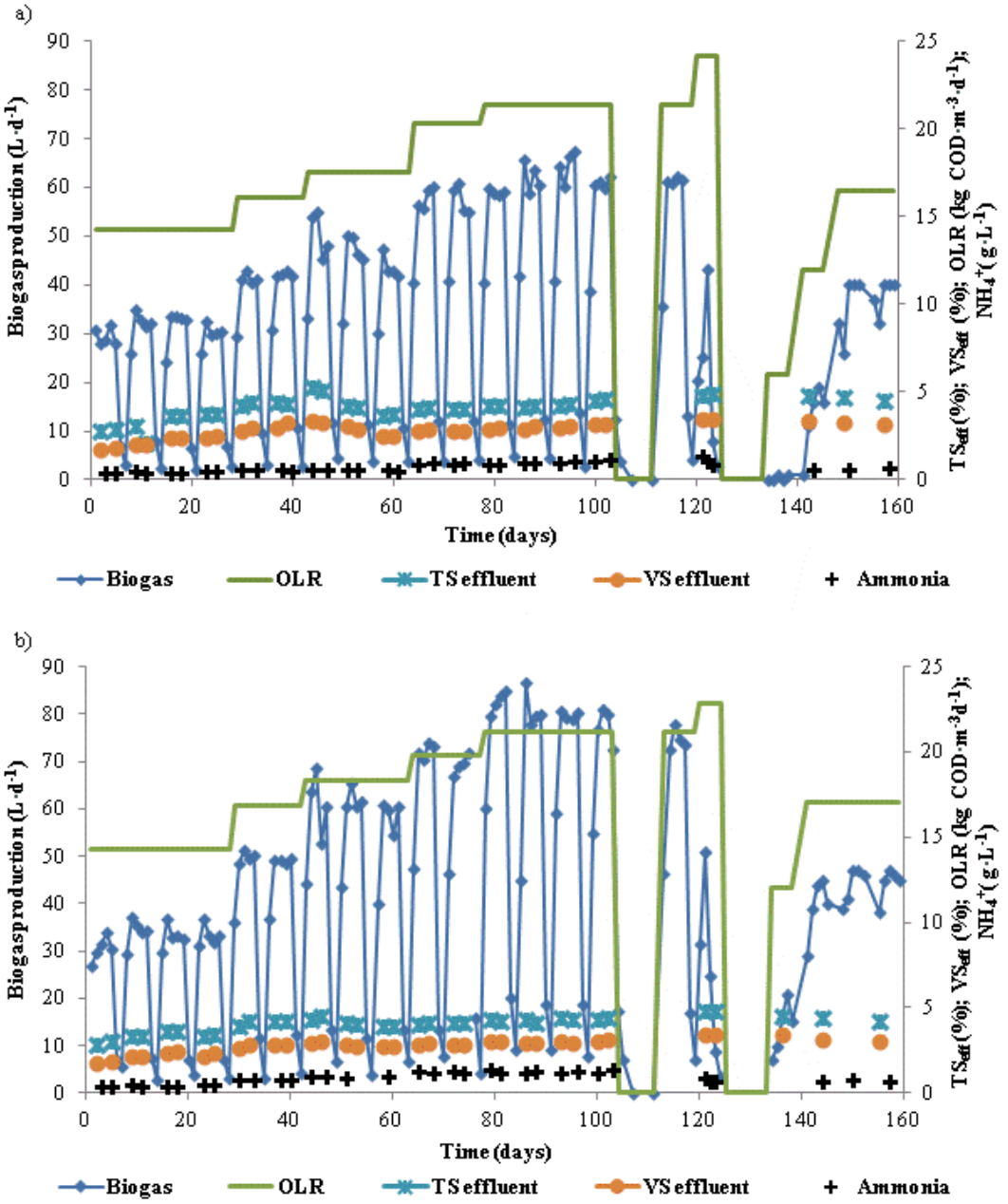

Figure 4.7 Reactor performance during Co-digestion of fresh biowaste suspension with bread suspension. Co-digestion of biowaste with WBS (Fig. 4.7 a) or RBS (Fig. 4.7 b).

Interruption of feeding for 8 days for process recovery (e.g. after VFA accumulation, days 105 - 115) stabilized the fermentation again and after resuming feeding at the same OLR as before previous gas productivity was obtained almost immediately. At an OLR of more than 22 kg $m^{-3}d^{-1}$ both co-

fermentation reactors began to fail due to the decreasing pH, caused by acetate, propionate and n-butyrate accumulation (day 125).

Table 4.5 Co-digestion of wheat bread suspension (WBS) in a biowaste digester (from Li et al., 2015 in press)

Days	Mode	Co-substrate addition ($L \cdot d^{-1}$)	HRT (d)	OLR ($kg \cdot m^{-3} \cdot d^{-1}$)	Additional OLR	BPR ($L \cdot d^{-1}$)	VBPR ($L \cdot L^{-1} \cdot d^{-1}$)	Improve-ment
1-28	Mono-digestion	-	8	14.3	-	30.56	3.8	-
29-42	Co-digestion	0.1	7.27	16.1	12.5 %	39.39	4.9	29%
43-61	Co-digestion	0.2	6.67	17.5	22.4%	45.67	5.7	49%
64-75	Co-digestion	0.3	6.15	20.3	42.0%	57.71	7.2	88%
78-98	Co-digestion	0.4	5.71	21.4	49.7 %	64.54	8.1	111%
106-110	Co-digestion	0.5	5.33	24.2	69.1%	19.92	2.5	-35%[1]

BPR = biogas production rate, VBPR = volume-related biogas production rate, [1] = Overload.

Titration to pH 7.2 with NaOH at day 125 and interruption of feeding for 7 days led to a recovery of the gas production at similar amounts as before at the same loading during days 45 – 60. Acetate and propionate levels fluctuated, whereas n-butyrate apparently was completely degraded and finally disappeared; thus the process was not yet completely in balance. In the first two weeks after addition of bread suspensions into biowaste digesters, TS and VS content of effluent slightly increased, but in the following time span the TS and VS content of effluent from the co-digesters was in the same range as in mono-digesters of biowaste, which indicated that bread suspensions could be nearly completely degraded during anaerobic co-digestion of biowaste and bread.

Table 4.6 Co-digestion of rye bread suspension (RBS) in a biowaste digester (Li et al., 2014 in press)

Days	Mode	Co-substrate addition ($L \cdot d^{-1}$)	HRT (d)	OLR ($kg \cdot m^{-3} \cdot d^{-1}$)	Additional OLR	BPR ($L \cdot d^{-1}$)	VBPR ($L \cdot L^{-1} \cdot d^{-1}$)	Improve-ment
1-28	Mono-digestion	-	8	14.3	-	32.65	4.1	-
29-42	Co-digestion	0.1	7.27	16.9	18.2%	46.84	5.9	43%
43-61	Co-digestion	0.2	6.7	18.3	28.0%	57.1	7.1	75%
64-75	Co-digestion	0.3	6.15	19.8	38.5%	65.97	8.3	102%
78-103	Co-digestion	0.4	5.7	21.2	48.3%	73.86	9.5	126%
106-110	Co-digestion	0.5	5.3	22.9	60.1%	23.88	3.0	-27%[1]

BPR = biogas production rate, VBPR = volume-related biogas production rate, [1] = Overload.

The biogas production during stepwise increase of the OLR by constant amount of FBW and increasing amounts of WBS or RBS is summarized in Table 4.5 and Table 4.6, in order to obtain a direct measure of the biogas that was derived from addition of the bread suspensions. Biogas production rates were determined as average values of biogas production during the working days. Addition of WBS and RBS not only increased the biogas production linearly with increasing OLR (Table 4.5 and 4.6), but also enhanced the biogas production rate. During co-digestion of FBS + WBS gas productivity at an OLR of 21.4 $kg \cdot m^{-3} \cdot d^{-1}$ increased by 89 % whereas during co-digestion of FBS + RBS at an OLR of 20.4 $kg \cdot m^{-3} \cdot d^{-1}$ gas productivity increased by 132 % as compared to mono-digestion of biowaste suspension. For co-digestion of FBS + WBS a specific gas productivity of 0.31 – 0.33 $L \cdot g^{-1}$ and for co-digestion of FBS + RBS

of 0.34 – 0.41 $L \cdot g^{-1}$ was calculated from the data of tables 4.5 and 4.6. The methane content in the biogas was 70 ± 1 %.

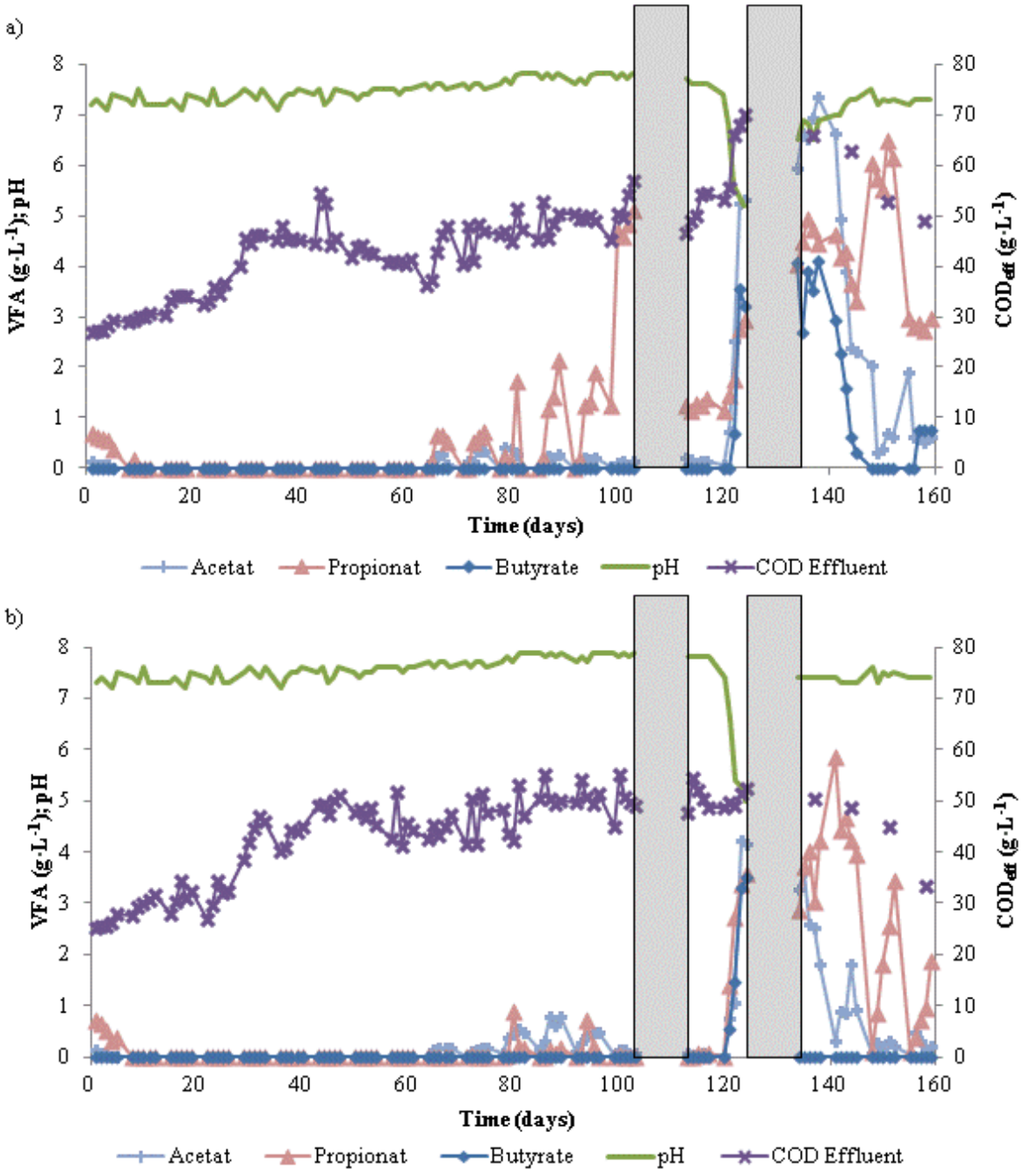

Figure 4.8 Variant of VFA concentration, pH value and COD in the reactor's effluent during co-digestion of fresh biowaste suspension with bread suspension: Fig. 4.8a) Co-digestion with WBS and Fig. 4.8b) Co-digestion with RBS. (Li et al., 2014 in press)

Figure 4.8 "depicts the residual VFA concentration, pH value and COD in effluent from the co-digester of biowaste with WBS and RBS. The COD in the

effluent of biowaste + WBS or RBS reactors 1 and 2 was not significantly different, but VFA concentrations were little higher in the effluents of reactor 1 with co-feeding of WBS than of reactor 2 with co-feeding of RBS. During co-digestion of FBS + WBS in reactor 1 or FBS + RBS in reactor 2 no VFAs accumulated in the digester liquid after feeding up to an OLR of 17.5 or 19 $kg \cdot m^{-3} \cdot d^{-1}$, and still less than 1 $g \cdot L^{-1}$ VFAs were found at OLRs of 20 or 22 $kg \cdot m^{-3} \cdot d^{-1}$, respectively, in both reactors. At OLRs > 20 $kg \cdot m^{-3} \cdot d^{-1}$ VFAs accumulated for a short while immediately after feeding due to exceeded degradation capacities of propionate-oxidizing and other acetogenic bacteria. Digestion of FBS with WBS or RBS as co-substrates failed however, when the OLR was raised to 24 $kg \cdot m^{-3} \cdot d^{-1}$ in reactor 1 and reactor 2 (Fig. 4.8a, 4.8b). VFA concentrations after feeding were higher in reactor 1 with WBS than in reactor 2 with RBS at the same pH, which was in accordance with the $K_{S4.3}$ values of the bread suspensions. After interruptions of feeding in both reactors from days 125 – 132 and resumption of feeding up to an OLR of 17.5 $kg \cdot m^{-3} \cdot d^{-1}$ a stable biogas production was obtained again, but acetate and propionate concentrations were still relatively high". (Cited from Li et al., 2015 in press)

OLR and HRT are two most important operational conditions during design of a full-scale anaerobic digester for waste treatment. The aim for a waste treatment plant is to handle as much waste as possible in the shortest possible time. However, occasional overloading with organic matter (co-substrates) or a too short HRT would lead to failure of anaerobic digestion. When the organic loading rates exceed the metabolic capacity of anaerobic bacteria and the buffer capacity of the substrate(s), the system faces an acidification risk. The generation time of methanogens is relatively long compared to hydrolytic bacteria and acetogenic bacteria. Low HRT may cause wash out of acetogenic bacteria and of methanogens and ruin the syntrophic interaction of fatty acid degrading acetogens with acetate and H_2/CO_2-utilizing methanogens. It is necessary to simulate the maximal OLR and the shortest possible HRT for a

full-scale digester prior to realization in a laboratory-scale reactor under “in praxi” conditions. This means using real suspensions and not model substrates, as often reported in the literature. During mono-digestion of biowaste suspensions taken form a full-scale plant for increasing OLRs up to 19 $kg \cdot m^{-3} \cdot d^{-1}$ a stable biogas process could be maintained, although a long-lasting VFAs accumulation occurred when the HRT was reduced to 5.7d (Gallert et al., 2003). Nayono et al. (2010) have reported the co-digestion of biowaste with increasing OLR by addition of food waste and press water. The biogas production increased by about 75 % when the OLR was increased e.g. from 12 to 20 $kg \cdot m^{-3} \cdot d^{-1}$. Similar as with bread suspensions, the biogas process with press water or food wastes as co-substrates in a biowaste reactor became unstable when the OLR was increased to 23 $kg \cdot m^{-3} \cdot d^{-1}$, the pH dropped and the process collapsed. The co-digestion of municipal solid waste with fats has been reported to increase the biogas production along with the increasing OLR up to 2.5 kg $VS \cdot m^{-3} \cdot d^{-1}$ at a fixed HRT of 17 days (Fernandez et al., 2005). Biogas production could also be increased during dry anaerobic digestion (DAD) of live stock wastes and a mixture of food + paper waste, but the process of DAD collapsed already above a solid retention time of 10 kg $TS \cdot m^{-3} \cdot d^{-1}$, presumably not because of a too low pH but due to inhibiting ammonia concentrations (Kim and Oh, 2011). In this study, the maximal OLR for stable operation was around 21 $kg \cdot m^{-3} \cdot d^{-1}$ at the HRT of 5.7 d.

To determine the maximal OLR for co-digestion of biowaste with bread at a fixed HRT, two biowaste digesters respectively with increasing addition of WBS and RBS were operated at HRT of 6.2 days. The reactors were initially fed with increasing amount of FBS till HRT of 6.2 days. Since then the reactors were fed with the mixture of FBS with WBS or RBS, HRT of both reactors was fixed at 6.2 days and OLR increased by increasing ratio of bread in feedstock. The variation of HRTs and the increase of OLRs during running of the experiment are represented in Figure 4.9.

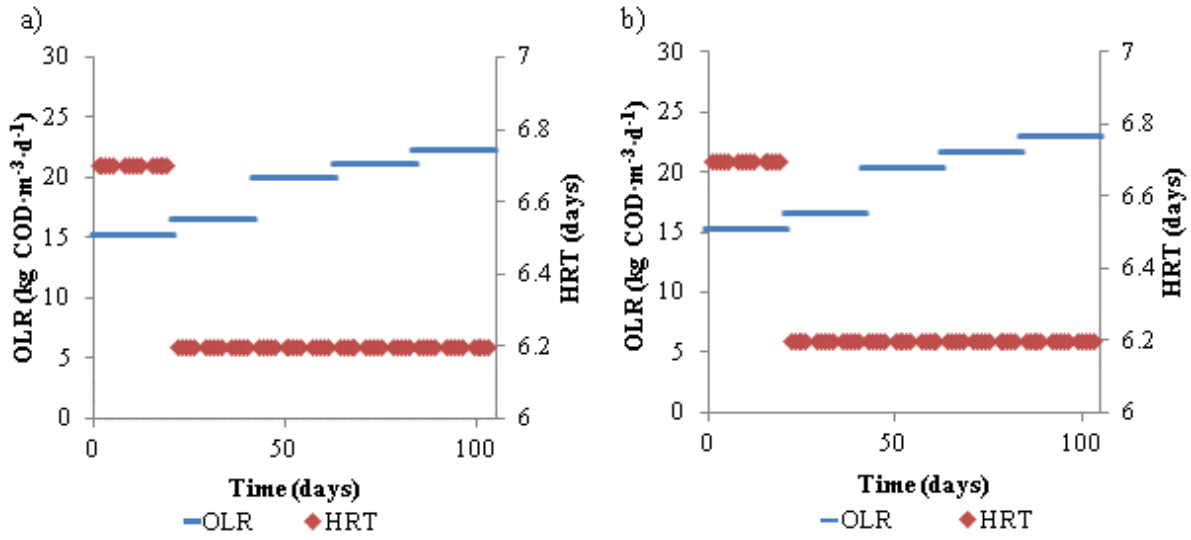

Figure 4.9 Increasing of OLR by addition of co-substrates during the co-digestion at fixed HRT. a) by addition of WBS; b) by addition of RBS.

Figure 4.10 depicts the biogas production and VFAs level during co-digestion of biowaste suspension with WBS and RBS at a fixed HRT. Two reactors were started in parallel as mono-digestion with biowaste feeding. In the first two week, acetate and propionate were both accumulating to a high level. VFAs were degraded to low concentration during the following weekly feeding. After reduction of the HRT from 6.7 to 6.2d by increasing of FBS loading rate, VFAS appeared only in the first week. The steady state condition was reached after three weeks. Since then the two reactors were fed with biowaste suspension and bread suspension as co-digestion process. HRT for both reactors was fixed at 6.2 days, the ratio of WBS and RBS amount in feeding was stepwise increased to obtain increment of OLR (Table 4.7 and 4.8). At the beginning of co-digestion, the VFAs accumulation has lasted for two weeks in co-digester with WBS, whereas VFAs appeared only in the first week in co-digester with RBS. In the first week after increment of OLR from 21.2 to 22.4 $kg \cdot m^{-3} \cdot d^{-1}$, a high and long-lasting VFAs accumulation was observed in co-digester of FBS with RBS, indicating instability of the digestion process.

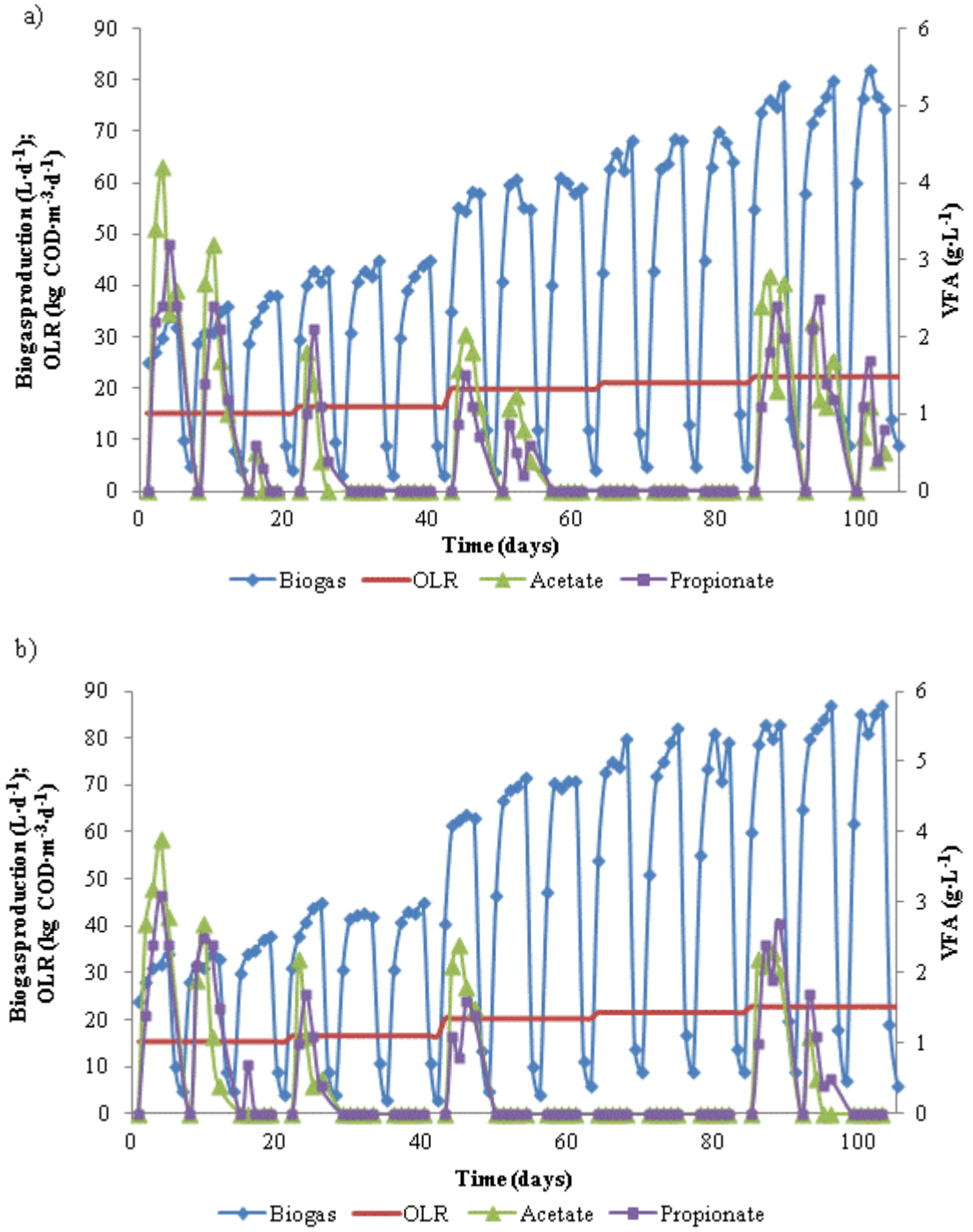

Figure 4.10 Reactor performance during Co-digestion of fresh biowaste suspension with bread suspension at fixed HRT. a) Co-digestion with WBS and b) Co-digestion with RBS.

From the Figure 4.10 it can be seen that the biogas production constantly increased during increment of OLR from 16.6 to 22.4 $kg \cdot m^{-3} \cdot d^{-1}$ in co-digestion of FBS with WBS and during increase OLR from 16.6 to 23 $kg \cdot m^{-3} \cdot d^{-1}$ in co-

digestion of FBS with RBS at fixed HRT of 6.2 d. The biogas production rates were calculated according to the average value of biogas production during the working days. The biogas production rates according to stepwise increase of OLR in this experiment are summarized in Table 4.7 and Table 4.8, to obtain the quantified relationship between biogas production and OLR increase.

Table 4.7 Co-digestion of wheat bread suspension (WBS) in a biowaste digester at fixed HRT

Days	Mode	Main-substrate ($L \cdot d^{-1}$)	Co-substrate ($L \cdot d^{-1}$)	HRT (d)	OLR ($kg \cdot m^{-3} \cdot d^{-1}$)	Additional OLR	BPR ($L \cdot d^{-1}$)	Improve-ment
1-21	Mono-digestion	1.2	-	6.7	15.3	-	32.3	-
22-42	Mono-digestion	1.3	-	6.2	16.6	-	39.9	-
43-63	Co-digestion	1	0.3	6.2	20	20.5%	47.8	20.1%
64-84	Co-digestion	0.9	0.4	6.2	21.2	27.7%	61.2	53.4%
85-105	Co-digestion	0.8	0.5	6.2	22.4	34.9 %	72.6	81.9%

BPR = biogas production rate

During co-digestion of FBS + WBS gas productivity at an OLR of 22.4 $kg \cdot m^{-3} \cdot d^{-1}$ increased by 81.9 % whereas during co-digestion of FBS + RBS at an OLR of 23 $kg \cdot m^{-3} \cdot d^{-1}$ gas productivity increased by 96.5 % as compared to mono-digestion of biowaste suspension at the same HRT. Due to the high solid content of bread suspension (approx. 20%), continued increase the ratio of bread in feedstock brought difficulty at operation of reactor as wet anaerobic digestion. During co-digestion with WBS at HRT of 6.2, the reactor became unstable at OLR of 22.4 $kg \cdot m^{-3} \cdot d^{-1}$ indicated by the long-lasting VFAs accumulation, OLR of 22.4 $kg \cdot m^{-3} \cdot d^{-1}$ was considered to be maximal OLR for co-digestion of FBS with WBS. Due to the stability of co-digestion with RBS at

OLR of 23 kg·m^{-3}·d^{-1}, it seemed that maximal OLR for co-digestion with RBS is higher.

Table 4.8 Co-digestion of rye bread suspension (RBS) in a biowaste digester at fixed HRT

Days	Mode	Main-substrate (L·d^{-1})	Co-substrate (L·d^{-1})	HRT (d)	OLR (kg·m^{-3}·d^{-1})	Additional OLR	BPR (L·d^{-1})	Improve-ment
1-21	Mono-digestion	1.2	-	6.7	15.3	-	32.1	-
22-42	Mono-digestion	1.3	-	6.2	16.6	-	40.1	-
43-63	Co-digestion	1	0.3	6.2	20.4	22.8%	54.4	35.7%
64-84	Co-digestion	0.9	0.4	6.2	21.7	30.7%	68.5	70.8%
85-105	Co-digestion	0.8	0.5	6.2	23	38.5 %	78.8	96.5%

BPR = biogas production rate

4.3 Co-digestion of biowaste suspension with propionic acid

(Modified from Li et al., 2015)

Propionate is a key intermediate of anaerobic digestion in general and of biowaste in particular (McMahon et al. 2004; Gallert and Winter 2003, 2005, 2008; Li et al. 2012; Moertelmaier et al. 2014). Main sources of propionate in bioreactors are odd numbered fatty acids from lipolysis of fat and oil as well as from carbohydrate and amino acid degradation (Gallert and Winter 2005). Propionate accumulates during disturbances of the anaerobic digestion process, caused by e.g., toxic substances, high dry matter content or high organic loading rates (OLRs) close to overload, when hydrolysis, acidogenesis, acetogenesis and methanogenesis, leading to biogas formation from complex organic matter, are no longer balanced (Gallert and Winter 2008, Moertelmaier et al. 2014, Li et al.

2014). Degradation of accumulated fatty acids such as propionate, however, is thermodynamically

unfavourable (Gallert and Winter 2013). Only a few, slow-growing and obligately syntrophic propionate-oxidizing bacteria (POB) can metabolize propionate to acetate, CO_2 and hydrogen in the absence of electron acceptors such as e.g., sulfate. Propionate oxidation under methanogenic conditions requires a hydrogen partial pressure of < 6.5 Pa in a narrow thermodynamically defined window. Thus, in many anaerobic digesters high propionate concentrations can persist for a long time without failure of methane production, e.g., after start up or at high load conditions (Gallert and Winter, 2008; Moertelmaier et al. 2014; McMahon et al. 2004).

In this part of my study biowaste suspension with little "background-propionate" (originating from acidification during home storage, collection and preparation) was digested with increasing amounts of propionate as a co-substrate in a semi-continuous digester to determine maximal propionate oxidation activity in biowaste. In addition a digester was fed with only biowaste during working days and only propionate over the weekend to maintain a stable biogas production. Propionate degradation rates and population shifts during biowaste and/or propionate digestion were also determined and compared.

4.3.1 Main characteristics of the biowaste substrate

Table 4.9 presents the main characteristics of biowaste suspensions used in this experiment. In this experiment 2 portions of biowaste suspension were collected from the biowaste treatment plant at different times. The COD of the biowaste suspension varied due to the quality-difference of municipal biowaste in the winter time (Table 4.9).

The propionic acid used in this experiment was from the life science technology company SIGMA-ALDRICH. The typical physical properties of this propionic acid are depicted in Table 4.10. For digestion of biowaste + propionate

respective amounts of concentrated propionic acid were mixed into the daily portion of fresh biowaste suspension. In one assay of this experiment, the reactor was fed with biowaste suspension during the working days and only propionic acid at weekends. In order to be able to continuously pump the propionic acid into the reactor and avoid acidification, propionic acid was 5-fold diluted. Effluent from the methane reactor of the biowaste treatment plant Karlsruhe was used as an initial inoculum for this experiment.

Table 4.9 Composition of biowaste suspensions (from Li et al., 2015)

Parameters	**Average values**
Total solids, TS (%)	6.1 ± 0.5
Volatile solids, VS (%)	5.2 ± 0.4
Chemical oxygen demand, COD ($g \cdot L^{-1}$)	94 – 113
Total Kjeldahl nitrogen, TKN ($g \cdot L^{-1}$)	2.2 ± 0.2
NH_4^+-Nitrogen ($g \cdot L^{-1}$)	0.5 ± 0.1
pH	4.5
Acetate ($g \cdot L^{-1}$)	3.1 ± 0.3
Propionate ($g \cdot L^{-1}$)	2.7 ± 0.3
n-Butyrate ($g \cdot L^{-1}$)	1.5 ± 0.2

Table 4.10 Physical properties of propionic acid

Property	**Values**
Molecular weight ($g \cdot mol^{-1}$)	74.08
Boiling point at 760 mmHg, 1.01 ar	104.9°C
Freezing point	-20°C
Vapor pressure	2.4 mmHg
Density ($g \cdot ml^{-1}$)	0.993
Assay (%)	> 99.5

“Four cylindrical glass reactors with a total volume of 10 L and a working volume of 8 L, wrapped with silicon tubing for warm water circulation from a thermostat to maintain 37 °C, were fed from Monday to Friday at 8 a.m. and 6 p.m. with fresh biowaste suspension, replacing 600 ml of digested biowaste. No biowaste feed was added on Saturday and Sunday as in the full-scale plant of Karlsruhe. The laboratory digesters were run at 12 or 14 kg $COD \cdot m^{-3} \cdot d^{-1}$ organic loading rate (OLR) with biowaste batch 1 or batch 2, respectively as a “basic

load". Reactor 1 was run as a control for 50 days at an OLR of 12 kg COD·m^{-3}·d^{-1} (biowaste batch 1). In reactor 2 the OLR was also 12 kg COD·m^{-3}·d^{-1}, maintained with biowaste suspension batch 1 until day 55 and then increased to 14 kg COD·m^{-3}·d^{-1}, when biowaste batch 2 was added. A constant OLR was maintained with biowaste, which was stepwise increased by addition of respective amounts of propionic acid (addition of 0.7 g propionic acid per L equals an OLR increase of 1 kg COD·m^{-3}·d^{-1}) to maximally 18 kg COD·m^{-3}·d^{-1}. During glass repairs (days 75 – 85 Fig. 4.11b) the reactor content was stored under anaerobic conditions but was not fed. Reactor 3 was run with a basic OLR of 3 kg COD·m^{-3}·d^{-1}, maintained by propionic acid addition all week to keep the propionate-oxidizing bacteria (POB) active. Biowaste suspension was available from Monday to Friday and was added to give an additional OLR of 11 kg COD·m^{-3}·d^{-1}. Reactor 4 was fed with biowaste suspension from Monday to Friday at an OLR of 11 kg COD·m^{-3}·d^{-1} and with propionic acid from Friday night to Monday morning at an OLR of 5 kg COD·m^{-3}·d^{-1}. Although the OLR with propionic acid was only 5 kg COD·m^{-3}·d^{-1}, propionic acid was completely degraded and for this feeding regime the same daily gas production as with biowaste, necessary for continuous operation of a generator, was obtained.

4.3.2 Performance of co-digestion of biowaste with propionic acid

Figure 4.9 depicts the biogas production and fatty acid accumulation in an anaerobic mono-digester of biowaste and in a co-digester of biowaste + propionic acid. During feeding of an anaerobic digester (reactor 1) with biowaste suspension from Monday to Friday and interruption of feeding on Saturday and Sunday, maintaining an OLR of 12 kg COD·m^{-3}·d^{-1}, propionate accumulated in the first 3 weeks after start during the 5 days with biowaste feeding, but was completely degraded during Saturday and Sunday, when no fresh biowaste was supplied. A steady state was reached 25–27 days after start with a high enough acetogenic and methanogenic activity to completely convert

propionate and acetate to methane. The biogas production decreased obviously only at weekends because of no feeding of fresh biowaste. The biogas production on Monday was much lower than on the following working days in the first 3 weeks after start. Thirty five days after start the metabolic activity of the population was stable and high enough so that after starvation over the weekend and resuming feeding on Monday only slightly less biogas (28–30 $L \cdot d^{-1}$) was produced than from Tuesday to Friday (31–36 $L \cdot d^{-1}$), respectively. During working days the biogas production ranged from 28 to 36 $L \cdot d^{-1}$, and the average daily biogas production was 34 $L \cdot d^{-1}$.

Another reactor (reactor 2) was operated with a basic OLR of 12 (day 1–55) or 14 kg $COD_{biowaste} \cdot m^{-3} \cdot d^{-1}$ (day 55 onwards) and the high basic load was stepwise increased to 18 kg $COD \cdot m^{-3} \cdot d^{-1}$ by co-feeding increasing amounts of propionate. After every increase of the OLR, during the week high amounts of propionate (higher than the added propionate) accumulated, which were degraded during the starvation periods of week ends. For each stepwise increased OLR, propionate accumulation during the second week of feeding was significantly lower than during the first week and no propionate or only very little propionate accumulated in the third week. With increasing OLR gas production increased with lower values on Monday than from Tuesday to Friday (Figure 4.11b, days 0–75), indicating some activity stagnation during the weekends without feeding. During 10 days interruption of the feeding for glass repairs (Figure 4.11b, day 75–85), POB lost much of their metabolic activity. After resuming biowaste + propionate feeding at almost the same OLR as before maintenance, when no propionate was detected in digester effluent, the highest propionate peak at all was measured (Figure 4.11b, day 89). Complete regeneration of the propionate degradation activity by POB took more than 40 days. Finally, at the very high OLR of 18 kg $COD \cdot m^{-3} \cdot d^{-1}$, maintained with biowaste + propionate feeding for 5 days per week with no feeding on Saturday and Sunday, steady state

conditions without residual fatty acids in the effluent were obtained (Figure 4.11b, days 130–140 and further on).

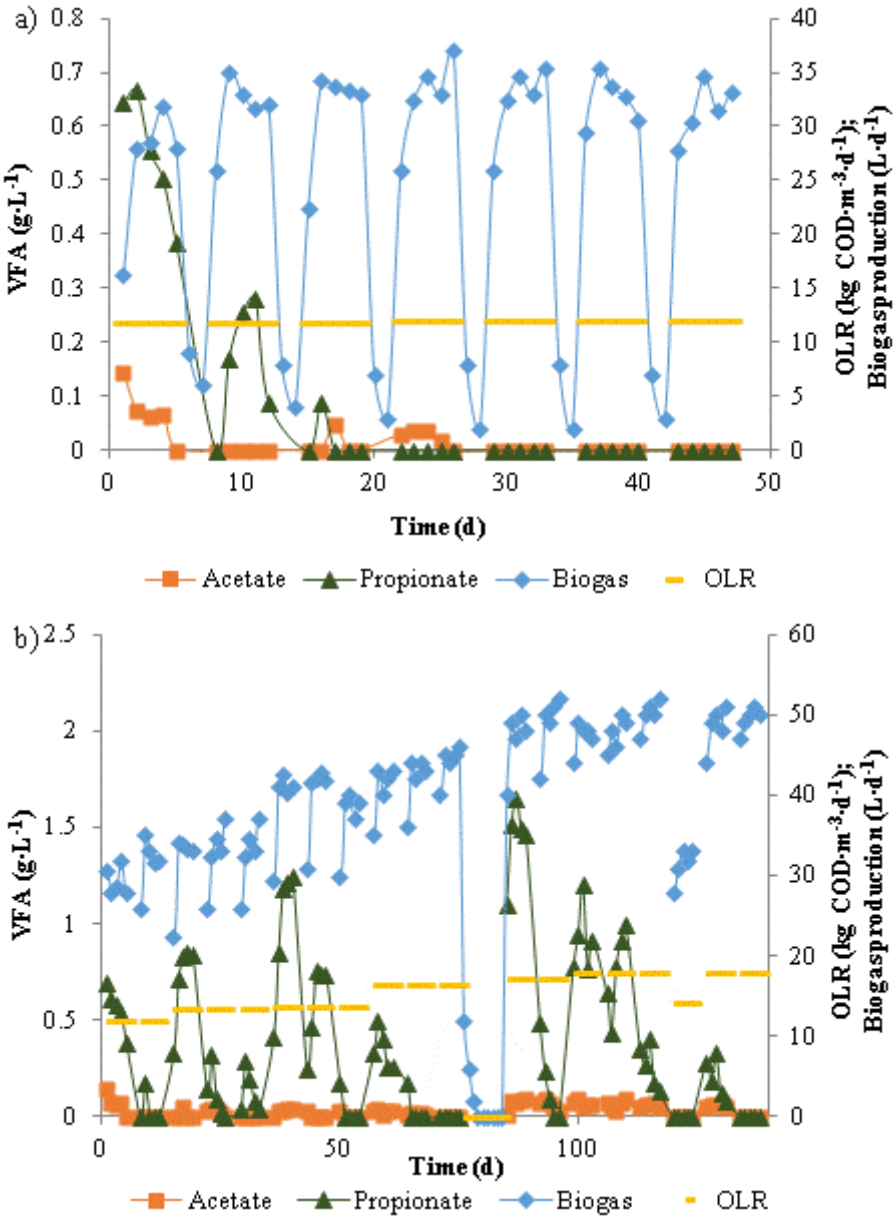

Figure 4.11 Biogas production and fatty acid levels in an 10 L biowaste digester (reactor 1) after start at an OLR of 12 kg $COD_{biowaste} \cdot m^{-3} \cdot d^{-1}$ (**a**) and for increasing organic loading rates up to 18 kg $COD \cdot m^{-3} \cdot d^{-1}$, maintained by 12 kg (day 1–55) or 14 kg (new batch biowaste from day 55 onwards) $COD_{biowaste} \cdot m^{-3} \cdot d^{-1}$ plus respective amounts of propionate (reactor 2, **b**). No feeding between days 75–85 due to maintenance works (Reproduced from Li et al., 2015).

Figure 4.12 presents the biogas production, VFA levels and COD elimination in reactor 3, which was operated with 3 kg $COD_{biowaste} \cdot m^{-3} \cdot d^{-1}$ as a "background" OLR all week to maintain a steadily high propionate oxidation activity. "From Monday to Friday biowaste suspension was additionally supplied to raise the OLR to 14 kg $COD \cdot m^{-3} \cdot d^{-1}$. The minimal biogas production with propionate alone during weekends was 16–18 $L \cdot d^{-1}$. It increased to more than 40 $L \cdot d^{-1}$, when the OLR was increased Monday to Friday by feeding fresh biowaste suspension. Compared to reactor 2 the biogas production in reactor 3 was improved obviously during the weekend, which may solve the shortage of biogas for electricity generation in full-scale plant at weekends. Only 2 weeks after start no propionate accumulated in the 10 L reactor during the week, when the OLR was raised from 3 kg $COD_{propionate} \cdot m^{-3} \cdot d^{-1}$ to 14 kg $COD \cdot m^{-3} \cdot d^{-1}$ by biowaste addition. Acetate in the effluent disappeared completely after 3 weeks". (Cited from Li et al., 2015)

To obtain an constant biogas production during the whole week, feeding of an anaerobic digester with biowaste suspension during the working days and with propionic acid at weekends was maintained in a laberatory scale reactor. Figure 4.13 depicts the biogas production with different OLRs in reactor 4, which was run with biowaste feeding at an OLR of 12 kg $COD \cdot m^{-3} \cdot d^{-1}$ from Monday until Friday and with propionate feeding at an OLR of 5 kg $COD \cdot m^{-3} \cdot d^{-1}$ from Friday night to Monday morning to maintain a constant gas production during seven days a week. The biogas production from Monday to Friday (26 – 28 $L \cdot d^{-1}$) with biowaste feeding was almost same as at weekend only with feeding of propionic acid (25 – 27 $L \cdot d^{-1}$). No propionate or acetate was detected in the digester effluent at any time. Thus the fermentation was stable, representing steady state conditions.

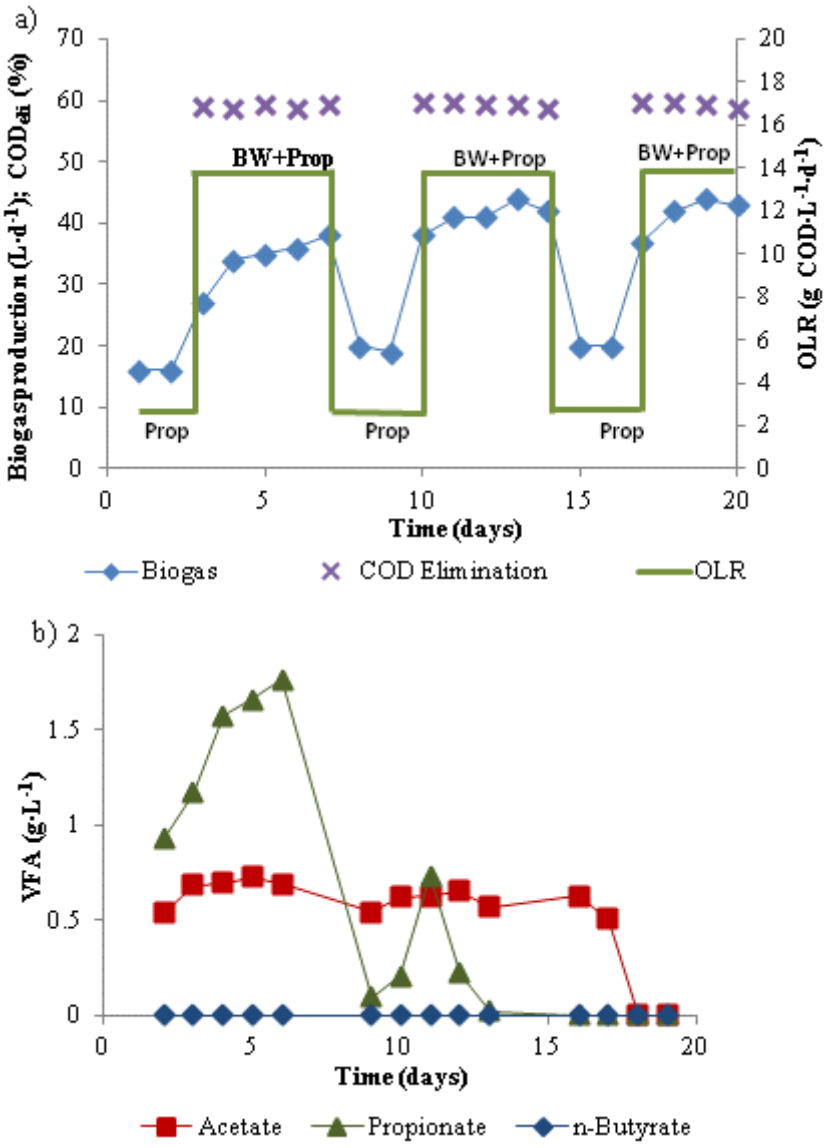

Figure 4.12 Biogas production (**a**) and fatty acid levels (**b**) in biowaste digester fed constantly with propionate (2.68 mM, 80 mL·d^{-1}) at an OLR of 3 kg COD·m^{-3}·d^{-1} and additionally with biowaste from Monday to Friday (1 L·d^{-1}) to reach an OLR of 14 kg COD·m^{-3}·d^{-1} (reactor 3).

"In Karlsruhe, Germany, source-sorted municipal biowaste is collected Monday to Friday and a biowaste suspension for anaerobic digestion prepared at the same day. Since the storage capacity for the biowaste suspension is limited, it is digested at the same day. Storage of freshly collected biowaste on Friday until Monday morning is avoided to prevent spoilage by biocide-forming *Phycomycetes* which might inhibit methanogenesis. No storage tank for digested suspension is available, requiring solid separation of the digested

suspension by centrifugation immediately after removal from the biowaste reactor. Thus, a constant biogas amount for operation of a gas engine and an electricity generator is only available during working days and only little biogas is produced on weekends without fresh feed. To overcome this restriction, removal of biowaste suspension to the minimum filling level on Friday night and addition of highly concentrated liquid wastes during the week end until the maximum filling level is obtained on Monday would allow a constant gas production, as shown here by laboratory experiments with propionic acid as a model liquid waste.

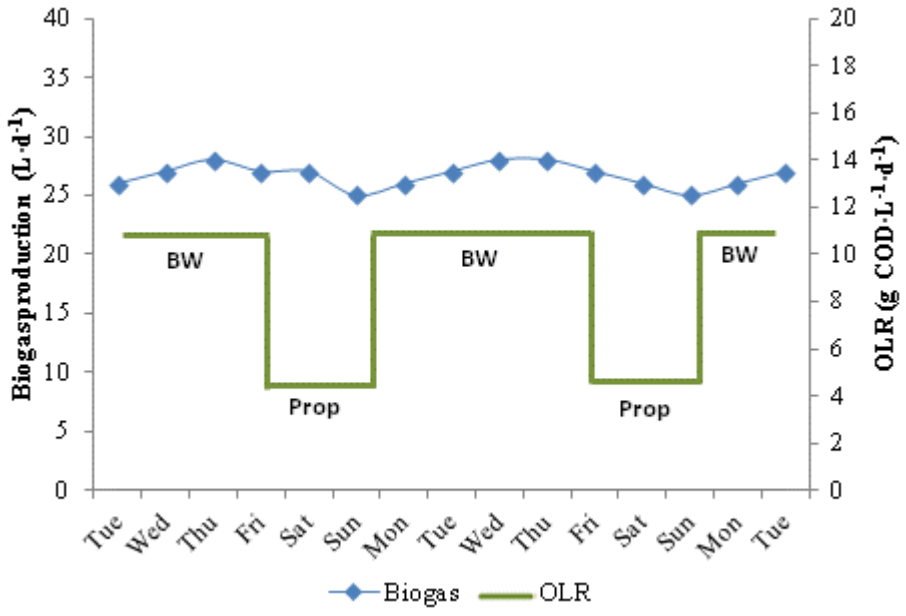

Figure 4.13 Periodic feeding of biowaste (BW, 1 $L \cdot d^{-1}$) and propionate (Prop, 2.68 M, 120 $mL \cdot d^{-1}$) to maintain an almost constant gas production in biowaste digester (reactor 4) over weekends, when no biowaste was available. No fatty acids were detected at any time (Reproduced from Li et al., 2015).

Nayono et al. (2009) reported already that highly concentrated food waste or press water from biowaste would be suitable substrates for automatic pump feeding of the digester during weekends. Those substrates are much more homogeneous than biowaste and would allow plant operation in the absence of

inspecting staff. Acidified liquid substrates with high concentrations of fatty acids such as acetate, propionate or n-butyrate might be used for automatic feeding of a biowaste reactor during the weekends, since these fatty acids, including propionate, can be rapidly degraded without a significant lag-phase after biowaste feeding, even if no fatty acids are found in the digester liquid during the week, as shown in this study. Since fatty acids such as propionate in acidified substrates are 100% biodegradable in syntrophic association of acetogenic and methanogenic bacteria, for a similar biogas production a much lower OLR can be maintained during feeding of highly concentrated acidified substrates than during feeding of complex biowaste suspensions, which are only degradable to an extent of 50–70%. (Nayono et al. 2009, Nayono et al. 2010)

4.3.3 Propionate oxidation rates

For analyses of the variation of propionate degradation rates with increased propionate feeding, duplicate assays in serum bottles, containing 40 ml effluent of reactor 2 during different OLR as sources of microorganisms and 800 ± 50 $mg \cdot L^{-1}$ propionate were incubated at 37 °C. During incubation sample were withdrawn with a syringe and the concentration of propionate was determined by gas chromatography. Mean degradation rates were calculated for logarithmic or linear degradation phases of the respective acid in two parallel assays.

Figure 4.14 depicts the propionate degradation process in effluent from reactor 2, which was sampled every second day per week and before an increase of propionate addition into the biowaste feeding. Propionate degradation rates were calculated and are summarized in Table 4.11. Propionate degradation rates (PDRs) at an OLR of 12 kg $COD_{biowaste} \cdot m^{-3} \cdot d^{-1}$ in reactor 2 were initially between 40.4 – 41.4 $mg \cdot L^{-1} \cdot d^{-1}$. The PDRs in reactor 2 with a high basic OLR of 12/14 kg $COD_{biowaste} \cdot m^{-3} \cdot d^{-1}$ plus increasing amounts of propionate until a final OLR of 18 kg $COD \cdot m^{-3} \cdot d^{-1}$ was reached, had increased to 109.2 $mg \cdot L^{-1} \cdot d^{-1}$. PDRs increased with addition of propionate.

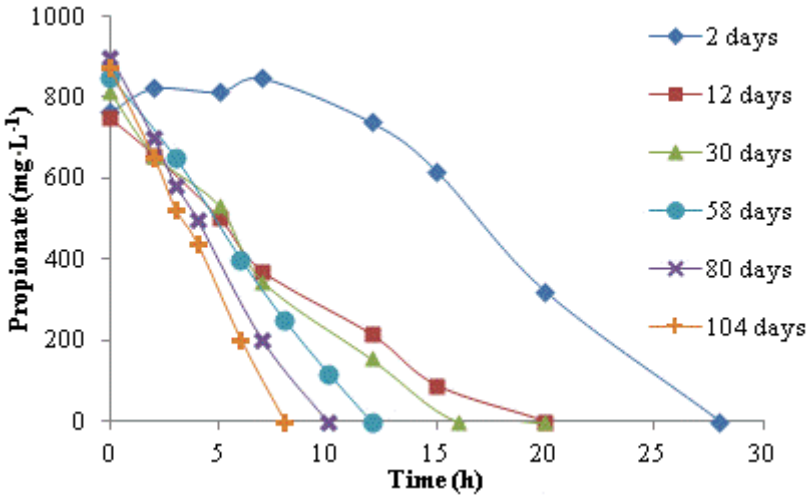

Figure 4.14 Propionate degradation activity in effluent of reactor 2.

Table 4.11 Propionate degradation rates in reactor 2 for biowaste at an OLR of 12/14 kg COD m^{-3} d^{-1} and after a stepwise increase of the OLR to finally 18 kg COD m^{-3} d^{-1} by co-feeding of propionate (from: Li et al., 2015)

Time (days)	OLR (kg COD·m^{-3}·d^{-1})	Propionate addition (g·L^{-1})	Degradation rate (mg·L^{-1}·h^{-1})
2	12	-	40.4 [b]
12	12	-	41.4 [c]
30	12 +1 [a]	0.7	54.8 [b]
72	14 + 2.5 [a]	1.5	70.8 [b]
94	14 + 3.0 [a]	1.9	99.9 [b]
117	14 + 4.0 [a]	2.5	109.2 [b]

Biowaste contributed 12 (batch 1 until day 30) or 14 kg COD m^{-3} d^{-1} (batch 2, after day 30). [a] Additional OLR by propionate addition, [b] Average propionate degradation rate (± 0.3) determined in parallel incubations. [c] Average propionate degradation rate of duplicate samples (± 0.3) of reactor 1 and reactor 2, as well as from the full-scale biowaste reactor of the City of Karlsruhe.

To determine the different propionate degradation rates after biowaste feeding and propionate feeding, the same assays with effluent of reactor 4 at Friday (after one week biowaste feeding) or on Monday (after propionate feeding during weekend) were carried out (Figure 4.15).

In reactor 4 with successive feeding of 12 kg $COD_{biowaste}$·m^{-3}·d^{-1} for 5 days and 5 kg $COD_{propionate}$·m^{-3}·d^{-1} for 2 days during the weekend, resulting in a similar daily gas productivity, sludge withdrawn after biowaste feeding had a PDR of 70 mg·L^{-1}·h^{-1} (Figure 4.15a) which was almost double as much as in the only

biowaste fed reactor at the same loading (70 compared to 40 $mg \cdot L^{-1} \cdot d^{-1}$) and sludge withdrawn after 2 days of only propionate feeding had a much higher PDR of around 100 $mg \cdot L^{-1} \cdot h^{-1}$.

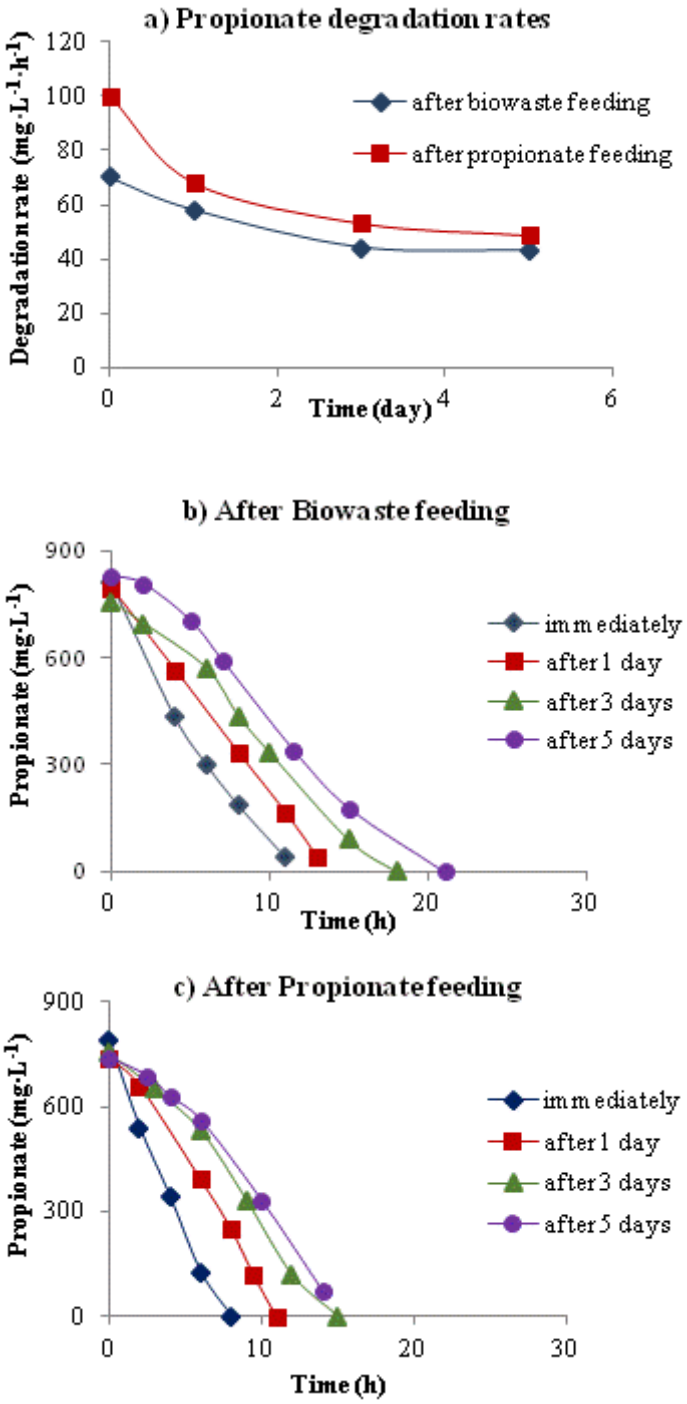

Figure 4.15 Propionate degradation rates (**a**) and degradation activity immediately and after 1–5 days of starvation in effluent of reactor 4 after biowaste (**b**) or propionate feeding (**c**) (from Li et al., 2015).

However, during the first day of starvation the PDR decreased much faster in the sludge fed propionate than in the sludge fed biowaste (Figure 4.15a). About 30–50% of the propionate oxidizing activity (POA) was lost during only 5 days starvation and already after 3 days starvation degradation of propionate in the assays started only after a lag phase of 4–7 h (Figure 4.15b,c). Propionate degradation was completed much earlier in propionate-fed sludge than in biowaste-fed sludge (Figure 4.15b, c). While during the first day of starvation POA in the propionate-fed sludge decreased much faster than in the biowaste-fed sludge (30% *versus* 13%), later on POA in both assays decreased at similar rates (Figure 4.15a).

Under steady state conditions during anaerobic digestion of biowaste, when no fatty acids remain in the digestion fluid, production rates of metabolites matched with their conversion rates to finally biogas. In the presence of inhibitors or at high loading close to overload conditions methanogenesis gets instable and fatty acids such as propionate begin to accumulate. Some propionate in the effluent must, however, not necessarily indicate already a disturbance or breakdown of biogas production. During phenol inhibition in a glucose-fed anaerobic digester for instance, 2.75 $g \cdot L^{-1}$ propionate and about 0.1 $g \cdot L^{-1}$ acetate and n-butyrate accumulated, but methanogenesis did not collapse (Pullammanppallil et al., 2001). At stepwise increase of the OLR in a biowaste reactor from 4.3 to 15 kg $COD \cdot m^{-3} \cdot d^{-1}$, propionate appeared in the reactor effluent after each increase of the OLR for a while (Gallert et al., 2003). When the reactor was run at the highest OLR of 15 kg $COD \cdot m^{-3} \cdot d^{-1}$, in the first week the propionate peak concentration reached to 3.4 $g \cdot L^{-1}$, but later on decreased to 1.4 $g \cdot L^{-1}$ at ongoing biogas production. All propionate was degraded during weekends without feeding, as similarly observed here. Amani *et al*. 2011 showed that propionate concentrations in an anaerobic digester significantly influenced propionate oxidation. When the propionate concentration was 3 $g \cdot L^{-1}$, 17 % less propionate was removed than at a lower concentration of 1.5 $g \cdot L^{-1}$.

In our experiments after each addition of propionic acid for a stepwise increase of the OLR in the biowaste reactor, propionate concentrations in effluent sharply increased during the first days, but the propionate was completely degraded during weekends without biowaste feeding. Less propionate accumulated in the second week after an increase of the OLR and almost no propionate accumulated in the third week of propionate feeding at the same OLR, indicating that the propionate degrading activity of the POB was improved. The maximum propionate accumulation reached 1.6 $g \cdot L^{-1}$ in reactor effluent after the addition of 1.9 $g \cdot L^{-1}$ propionate with the feed. Biogas production increased with time and the pH in digester remained stable throughout the stepwise increase of the OLR up to 18 kg $COD \cdot m^{-3} \cdot d^{-1}$ with biowaste and propionate. There was a strong Pearson´s correlation of added propionate and biogas production of 0.86 until day 90 for an OLR of 14 kg $COD_{biowaste}$ + 2.5 kg $COD_{propionate} \cdot m^{-3} \cdot d^{-1}$. When more propionate was added (Figure 1b, day 85–140), biogas production did no longer increase, indicating not only a reduced biowaste conversion to biogas but also a reduced acidification efficiency, since no fatty acids were accumulating and the previously accumulated propionate was apparently degraded with time. Thus the addition of 2.5 kg $COD_{propionate} \cdot m^{-3} \cdot d^{-1}$ together with 14 kg $COD_{biowaste} \cdot m^{-3} \cdot d^{-1}$ was the maximum amount for co-digestion at maximal degradation efficiency and maximal biogas production" (Li,et al., 2015).

4.4 Effect of moisture content on dry anaerobic digestion of biowaste (Modified from Li et al., 2014)

"The separately collected biowaste fraction of municipal waste can be handled either with addition of process water for wet anaerobic digestion or directly by dry anaerobic digestion with high solid content (De Baere, 2000; Lunning et al. 2003). Dry digestion is considered as energy-saving technique due to less pre-treatment and process water addition. However, at the usual moisture content of

separately collected biowaste fraction of ±30%, the anaerobic digestion may encounter mass transfer limitation caused presumably by lack of bio-available water (Abbassi-Guendouz et al. 2012). Total solid content is the main parameter to regulate the biogas production. It strongly interacts with the particle size of substrates and also indicates the important role of water availability for dry anaerobic digestion (Motte et al.2013). The biogas production was slightly decreased for increasing DM content from 10 to 25 %. At 30 % DM content methanogenic activity was no longer stable and at 35 % DM content methanogenesis failed completely (Fernández-Rodríguez et al. 2012). Even at thermophilic incubation conditions, where more moisture is bio available due to an increased "fluidity" and a hygienized product is obtained, bioavailability of water is not sufficient for high methane yields (Fernández-Rodríguez et al. 2013). Specific growth rates were 27-60 % higher during thermophilic than during mesophilic methanogenesis, which could have been due to an increased water activity. More water of the moisture was apparently bio-available at 55^{o}C than at 37^{o}C.

As the development towards dry anaerobic digestion is relatively new, not very many data on the behavior of dry anaerobic digestion during start-up are available as yet. The start-up of reactors for anaerobic digestion is a sensible, time consuming process and depends on the activity of the inoculums, which ideally should come from digestion of the same or of a similar substrate. A major problem during the start-up phase may be the accumulation of volatile fatty acids, especially of propionate by the fast-growing heterotrophs (Gallert and Winter, 2008; Felchner-Zwirello et al., 2012, 2013), which leads to an acidification of the reactor content and, if no counteractions are taken, to failure. As during the early days of a start-up procedure no or only very little methane is produced, a rapid acidification by fast-growing acidogenic bacteria must be prevented, so that acetogens and methanogens can cope with volatile fatty acid production and thus prevent fatty acid accumulation and a pH drop (e.g. Gallert

et al., 2003; Gallert and Winter, 2005). Monitoring of fatty acids and pH value is considered helpful for a successful start-up of bioreactors.

In this part of the study the optimal water content for dry anaerobic digestion was determined and the influence of a temperature change from ambient to mesophilic and thermophilic temperatures during start-up period was investigated.

4.4.1 Main characteristics of substrate

Source-sorted biowaste was collected with rotating drum trucks by City authorities of Karlsruhe, Germany for large-scale wet anaerobic digestion (WAD) (Gallert et al., 2003; Nayono et al., 2009). For lab-scale dry anaerobic digestion (DAD) experiments woody material, ornamental plant soils as well as paper, plastic foils, broken glass and metals were manually sorted out from the collected biowaste fraction before shredding in a cutter (ZG Raiffeisen, Karlsruhe) to 1 cm length. Table 4.12 presents the main characteristics of the fresh biowaste. The dry matter (DM) content of the sorted biowaste (triplicate analyses) was 30.9 ± 0.6 % (first batch for start of experiments) and 30.3 ± 0.6 % (second batch for the re-feeding experiments). In both batches the organic dry matter content (ODM, duplicate analyses) was 65 – 67 % of the DM content. As a source of microorganisms solid residues of digested biowaste suspensions were taken from the extrusion pipe of the sludge centrifuge at the WAD plant of the City of Karlsruhe. This inoculum contained 33.7 ± 0.6 % DM of which 62 % were organic material (bacteria and undigested/non-digestible biowaste particles).

Portions of 10 kg of shredded fresh biowaste and of 10 kg solid residues of digested biowaste suspension were mixed thoroughly. Little water was added to obtain a calculated DM content of 30 %, which was confirmed by analysis. To obtain biowaste fractions with 25 or 20 % DM content, the above mixture was accordingly diluted with little water. Parallel DAD experiments with two kg of

the above prepared biowaste fractions that contained 30, 25 or 20 % DM were started in 3 L glass reactors. The total weight, solid content and volatile solid content in every digester are represented in Table 4.13. All reactors are initially incubated at room temperature and later changed to mesophilic (37^{o}C) and thermophilic (55^{o}C) temperature. At the beginning of the experiment the pH value in all reactors was adjusted to above 7.5 with 5M NaOH and after acidification the pH value of the reactors was adjusted again by addition of NaOH.

Table 4.12 Main characteristics of fresh biowaste and digested residue

	Fresh biowaste	Digested residue
Total solids, TS (%)	30.6 ± 0.6	33.7 ± 0.6
Volatile solids, VS (%)	23.2 ± 0.6	20.5 ± 0.6
pH	4.0	7.8
Acetate ($g \cdot L^{-1}$)	1.66	1.67
Propionate ($g \cdot L^{-1}$)	0	0.16

Table 4.13 Mass data of reactors for dry anaerobic digestion (DAD) of biowaste with 30, 25, 20% dry matter content at start of digestion

Time	t_0 (start)		
Biowaste	T_{total} (kg)	TS (%)	VS (%)
DM 30% R1	5.1	30.9	20.1
DM 30% R2	5.04	30.9	20.1
DM 25% R1	5.54	25.2	16.3
DM 25% R2	5.72	25.2	16.3
DM 20% R1	6.14	21.2	13.8
DM 20% R2	6.02	21.2	13.8

DM = dry matter, R1, R2 = reactor 1 and reactor 2 at the same or different temperature regime

4.4.2 Influence of moisture content of substrate on dry anaerobic digestion

The biogas production from dry anaerobic digestion of source-sorted municipal biowaste at its original dry matter content of 20%, as well as the temperature change and the variation of VFA levels for almost 1 year is depicted in Figure 4.16. After 5, 10 and 25 days pH value of those two reactors decreased below 6.7 and was adjusted to nearly 8 with 5M NaOH. Later on no pH correction was necessary, even though volatile fatty acid (VFA) such as acetate, propionate and n-butyrate were still increasing. The biogas production started after a lag phase of 25 days. In first 150 days 0.33 m^3 methane·kg^{-1} ODM and 0.32 m^3 methane·kg^{-1} ODM was, respectively, released from reactor 1 and 2. Only little more gas was produced upon further incubation at room temperature. When the gas production had ceased after 210 days at room temperature, the reactor temperature increased in reactor 1 to mesophilic temperature (37^oC) and in reactor 2 to thermophilic temperature (55^oC). The temperature change did not lead to a significant further degradation and only little more gas was produced in both reactors. The VFA accumulation in total or distinguished as acetate, propionate and n-butyrate were similar in both reactors. The maximal n-butyrate concentration reached about 10 g·L^{-1} and was completely degraded after 70 days. The degradation of up to 20 g·L^{-1} acetate to the low final steady state level spent 120 – 150 days.

Propionate concentrations in the reactors increased to 15 – 18 g·L^{-1} during n-butyrate and acetate degradation and reached their low steady state level of 0.5 ± 0.25 g·L^{-1} only after 150 – 180 days. These VFA concentrations were in the same range as reported by Zahedi et al. (2013) for thermophilic DAD in a stirred tank reactor. The moisture content of biowaste for DAD and the incubation temperature are important factors for anaerobic digestion to proceed at all and for the final efficiency of digestion. In biowaste DAD reactors with 20

% DM content there was apparently enough bio-available water for non water limited biogas formation by the established micro flora at 20, 37 and 55°C.

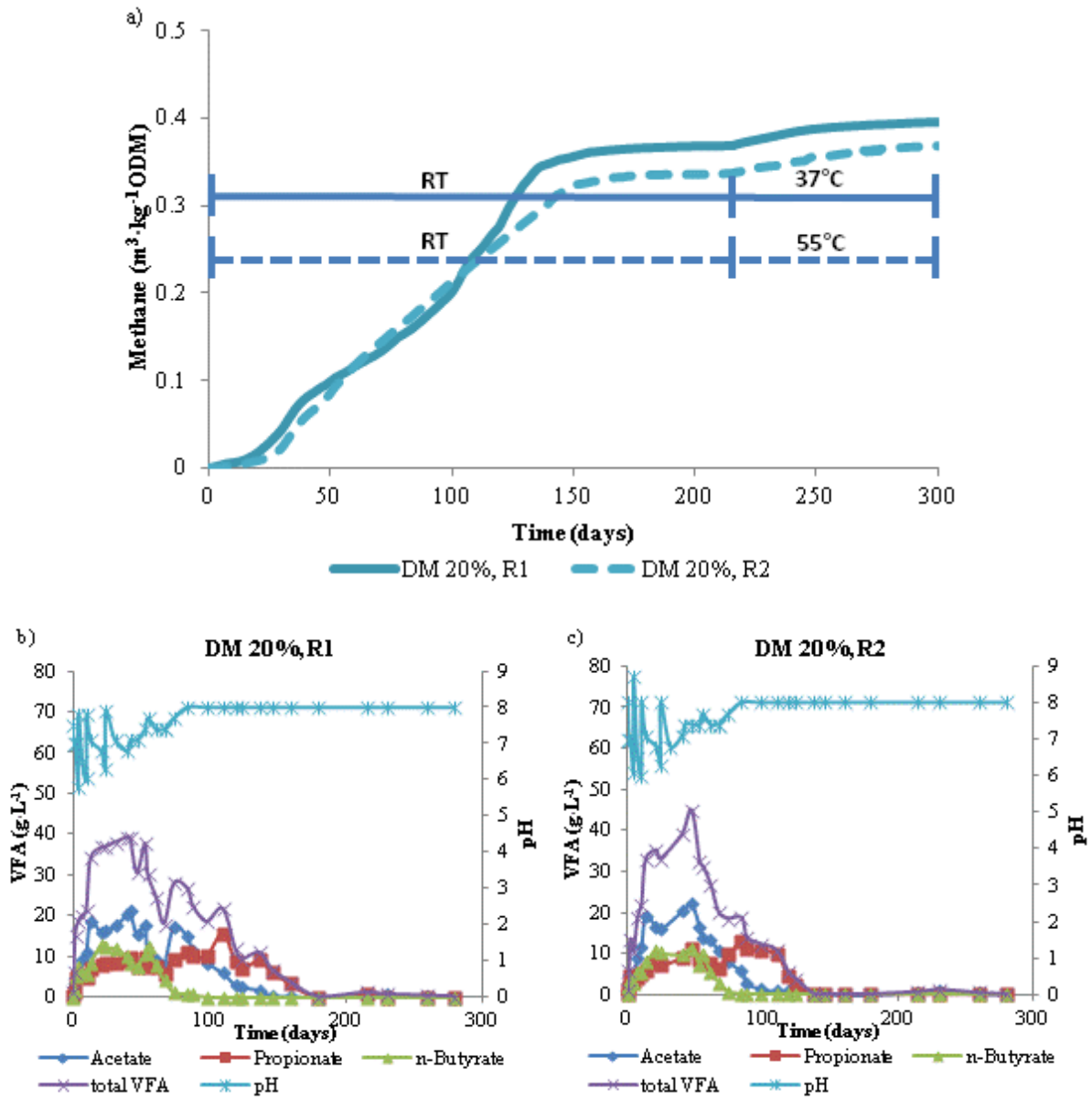

Figure 4.16 Biogas production in reactor R1 an R2 (Fig. a) at room temperature (RT), pH and volatile fatty acid (VFA) concentrations (Fig. b, c) during digestion of biowaste with 20 % DM content. Raising the temperature to 37°C or 55°C after 220 h (Fig. 1a) did not cause significant more biogas generation.

The biogas production from dry anaerobic digestion of source-sorted municipal biowaste at its original dry matter content of 25%, as well as the temperature change and the variation of VFA levels during the whole process is depicted in

Figure 4.17. As in the reactors with 20 % DM content VFA were accumulating initially and the pH was adjusted to 8 three times in the first 30 days. The gas production at 20 ± 1°C in reactor 1 started only after 140 days and proceeded for about 100 days. The total methane amount reached 0.17 $m^3 \cdot kg^{-1}$ ODM, which was only about 50 % of the gas amount that was obtained in reactors with biowaste that contained 20 % DM. In reactor 2 at 37°C biogas was produced much faster than at 20 ± 1°C, the total biogas amount was similar as the gas amount that was obtained in reactors with biowaste that contained 20 % DM. When the gas production ended in the reactor that was incubated at 37 °C the temperature was further increased to 55°C. About 15 % more biogas were produced at the higher incubation temperature. The VFA accumulation in reactor 1 was almost identical to in reactor 2. Acetate, propionate and n-butyrate were accumulating in the first 60 days respectively to 25 $g \cdot L^{-1}$, 12 $g \cdot L^{-1}$ and 10 $g \cdot L^{-1}$ and then were slowly degraded. Butyrate degradation apparently led to acetate formation and an increased propionate concentration. Acetate degradation also caused an increase of propionate concentration, which finally was degraded after 220 days. In biowaste that contained 25 % DM content the bio available water at 20°C was apparently still enough to allow rapid hydrolysis and acidification, but the biogas production by syntrophic interaction of acetogenic and methanogenic bacteria was significantly delayed and did not proceed to completion. Since a_W is temperature dependent (Starzak and Mathlouthi, 2006) a temperature shift from 20°C to 37°C may have increased the amount of bio available water so that methanogenesis in the biowaste reactor with 25 % DM content could proceed to completion.

The biogas production from dry anaerobic digestion of source-sorted municipal biowaste at its original dry matter content of 30%, as well as the temperature change and the variation of VFA levels during the whole process is depicted in Figure 4.18. After start of the experiment reactors were rapidly acidified and the pH was adjusted to 8 four times in the first 70 days. Later on pH did not

decrease any more, however, methanogenesis in both reactors could not start within 210 days of incubation at room temperature. Even when the temperature in reactor 2 was raised to 37°C at day 90 gas production did not start within the next 40 days. Only when the temperature was raised from 20 ± 1 °C to 55 °C in reactor 1 or from 37°C to 55°C in reactor 2 biogas production began.

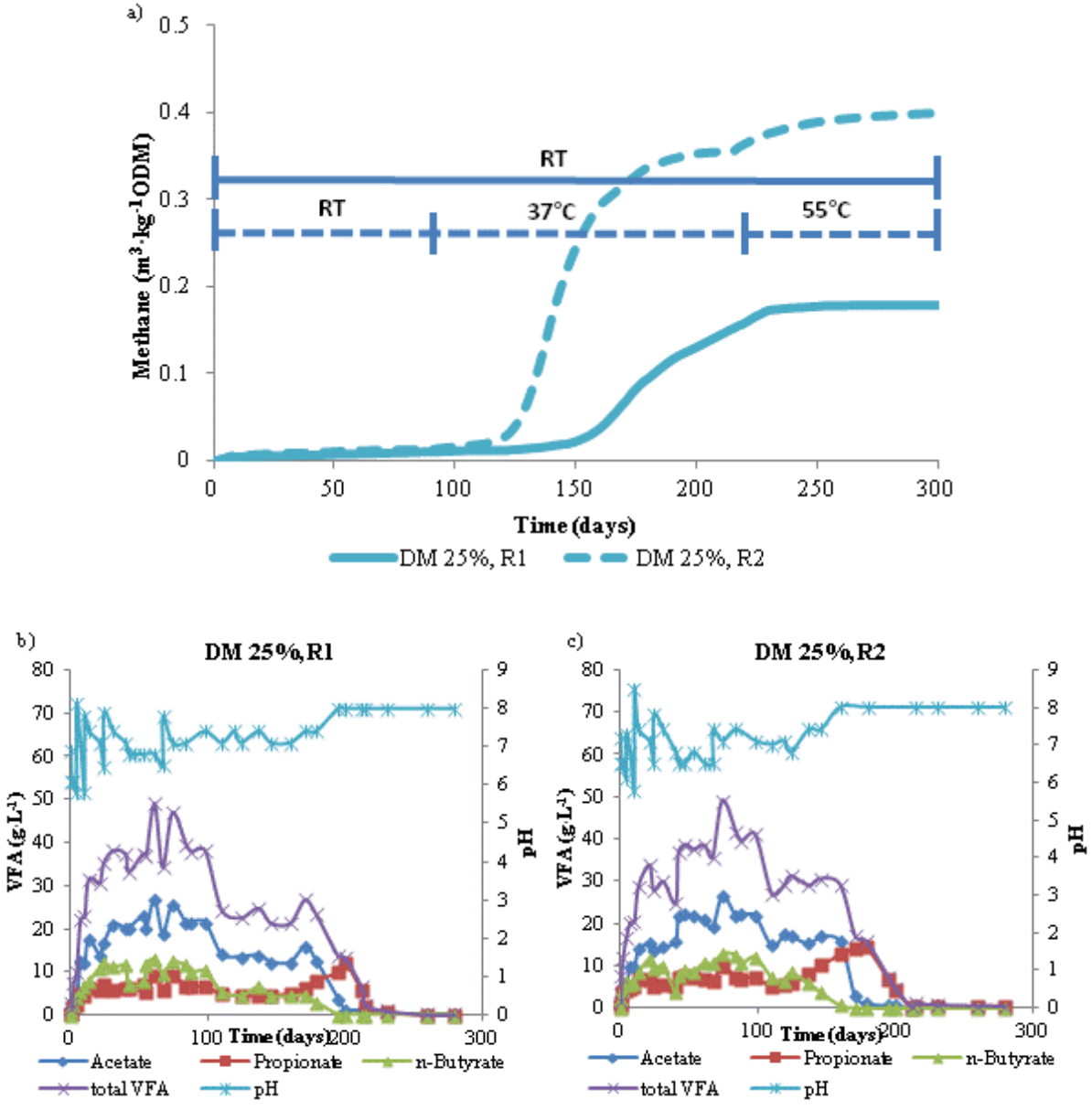

Figure 4.17 Biogas production in reactor R1 (300 d at room temperature) an R2 (room temperature → 37°C → 55°C, Fig. a), pH and volatile fatty acid (VFA) concentrations (Fig. b,c) during digestion of biowaste with 25 % DM content.

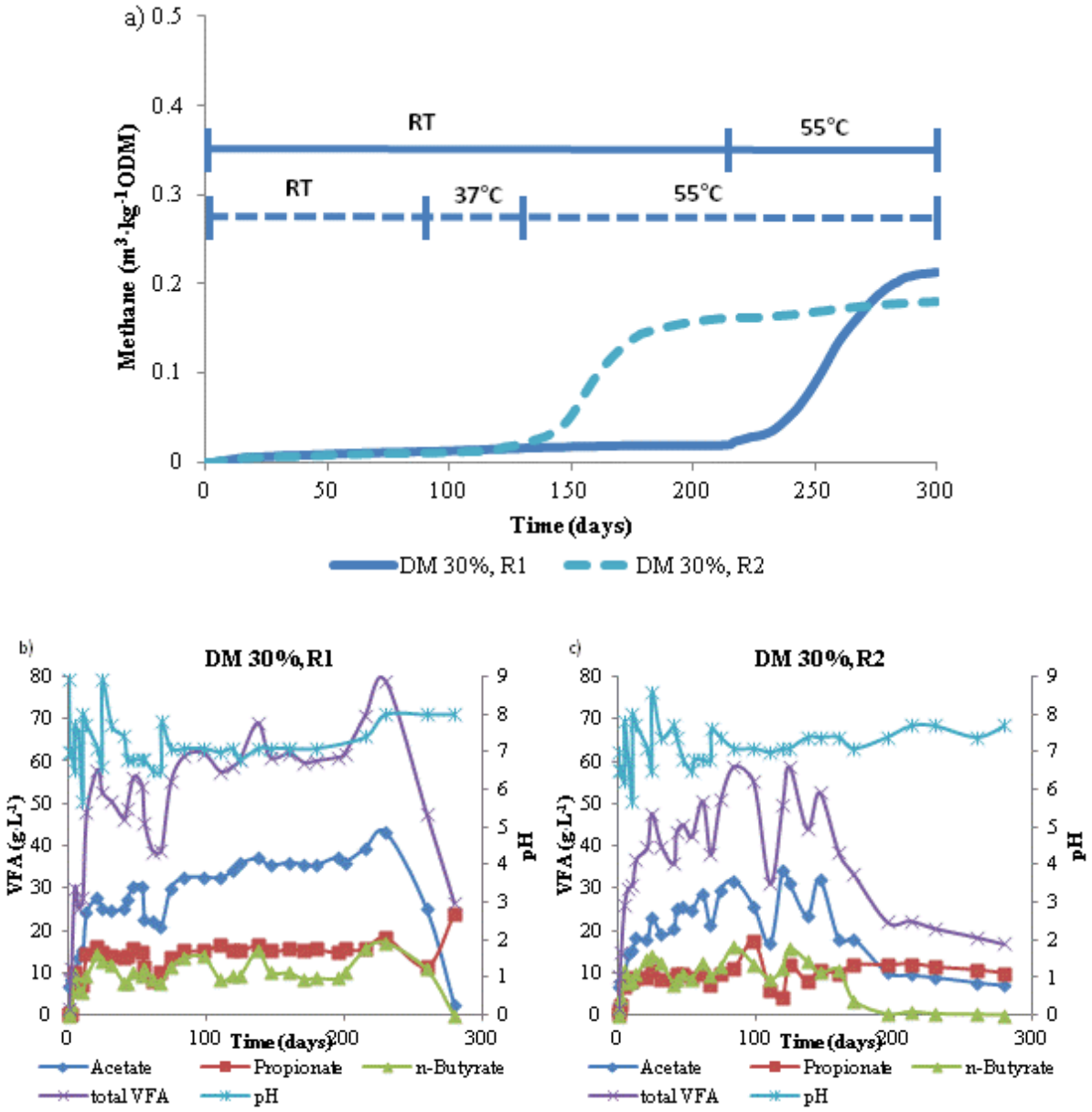

Figure 4.18 Biogas production in reactor R1 (room temperature →55°C) an R2 (room temperature → 37°C → 55°C, Fig. a), pH and volatile fatty acid (VFA) concentrations (Fig. b,c) during digestion of biowaste with 30 % dry matter (DM) content.

Compared to the gas production in the biowaste reactors with 20% DM content (0.35 – 0.38 $m^3 \cdot kg^{-1}$ ODM), only about half of methane yield (0.18 – 0.22 $m^3 \cdot kg^{-1}$ ODM) was finally obtained in the reactors with 30% DM content, although more digestible substrate was available. VFAs were accumulating at higher level in reactor with 30 % DM content than in reactor with 20% and 25% DM content. The acetate concentration in reactor 1 during long time incubation

at room temperature exceeded 40 $g \cdot L^{-1}$ and reached 30 $g \cdot L^{-1}$ in reactor 2 that was incubated at room temperature followed by mesophilic temperature. The propionate and n-butyrate concentration in both reactors maintained at above 30 $g \cdot L^{-1}$. All VFAs were degraded only after biogas production began at thermophilic temperature. The water activity in biowaste with 30 % DM content apparently still allowed acidification but no longer biogas production. Even the increased water activities at 37 or 55^{o}C seemed to be not high enough for non water limited methanogenesis as in the 20 % DM assays. Staley et al. (2011) and Abbassi-Guendouz et al. (2012) have also reported that DM-contents higher than 30% inhibited AD performance with failure in methane production and accumulation of VFAs. Metabolic pathways were also influenced by the DM content of biowaste during digestion by DAD. A metabolism shift unfavorable for hydrogen production (and in conseqzence for methane production) was reported at 28% DM (Motte et al. 2013).

4.4.3 Re-feeding of the reactors

After 300 days biogas production ceased in the parallel biowaste reactors with 20, 25 and 30 % DM content, 1 kg digestion residue of each reactor was mixed with 1 kg fresh biowaste and diluted with water to the desired moisture content of the following experiment. The content of the reactors, incubation temperature and the accurate DM content in every digester after re-feeding are represented in Table 4.14. The moisture content was re-adjusted to approx. 20%, 25% and 30%. One of two biowaste reactor with 20, 25 and 30 % DM-content was incubated at 37^{o}C and the other at 55^{o}C, respectively.

The biogas production from dry anaerobic digester with dry matter content of 20%, 25% and 30% and the variation of VFA levels in all digesters after re-feeding with fresh biowaste is depicted in Figure 4.19. At the beginning VFAs accumulated and the pH value had dropped below 6.5. After adjustment with 5M NaOH twice at 3 and 12 days pH value maintained steadily above 7.

Table 4.14 Re-feeding of dry anaerobic reactors for mesophilic and thermophilic methanogenesis

Reactor designation after re-feeding	Content	Incubation Temperature	Water addition	DM[1]
DM 30%,R1*	1 kg residue DM 30% R1 + 1kg fresh biowaste	37°C	n.a.	28.6%
DM 30%, R2*	1 kg residue DM30% R2 + 1kg fresh biowaste	55 °C	n.a.	28.6%
DM 25%, R1*	1 kg residue DM 25 % R1 + 1kg fresh biowaste	37°C	n.m.	24.9%
DM 25%,R2*	1 kg residue DM 25 % R2 + 1kg fresh biowaste	55°C	n.m.	24.8%
DM 20%, R1*	1 kg residue DM 20 % R1 + 1kg fresh biowaste	37°C	250 ml	20.3%
DM 20%, R2*	1 kg residue DM 20 % R2 + 1kg fresh biowaste	55°C	250 ml	20.0%

[1]DM= dry matter content at start, n.a. = no addition, n.m. = not measured.

Within 60 days incubation only about 0.03 and 0.075 $m^3 \cdot kg^{-1}$ ODM methane respectively released from the DAD reactors with 30% DM content at 37°C and 55°C. After 60 days propionate dominated the reactor with 30% DM content incubated at 37°C, whereas acetate dominated the reactor with 30% DM content incubated at 55°C. The VFAs level in reactor incubated at 37°C was higher than at 55°C. In the DAD reactors that contained biowaste with 25% DM content the methane production of 0.24 $m^3 \cdot kg^{-1}$ ODM at 37°C was significant less than the methane production of 0.35 m^3 kg^{-1} ODM at 55°C. 10 g L^{-1} propionate also remained un-degraded in reactor at 37°C, whereas all VFAs were completely degraded in reactor at 55°C. In both DAD reactor with 20% DM content respectively incubated at 37°C and 55°C 0.35 m^3 methane$\cdot kg^{-1}$ ODM was produced and VFAs completely degraded after 60 days. During the start-up phase biogas production at 20 ± 1°C in the assays with 20 % DM containing biowaste started only after 20 days and continued until day 150. Gas production

after re-feeding and incubation at either 37°C or 55°C started almost immediately and ended after only 30 – 40 days.

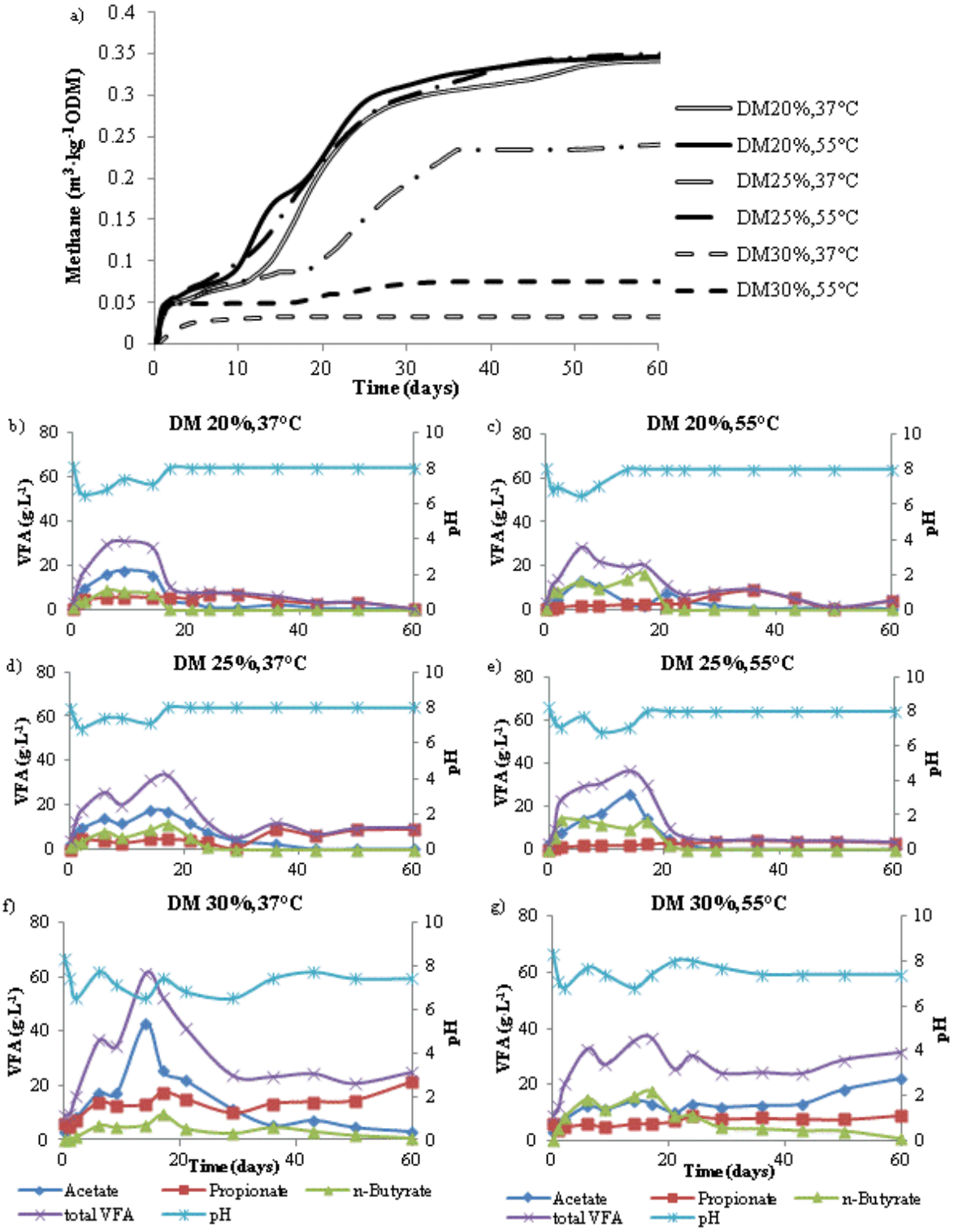

Figure 4.19 Biogas production in biowaste reactors with 20, 25 and 30 % DM content (Fig. a), pH and volatile fatty acid (VFA) concentrations after re-feeding the reactors at mesophilic (37°C; Fig. 4.19b, d, f) or thermophilic (55°C; Fig. 4.19c, e, g) temperatures.

The methane production rates of dry anaerobic digestion of biowaste with 20%, 25% and 30% DM at different temperature during the first and second feeding are summarized in Table 4.15. Methane production rates of 0.9 – 3 $L \cdot kg^{-1} \cdot d^{-1}$ from biowaste with 20 – 30 % DM (13 – 19.5 % ODM) were calculated for the initial batch DAD assays at 20, 37 and 55 ^{o}C. After re-feeding of the DAD reactors methane production rates were much higher, 4.3 and 5.8 $L \cdot kg^{-1} \cdot d^{-1}$ at 37^{o}C and 5.8 $Lkg^{-1}d^{-1}$ 55^{o}C in the reactors with 20 or 25 % DM content. as well as at 55^{o}C in the reactor with 25 % DM content. At 37^{o}C the methane production rate in the DAD reactor with 25 % DM content was lower (4.3 instead of 5.8 $L \cdot kg^{-1} \cdot d^{-1}$), presumably due to a too low a_W value.

Table 4.15 Methane production rates during dry anaerobic digestion of biowaste at 20, 37 and 55^{o}C

20 ± 1^{o}C		37 ± 0.5^{o}C		55 ± 0.5^{o}C	
DM %	CH_4 $L \cdot kg^{-1}$ODM$\cdot d^{-1}$	DM %	CH_4 $L \cdot kg^{-1}$ODM$\cdot d^{-1}$	DM %	CH_4 $L \cdot kg^{-1}$ODM$\cdot d^{-1}$
First Feeding					
21.2	1.7	21.2	n.d.	21.2	n.d.
25.2	0.9	25.2	3.0	25.2	n.d.
30.9	No gas	30.9	No gas	30.9	1.7
Re-feeding					
20.2	n.d.	20.2	5.8	20.2	5.8
24.9	n.d.	24.9	4.3	24.9	5.8
28.6	n.d.	28.6	Neg.	28.6	Neg.

DM = dry matter, ODM = organic dry matter, n.d. = not determined, neg. = negligible. Lines 1-3: Rates calculated from Fig. 4.16a-4.18a for logarithmic/linear CH_4 production phases and 70 % methane in the biogas. Lines 4-6: Rates calculated from Figure 4.19a as mentioned above.

Almost no methane was produced in any DAD reactor with 30 % DM content, although the water activity at 55^{o}C should be higher than at 20 or 37^{o}C. At this

high DM content most of the moisture apparently was tightly bound to particles and increasing the temperature could not increase bio-availability far enough for acetogenic and methanogenic bacteria. The temperature shift apparently had a more severe effect on the activity of acetogenic and methanogenic bacteria than on acidogenic activity. The reason for this may be a broader range of still growth allowing temperatures of the biowaste hydrolyzing and VFA-producing bacteria.

Methane production rates and, similarly important, total biogas yields are the main criteria for either WAD or DAD of organic wastes (de Baere, 2000). A comparison of the maximal biogas productivity of the biowaste fraction of the City of Karlsruhe for WAD and DAD revealed that during WAD of biowaste with 5 – 6 % DM content the same amount of biogas per gram (590 $ml \cdot g^{-1}$ VS; Nayono et al., 2009) at a hydraulic retention time (HRT) of 7 d than during batch DAD of biowaste with 20 - 25 % DM content during an almost ten times longer time span (60 d; 530-590 $ml \cdot g^{-1}$ VS, this paper) was generated. This shows that the final biodegradation efficiency of municipal biowaste with 20 or maximally 25 % DM content in box-reactors for DAD may be as good as in completely mixed reactors for WAD with 5 - 6 % DM content. The average methane content in the biogas from WAD was 62 - 70 % (Gallert et al., 2003; Nayono et al., 2009) as compared to DAD, where it was 70 - 75 %, due to a higher pH, The space loading for stable WAD in laboratory and in full-scale was 15 $kg \cdot m^{-3} \cdot d^{-1}$ for a HRT of 6 days (Gallert et al., 2003) and thus was in the same order as in all the references for DAD mentioned by Zahedi et al. (2013). Even in their own work total volatile solids accumulation began at a HRT of 6.6 days, equivalent to a space loading of 13 $kg \cdot m^{-3} \cdot d^{-1}$, although methane productivity apparently was still stable."(Cited from Li et al. 2014)

Chapter 5

Summary

Anaerobic digestion of the organic fraction of source-sorted municipal waste (biowaste) was investigated in this study with two different process technologies – wet anaerobic digestion and dry anaerobic digestion. To find out appropriate co-substrates for improvement of the wet anaerobic digestion of biowaste, the main characteristics and the biogas production potential of several "candidates" were determined. Anaerobic co-digestion of biowaste with old bread for the improvement of biogas production was investigated in a semi-continuous mode. The variation of propionate degradation rates during the addition of propionic acid into an anaerobic biowaste digester was examined in order to better understand the effect of some important intermediates and the conditions under which stable anaerobic digestion is possible. For dry anaerobic digestion, the effect of moisture on anaerobic digestion of biowaste at different temperatures was investigated in this study.

Potential of different organic materials as co-substrates for anaerobic digestion

The biogas production potential of biowaste, sewage sludge, old bread, yoghourt and food waste was determined in batch assays. The maximal biogas production from sewage sludge and yoghurt was either lower or similar as that of biowaste, whereas the biogas production potential of old bread and food waste was much higher than that of biowaste. The co-digestion of biowaste with such different organic materials was operated for a short time in continuous mode to examine the suitability of those co-substrates for anaerobic biowaste co-digestion. The average biogas production rate during co-digestion of biowaste with old bread, yoghurt and food waste was obviously better as during mono-digestion of biowaste. During co-digestion of biowaste with old bread more biogas was produced than with all other substrates for co-digestion. Old

bread seemed to be a storable, appropriate co-substrate for anaerobic digestion of biowaste.

Anaerobic co-digestion of biowaste with bread for improvement of biogas production

Acidification and buffer capacities of wheat bread, rye bread and biowaste suspensions were investigated. The acidification of bread suspensions was mainly caused by lactate. For the same pH decrease the VFA content of acidified RBS was much higher than the VFA content of WBS at the same lactic acid content. The buffer capacity of RBS apparently was twice higher than that of WBS. By addition of increased amounts of much higher concentrated bread suspension than biowaste suspension to the digesters the OLR was increased proportionally higher than the HRT. Co-digestion of biowaste with wheat or rye bread was stable up to an OLR of 20 $kg \cdot m^{-3} \cdot d^{-1}$ and at a HRT of 5.7 days. Maximally 10 m^3 $biogas \cdot m^{-3} \cdot d^{-1}$ was produced. Reactors failed above an OLR of 22 $kg \cdot m^{-3} \cdot d^{-1}$. In order to determine the maximal OLR for co-digestion of biowaste with bread at a short, practically applicable HRT, the reactors were fed with the mixture of FBS plus WBS or RBS at a fixed HRT for both reactors of 6.2 days. The OLR was increased by increasing the ratio of bread in the feedstock. Biowaste and wheat bread co-digestion at a HRT of 6.2 d became unstable at an OLR higher than 22.4 $kg \cdot m^{-3} \cdot d^{-1}$, whereas biowaste and rye bread co-digestion became unstable at an OLR higher than 23 $kg \cdot m^{-3} \cdot d^{-1}$.

Co-digestion of biowaste suspension with propionic acid

"Anaerobic digestion of biowaste was operated with addition of propionic acid in three different ways. Propionate degradation rates (PDRs) were determined during increasing of the OLR by addition of biowaste and biowaste plus propionic acid. The variation of PDRs during change of feeding from biowaste to only propionate was also investigated. Propionic acid as a model substrate for highly acidified organic liquids in a biowaste reactor could immediately

substitute biowaste suspension. Addition of little propionate to biowaste suspension improved the propionate oxidation activity in a biowaste reactor at increasing OLR. An almost constant daily biogas production could be maintained during feeding of manually prepared biowaste suspension twice a day from Monday to Friday (regular biowaste collection period) and continuous pump feeding of propionic acid as a concentrated model substrate for acidified liquid wastes on Saturday and Sunday, when no biowaste was collected. Propionate degradation rates (PDRs) were much higher after propionate feeding than after biowaste feeding. During 5 days starvation the PDR decreased by 50 %, but remained higher in biowaste suspensions after propionate feeding than after biowaste feeding" (Slightly modified from Li et al., 2014 in press).

Effect of moisture content on dry anaerobic digestion of biowaste

Dry anaerobic digestion of biowaste containing 20%, 25% and 30% DM content was investigated during temperature change from ambient to mesophilic and/or thermophilic temperatures. Dry anaerobic digestion at 20 – 55 ^{o}C was feasible with 20 % DM-containing biowaste. The biogas production from dry anaerobic digestion of 25% DM-containing biowaste was restricted and incomplete at 37^{o}C, whereas it proceeded to completion at 55^{o}C. Dry anaerobic digestion of 30% DM-containing biowaste could not be operated under stable conditions at any of the three temperature ranges. At 55^{o}C the methane production rate in the DAD reactor with 25 % DM content was higher than at 37^{o}C, whereas the methane production rate in the DAD reactor with 20 % DM content was the same at both temperatures.

Chapter 6

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