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ANAEROBIC MONO-DIGESTION OF MAIZE AND CELLULOSE AT DIFFERENT TEMPERATURES AND OPERATING MODES

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Konz in December 2011
Katarzyna Gołkowska

If we knew what it was we were doing,

It would not be called research, would it?

Albert Einstein (1879–1955)

Abstract

The purpose of this research project was to improve the understanding of anaerobic digestion of energy crops. Fermentation of a model substrate (microcrystalline cellulose) and agricultural one (maize silage) was investigated in the study. 18 batch, 3 semi-batch and 3 continuous fermentation series were conducted under mesophilic (38°C) or thermophilic (55°C) conditions in laboratory scale. A comprehensive analysis of both fermentation product (biogas) as well as the content of the reactor (liquid phase) were conducted during the study. The determined analytical parameters were additionally applied to model substrate degradation with help of 1st order and Monod equations.

The results deal with 3 different digestion aspects: response of bacterial biocenosis to elevated single organic loading rates, influence of the substrate composition and temperature on performance of a biogas reactor and impact of operating mode on the fermentation progress. The major findings are:

a) Even extremely high but single charges of cellulose under thermophilic conditions did not lead to the collapse of the system or to acidosis.
b) More stable digestion and higher methane content in biogas were observed under mesophilic conditions.
c) No explicit difference in biogas yield was measured for both temperature modes.
d) Contradictory to the biogas praxis faster digestion took place under mesophilic conditions.
e) Mechanism of maize fermentation, with high concentrations of acetic/butyric acid as intermediate, differed from cellulose being degraded mainly over acetic/propionic acid.
f) The development of reversible (acetic/propionic acid) and irreversible (acetic/butyric acid) inhibition was observed in thermophilic experiments.
g) An extremely high adaption level of anaerobic biocenosis to elevated organic loading rates or increased feeding frequency at 55°C was observed in both semi-batch and continuous mode.
h) A 5% correction of methane content in biogas is necessary if transferring the results of substrate degradability test from batch to continuous mode.

The results show a higher efficiency of mesophilic digestion in every degradation aspect and a huge adaption possibility of anaerobic biocenosis to increased organic loading rates. The revealed different inhibition mechanisms and degradation pathways than those assumed in the literature show an immense research potential in the field of anaerobic digestion with the focus on energy crops.

Keywords: anaerobic digestion, maize silage, cellulose, thermophilic, mesophilic, batch, semi-batch, continuous, fermentation mechanism, methane production, inhibition, high organic loading rates, butyric acid

Kurzfassung

Das Ziel dieser Arbeit war ein besseres Verständnis der Vorgänge bei der anaeroben Vergärung von nachwachsenden Rohstoffen zu schaffen. Zwei Substrate wurden im Rahmen der Studie untersucht: das Modelsubstrat mikrokristalline Zellulose und das landwirtschaftliche Substrat Maissilage. Insgesamt wurden 18 Batch-, 3 quasi-kontinuierliche und 3 kontinuierliche Versuchsserien im Labormaßstab unter mesophilen (38°C) oder thermophilen (55°C) Bedingungen durchgeführt. Sowohl die Gärmasse als auch das erzeugte Biogas wurden in verschiedenen Vergärungsstadien umfassend analysiert. Die gesammelten Daten wurden eingesetzt, um den Substratabbau mit dem Reaktionsgeschwindigkeitsansatz erster Ordnung bzw. von Monod zu modellieren.

Drei Hauptaspekte wurden im Rahmen der Studie untersucht: Gärverhalten bakterieller Biozönose unter erhöhten Einzelbelastungen, Einfluss von Substratzusammensetzung und Temperatur auf den Gärprozess, sowie Vergleich des Gärablaufs bei unterschiedlichen Betriebsweisen (Batch, quasi-kontinuierlich bzw. kontinuierlich). Die wichtigsten Untersuchungsergebnisse sind:

a) Eine sehr hohe aber nur Einzelbelastung führte unter thermophilen Bedingungen zu keiner dauerhaften Systemstörung oder Versäuerung des Reaktorinhalts.
b) Ein stabiler Fermentationsvorgang und ein höherer Methangehalt im Biogas wurden unter mesophilen Bedingungen beobachtet.
c) Der auf die Substratzufuhr bezogene spezifische Biogasertrag war für beide untersuchten Temperaturen gleich.
d) Im Gegensatz zu Berichten aus der Literatur, erfolgte unter mesophilen Bedingungen ein schneller Substratabbau.
e) Der Vergärungsmechanismus war unterschiedlich für Maissilage und Zellulose: der Abbau von Maissilage erfolgte verstärkt über Buttersäure als Zwischenprodukt während der von Zellulose hauptsächlich über die Essig- und Propionsäure stattfand.
f) In der thermophilen Betriebsweise konnten zwei Mechanismen der Inhibierung beobachtet werden: Inhibierung bedingt durch erhöhten Essig-/Propionsäuregehalt (reversibel) und Essig-/Buttersäuregehalt (irreversibel).
g) Sowohl in quasi-kontinuierlichen als auch in kontinuierlichen Betrieb unter thermophilen Bedingungen zeigte die bakterielle Biozönose eine hohe Anpassungsfähigkeit bei erhöhten Reaktorbelastungen bzw. häufiger Beschickung.
h) Bei der Übertragung des Ergebnisses eines Biogasertragstests vom Batch auf kontinuierlichen Betrieb ist eine Reduzierung des Methangehalts im Biogas um 5% vorzunehmen.

Die Ergebnisse zeigen Vorteile der mesophilen gegenüber der thermophilen Betriebsweise bezüglich des Fermentationsablaufs insgesamt aber auch eine große Anpassungsfähigkeit der anaeroben Biozönose gegenüber einer erhöhten Reaktorbelastungen. Die beobachteten zwei unterschiedlich bedingte Inhibierungsarten und Differenzen in den Abbauwegen für Maissilage und Zellulose weisen auf einen weiteren Forschungsbedarf im Bereich anaerober Vergärung von nachwachsenden Rohstoffen hin.

Schlüsselworte: anaerobe Vergärung, Maissilage, Zellulose, thermophil, mesophil, Batch, quasi-kontinuierlich, kontinuierlich, Vergärungsmechanismus, Methanbildung, Inhibierung, erhöhte Reaktorbelastung, Buttersäure

Résumé

Le but de ce projet de recherche était de contribuer à une meilleure compréhension de la fermentation anaérobie des plantes énergétiques. La fermentation de deux substrats a été étudiée : le substrat modèle de cellulose microcristalline et le substrat courant d'ensilage de maïs. Les mesures réalisées au laboratoire ont porté sur 18 essais de type batch, 3 de type semi-batch et 3 de type continu en régime mésophile (38°C) et thermophile (55°C).

L'avancement de la fermentation à différents instants a été caractérisé par une analyse complète du biogaz ainsi que du contenu du réacteur (phase liquide). Les paramètres analytiques ainsi déterminés ont été utilisés en vue de modéliser la dégradation du substrat ; deux modèles cinétiques (premier ordre et de Monod) ont été comparés.

Les trois aspects principaux de cette étude sont : la biocénose bactérienne à des taux de charge organique élevés, l'influence de la composition du substrat et de la température sur le processus de fermentation et l'impact du mode de fonctionnement sur le déroulement de la fermentation. Les conclusions principales sont :

a) Une charge très élevée mais unique de cellulose dans des conditions thermophiles n'a pas conduit à la perturbation permanente du système ou à l'acidose.
b) La digestion était plus stable avec un contenu de méthane plus élevé sous des conditions mésophiles.
c) Le rendement de biogaz était quasiment identique pour les deux modes de température.
d) Contrairement aux observations dans la littérature, une digestion plus rapide a été observée sous les conditions mésophiles.
e) Les mécanismes de la fermentation de maïs et de cellulose étaient différents : la dégradation de l'ensilage de maïs se passait principalement par la formation de l'acide butyrique comme produit intermédiaire tandis que la cellulose se dégradait principalement via les acides acétique et propionique.
f) L'apparition d'une acidose réversible (acides acétique/propionique) et d'une acidose irréversible (acides acétique/butyrique) a été observée dans les expériences thermophiles.
g) La biocénose anaérobique sous des conditions thermophiles montrait une adaptation forte aux taux de charge organique élevés ou aux fréquences d'alimentation élevées dans les essais semi-batch et en mode continu.
h) Pour transférer les résultats d'un test de dégradation obtenu en batch au mode continu, il est nécessaire de réduire le contenu en méthane dans le biogaz de 5%.

Les résultats montrent des avantages de la digestion mésophile dans le déroulement de la dégradation elle-même et montrent une forte adaptation de la biocénose anaérobique aux taux de charge organique élevés. Les mécanismes d'inhibition diffèrent de ceux décrits dans la littérature et révèlent un potentiel de recherche immense dans le domaine de la digestion anaérobique en mettant l'accent sur les plantes énergétiques.

Mots-clés: digestion anaérobique, ensilage de maïs, cellulose, thermophile, mésophile, batch, semi-batch, continu, mécanisme de la fermentation, méthane, acidose, taux de charge organique élevé, acide butyrique

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List of Abbreviations

ADF	Acid detergent fiber
ADL	Acid detergent lignin
ADM1	Anaerobic Digestion Model No. 1
B	Biogas produced by the time t
B_{end}	Biogas produced by time t_{end} (total biogas produced)
COD	Chemical oxygen demand
CSTR	Continuous stirred-tank reactor
DM	Dry matter
DM_K	Corrected dry matter
DM_N	Measured dry matter
FID	Flame ionization detector
FM	Fresh mass
GC	Gas chromatograph
GP	Gas production
GPR	Gas production rate
HAc	Acetic acid
HBu	Butyric acid
HPr	Propionic acid
HVa	Valeric acid
IR	Infra red
K	First order kinetic constant (1^{st} order model)
K_m	Monod maximum specific uptake rate
K_s	Substrate half saturation constant (Monod model)
LCFA	Long chain fatty acids
m_c	Mass of cellulose
m_M	Mass of maize silage
M_C	Molar mass of C
M_H	Molar mass of H
M_O	Molar mass of O
MZ I	Maize silage of 1^{st} harvest
MZ II	Maize silage of 2^{nd} harvest
μ_{max}	Monod maximal specific growth rate of bacteria
NDF	Neutral detergent fiber
NFC	Non fibrous carbohydrates
OLR	Organic loading rate
ORP	Oxidation-reduction potential
S	Substrate concentration at time t
S_0	Initial substrate concentration

sGP	Specific gas production
sGPR	Specific gas production rate
t	Time
t_{end}	Total time
TIC	Titrated inorganic carbon (carbonate buffer)
TVA	Titrated volatile acids
VFA	Volatile fatty acids
VS	Volatile solids
VS_i	Volatile solids in inoculum
VS_s	Volatile solids in substrate
x	Microbial concentration (Monod model)
x_0	Initial microbial concentration (Monod model)
XL	Fraction of crude lipids
XP	Fraction of crude proteins
X_{VFA}	Fraction of volatile compounds in the silage
y	Microbial yield coefficient (Monod model)
Y_B	Biogas yield

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1 Introduction and motivation

Anaerobic digestion in agricultural biogas plants gained more attention in last years due to its environmental benefits (CO_2 neutral energy production) as well as the political and financial support provided in many European countries (WEILAND, 2003a). In particular the energy crops fermentation has developed rapidly in recent time. Fermentation of agricultural feedstock allows activating abandoned agricultural land and constructing more compact and therefore more efficient plants than digestion of manure/slurry only. The predominant number of biogas plants nowadays is run mesophilic (36-38°C), while thermophilic digestion mode (55°C) is widespread mainly in Scandinavian countries.

Although lots of research was done in the field of anaerobic digestion, the most findings are based on the experiments conducted with waste water (e.g. WIEGANT ET AL., 1986; VAN HAANDEL & LETTINGA, 1994; KAYHANIAN & RICH, 1995; GALLERT & WINTER, 2008), manure (e.g. ANGELIDAKI & AHRING, 1994; AHRING ET AL., 1995; PIND ET AL., 2003) and solid waste (e.g. KIELY ET AL., 1997; LIU ET AL., 2008; RINCON ET AL., 2008) or co-digestion of both (e.g. CALLAGHAN ET AL., 2002; ANGELIDAKI & ELLEGAARD, 2003; VAVILIN ET AL., 2003; GELEGENIS ET AL., 2007). In general there was not much published on mono-fermentation of energy crops by now (HINKEN ET AL., 2008; KLOCKE ET AL., 2008; LEBUHN ET AL., 2008; RAPOSO ET AL., 2006; KRAKAT ET AL., 2010; WICHERN ET AL., 2010; POBEHEIM ET AL., 2010 & 2011; DEMIREL & SCHERER, 2011). Similar to the industrial trends mesophilic conditions have been better reviewed in the literature over the years while thermophilic ones have been mainly a subject of more recent studies.

Originally it was assumed that the degradation pathways and the performance of process parameters for agricultural feedstock digestion must be similar to that observed for anaerobic digestion of waste water and manure. The last findings reveal that this is not always the case. Many further aspects of anaerobic digestion for energy crops have not been investigated by now. Some of them, such as the impact of micro nutrients on the anaerobic digestion of energy crops (HINKEN ET AL., 2008; LEBUHN ET AL., 2008; POBEHEIM ET AL, 2010 & 2011; DEMIREL & SCHERER, 2011), the exact microbiological composition of bacterial biocenosis (KLOCKE ET AL., 2008; NETTMANN ET AL., 2008; KRAKAT ET AL., 2010), degradation pathways (NETTMANN ET AL., 2008; KRAKAT ET AL., 2010; LAUKENMANN ET AL., 2010) and complex modelling e.g. with ADM 1 (BATSTONE ET AL., 2002; FENG ET AL., 2006; KALFAS ET AL., 2006; LÜBKEN ET AL., 2007, BOUBAKER & RIDHA, 2008; WICHERN ET AL., 2010) are in the focus of current research.

Batch studies of substrate degradation combined with a frequent analysis of the digestion parameters give more details about the digestion progress than frequently charged continuous reactors. For that reason a complex batch fermentation study of maize silage (agricultural substrate) was conducted under thermophilic and mesophilic conditions and different organic loading rates (OLR). The results were additionally compared with cellulose (a model substrate) being the most ubiquitary plant cell component. Further, since most research projects concentrate on one operating mode (batch or continuous), or one temperature (mainly mesophilic), there is no literature on mono-fermentation of energy crops comparing extensively performance of the same bacterial biocenosis for different operating modes, temperatures and OLRs. This thesis should close the research gap and improve the understanding of energy crops fermentation under mesophilic and thermophilic conditions.

During the experimental series run under thermophilic and mesophilic conditions in different operating modes the following aspects were investigated:

- **Response of bacterial biocenosis to elevated single organic loading rates:** A series of 6 cellulose batch tests was run at different OLRs under thermophilic conditions with the research focus on flexibility of high OLR adaption, response of measured parameters, optimum OLR and indicators of inhibition.
- **Influence of digestion temperature (38°C and 55°C) on the performance of a biogas reactor**: In 12 batch experiments differences in fermentation were investigated for maize and cellulose focusing on substrate degradability, changes in biogas parameters (yield and quality) and comparison of temperature related performance of bacterial biocenosis.
- **Impact of the substrate (cellulose and maize) on anaerobic digestion patterns**: The core area of the study in 12 batch reactors under mesophilic and thermophilic conditions was the comparison of degradation pathways and performance of the analytical parameters.
- **Change of operating mode and its consequence for the fermentation progress**: Thermophilic experimental series in batch, semi-batch and continuous mode with maize were supposed to unveil bacterial adaption possibilities for the shortening feeding intervals. The performance of system parameters with the focus on those regarded as inhibition indicators was monitored and compared with the literature. Further the setting of inhibitory conditions was observed and described in the experiment.

A comprehensive analysis of both fermentation product (biogas) as well as the content of the reactor (liquid phase) were conducted during the study. The collected analytical parameters not only gave a direct insight into subsequent fermentation steps but were additionally applied to model substrate degradation with help of 1st order and Monod equations. This allowed the comparison of digestion kinetics for different temperatures, substrates and operating modes.

2 Biogas technology

2.1 Historical development

Biogas was already known as energy source before even one could define what its composition was. Already 1884 under supervision of Pasteur the first extensive studies on horse dung fermentation to produce biogas and further electricity were done. At the end of 19th century the first waste water treatment plant producing hospital illumination was put into operation in Mumbai, India, (SCHULZ & EDER, 2001). At the beginning of 20th century methane gas was for the first time introduced to the public gas works. Until the World War II the biogas technology was mainly installed for the waste water treatment and the produced biogas was used to produce electricity or heat. The rediscovery of dung as biogas source did not happen before the beginning of 1950s. The fermentation of manure allowed not only producing energy but also reducing the odor contamination as well as eliminating the atmospheric emissions of climate-relevant gases from the dung.

However since 1950s the profitability of energy production from biogas was strongly influenced by an excess or shortage of fossil fuels. The oil crisis in the 1970s as well as continuously increasing crude oil prices since 2000 strongly encouraged investments in biogas industry. Further impulses were given by the authorities, trying to enhance the energy production from renewable resources. Since 1991 in Germany and since 1994 in Luxemburg the energy or natural gas production from biomass has been consequently supported with different forms of subsidy and complex bonus systems (AMON ET. AL, 2002). The overview of the existing bonus systems in Luxemburg and Germany for the agricultural biogas plants is presented in Tab. A.1 (Attachment A).

After 1990s new trends in biogas technology appeared: fermentation of energy crops and the implementation of biogas technology in the landfills (DEUBLEIN & STEINHAUSER, 2008). The new substrates contributed to the further technological development in the direction of dry digestion. The dry digestion technology is not clearly defined in the literature and among the biogas experts. Weiland (2004) reported of the term being used for digestion of substrate with dry matter (DM) content higher than 25% FM, while Wilfert et al. (2004) regards the fermentation of stackable substrates with DM content higher than 15% FM as dry digestion as well.

The meaning of biogas as environmental friendly and CO_2-neutral energy source is gaining in importance. Many agricultural biogas plants constructed lately can be regarded more in categories of power plants than recycling plants. Their main goal is no

longer the waste utilization but the biogas production, its converting to heat and electricity or its treatment and feeding into natural gas pipelines. The historical development of biogas technology is presented in a compact form in Fig. 2.1.

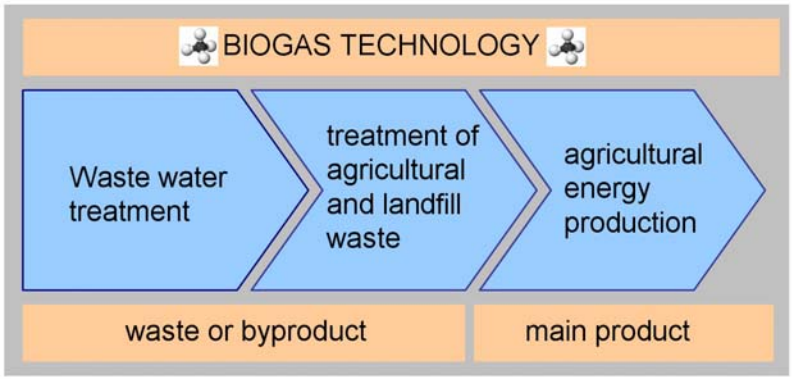

Fig. 2.1 Evolution of biogas industry

2.2 Agricultural biogas plants

The following substrates are utilized in agricultural biogas plants: agricultural waste (dung, manure and agricultural residues), organic waste (of industrial and municipal origin) as well as energy crops (usually cultivated for that purpose). Depending on the substrate properties a different digestion technology can be applied. If the dry matter (DM) content of biomass does not exceed 13% the substrate can be handled with conventional pumps and mixing aggregates, this is the so called wet digestion. Except of laboratory tests wet fermenters are always operated in continuous mode. A semi-moist, stackable and free-flowing substrate with a DM content of 20-35% can be degraded in so called dry digestion fermenters[1]. These are operated both in continuous or discontinuous mode (WEILAND, 2005). However this strict classification is often revised in praxis. In 2003 93% of German biogas plants were operated in so called co-fermentation mode. On average the co-substrate (mainly maize, ensilaged corns or grass silage) made out 10-25% of their input biomass (WEILAND, 2003b). Considering the biogas plants put into operation between 2004 and 2006, nearly 20% of them are run with 1 co-substrate, 31% with 2 and 33% with 3 co-substrates. 12% of the biogas plants put into operation between 2004 and 2006 are running in dry fermentation mode (Weiland, 2007). While in last 7 years the total number of biogas plants in Germany increased from 1760 (2003) up to 7000 (2011), the total electric power increased from

[1] Digestion in biogas reactor run in dry digestion mode takes place only under aqueous conditions and has still half-liquid consistence.

190 MW_{el} up to 2728 MW_{el}. This means 4 times higher number of biogas plants able to produce 14 times more energy (FNR, 2011).

Most of the co-fermentation biogas plants are operated in the range of 2-4 kgVS/m³·d (WEILAND, 2010). In the biogas industry the organic loading rate (OLR) of 4 kgVS/m³·d is regarded as an absolute upper limit for stirred tank reactors operated in continuous mode without micro nutritional additives (EDER & SCHULZ, 2007; GERSTL, 2008; ZOSEL ET AL., 2008; SCHOLWIN ET AL., 2009; WEILAND, 2010). With addition of supportive micro elements or application of different fermenter types even OLR between 10-14.5 kgVS/m³·d are reported to be possible (LIEBENEINER, 2010; WEILAND, 2010).

2.3 Biomass cycle

Natural digestion of biomass to produce biogas takes place in the stomachs of ruminant animals. However in this case a great part of energy saved in the substrate is transformed to support growth and existence of the animal as well as the milk production. A single cow is capable of producing up to 310 ± 60 litres of methane emissions per day (BERRA ET AL., 2008). These huge amounts of climate relevant gas daily

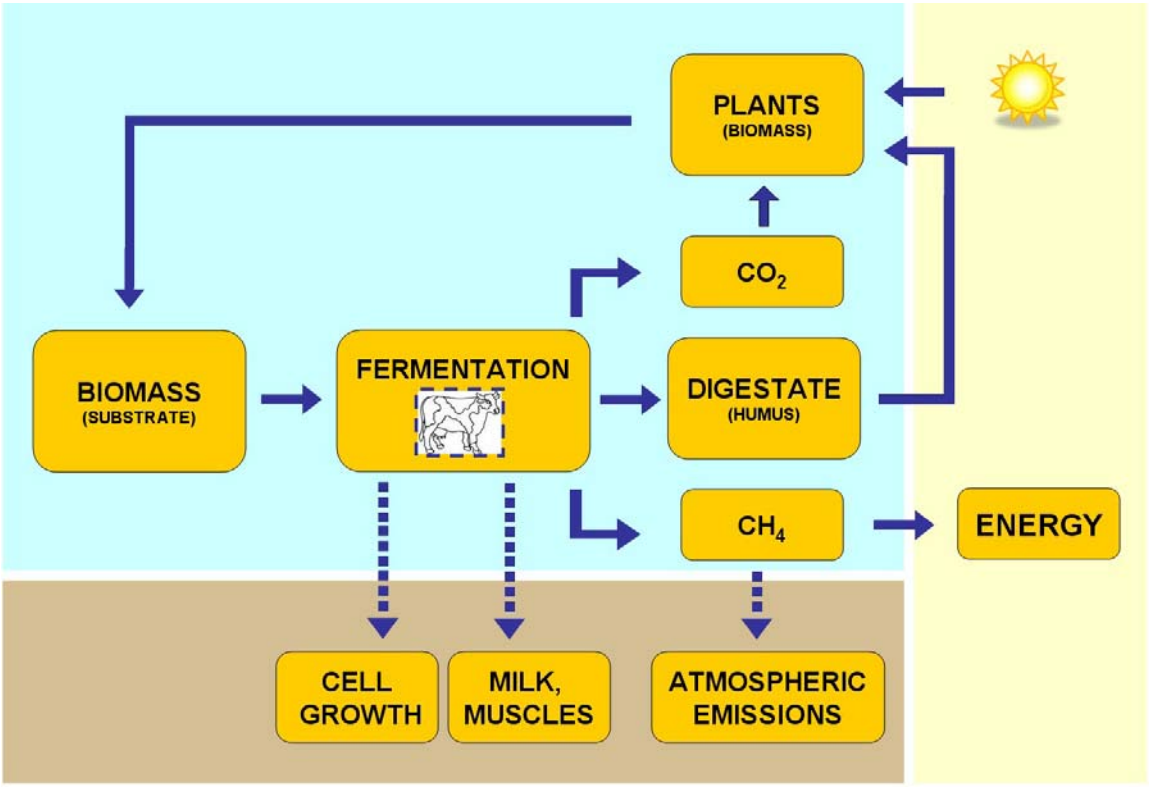

Fig. 2.2 Biomass cycle for fermentation of energy crops in an anaerobic reactor (normal arrows) and for fermentation in the stomachs of ruminant animals (normal + dashed arrows)

produced by the animals are released into atmosphere without energetic recovery, which additionally strongly increases human conditioned greenhouse effect. Biomass residues (digestate) of animal digestion, which still contain anaerobic degradable components, are a source of odour emissions and therefore can only to some extent be used as fertilisers.

Anaerobic reactors producing biogas follow a pattern similar to animal digestion: a degradable part of vegetable substrate is converted into biogas. In this case almost 90% of degradable substrate is transformed into energy saved in biogas as the energy consumption of bacteria is very low in comparison to ruminant animals. Unlike the digestate produced by ruminant animals, the biomass residues after anaerobic reactor fermentation are characterised by low content of VS and produce less odour emissions. Furthermore the animal digestate can also be used as substrate in fermenting reactors. The different ways of energy crops degradation, performed either by ruminant animals or without their contribution, are given in Fig. 2.2.

Depending on the substrate characteristics different biogas yields can be achieved. Fermentation of agricultural waste is characterised by rather low biogas yield, while digestion of silages gives 0.5 to 2 times higher biogas yield. The different substrates used in biogas industry together with their DM and VS characteristics and the expected biogas yields (Y_B) are given in Tab. 2.1.

Tab. 2.1 Characteristics of different substrates for biogas production (BISCHERT ET AL., 2006)

Substrate	Form	DM [%]	VS [% of DM]	Y_B [l_N/kgVS]
cattle manure	with straw	25	85	450
pig manure	with straw	35	85	370
pig manure	liquid	7	75	420
rye	ensilaged	33	93	730
barley	ensilaged	25	93	920
grass	ensilaged	35	91	540
maize	ensilaged	29-37	96-97	680-860

2.4 Biological basics

Biogas is a product of anaerobic mineralization of organic matter. This complex multi-stage process is performed by wide variety of bacteria living in symbiotic relationship though requiring different living conditions. Four digestion phases can be distinguished: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The biomass degradation schema as well as the optimal conditions and generation times for different bacteria

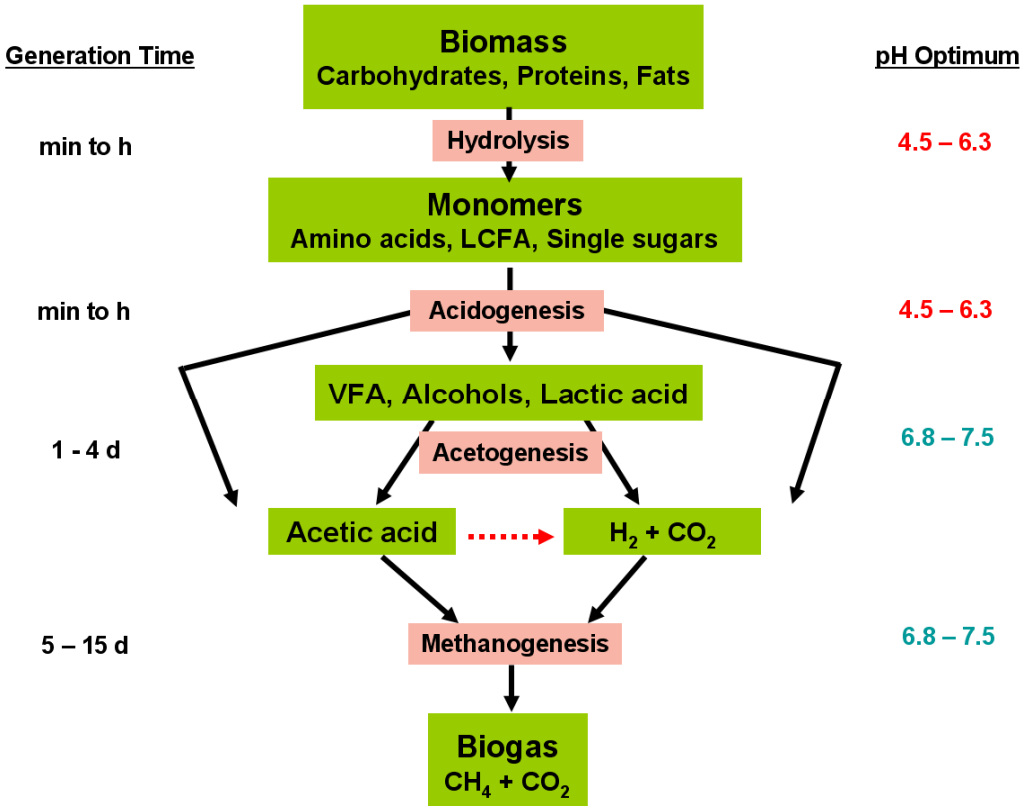

Fig. 2.3 Degradation of biomass to biogas, generation time and pH optima for different bacteria groups (red dashed arrows show the new degradation paths discovered by LAUKENMANN ET AL. (2010), NETTMANN ET AL., (2008) AND KRAKAT ET AL. (2010).

groups within a monogenic biocenosis are given in Fig. 2.3. The process of anaerobic biomass degradation was reviewed extensively by Baader et al. (1978), KALTWASSER (1980), MUDRACK (1983), FRITSCHE (1990), KALTSCHMITT ET AL. (1993), WELLINGER ET AL. (1991), AMON ET AL. (2002), SCHULZ & EDER (2001), SCHATTAUER & WEILAND (2006).

Hydrolysis

In the hydrolysis phase the suspended organic matter consisting of complex polymers: proteins, fats and carbohydrates is degraded to monomer such as amino acids, long chain fatty acids (LCFA) or single sugars (mono- and disaccharides). The detailed information about differences in the hydrolysis for various substrates is given by DAHLHOFF (2007). The hydrolytic bacteria are facultative anaerobe and excrete exo-enzymes. The fermentation takes place outside of the bacterial cell (VAN HAANDEL & LETTINGA, 1994). In the subsequent steps (acidogenesis, acetogenesis and methanogenesis) monomers are being converted to biomass following biochemical reactions listed in Tab.2.2.

Tab.2.2 Possible stoichiometric VFA production and degradation reactions during conversion of carbohydrates and fats (during digestion of proteins amino acids are mainly degraded to acetic acid in the complex Stickland reactions (BATSTONE ET AL., 2002))

	Substarte	Product	Reaction	Source
1	glucose	HAc, CO_2, H_2	$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$	1, 3
2	glucose	HPr	$C_6H_{12}O_6 + 2H_2 \rightarrow 2C_2H_5COOH + 2H_2O$	1, 3
3	glucose	HAc, HPr, CO_2	$3C_6H_{12}O_6 \rightarrow 4C_2H_5COOH + 2CH_3COOH + 2CO_2 + 2H_2O$	1
4	glucose	HBu, CO_2, H_2	$C_6H_{12}O_6 \rightarrow C_3H_7COOH + 2CO_2 + 2H_2$	1, 3
5	palmitic acid	HAc, H_2	$C_{16}H_{31}COOH + 14H_2O \rightarrow 8CH_3COOH + 14H_2$	1
6	acetic acid	CH_4, CO_2	$CH_3COOH \rightarrow CH_4 + CO_2$	3, 2
7	butyric acid	HAc, H_2	$C_3H_7COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$	1, 2, 3
8	propionic acid	HAc, CO_2, H_2	$C_3H_7COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$	1, 2, 3
9	CO_2, H_2	CH_4, CO_2	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	1, 3

1 - BATSTONE ET AL.., 2002; 2 - PIND ET AL., 2003; 3 - DENAC ET AL., 1988

Acidogenesis

The products of hydrolysis are converted into C_1- C_5 molecules such as volatile fatty acids (VFA) e.g acetic acid (HAc), propionic acid (HPr), butyric acid (HBu) as well as alcohols, H_2, CO_2 and depending on the substrate components H_2S and NH_3 (reactions 1-5, Tab.2.2). Both the hydrolytic and the acidogenic bacteria work optimal under acid conditions at pH 4.5 – 6.3. Lower hydrogen partial pressure encourages production of highly reduced compounds such as HAc, whereas higher partial pressure of hydrogen favors the conversion into less reduced intermediates. Double as much hydrogen is formed during acidogenesis of HAc than of HBu, while the production of HPr allows reducing concentration of hydrogen in the system. In this way the redox potential (ORP) is controlled by the bacteria especially in case of heavy surge organic loads (MOSEY, 1983).

Acetogenesis

In the further step the HBu and HPr are being degraded to HAc, hydrogen and CO_2 (reactions 7-8, Tab.2.2), which constitute a direct substrate for the methanogenesis. For both acetogenesis and methanogenesis the neutral pH conditions of 6.8 – 7.5 are necessary. The acetic bacteria tolerate only very low partial hydrogen pressure, thus they are forced to live in symbiotic relationship with hydrogenotrophic methane bacteria (KALTWASSER, 1980; MÄRKL ET AL., 1983; WELLINGER ET AL., 1991, GERBER & SPAN, 2008). From the energetic point of view the direct monomer degradation to HAc (skirting the acetogenesis reactions 7-8, Tab.2.2) is the most convenient for the bacteria and

therefore preferably chosen. The appearance of HBu and HPr are the bacterial response to the elevated hydrogen concentrations (MOSEY, 1983).

Methanogenesis

In the last step methane and CO_2 are formed by methane bacteria under strictly anaerobic atmosphere and neutral pH conditions. Two methane production paths are known and methane bacteria are divided into two groups: (i) acetotrophic (acetoclastic) bacteria producing methane from HAc (Reaction 6, Tab.2.2) and (II) hydrogenotrophic bacteria reducing CO_2 by hydrogen to produce methane (Reaction 9, Tab.2.2). All so far known methanogenics are hydrogenotrophic, but only few bacteria groups can utilize acetate (KUNST, 2005). So far it was assumed that 70% of methane is produced over acetate degradation path (ROEDIGER ET AL., 1990). This assumption was based mainly on the results of tests conducted with sludge from a sewage treatment plant (STADTMAN & BARKER, 1949; JERIS & MCCARTY, 1965; SMITH & MAH, 1966) or with inoculum adapted to acetate fermentation (BUSWELL & SOLLO, 1948). However the latest scientific studies of KRAKAT ET AL., (2010) and NETTMANN ET AL., (2008) performed on sludge from an agricultural biogas plant reveal the dominance of hydrogenotrophic methanogenesis especially under thermophilic conditions. According to the results hydrogenotrophic methanogenesis can embody 90-100% of methane production even. Therefore different degradation paths should be considered. According to KRAKAT ET AL., (2010) full substrate conversion to CO_2 and H_2 and the subsequent conversion to CH_4 and H_2O can be presumed. This hypothesis confirms LAUKENMANN ET AL. (2010). His studies of anaerobic acetate degradation with help of heavy stable isotope of carbon (^{13}C) show the preceding total conversion of acetate into CO_2 and only later CO_2 conversion to CH_4. These revolutionary results call into question heretofore assumed methane production paths and reveal a new challenge for the scientific research. It needs to be cleared whether methanogenic pathways depend on the temperature mode or maybe also on the inoculum characteristics.

Rate limiting step

Under not inhibitory conditions and especially for substrates of lower bioavailability (e.g. maize, cellulose) hydrolysis is considered as the slowest stage of anaerobic digestion, so called rate limiting step (NOIKE ET AL., 1985; TOMEI ET AL., 2008, VAVILIN ET AL., 1996). This means that the rate of monomer production from the substrate as the slowest one affects and limits the rate of further conversion steps in anaerobic digestion chain. Due to the longer generation time (s. Fig. 2.3), but also depending on the reactor conditions

and the bioavailability of the substrate also acetogenesis or methanogenesis may turn out to be rate limiting for anaerobic digestion (SEYFRIED ET AL.,1994; VAVILIN ET AL., 2008)

2.5 Process parameters

Different process parameters influence the process of anaerobic biomass-to-methane fermentation.

2.5.1 Temperature

In general the rate of chemical processes grows with increasing temperature. However this is not always valid for biological reactions catalyzed by enzymes, like the anaerobic digestion (WELLINGER ET AL., 1991). The hydrolytic and acetogen part of fermentative bacteria are known to be less sensitive to temperature change (KROISS & SVARDAL, 2005). The studies of ZOETEMEYER ET AL. (1982) reveal the highest acid production rate to be achieved between 48°C and 55°C. However DEUBLEIN & STEINHAUSER (2008) refers to temperature range of 25-35°C as optimal for hydrolysis and acidogenesis.

As the methane formation process is much more prone to temperature changes than other degradation steps, it is finally methanogenesis which determines the running temperature of a fermenter. MATA-ALVAREZ (2003) followed by other authors (KROISS & SVARDAL, 2005; SCHATTAUER & WEILAND, 2006; DEUBLEIN & STEINHAUSER, 2008) mentioned two temperature windows as optimal for methanogenesis: 30-40°C (mesophilic) and 50-58°C (thermophilic). However the most methanogen bacteria are known to be mesophilic. The thermophilic methanogens are regarded as faster biomass converter even though they are more sensible to temperature changes. Consequently most of biogas plants are run at 38-40°C while there are only few operated at 53-55°C.

2.5.2 Buffering system and pH

Optimal pH conditions for all groups of the methanogenic biocenosis are presented in Fig. 2.3. The hydrolytic and acidogenic bacteria have their optimum at lower pH values of 4.5-6.3 but are capable of working at neutral pH conditions as well. For acetogen and methanogen groups the pH of 6.8- 7.5 is strictly required. If the pH value decreases below 6.5 the enhanced acid production leads to further pH drop and the cease of methane production. For all degradation steps conducted in one fermentation tank (single stage process) a neutral pH value is necessary. It usually sets automatically due to self-regulatory mechanism: a double buffering system of the carbon dioxide and ammonia (s. Eq. 1), (ROEDIGER ET AL., 1990).

$$NH_3 + CO_2 + H_2O \rightarrow NH_4^+ + HCO_3^- \quad \text{Eq. 1}$$

A too strong acidification of the fermenting biomass can be avoided mainly due to the $CO_2/HCO_3^-/ CO_3^{2-}$ buffering system (s. Eq. 2 and Fig. 2.4 left). The CO_2 produced during anaerobic digestion dissolves in the inoculum. For the pH values between 6.5 and 8.5 90-99% of CO_2 converts to the hydrogen carbonate form (HCO_3^-) neutralising the hydrogen ions released during acidogenesis, so that the pH value does not drop (HECHT, 2008).

$$H_2O + CO_2 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+ \leftrightarrow H_2CO_3 \quad \text{Eq. 2}$$

The additional support for the carbon dioxide buffer is given by the presence of ammonia/ammonium buffer. The lower pH value is, the higher percentage of ammonia dissolved in the reactor dissociates to form ammonium. In this way the hydroxyl ions are formed (s. Eq. 3, Eq. 4 and Fig. 2.4 right), which improves the stability of the system (HECHT, 2008).

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \quad \text{Eq. 3}$$

$$NH_3 + H^+ \leftrightarrow NH_4^+ \quad \text{Eq. 4}$$

Due to high buffer capacity in anaerobic fermenter the negative influence of increased concentrations of VFA on digestion can be reduced. High buffer capacity and a stable pH value prevent the increase in concentration of undissociated acids which have a strongly inhibitory influence on digestion.

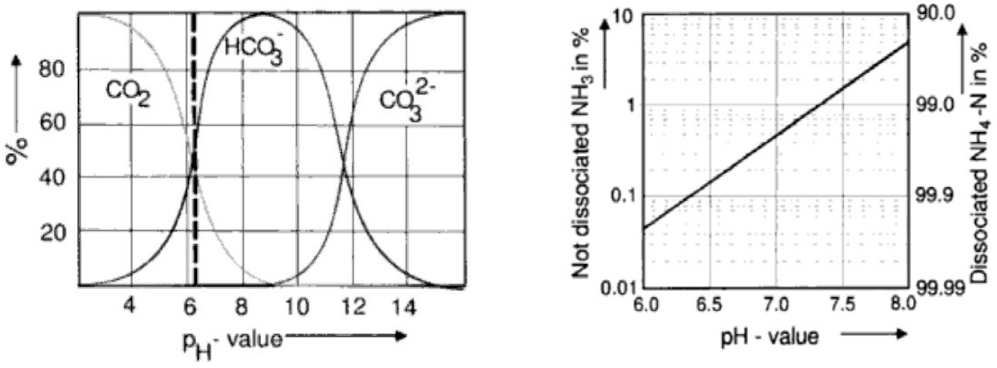

Fig. 2.4 Carbon dioxide/hydrogen carbonate/carbonate buffer system (left); Ammonia dissociation in aqueous media depending on the pH (right), (DEUBLEIN & STEINHAUSER, 2008)

2.5.3 Oxidation-reduction potential

All metabolic processes are characterised by redox reactions. For pure methanogenic cultures the optimal oxidation-reduction potential (ORP) values range between -300 mV and -330 mV (DEUBLEIN & STEINHAUSER, 2008; SCHOLWIN ET AL., 2009). For acetogenesis and methanogenesis in mixed culture, ORP smaller than -250 mV (KARPENSTEIN-MACHAN, 2005) or -300 mV (UTEC, 2003) are to be expected. Hydrolysis and Acidogenesis proceed at +400 mV to -300 mV (KARPENSTEIN-MACHAN, 2005). Therefore it is not surprising for a stirred biogas reactor to detect the ORP values even up to 0 mV (DEUBLEIN & STEINHAUSER, 2008; SCHOLWIN ET AL., 2009).

2.5.4 Type of substrate

The substrate composition determines the rate of anaerobic digestion. For different organic compounds (proteins, carbohydrates and fats) different degradation time is necessary to reach a similar conversion level into methane. Simple carbohydrates are the first to be degraded while the complex ones like cellulose-lignin-aggregate or lignin need more time and in some cases cannot be degraded at all under anaerobic conditions. Furthermore also the quality of biogas changes depends on the substrate composition. The highest methane concentration is achieved during digestion of fats and the lowest one for degradation of carbohydrates. If the chemical substrate formula is known, the equations of BUSWELL & MUELLER (1952) and BOYLE (1976) allow calculating the theoretical biogas composition (s. Eq. 5 and Eq. 6 respectively).

$$C_aH_bO_c + \left(a - \frac{b}{4} - \frac{c}{2}\right)H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4}\right)CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4}\right)CO_2 \qquad \textbf{Eq. 5}$$

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right)H_2O \rightarrow \qquad \textbf{Eq. 6}$$

$$\rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right)CO_2 + dNH_3 + eH_2S$$

Tab. 2.3 presents the theoretical biogas yields (Y_B) and methane content in biogas expected for carbohydrates, fats and proteins.

Another important aspect is the bioavailability of the substrate. Higher specific surface of material improves significantly the accessibility of the substrate (HILLS & NAKANO, 1984, SHARMA ET AL., 1988). For hardly degradable materials like cellulose-lignin-

complex the comminution by cutting, grinding or bio-extrusion (thermo mechanical disintegration) considerable increases the specific biogas production (even by 25%) and/or reduces the degradation time (PALMOWSKI & MÜLLER, 1999, LEHMANN, 2009). Also sonication can be applied to increase the degradation efficiency or to reduce the time of gasification (GRÜNING & ORTH, 1999; NEIS ET AL., 2000; NEIS ET AL., 2001; NICKEL, 2002). However this method of substrate disintegration is mainly applied for the solid sewage sludge (CLARK & NUJJOO, 2000; ONEYCHE ET AL., 2002, TOMEI ET AL., 2008). An extensive literature review on the investigated pre-treatment methods is given by DELGENES ET AL. (2003).

Tab. 2.3 Theoretical specific biogas production and composition depending on the substrate type (VDI, 2004)

substrate type	chemical formula	theoretical SGP	theoretical biogas composition	
		l_N/kgVS	vol % CH_4	vol % CO_2
carbohydrates	$(CH_2O)_n$	746	50	50
fats	$C_{16}H_{32}O_2$	1390	72	28
proteins	$C_{13}H_{25}O_7(N_3S)$	800	60	40

2.5.5 Trace elements

Certain elements such as Fe, Ni, Co, Mo, Se and W are necessary to perform a stable anaerobic digestion. The influence of trace elements on the methane fermentation has been subject of scientific studies since the early 80s. An extensive literature overview is given by TAKASHIMA & SPEECE (1990) and DEMIREL & SCHERER (2011). Many publications deal with general influence and the impact of trace elements deficiency on anaerobic digestion of municipal solid waste or animal dung (RAO & SEENAYYA, 1994; ZHANG ET AL., 2003; KAYHANIAN & RICH, 2005; KUNST, 2005; KUMAR ET AL., 2006). However for those substrates the main part of trace elements is delivered with the substrate itself. The problem escalates for digesters operated only with energy crops e.g. with grass and maize only. The loss of trace elements after exceeding of one year operation time is reported. An increased propionic acid concentration, which cannot be reduced over longer periods of time, as well as decreased biogas production are regarded to be the signs of the trace element shortage (LEMMER, 2007; ANONYMOUS, 2009). As comprehensive studies in this field are still ongoing, only few publications dealing with digestion of energy crops could be found (HINKEN ET AL., 2008; LEBUHN ET AL., 2008; POBEHEIM ET AL., 2010 & 2011; DEMIREL & SCHERER, 2011).

Scientific studies on the role of trace elements revealed that their addition may also allow digestion at extremely high OLRs or under conditions normally regarded as suppressive (RAO & SEENAYYA, 1994; CLIMENHAGA & BANKS, 2008, LEBUHN ET AL., 2008). This

aspect is of great interest especially for the industry. Many of them performed or keep performing trials on full scale biogas plants. The only perceivable result of these tests is a wide variety of trace element supplements available on the market (e.g. by Bioreact, Schmack, ABEL ReTec) without any possibility to have insight into the experiments.

2.5.6 Inhibitors

The substances such as heavy metals, ammonia, nitrate, organic acids (especially in their undissociated form) and hydrogen sulphide can be found in every biogas plant. However if their concentration exceed certain threshold, they may act as inhibitors especially to sensitive methanogenic bacteria. An overview of the inhibitory thresholds for the above-mentioned substances is given among others by SCHATTAUER & WEILAND (2006), DEUBLEIN & STEINHAUSER (2008). However the inhibitory capability is not only caused by the exceeding of certain concentration limits. Continuous or sudden flow of inhibitor, interactions between inhibitors and other compounds, adaption ability of bacteria as well as different temperature or pH conditions – all those parameters affect the reaction to the potential inhibitory compounds (DEUBLEIN & STEINHAUSER, 2008; MATA-ALVAREZ, 2003). KROISS & SVARDAL (2005) emphasize, that exceeding inhibitory thresholds can directly cause a serious inhibition (metabolic cease) only if bacteria are working at their maximal turnover rate.

2.6 Indicators of inhibited digestion

The term "inhibited digestion" is usually applied if the total GP resulting from digestion of certain substrate is much lower than expected by terms of substrate composition and biodegradability. This low GP is always a consequence of digestion disturbance (or insufficient inoculum activity in case of batch experiments) and accompanied by further changes in VFA rate in reactor. In the biogas literature four different indicators are applied to define the boundaries of non-inhibited digestion:

(1) **VS_s/VS_i:** According to VDI (2004) the VS ratio of substrate to inoculum in a batch experiment should not exceed 0.5. However if the VS content of inoculum is too low the critical VS_s/VS_i – ratio can be easily exceeded. That is why the additional requirements for VS_i were established (s. Eq. 7 and Eq. 8).

$$VS_I > 50\%\ DM_i \qquad \textbf{Eq. 7}$$

$$1.5\%\ FM < VS_i < 2\%\ FM \qquad \textbf{Eq. 8}$$

These requirements may be helpful to prepare batch tests under non-inhibitory conditions, but their implementation is not a necessary and sufficient condition to

perform the not-inhibited digestion experiments. The overview of all batch experiments (s. Chapter 3.2) reveals that particularly the third requirement of keeping VS content of inoculum ranged between 1.5 and 2% of FM is difficult to fulfill.

(2) **TVA/TIC**: The ratio of titrated volatile acids (TVA) and titrated inorganic carbon (TIC) is considered as indicator of acid inhibition and overloading. It should be kept under 0.3 (CECCHI ET AL., 2003; TELSCHOW, 2007) or 0.4 (LOSSIE & PÜTZ, 2008) during the fermentation process to avoid any disturbances.

(3) **HPr/HAc and the sum of VFA**: Already 1988 HILL & HOLMBERG defined HPr/HAc ratio of 0.25 together with the sum of VFA of 2000 mg/l as the limits of uninhibited fermentation. But according to the latest research (HECHT ET AL., 2007; LEMMER, 2007), HPr/HAc ratio higher than 0.5, with simultaneously exceeded 3500 mg/l of VFA are considered as the signs of system instability due to acidosis.

(4) **C_4-C_5 VFA**: Increased production of long LCFA is reported to be an indicator of digestion problems (CHEN & DAY, 1986; DAHLHOFF, 2007). But particularly presence of branched fatty acids such as iso-butyric (iso-HBu) and iso- valeric acid (iso-HVa) is regarded as inhibitory already at the concentrations higher than 15 mg/l (HILL & HOLMBERG, 1988) or 50 mg/l (SCHATTAUER & WEILAND, 2006).

2.7 Operating mode

Both in laboratory scale and in the industry anaerobic digestion of biomass is being conducted in different operating modes. In batch mode the reactors are charged only once and digested for a certain period (usually until the gas production cease is reached). This method is used in laboratory scale to investigate the biogas yields of different substrates (BADGER ET AL., 1979). In the biogas industry batch fermentation is mostly applied in the fermenter box method (WEILAND, 2006 & 2010), even though it did not gain too much popularity. The most common digestion methods are continuous or semi batch digestion. In these operating modes substrate is fed to the reactor either in continuous way or with a constant feeding raster. These promote reproducible and stable GP and allow automation of the fermentation process (WEILAND, 2000). However the maximal biogas yields can only be obtained in batch experiments. This is due to VS removal via effluent in semi-batch and continuous mode (SCHLATTMANN ET AL., 2004; SCHUMACHER ET AL., 2006). Already first comparisons of batch and continuous method for agricultural manure (HASHIMOTO, 1982 &1983) revealed the decrease of biogas yields for continuous digestion. The biogas yield decrease is also to be expected with the increase of reactor volume (SCHLATTMANN ET AL., 2004). Nevertheless in the literature registered

biogas yield results are so spread that the expected trends cannot be observed. Further in many cases the detailed information about the experimental set up is missing e.g. whether the laboratory or industrial scale experiment was conduced, in what fermentation mode a biogas yield was obtained, or if the GP was standardized or not (and if not under what temperature and pressure condition the GP was measured). The unanswered questions often make the results incomparable. This problem was extensively discussed by MÄHNERT (2007).

2.8 Modeling

Unlike aerobic digestion the anaerobic fermentation can be characterised by a lower microbial energy consumption and biomass growth. The energy produced during substrate conversion is saved in form of methane, which can be further utilized as an energy source. The anaerobic systems tend to instabilities caused mainly by overloading or other inappropriate operating conditions. Models help to describe and understand the processes within a fermenter. Therefore they may be used to improve the design and operation of the biogas reactors. Models of lab scale tests deliver helpful information for the scale-up. Further, in batch mode with much less effort an extreme performance of the anaerobic fermenter can be reached and modelled. But the kinetic data of bacterial growth from a batch reactor cannot be directly used for steady state processes (WOLF, 1991).

The complexity of a model is always defined by how accurate the different processes of the system should be described and what the model destination is. In modelling of anaerobic digestion the mathematical equations known from describing aerobic processes are applied.

The simplest model to describe the biomass degradation by microbial culture is the first order model. In its equation it defines the substrate utilisation rate as function of substrate concentration only (for details s. Eq. 15 in Chapter 3.5.3). Many aspects such as heterogeneity of the substrate, microbial growth and decay, as well as any sort of inhibition are not included in the equation. The 1st order kinetics is widely applied in the literature to model the hydrolysis step in anaerobic digestion (BATSTONE et al., 2002).

If assumed that the growth of bacterial biocenosis is similar to the growth of a pure bacterial culture, a model of Monod-type can be applied (MONOD, 1950; KNIGHTES & PETERS, 2000). The basics of Monod model are given by Eq. 13, 14 and 15 (Chapter 3.5.3). The model is a function of substrate concentration but includes the influence of the bacterial growth and decay on the digestion process. Neither

degradation of complex substrates (TE BOEKHORST ET AL., 1981; PFEFFER, 1974) nor the lag phase or inhibited digestion can be described by the model (STRIGUL ET AL., 2009).

To improve the accuracy of MONOD model further upgrades concerning effects of adaption of microorganisms to steady state processes by mutation (MOSER, 1958), influence of mass transfer limitations on the microbial population (CONTOIS, 1959), diffusion and permeation of substrate through the cell walls (POWELL, 1967), cell concentration as a function of initial substrate concentration (CHEN & HASHIMOTO, 1980), deceleration during the lag phase (BERGTER, 1983), or dependence of specific growth rate on gas production (MITSDÖRFFER, 1991) were introduced. The modified MONOD models can also be extended by various inhibition terms including substrate or product inhibition as well as other physical factors. Different kinetic models are reviewed extensively by PAVLOSTATHIS & GIRALDO-GOMEZ (1991), LYBERATOS & SKIADAS (1999), GARCIA-HERAS (2003) and GERBER & SPAN (2008).

Anaerobic Digestion Model No. 1 (ADM1) is the most comprehensive model applied in wastewater. It was created as a tool allowing predictions of sufficient accuracy to be used in process development, operation and optimisation. The model describes disintegration, hydrolysis, acidogenesis, acetogenesis and methanogenesis. Considering 19 biochemical kinetic processes, it combines the simple first order kinetics for hydrolysis step with MONOD-type kinetics used for description of all intracellular biochemical reactions and further inhibition functions including pH, hydrogen and free ammonia influence. Apart from biologically mediated processes two very important groups of physico-chemical reactions are incorporated in the model: processes taking part in the liquid phase (i.e. ion association or dissociation, buffering) and liquid-gas processes (i.e. liquid-gas transfer), (BATSTONE et al., 2002).

Although a lot of research has been done on modeling of anaerobic digestion steps especially for solid waste (CHEN & HASHIMOTO, 1980; KIELY ET AL., 1997; KALFAS ET AL., 2006; SOSNOWSKI ET AL., 2007; BOUBAKER & RIDHA, 2008; LIU ET AL., 2008; VAVILIN ET AL., 2008; QU ET AL., 2009) or waste water treatment (BATSTONE ET AL., 2002; FENG ET AL., 2006; TOMEI ET AL., 2008; BOUBAKER & RIDHA, 2008), not so many results can be found on modeling of agricultural biogas production (HILL & BARTH, 1977; SIMEONOV ET AL., 1996; ANGELIDAKI ET AL., 1999) and only a few consider the digestion of energy crops (WICHERN ET AL., 2008 & 2009). No models dealing with anaerobic digestion of energy crops under thermophilic conditions can be found. Further there is no extensive comparative study on influence of change of operating mode (batch, semi-batch, continuous model) on the kinetics of anaerobic digestion. The results of this study

should help to close the research gap in the field of energy crops digestion under mesophilic and thermophilic conditions and for different operating modes using very simple modelling fits.

3 Materials and methods

3.1 Substrate

Two substrates were used in the study: pure microcrystalline cellulose and agricultural maize silage. Samples of both substrates are presented in Fig. 3.1.

Fig. 3.1 Digestion substrates: samples of maize (left) and cellulose (right)

Cellulose

A microcrystalline cellulose powder of pharmaceutical grade (Euro OTC Pharma GmbH) with VS = 98% DM was used as a model substrate for batch tests investigating the influence of OLR on the digestion performance. Digestion of pure cellulose, if 100% conversion is considered, delivers 827 l_N/gVS of biogas.

Maize silage

Maize silages of two harvests were applied in the study: MS I- for thermophilic batches, semi-batch tests and MS II – for mesophilic batches and thermophilic continuous tests (s. Fig. 3.1). The ensilaged maize was stored frozen and defrosted at low temperatures (4°C) about 24h before charging of the fermenters. Both silages were characterised by Van Soest and Weende analysis (for the method details and the results s. Chapter 3.4 and Tab. B.1, Attachment AB.1). Further corrections on the DM-content of maize were done according to WEIßBACH & KUHLA (1995). This allowed the calculation of corrected DM content (DM_K) of the substrate increased by the amount of volatile compounds lost by volatilization during DM determination according to Eq. 9.

$$DM_K = 2.22 + 0.960\ DM_N \qquad \text{Eq. 9}$$

Where:

DM_N	[%]	measured DM value
DM_K	[%]	corrected DM value

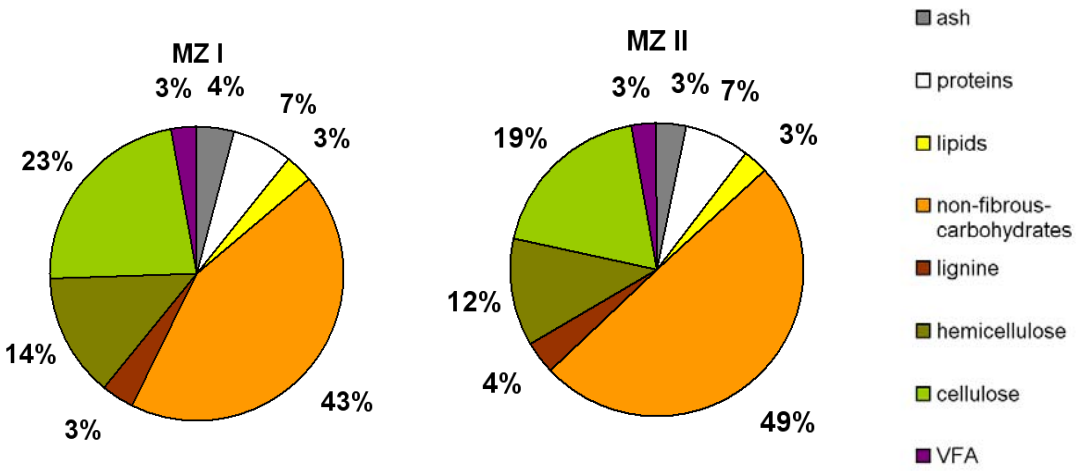

Fig. 3.2 DM Composition of both maize silages used in the tests (calculated from Van Soest results including the VFA corrections according to Chapter 3.1)

The composition of maize silages corrected according to Eq. 9 is presented in Fig. 3.2. Lignin was considered as non-degradable component of maize, so that the total degradability of maize was calculated according to formulas given in Tab. 2.3 (s. Chapter 2.5.4), excluding the lignin content. The calculated biogas yield from the silages equalled 742 l_N/gVS for MZ I and 743 l_N/gVS for MZ II.

Since maize silage is prepared by cutting and natural acidifying of the whole plant mass (inclusive leaves, stalks and corn-cobs) its composition represents the global plant content. Consequently a sample containing more stalk content (with high percentage of lignin) would give less biogas than the one consisting mainly of ensilaged leaves. This natural inhomogeneity effect can to some extent be reduced by fine cutting and mixing of the silage but some uncertainty will always be present in the results which refer to the silage composition e.g. biogas yield (Y_B). This aspect is also addressed in the discussion chapter.

Maximal expected biogas yield

During biomass to biogas conversion the majority of the organic carbon captured in the substrate is being transferred into biogas. Only maximum 10% of carbon is considered as lost during the conversion (GREPMEIER, 2002). The carbon balance for anaerobic digestion is presented in Fig. 3.3.

Similar to VDI (2004) and GREPMEIER (2002) the maximal expected biogas yield was calculated by reducing the theoretical biogas yield by 10%. This followed the assumption of 10% biomass loss due to microbial growth (5%) and undigested carbon residuals in effluent (5%). Consequently the maximum expected biogas yield values of 752 l_N/gVS for cellulose and 669 l_N/ gVS for MZ I and MZ II were assumed

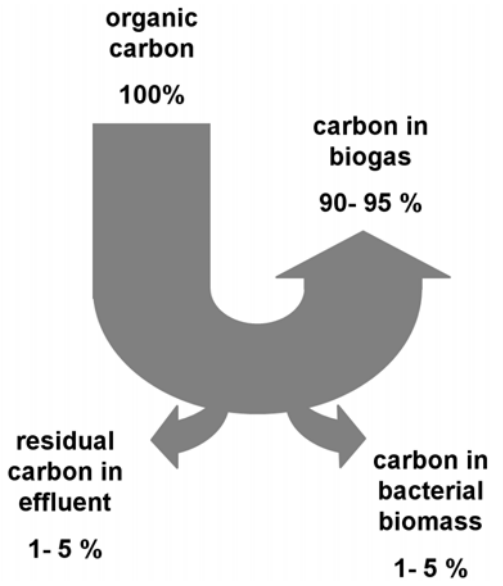

Fig. 3.3 Carbon balance of anaerobic digestion (GREPMEIER, 2002)

3.2 Inoculum

The inoculum for the thermophilic (55°C) batch tests was obtained from 50 l continuously fed research fermenter. This plug-flow reactor was operated in a dry fermentation mode (fed with maize and grass silage mix only) under thermophilic regime. Mesophilic inoculum (38°C) was retrieved from biogas plant in Beckerich. This biogas plant is being fed with agricultural slurry and energy crops mix.

The biomass was filtered through a kitchen strainer prior to the test. The homogenous filtrate was applied in the tests to accomplish the reproducible conditions for each batch. The DM of the inocula ranged between 1.9 and 4.5 % of FM and the VS between 45 and 61% of DM (1.0 – 2.7 % of FM). The inocula were thermostated for 2-3 weeks before each test to remove the residues of the previous trials. Guidelines for the fermentation of organic material were followed (VDI, 2004). The subsequent batches were performed with the same inoculum to achieve the optimal substrate adaption to the different OLR. The detailed history of the inoculum used is presented in Tab. B.2 (Attachment B).

3.3 Experimental set up

Six groups of experiments were performed during the study: (1) thermophilic cellulose fermentation in batch mode, (2) thermophilic maize fermentation in batch mode, (3) thermophilic maize fermentation in semi-batch mode, (4) thermophilic maize

Tab. 3.1 Test series performed during the study

Temp.	substrate	mode	single feeding OLR [kgVS/m^3]							Feeding frequency
			4.1	5.4 ± 0.5	11.2 ± 0.4	17.0 ± 1.0	22.9	28.6	34.3	
55°C	cellulose	batch	-	x	x[a]	x[a]	x	x[a]	x	1 per test
	maize (MZ I)	batch	-	x	x	x	-	-	-	1 per test
	maize (MZ I)	semi-batch	-	x	x	x	-	-	-	1 per 3 days 10 times
	maize (MZ II)	conti	x	x	x	-	-	-	-	1 per day 10 times
38°C	cellulose	batch	-	x	x	x	-	-	-	1 per test
	maize (MZ II)	batch	-	x	x	x	-	-	-	1 per test

[a] without on-line pH and redox potential registration system

fermentation in continuous mode, (5) mesophilic maize fermentation in batch mode and (6) mesophilic cellulose fermentation in batch mode. Tab. 3.1 summarizes all test series.

In the batch experiments listed in Tab. 3.1 the indicated single feeding OLRs corresponded also to the total OLRs. The precise definition of OLR for semi-batch and continuous mode is much more complex especially where it comes to direct comparison of some experimental parameters between different operating modes. Due to differences in feeding frequency the OLRs for these operating modes can be defined on three ways: (i) the OLR charged at every feeding event, (ii) total OLR introduced into fermenter during the experiment and (iii) the daily OLR calculated by dividing of total OLR by the length of the feeding period. An overview of the OLRs for semi-batch and continuous digestion corresponding to different OLR definitions is given in Tab. 3.2. In further chapters the single feeding OLRs was used if certain experimental series were mentioned. However for comparison purposes both single feeding and daily calculated OLR values were used in Chapters 4.3.1and 5.3.

Batch experiments

Batch experiments were conducted in 1 l fermenting bottles filled with 700g of inoculum each. Depending on the operating temperature (38°c or 55°C) the inocula of different origin were applied (s. Chapter 3.2). In all batch series the reactors were opened only once at the beginning of the experiment as the substrate was introduced into reactor. For each experimental series 12 - 18 reactor bottles were prepared and run parallel under the same conditions. The applied number of reactors was adjusted to the OLR. The higher OLR investigated, the more reactors were run in parallel. This was essential due to a longer GP period creating the necessity of more reactor samplings.

Tab. 3.2 Summary of OLR data for semi-batch and continuous mode

operating mode	single feeding OLR [kgVS/m^3]	total OLR [kgVS/m^3]	feeding period [d]	calculated daily OLR [kgVS/m^3]
semi-batch	5.9	59	30	2.0
	11.7	117	30	3.9
	17.6	176	30	5.9
conti-nuous	4.1	41	10	4.1
	5.9	59	10	5.9
	11.7	70[a]	6	-

[a]the experiment was stopped after feeding 6 due to acidosis of the fermenmter

For each series a separate reactor was run with the inoculum only to qualify the background production. Two further reactors were used for on-line monitoring of pH and ORP[2]. Depending on the investigated OLR 6-13 reactors were used for sampling purposes. On particular stages of digestion the reactors were stopped, the digestate was homogenised[3] and sampled for analysis of VFA, TVA and TIC. For all thermophilic series with cellulose and maize additionally DM and VS content of the digestate was measured. However the results were not accurate enough to be applied for the further data analysis. For that reason the DM and VS analysis of reactor content was abandoned for further batches (for details s. Chapter 3.5.1). Each time only one reactor was stopped for the sampling purposes. Once stopped, the reactor was not used for the further study to prevent the disruption of digestion by air inflow. A schematic diagram of experimental set up and a picture of typical fermentation series is shown in Fig. 3.4.

The daily and total mean GP value, as well as the statistical significance of the GP (expressed in standard deviation), were calculated basing on the GP received from 2-3 biogas reactors. The digestion was regarded as completed when the GP smaller than 5 ml was produced within a period of 3 days. The details on applied analytical methods and biogas measurements are given in Chapter 3.4.

Semi-batch and continuous experiments

The experimental series in semi-batch and continuous mode were prepared in the similar way. Fermentation took place in 2 l fermenting bottles filled with 700g of

[2] except for the series, in which the on-line system was not available (s. Tab. 3.1)

[3] The reactor content was shaken so that the remaining substrate and the intermediary digestion products were regularly distributed in the reactor content.

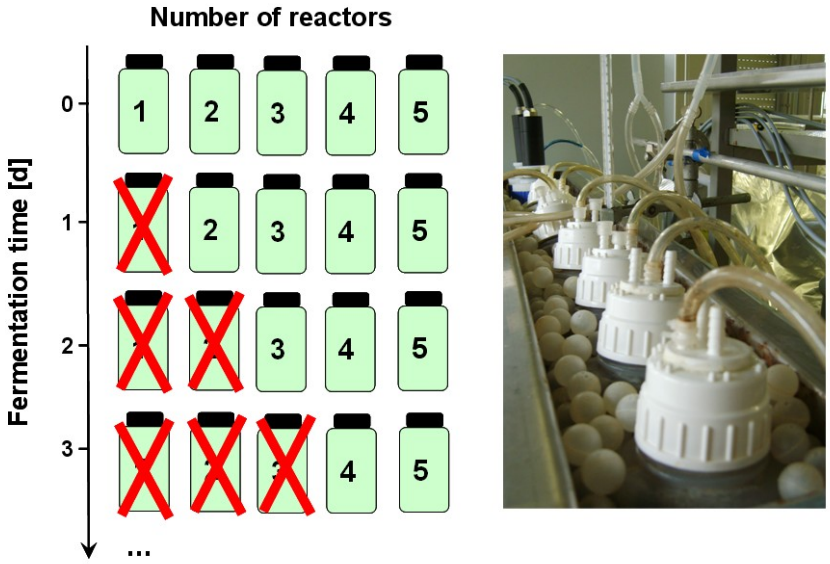

Fig. 3.4 Experimental set up for a batch series: with each day one reactor was stopped and analyzed (left), bottle fermenters during digestion (right).

inoculum each. Both series were conducted under thermophilic conditions (55°C). The inoculum applied in the tests was from the recovery after thermophilic batch digestion but was originally retrieved from 50 l continuously fed research fermenter of the university (s. Chapter 3.2). In each series all reactors were charged 10 times: in semi-batch every third day while in continuous mode every day. This means that the full charging period in semi-batch lasted for 30 days while in continuous mode after 10 days the feeding period was terminated[4].

For each test series and experimental mode a set of 8 fermenter bottles was run in parallel. One reactor was used for capturing the background production and further 2 for online measurements of pH and ORP. The GP from 2 reactors was measured and used to calculate the mean and total GP as well as the statistical significance of the measured values (expressed in standard deviation). The three remaining reactors were used for sampling purposes: The sampling took place in a rotation mode. The reactors were reopened for the feeding purposes. The samples were taken always directly prior to the substrate recharge (which means every third day in semi-batch and every day in continuous mode). On the selected days 40 ml of digestate were extracted from only one reactor bottle. The sample was directly filtered through a sieve with 1 mm mesh size and the digestate remaining on the sieve was returned to the reactor. The filtrate was used for TVA, TIC and VFA analysis. Further 40 ml of activated inoculum were added

[4] During continuous digestion of maize at OLR of 11.7 kgVS/m³ several signs of digestion disturbance (the olfactometric analysis of the reactor content, the low gas production and its final cease) caused the experiment being stopped already after the feeding 6.

to the sampled reactor to replace the volume reduction due to multiply reactor sampling. After the feeding period the substrate was left in the reactors until the GP ceased, which was assumed when the GP did not exceed the sum of 5ml for a 3-day period. The details on applied analytical methods and biogas measurements are given in Chapter 3.4.

Independent on the operating mode the content of only one reactor per day was analyzed. However the observation of parallel GP from simultaneously started reactors reveal that the reproducibility of the subsequent fermentation stages was quite high if the described inoculum preparation procedure was strictly followed. The observed differences in the GP did not exceed 10%, which can be assumed as a natural consequence of the biological diversity typical for experiments conducted on living organisms (HELFFRICH & OECHSNER, 2003).

3.4 Analytical methods

During the fermentation biogas volume and quality data were collected with help of gas meter (a saline water trap) and biogas analyzer. ORP and pH were continuously measured in the reactor during the process. Digestate from the fermenter was sampled and analysed to quantify DM, VS, VFA, TVA and TIC. The input material (cellulose and maize) was characterised in terms of DM and VS. The exact composition of maize silage was additionally characterized by Van Soest and Weende analysis. The scheme of a typical fermenting and sampling system is presented in Fig. 3.5.

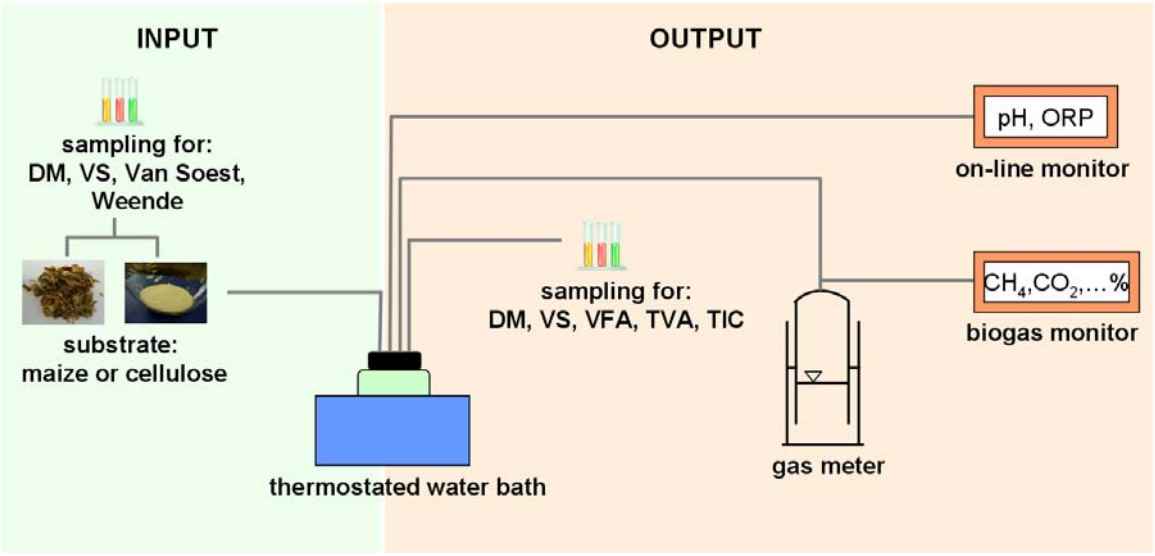

Fig. 3.5 Schema of the fermenting system and the analysis being done on its content

Monitoring of pH and oxidation-reduction potential (ORP)

ORP and pH data acquisition during all test series[5] was carried out with help of the WTW on-line monitoring system (Quadroline pH 296 with SensoLyt SEA and PtA, WTW) developed for heavy loaded waste. Parallel pH measurements of every reactor content were performed directly after being opened (PH3210 with SenTix 41, WTW) or at the beginning of the sample titration (Titroline easy with BlueLine 12, Schott).

The information about performance and life-span of the on-line pH and ORP electrodes in a biogas fermenter was additionally collected during the study (for details s. Attachment G). The pH and ORP on-line monitoring system is presented in Fig. 3.6

Fig. 3.6 ORP and pH on-line system applied in the test: the electrode adapters in the fermenters (left), the monitoring displays (central), an ORP electrode (right).

Biogas analysis

The biogas was collected in a gas trap by displacement of saturated saline water. For each experimental series GP was measured parallel on 2 reactors. The gas volume was measured until the GP smaller than 5 ml was measured for a period of 3 days. Such GP assessment method is more accurate than the one proposed by VDI (2004)[6]. Measured biogas volume was converted into standard temperature and pressure conditions (273.15K; $1013.25 \cdot 10^2$Pa). The daily and total mean GP and its statistical significance (expressed in standard deviation) were calculated and used for the further analysis of the results. The observed differences in the GP did not exceed 10%, which can be assumed as a natural consequence of the biological diversity typical for experiments conducted on living organisms (HELFFRICH & OECHSNER, 2003).

Biogas composition was analyzed with an IR (CH_4, CO_2) and electro chemical (O_2 and H_2S) gas monitor (Biogasmonitor BM2000, Ansyco). At high gas production rates the analysis were performed daily, at lower rates not until the minimal gas volume necessary for the analysis (between 250 and 300 ml) was collected. The biogas monitor delivers

[5] except for thermophilic 11.4, 17.1 and 28.6 kgVS/m³ cellulose in batch

[6] According to VDI (2004) guidelines the experiment can be stopped when the daily GP is lower than 10% of the volume of total produced biogas.

CH_4 and CO_2 results with 3%, O_2 with 1% and H_2S with 50-100[7] ppm accuracy. The reproducibility of the results reached 0.2% for CH_4 and CO_2, 0.5% for O_2 and 50 ppm for H_2S. Due to lower accuracy and precision of H_2S measurements, H_2S results were only compared and analysed if extreme parameter values were measured (s. Chapter 4.1.1). The equipment used for biogas analysis is shown in Fig. 3.7.

Fig. 3.7 The gas meters (left) and the biogas monitor (right) used in the studies

Dry matter and volatile solids content

DM and VS of substrates and inoculum were determined according to DIN EN ISO 12879 and 12880. The homogenous reactor content or substrate sample was dried 24 h (if necessary longer) at 105°C for DM analysis and carbonized 24 h at 550°C for VS determination. Each sample was analyzed twice. The reproducibility of the DM and VS results for substrates and reactor samples was not lower than 95%.

Van Soest and Weende analysis

The composition of maize silage was determined according to Van Soest and Weende method (VAN SOEST, 1967, VAN SOEST et al., 1991). The following substrate components were determined by the method: crude ash, crude protein, crude fat as well as the non fibrous carbohydrates (NFC) and fibrous components – neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). An overview of biomass composition and the parameters analyzed by Van Soest and Weende method is given in Fig. 3.8.

[7] For H_2S sensor the exact accuracy of the device cannot be defined as the results may be influenced by cross reactions with other biogas compoments

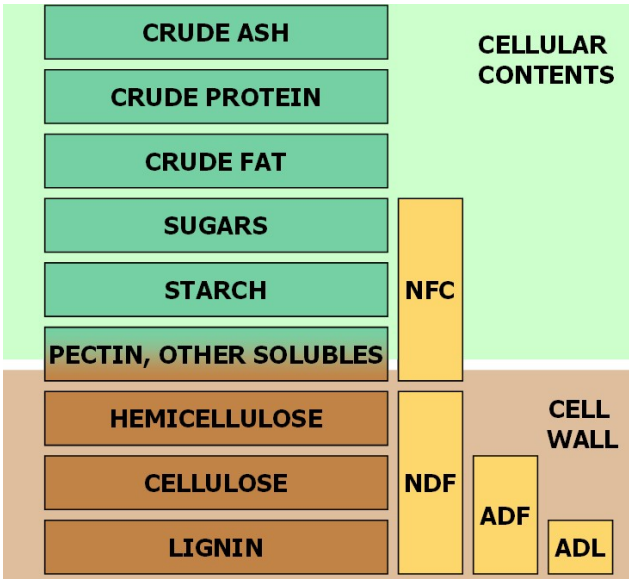

Fig. 3.8 Biomass composition and the parameters analyzed by Van Soest and Weende method

Substrates for the analysis were dried at 60°C. Chemical analysis was conducted at the University of Kassel (Germany). Each silage sample was investigated twice. The results of Van Soest and Weende analysis for maize silages are listed in Tab. B.1 (Attachment B.1). The reproducibility of the results for separate fractions (ADF, NFC, etc.) was not lower than 97%. However the results can be strongly influenced by the natural inhomogeneity of the maize silage (s. Chapter 3.1 and 5).

Titrated volatile acids/ titrated inorganic carbon

Titrated inorganic carbon (TIC) describing the buffer capacity of the system and the titrated volatile acids (TVA) characterising the fermentation progress were obtained titrimetricly (Titroline easy with BlueLine 12, Schott) following the Nordmann procedure (NORDMANN, 1977; TELSCHOW, 2007). Immediately after sampling the homogenized reactor content was filtered through a kitchen sieve with mesh size of 1mm. In the following step 20 ml of filtrate were titrated with sulphuric acid (0.1 mol/l) in two-point titration mode (with end points at pH 5.0 and 4.4). Only one sample was taken for each investigated reactor. TIC and TVA were calculated according to Eq. 10 and Eq. 11.

$$TIC = \frac{20ml}{Vml} \cdot A \cdot 250 \qquad \text{Eq. 10}$$

$$TVA = \left(\frac{20ml}{Vml} \cdot B \cdot 1.66 - 0.15\right) \cdot 500 \qquad \text{Eq. 11}$$

Where:

V [ml] sample volume;
A [ml] consumption of 0.1 mol/l H_2SO_4 during titration to pH 5.0;
B [ml] consumption of 0.1 mol/l H_2SO_4 during titration from pH 5.0 to pH 4.4

Volatile fatty acids

Complementary to the sum parameter TVA the concentration of each C_2-C_5 volatile fatty acids (VFA) was measured. The VFA purification method similar to KITTELMANN ET AL. (1983), PECHER (1989) and PIND ET AL. (2003) was developed. For each investigated reactor only one VFA sample was taken. The reactor samples were centrifuged at 12 000g (MiniSpin, Eppendorf). The supernatant was acidified with an acid reagent in the proportion 9:1 and passed through a nylon 0.45-µm-pore-size filter (Rotilabo, Carl Roth). Composition of the acid reagent is given in Tab. 3.3. The 4-methylpentane acid was applied in the reagent as a standard, which gave a direct possibility to compare the quality of each single measurement with the others.

Tab. 3.3 Chemical composition of acid solution applied for pre-treatment of the samples for gas chromatographic analysis of VFA

chemical component	volume applied [ml]
acetone	79.8
H_3PO_4	20.0
4-methylpentane acid	0.2
total volume	100.0

The VFA samples were analysed by gas chromatograph (Focus GC with FID, Interscience) equipped with capillary columns (Econo-Cap™-1000, Grace; Polar Deactivation Guard Column, Restek). Each sample was analysed 5-10 times by GC. The received mean value was used for the further analysis. The general accuracy of the VFA measurements reached 99%, while the sample reproducibility amounted to 98-99% if pure acids were analyzed. The lower detection limit for pure acid concentrations reached 0.001 g/l. Both the lower limit of detection and the analytical precision were strongly influenced by the inoculum matrix even after sample pre-treatment and differed depending on the acid being analysed. It was observed that for biogas reactor samples the procedure provides the results for HAc and HPr with 95-99% precision while for iso-, n-HBu and iso-, n-HVa with 80-95% precision. The lower limit of detection varied between 0.01 g/l and 0.001 g/l depending on the sample and acid.

3.5 Model development

3.5.1 Volatile solids data

In the initial batch tests[8] the VS of the reactors was regularly measured during degradation. These values were used to calculate substrate degradation level, however the results were inconsistent and varied strongly (s. Fig. 3.9. For some tests many measured VS values were not following a decreasing trend (e.g. 5.7 kg VS/m^3 maize or 11.4 kg VS/m^3 cellulose). Furthermore all data collected for cellulose batches suggested much higher final degradation level than possible. These difficulties occurred independent of the substrate applied and did not allow applying the VS substrate degradation data in further analysis. The observed problems were connected to the specifics of the material (both substrates and inoculum) used in the study. In the reactors cellulose used to sediment whereas maize was floating on the inoculum surface during sampling. Furthermore, as noticed already by CHEN & HASHIMOTO (1978), some VS volatilise during DM and VS determination, which causes additional errors in the measured VS amount.

Consequently the VS values were not exact enough and caused a severe calculation inaccuracy. Similar problems were already reported by CHEN & HASHIMOTO (1978), who developed an equation allowing conversion of measured methane volume into substrate degradation. The equation follows the assumption that for non-inhibitory anaerobic digestion the methane production is directly linked to substrate reduction and no other way of substrate degradation is possible.

Chen & Hashimoto equation has been widely applied for calculation of different substrates especially if GP is the only reliable data characterising the digestion process. However the equation in the form proposed by the authors did not enclose the substrate degradation selectivity towards methane and therefore assumes the constant fraction of CH_4 in biogas. While such estimation could be accepted for continuous and steady state experiments, it is definitely invalid for semi-batch and even more for batch experiments, in which the CH_4/CO_2 ratio varies seriously for at least 3 initial days of digestion. To improve the accuracy of the results the Chen & Hashimoto equation was applied, however not the methane but biogas data was implemented in the model (s. Eq. 12). This approach allowed more accurate substrate-product conversion.

[8] The VS of complete reactor series was measured only for thermophilic cellulose and maize batches. In the following tests the procedure was abandoned due to variation of the results.

$$\frac{S}{S_0} = \frac{B_{end} - B}{B_{end}} \qquad \text{Eq. 12}$$

Where:

S	[g/l]	substrate concentration at time t
S_0	[g/l]	initial substrate concentration at time t_0
B	[ml_N]	biogas produced at time t
B_{end}	[ml_N]	total biogas production at time t_{end}

The comparison of VS substrate degradation calculated from measured VS values and from biogas is presented in Fig. 3.9. Similar biogas based degradation curves were applied in further analysis to model the substrate degradation.

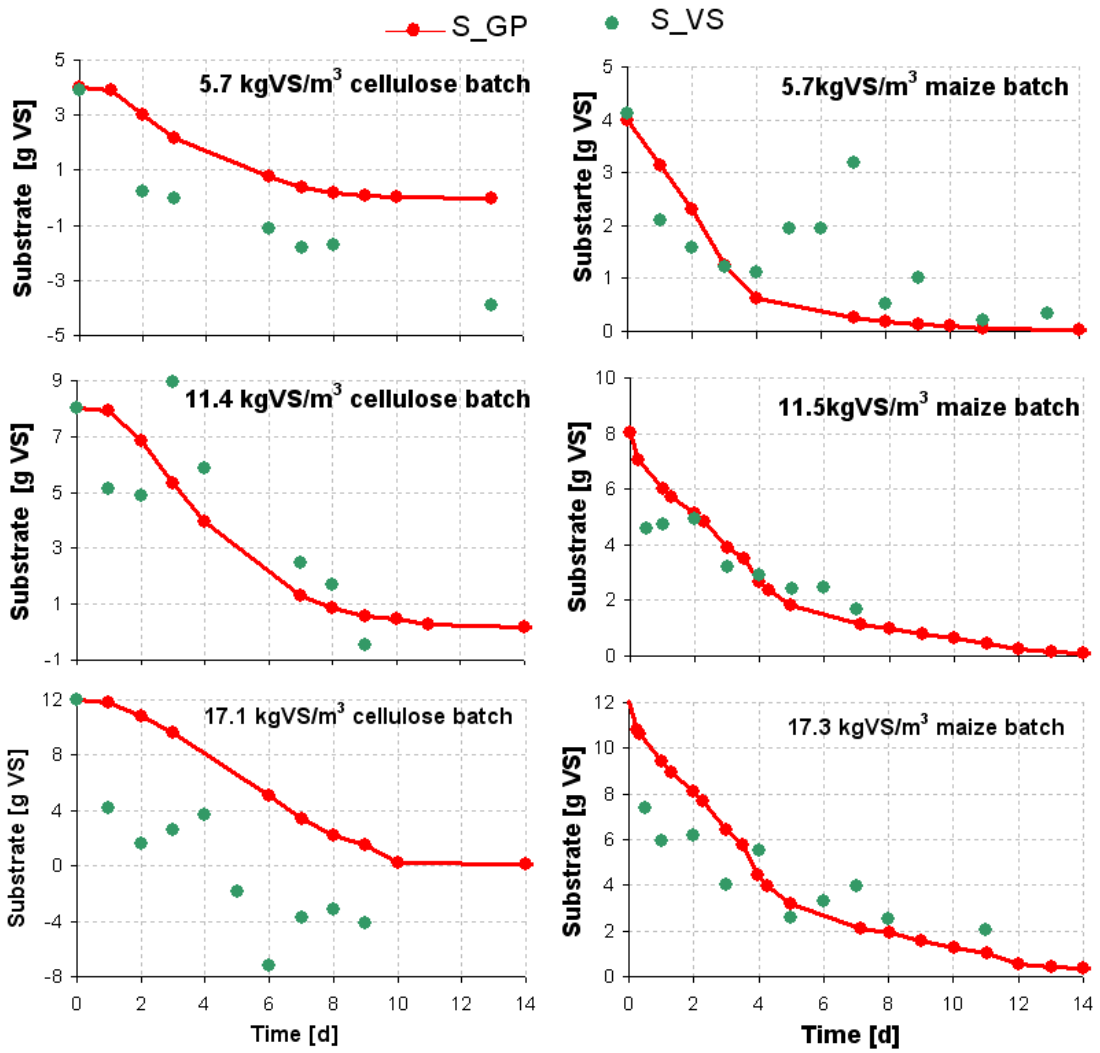

Fig. 3.9 Substrate degradation calculated from measured VS content (S_VS) or from gas production (S_GP) according to Chen & Hashimoto (Eq. 12) for thermophilic cellulose and maize silage degradation on batch mode.

3.5.2 Calculation of substrate carbon content and uptake

The chemical oxygen demand (COD) is a mass balance parameter commonly applied in anaerobic waste water modelling. However the use of COD was not feasible due to technical difficulties in measuring COD of the solid substrate and of the digestate. Instead the initial substrate concentration in the model was calculated in g carbon [g C] from its composition. The carbon content of cellulose was calculated according to its molecular formula (Tab. 3.4) with the Eq.13.

$$m_{CC} = \frac{5 \cdot M_C}{5 \cdot M_C + 10 \cdot M_H + 5 \cdot M_O} \cdot m_{CVS} \qquad \text{Eq. 13}$$

Where:

m_{CC}	[g]	carbon content of cellulose
m_{CVS}	[g]	volatile solids content of cellulose
M_C, M_H, M_O	[g/mol]	molar mass of C, H or O

The calculation of carbon content in maize was based on the results of Weende and Van Soest analysis of the silage. Additionally the correction of DM silage content was done following WEIßBACH & KUHLA (1995). For the purpose of carbon calculation the volatile compounds lost by volatilization during DM determination were assumed to be lactic acid only as this compound is responsible for the silage preservation. Using the general stoichiometric formula of lipids, proteins, carbohydrates and lactic acid given in

Tab. 3.4 as well as their fraction in maize silage (s. Tab. B.1, Attachment B.1) the initial carbon content of the silage was calculated with the Eq. 14. The time progress of substrate concentration was calculated from GP using the Eq. 12 (s. Chapter 3.5.1).

$$m_{MC} = \left[XL \cdot \frac{6M_C}{6M_C + 32M_H + 2M_O} + XP \cdot \frac{13M_C}{13M_C + 25M_H + 7M_O} + \right. \qquad \text{Eq.14}$$

$$\left. + (NFC + NDF - ADL) \cdot \frac{M_C}{M_C + 2M_H + M_O} + X_{VFA} \cdot \frac{3M_C}{3M_C + 6M_H + 3M_O} \right] \cdot m_{MDM}$$

Where:

m_{MC}	[g]	carbon content of maize silage
M_{MDM}	[g]	dry matter content of maize silage
M_C, M_H, M_O	[g/mol]	molar mass of C, H or O
XL		fraction of crude lipids
XP		fraction of crude proteins
NFC		fraction of non fibrous carbohydrates
NDF		fraction of neutral detergent fiber
ADL		fraction of acid detergent lignin
X_{VFA}		fraction of volatile compounds in the silage

Tab. 3.4 Stoichiometric equations used for calculation of carbon content of the substrate (VDI, 2004, modified)

Substance	Stoichiometric Equation
cellulose	$(C_5H_{10}O_5)_n$
carbohydrates	$(C_5H_{10}O_5)_n$
lipids	$C_6H_{32}O_2$
proteins	$C_{13}H_{25}O_7$
lactic acid	$C_3H_6O_3$

3.5.3 Substrate degradation model

In the study the basic mathematic functions of ADM1 were applied: (1) the first order kinetics (s. Eq. 15) usually used to model hydrolysis or degradation of slowly biodegradable substrates and (2) the Monod kinetics (s. Eq. 16 and 17) mainly applied for anaerobic digestion of easily biodegradable substrates such as lipids or some carbohydrates.

$$\frac{dS}{dt} = -kS \qquad \text{Eq. 15}$$

$$\frac{dS}{dt} = -\frac{\mu_{\max} \cdot S}{K_S + S} \cdot \frac{x}{y} \qquad \text{Eq. 16}$$

$$K_m = \frac{\mu_{\max}}{y} \qquad \text{Eq. 17}$$

$$\frac{dx}{dt} = -y\frac{dS}{dt} \qquad \text{Eq. 18}$$

Where:

S– Substrate concentration [g C/l]
t– Time [d]
k – First order kinetic constant [d^{-1}]
K_s – Substrate half saturation constant [g C/l]
y – Microbial yield coefficient [g C/g C]
x – Microbial concentration [g C/l]
K_m – Monod maximum specific uptake rate [g C/(g C·d)]
μ_{max}– Monod maximal specific growth rate of bacteria [d^{-1}]

The Monod model includes simulation of the bacterial growth (s. Eq. 18) with the assumed initial bacterial concentration of x_0. The relationship between the parameter from the Monod equation (Monod maximal specific growth rate of bacteria μ_{max}, substrate half saturation constant K_s) is presented in Fig. 3.10.

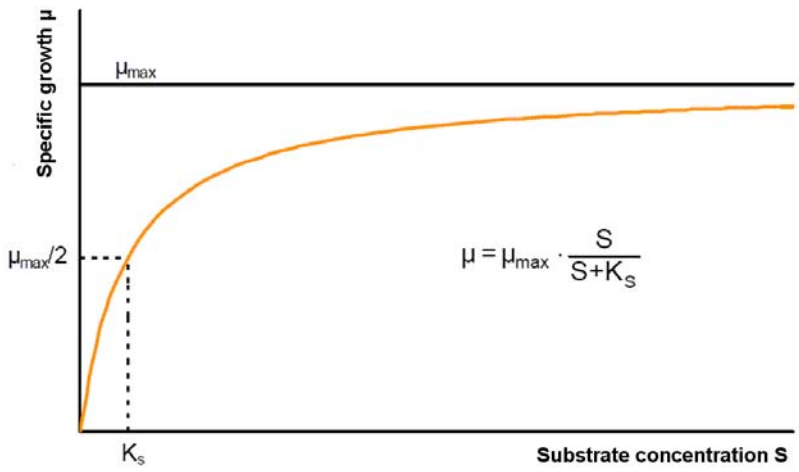

Fig. 3.10 Dependence of specific bacterial growth (μ) on substrate concentration (S) in Monod kinetic, where Ks is substrate half saturation constant (Gerber, 2009)

In the following approach no intermediate reactions were considered but only substrate degradation calculated from GP with Chen & Hashimoto formula was modelled (s. Eq. 12, Chapter 3.5.1).

For majority of 1st order and Monod curves the fitting of the measured points (values calculated from gas production with the help of Eq. 12) was done following the least squares method. This approach tries to find the best curve fit for all the points measured. However, in particular experiments there were much more points available for the final digestion stage characterizing the degradation of smaller quantities of substrate. Consequently, the curve of the least squares was in some cases showing a nearly perfect fit for the final steps but a worse fit for the initial degradation period, in which the largest part of the substrate was converted. To remove this undesired effect for some curves within the Monod or 1st order approach, the least squares fitting was improved manually, so that the focus was put mainly on the points measured in the initial part of the digestion.

In the first step all degradation curves were tried to be fitted with the same set of parameters. These turned out to be impossible. Consequently the kinetic parameters were adapted for each degradation curve separately. The extensive overview of first order and Monod kinetic parameters obtained for degradation of similar substrates to those applied in this study is presented in Tab. D.1 (Attachment D) and Tab. E.1 (Attachment E). For all Monod fits the initial microbial concentration (x_0) of 0.001 g C/l and microbial yield coefficient (y) of 0.05 g C/g C were applied. The value of x_0 was estimated basing on the VS content of the inoculum. The further bacterial growth was simulated by the model following Eq. 17. The value of y was chosen similar to that

commonly applied for acetoclastic methanogenesis and acetogenesis (BATSTONE ET AL., 2002; VAVILIN ET AL., 2008). The sensitivity of both x_0 and y was very low within the range considered as realistic for the parameters. The sensitivity analysis for the Monod model was performed with the local approach following the method of step variation of single parameter (KIM ET AL., 2006). Each reference parameter value was changed separately stepwise by 10% within the range of 50-200%. The detailed results of sensitivity analysis for locally highly sensitive μ_{max} and slightly sensitive K_s are given in Fig. F.1 to Fig. 4.14 (Attachment F). x_0 and y turned out to be insensitive within the investigated parameter range.

4 Results

4.1 Influence of OLR on thermophilic digestion of cellulose in batch mode

The study compares the results of 6 thermophilic cellulose batch series. The OLR between the subsequent series was increased stepwise by 5.7 kgVS/m³ from initial 5.7 kgVS/m³ to the final value 34.3 kgVS/m³. The objective of the experiment was the comparison of the key process parameter performance for anaerobic digestion of a model substrate (cellulose) under extreme conditions particularly with regard to inhibition signs. The VS ratio of 0.5 suggested by VDI (2004) for uninhibited batch tests was deliberately exceeded in 4 of 6 experimental sets to produce the instability in the reactors.

4.1.1 Biogas production

Independent of the OLR stable specific GP of 605–667 l_N/kgVS was observed in all conducted experimental series. This corresponds to 80–89% of the maximum expected biogas yield from the substrate. Methane content in total biogas of all tests varied between 51–55%. The summarized biogas data are presented in Tab. 4.1.

A considerable extension of total degradation time was observed with increasing OLR (s. Fig. 4.1). In the tests with 5.7–22.9 kgVS/m³ a total degradation time of 9–16 days

Tab. 4.1 Test parameters and summarized biogas results for digestion of cellulose under thermophilic conditions.

parameter		unit	cellulose					
organic loading rate		kgVS/m³	5.7	11.4	17.1	22.9	28.6	34.3
DM content inoculum		% FM	4.5	4.5	4.5	2.8	2.3	2.6
VS content inoculum		%DM	60	54	54	55	46	61
VS substrate/ VS inoculum		-	0.22	0.47	0.71	1.48	2.69	2.1
total specific GP		l_N/kgVS	605	656	654	667	643	662
methane in total biogas		%	55	53	52	50	51	54
% of max. possible biogas yield		%	80	87	87	89	86	88
degradation time	t_{50}	d	3.0	3.8	5.3	6.0	9.0	9.3
	t_{90}	d	7.0	7.5	9.5	12.0	20.0	21.0
	t_{50}/t_{90}	-	0.43	0.51	0.56	0.50	0.45	0.44
daily GPR	max. value[a]	l_N/(kgVS·d)	148 ± 9	129 ± 21	94 ± 2	99 ± 11	45 ± 1	69 ± 12
	day	-	2	3	6	5	6	5

was obtained whereas in batches with 28.6–34.3 kgVS/m³ the total decomposition time doubled in comparison to the lower OLRs. Although the OLR was increased six times, both the time needed in test to produce 50% (t_{50}) and 90% (t_{90}) of the total biogas have tripled between the lowest and the highest OLR. The different OLRs can be divided into 3 groups concerning their degradation times: (1) 5.7–11.4 kgVS/m³, (2) 17.1–22.9 kgVS/m³ and (3) 28.6–34.3 kgVS/m³. Both t_{50}– and t_{90}–values increased proportionally for the subsequent OLR groups, though for group (2) a considerable difference of t_{90} was observed.

The time progress of sGP, sGPR and changes of methane content in biogas are displayed in Fig. 4.1. For the OLR higher than 11.4 kgVS/m³ the maximum sGPR was attained between 5th and 6th day. A gradually decreasing trend of sGPR curves was observed with the increase of OLR, except for 34.3 kgVS/m³. The highest sGPR value of nearly 150 l_N/(kgVS·d) was achieved for 5.7. For 5.7 – 17.1 kgVS/m³ the sGPR dropped almost to 0 after day 12 while for 22.9 kgVS/m³ after day 16. For two highest OLRs the sGPR ranged between 20 and 30 l_N/(kgVS·d) for days 10–20 and finally dropped to 0 on day 30.

A decrease of measured final CH_4 content in biogas was observed with increasing OLR. For the lowest OLR the final content of CH_4 in biogas reached the level of 83%, while for

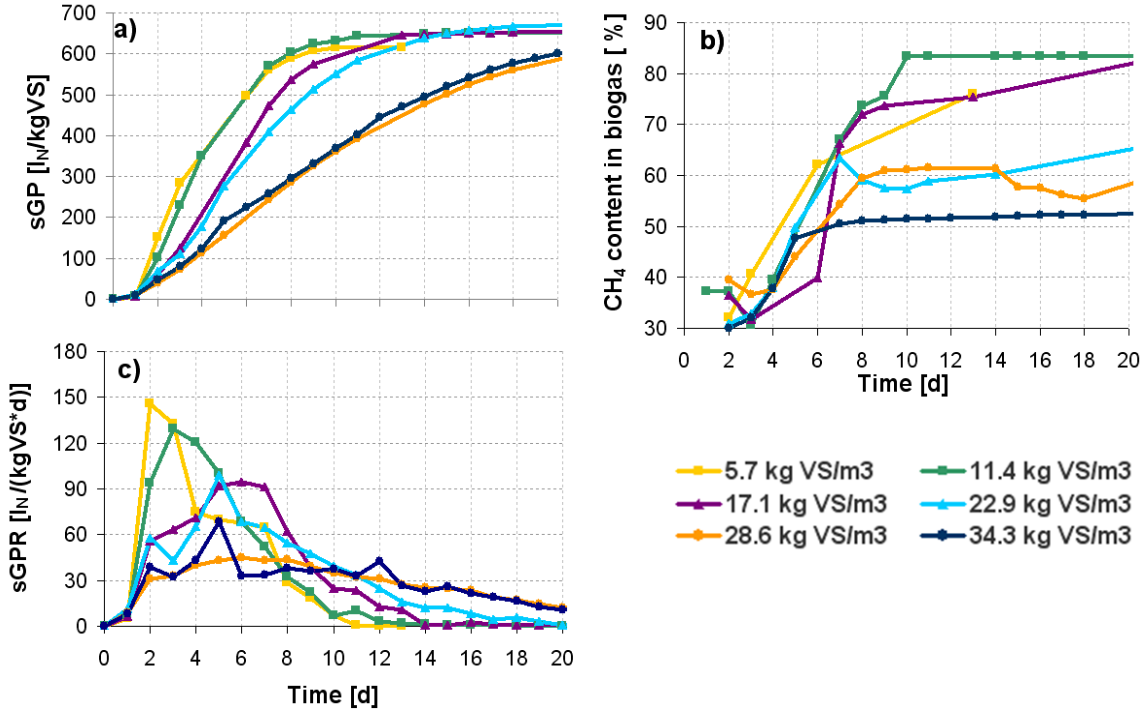

Fig. 4.1 Thermophilic cellulose batches with increasing organic loading rates: (a) cumulative biogas production; (b) changes of CH_4 content in biogas, and (c) changes of daily measured biogas production rate over total degradation time

the highest OLR only 52% were obtained towards the end of the test. The period of lower but constantly increasing CH_4 concentrations associated with hydrolysis and acetogenesis was observed in the first 7 days of digestion for all batches of the experimental study. In the tests with 5.7–22.9 kgVS/m³ at least 60% of biogas was already produced in this degradation phase. For higher OLRs almost 65% of biogas was produced after the final stable methane content of 52% was reached.

In the first step of decomposition (days 2–3), the elevated values of H_2S (300–400 ppm) occurred. Only for 34.3 kgVS/m³ a higher concentration of 1700 ppm was observed. In the subsequent decomposition step H_2S–concentrations dropped to 100–200 ppm.

4.1.2 Parameter changes within the reactor content

An overview of maximum and minimum parameter values as well as the examples of parameter performance over time is given in Tab. 4.2 and Fig. 4.2.

The highest pH was always observed at the beginning of the experiment. For all OLRs a subsequent pH drop associated to VFA production was noticed. For 5.7 –17.1 kgVS/m³ a

Tab. 4.2 pH, titrated volatile acids (TVA), titrated inorganic carbon (TIC), volatile fatty acids (VFA) measured for different organic loading rates for digestion of cellulose under thermophilic conditions

Parameter		Unit	cellulose					
OLR		kg VS /m³	5.7	11.4	17.1	22.9	28.6	34.3
pH	start value	-	8.12	8.27	8.43	8.01	8.34	7.95
	min. (day)	-	7.13 (3)	7.21 (2)	7.21 (5)	6.98 (4)	7.00 (4)	6.83 (3)
	final value	-	8.04	8.11	7.96	7.44	7.82	7.49
TVA	min. (day)	g/l	1.75 (0)	2.13 (0)	2.33 (0)	1.19 (0)	1.10 (1)	1.09 (0)
	max. (day)	g/l	2.98 (1)	5.44 (4)	5.44 (4)	4.57 (4)	4.93 (6)	3.04 (4)
	Δ	-	1.23	3.31	3.11	3.38	3.83	1.95
TIC	min. (day)	g/l	8.14 (2)	7.01 (3)	7.48 (4)	6.05 (3)	4.46 (6)	3.68 (4)
	max. (day)	g/l	10.04 (1)	9.39 (1)	10.38 (0)	8.81 (0)	7.61 (1)	5.43 (2)
	Δ	-	1.90	2.38	2.90	3.24	3.15	1.75
maximum TVA/TIC ratio		-	0.36	0.76	0.73	0.82	1.11	0.82
maximum VFA concentration[a]	acetic	mg/l	1577 ± 54	3993 ± 306	3999 ± 105	3527 ±169	4876 ± 229	2640 ± 103
	propionic	mg/l	258 ± 2	525 ± 25	582 ± 14	850 ± 32	685 ± 130	255 ± 10
	iso-butyric	mg/l	19 ± 1	76 ± 3	118 ± 15	126 ± 6	159 ± 10	105 ± 12
	n-butyric	mg/l	10 ± 1	55 ± 2	105 ± 11	159 ± 10	100 ± 1	95 ± 1
	iso-valeric	mg/l	20 ± 2	78 ± 5	155 ± 15	105 ± 12	91 ± 8	52 ± 1

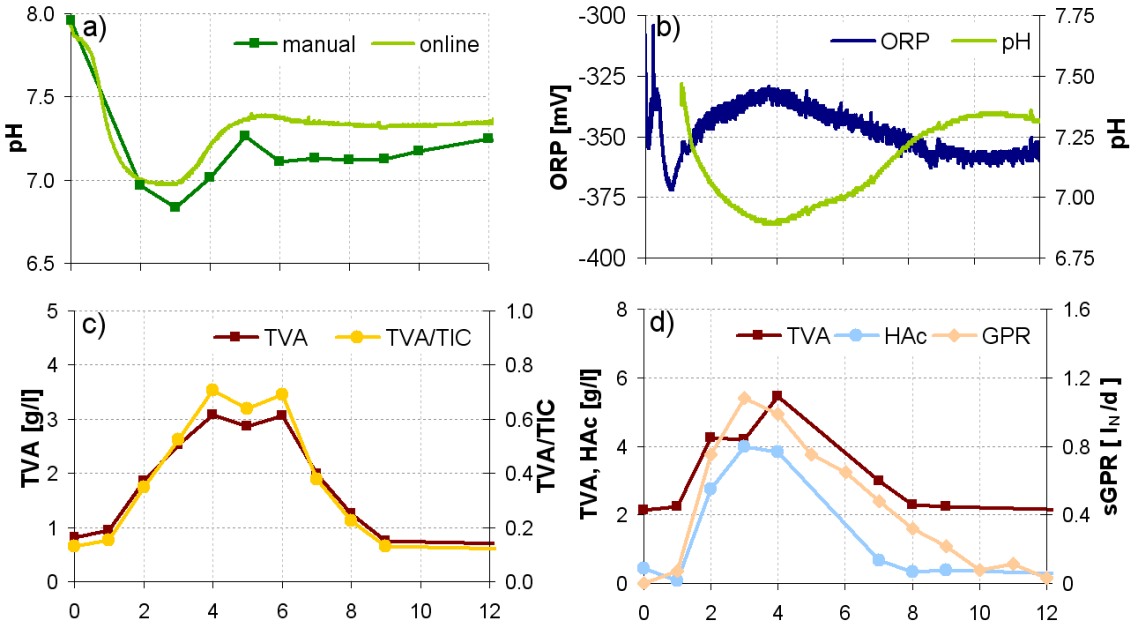

Fig. 4.2 Time evolution of (a) online and manually measured pH values for 34.3 kgVS/m³; (b) oxidation-reduction potential (ORP) and pH for 22.9 kgVS/m³; (c) titrated volatile acids (TVA) and the ratio of titrated volatile acids to inorganic carbon (TVA/TIC) for 22.9 kgVS/m³; (d) TVA, acetic acid (HAc) and specific gas production rate (sGPR) for 11.4 kgVS/m³ during thermophilic digestion of cellulose in batch mode

minimum pH of 7.2 ± 0.1 was observed. For the higher OLRs a minimum pH of 6.9 ± 0.1 was detected. The manually and online measured pH values differed slightly for the period of the minimum drop of the pH (s. Fig. 4.2a). Nevertheless the general pH pattern was similar and the differences were not meaningful considering that the pH values were not measured on the same reactor.

For all tests the ORP value ranged between -330 and -400 mV independent of the OLR. The curves of pH and ORP performed inversely.

A similar trend was observed for TVA and TIC patterns (s. Fig. C.5 and Fig. C.6, Attachment C.2). The maximum or minimum peaks of pH, ORP, TVA, TIC and TVA/TIC-ratio were measured at the same time points (Fig. 4.2b-c). General TVA and TIC pattern was similar for all OLRs. However greater differences between the maximum and minimum could be observed for higher OLRs beginning with 11.4 kgVS/m³ (s. Tab. 4.2). This trend did not continue for 34.3 kgVS/m³. A slighter increase of TVA and drop of TIC were registered for 34.3 kgVS/m³, similar to those observed for 5.7 kgVS/m³. Beginning with 11.4 kgVS/m³ the maximum TVA/TIC-ratio exceeded the value of 0.3-0.4 for all OLRs.

The time progression of GPR plotted with simultaneously measured HAc and TVA values for 11.4 kg VS/m³ is presented in Fig. 4.2d. The increase of the total concentration of

TVA (especially of HAc) and the increase of GPR were observed at exactly the same time. Such parameter coherence could be observed for all OLRs. The high biogas production was always accompanied by elevated values of TVA, VFA and ORP or diminished values of pH.

Changes of VFA patterns and the HPr/HAc ratio during the tests with different OLRs are presented in Fig. 4.3. The measured values of n-HVa were negligible for all experiments (max. 5 mg/l) and did not vary considerably with time.

A typical acid pattern of non-inhibitory decomposition was observed for 5.7 kgVS/m³. HAc and HPr concentrations stayed increased for maximal 5 days with the maximum

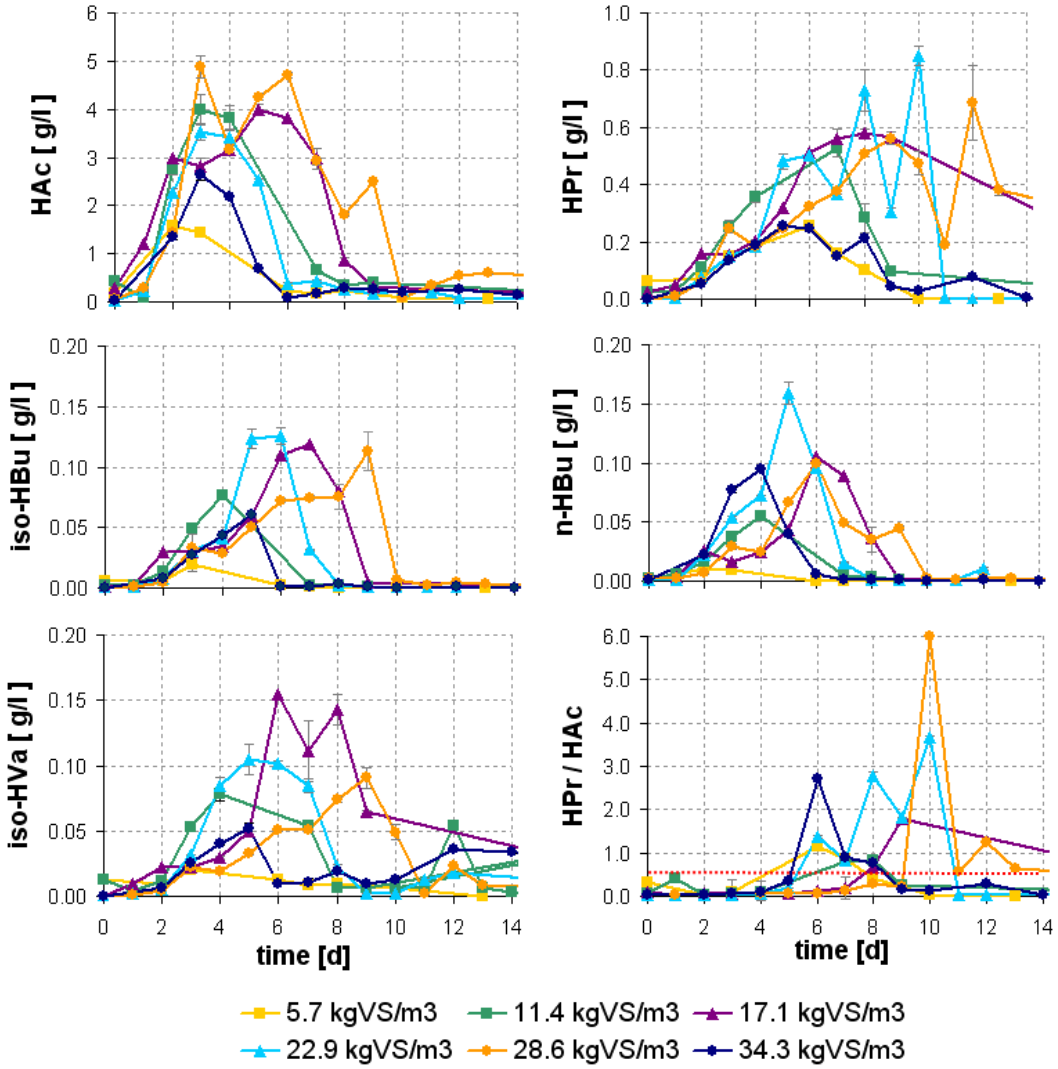

Fig. 4.3 Concentration changes of acetic (HAC), propionic (HPr), iso-butyric (iso-HBu), n-butyric (n-HBu) and iso-valeric acid (iso-HVa) as well as propionic to acetic acid ratio (HPr/HAc) measured for thermophilic cellulose batches. The measured values of n-valeric acid n-HVa were negligible for all experiments (max. 5 mg/l) .

values of 1.6 and 0.3 g/l respectively (s. Tab. 4.2). The other short chain fatty acids remained at the low concentration level with no more than 30 mg/l (s. Fig. 4.3 and Tab. 4.2).

For OLRs of 5.7, 11.4, 22.9 and 34.3 kg VS/m³ the increased concentrations of HAc were observed between day 1 and 6. For 17.1 and 28.6 kg VS/m³ the total degradation time of HAc increased up to 10 days. Generally the concentrations of iso-HBu, n-HBu, iso-HVa, n-HVa did not exceed 160 mg/l in any of the tests (s. Tab. 4.2). The highest maximum concentrations of C_4-C_5 VFA were not observed for the highest OLR but for 17.1 kgVS/m³ or 22.9 kgVS/m³. HPr increased parallel to HAc; however its subsequent degradation was slower than for HAc. In consequence an elevated HPr/HAc ratio was observed for all OLRs towards the end of the test, while for the first 5 days the critical value of 0.5 was not exceeded in any of the test series (s. Fig. 4.3).

4.1.3 Modeling of substrate degradation

In the first step all degradation curves were tried to be fitted with the same set of parameters. These turned out to be impossible. Consequently the kinetic parameters were adapted for each degradation curve separately. For Monod model the values of Ks and μ_{max} were adjusted, while x_0 and y were kept constant. But even for that case the application of both 1st order and Monod model to fit thermophilic degradation of cellulose in batch mode was possible only with certain limitations.

1st order

Unlike for maize silage, for modelling of cellulose degradation with 1st order equation problems with the fitting of the initial 1-4 days[9] of the digestion occurred. Consequently another approach was chosen: the initial 1-2 days of digestion with almost no changes of substrate concentration were excluded from the modeling procedure. However beginning with 22.9 kgVS/m³ the elimination of additional 1 or 2 days was necessary to achieve an acceptable data response to the model curve. An exemplary 1st order fitting curve for thermophilic digestion of cellulose in batch mode is given in Fig. 4.4, while the complete set of cellulose modelling graphics is presented in Fig. D.1 and Fig. D.2 (Attachment D).

The details on the excluded days of digestion and the not modeled fraction of substrate are summarized in Tab. 4.3. For three out of six cellulose fits the degradation of initial

[9] depending on the OLR

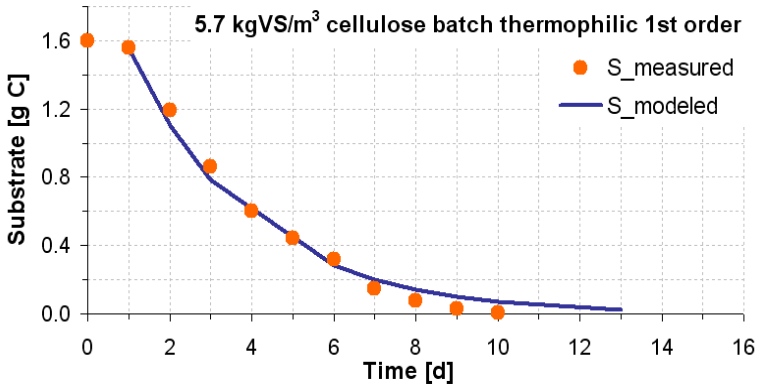

Fig. 4.4 Cellulose degradation under thermophilic conditions in batch mode measured and modeled with 1st order equation. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1).

17–19% of substrate could not be modeled with the 1st order curve. These were all the high OLR experiments with more than 17.1 kgVS/m³ of substrate load. The comparison of 1st order constants reveals the decreasing trend with OLR increase with the k values similar to those observed for maize (s. Chapter 4.3.3) especially for the two lowest OLR (regarded as a good fit).

Tab. 4.3 Summary of 1st order parameters obtained for thermophilic digestion of cellulose in batch mode (the OLRs which could be fitted well are marked grey). Measured values are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1).

OLR [kgVS/m³]	not modelled initial days	modelled fraction of S % of S	S_0 measured [gC]	S_0 modelled [gC]	k [d^{-1}]
5.7	1	97	1.56	1.55	0.34
11.4	1	99	2.73	2.84	0.29
17.1	2	90	4.32	4.73	0.25
22.9	3	83	5.33	5.49	0.20
28.6	4	83	6.62	6.93	0.12
34.3	4	81	7.75	8.23	0.14

Monod

For all fits the initial microbial concentration (x_0) of 0.001 g C/l and microbial yield coefficient (y) of 0.05 g C/g C were applied. The value of x_0 was estimated basing on the VS content of the inoculum. The y was chosen similar to the value commonly applied for acetoclastic methanogenesis and acetogenesis (BATSTONE ET AL., 2002; VAVILIN ET AL., 2008). The sensitivity of both x_0 and y (calculated basing on the local approach) was very low within the range considered as realistic for the parameters.

For the methodical approach chosen in the Monod model (similar x_0 and y parameters for all curve sets as well as Ks and U_{max} ranging within certain limits) problems with the fitting of the final stage of the digestion occurred. Such model was not able to predict the retarded substrate degradation on the final phase (s. Fig. 4.5). Nevertheless a good fit was obtained for the degradation of initial 60-71% of the substrate. Two highest OLRs of cellulose in thermophilic mode could not be modeled with such Monod approach (s. Fig. 4.5). In these experiments a good fit was obtained only for the degradation of the initial 44-47 % of the cellulose while the degradation of the residual biomass proceeded much slower than predicted by the model.

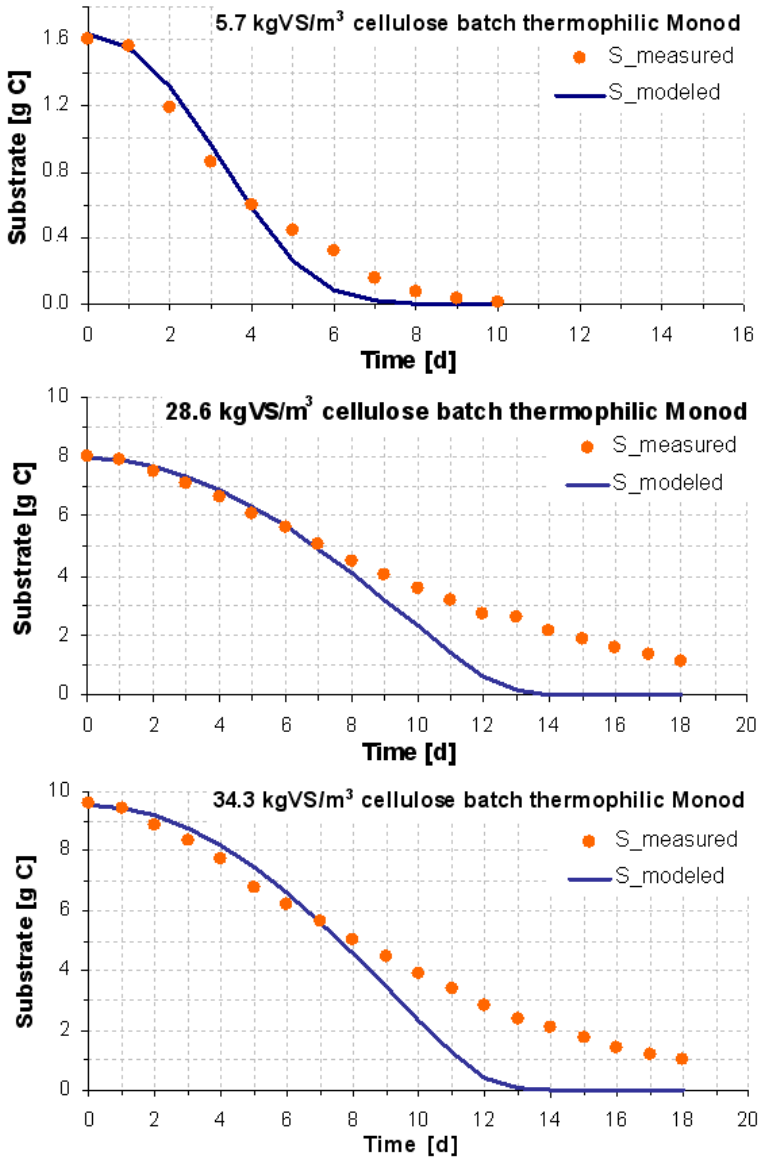

Fig. 4.5 Examples of modeled and measured cellulose degradation under thermophilic conditions in batch mode with Monod fit. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1).

The summary of the kinetic parameters for all batch series is given in Tab. 4.4. However, due to invalidity of the model for higher OLRs of cellulose experiments the following parameter analysis considers only thermophilic cellulose batches at 5.7-22.9 kgVS/m^3.

A quite stable K_S ranging between 0.41 and 0.49 was fit for the degradation series. Both K_m and μ_{max} showed an increasing trend with OLR decrease. K_m was within a range 0.66-2.75, while μ_{max} ranged between 0.03 and 0.14. The local sensitivity analysis revealed half saturation constant Ks as a slight sensitive parameter and the Monod maximum specific growth rate of bacteria μ_{max} as a highly sensitive one. The detailed results of sensitivity analysis are put together in Attachment F. The changes of K_s within a range (-40%; +100%) caused only minor changes of substrate uptake curve while for μ_{max} already the increase or decrease of the parameter by 20% resulted in serious changes of the curve progression.

Tab. 4.4 Summary of Monod parameters obtained for thermophilic digestion of cellulose in batch mode (the OLRs which could be fit well are marked grey). A literature overview of comparable parameters is given in Tab. E.1 (Attachment E). For all fits the initial microbial concentration (x_0) of 0.001 g C/l and microbial yield coefficient (y) of 0.05 g C/g C were applied.

OLR [kgVS/m^3]	S_0 calculated	modelled		Monod constants			
		days	fraction of S % of S	S_0	μmax	Km	Ks
5.7	1.6	0-4	63	1.63	0.14	2.75	0.49
11.4	3.2	0-5	64	3.23	0.08	1.52	0.41
17.1	4.8	0-8	82	4.80	0.04	0.80	0.41
22.9	6.4	0-7	60	6.20	0.03	0.66	0.42
28.6	8.0	0-6	44	8.00	0.02	0.36	0.41
34.3	9.6	0-7	47	9.60	0.02	0.38	0.43

4.2 Substrate and temperature influence on digestion in batch mode

The objective of the study was the analysis of the influence of different substrates or temperatures on anaerobic digestion performance. The results of 12 experimental series are compared in the following chapter. All tests were conducted in batch mode. Two substrates and two temperature modes were examined. Performance of anaerobic biocenosis was investigated for 3 analogue OLRs in each temperature mode and with each substrate.

4.2.1 Biogas production

The time evolution of total sGP and specific biogas production rate (sGPR) for selected batches of maize and cellulose is displayed in Fig. 4.6 and Fig. 4.7. The complete set of the graphics is put together in Fig. C.3 and Fig. C.4 (Attachment A), while more detailed digestion results are listed in Tab. 4.5. For all experiments certain trends related to the degraded substrate or digestion temperature were observed[10].

Degradation trends linked to the substrate

For maize degradation the differences between sGP patterns for different OLRs were not that considerable, while for cellulose a clear flattening of the sGP curve was observed for

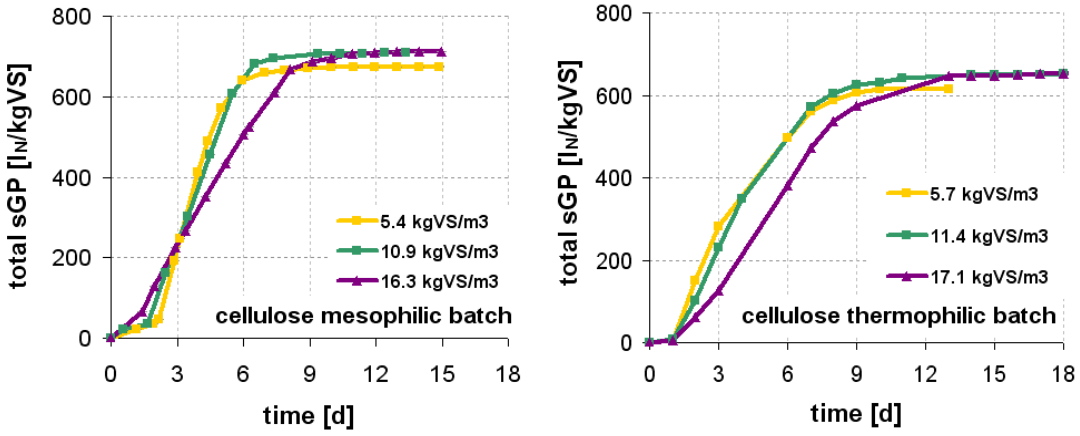

Fig. 4.6 Time progress of specific biogas production (sGP) for cellulose in mesophilic and thermophilic batch tests

[10] Both sGP and sGPR for 5.7 kgVS/m³ maize in thermophilic mode proceeded untypical, which is being associated to the too low VS content of the C.1 inoculum (s. Tab. B.2, Attachment B.2) and therefore not included in the further analysis.

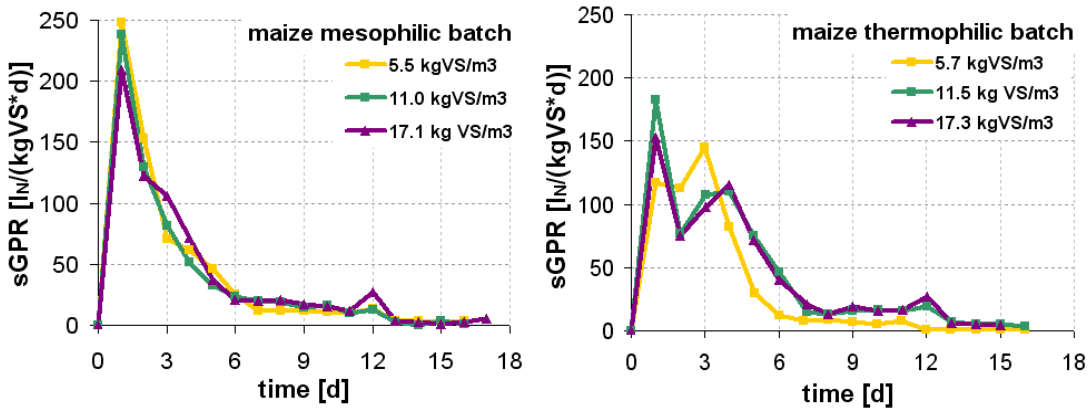

Fig. 4.7 Time progress of specific biogas production rate (sGPR) for maize degradation in mesophilic and thermophilic batch tests

the highest compared OLRs in both temperature modes[11]. The maximum sGPR was always measured for maize independent of the temperature and was observed on the first day of digestion. For cellulose the maxima occurred later than for maize but the enhanced sGPR values were registered generally for a longer time period. For maize at both temperatures (38°C and 55°C) an increase of sGPR on day 12 was registered but explicit for the higher OLRs. Both sGPR and sGP showed a delay in cellulose degradation more pronounced for thermophilic conditions.

Higher substrate degradation level and higher CH_4 content was measured for degradation of maize than for cellulose (s. Tab. 4.5). The degradation of cellulose was slower in the first stage (t_{50}) but outrun maize (t_{90}) for the second digestion period. The t_{50} range for different OLR of maize was narrower than for cellulose while for t_{90} the contrary was observed.

Degradation trends linked to the temperature mode

Higher sGPRs were measured at 38°C. Mesophilic conditions supported faster degradation with clear one-peak sGPR pattern. In thermophilic mode the sGPR peaks flattened, while for maize digestion even a multiple rate maxima could be distinguished.

In general, higher total biogas yield was obtained for mesophilic temperature ranges[12]. In 4 out of 6 cases for similar substrate and corresponding OLRs the CH_4 content in total biogas was higher for mesophilic experiments. 90% of total GP were reached later at 38°C whereas for t_{50} the trend was not that clear.

[11] cellulose digested at 16.3 kgVS/m³ in mesophilic and 17.1 kgVS/m³ in thermophilic

[12] Except for maize at 11.0 kgVS/m³ (mesophilic) and at 11.5 kgVS/m³ (thermophilic)

Tab. 4.5 Summarized biogas data for digestion of maize and cellulose under mesophilic and thermophilic conditions in batch mode

temperature mode	substrate	OLR [kgVS/m^3]	total sGP [l_N/kgVS]	CH_4 in total biogas [%]	% of max. possible biogas yield	t_{50} [d]	t_{90} [d]
mesophilic	cellulose	5.4	633	55	84	3.4	4.9
		10.9	680	58	90	3.8	5.8
		16.3	700	56	93	4.3	7.8
	maize	5.5	646	60	97	1.5	7.0
		11.0	647	59	97	1.5	8.0
		17.1	690	55	103	2.1	9.0
thermophilic	cellulose	5.7	605	55	80	3.0	7.0
		11.4	656	53	87	3.8	7.5
		17.1	654	52	87	5.3	9.5
	maize	5.7	585	56	87	2.5	7.0
		11.5	706	57	106	3.0	9.0
		17.3	680	56	102	3.5	11.0

4.2.2 Parameter changes in digestate

VFA trends

A complete set of graphics presenting performance of VFA measured on the reactor content for batch fermentation of maize and cellulose under mesophilic and thermophilic conditions is put together in Fig. 4.8 and Fig. 4.9.

For maize at higher OLRs (17.1 kgVS/m^3 mesophil and 11.5 and 17.3 kg VS/m^3 thermophil) HAc attained very high concentrations (s. Fig. 4.8). The C_4-C_5 VFA did not exceed 200 mg/l except for n-HBu. For all maize batch series n-HBu reached very high concentrations (higher in thermophilic than mesophilic mode) similar to the values typical for HAc (s. Fig. 4.9). Both n-HBu and HPr were increased simultaneous to enhanced HAc, however HPr raise followed after n-HBu degradation. HPr concentration dropped after HAc uptake. In general VFA trends for 17.1 kgVS/m^3 in mesophilic mode were even more extreme than the patterns observed at high OLRs for thermophilic conditions.

Unlike maize, VFA degradation peaks for cellulose were comparable for single C_3-C_5 VFA independent of the OLR and temperature mode. The typical VFA performance with increased HAc and only moderately enhanced HPr (s. Fig. 4.8) was observed for all investigated OLRs and temperatures. Contrary to maize the differences between HAc and HPr patterns for two highest OLRs of cellulose in thermophilic mode were not that

pronounced. Only insignificant amounts of n-HVa were measured, while the remaining C_4-C_5 VFA did not exceed 50mg/l for mesophilic and 150 mg/l for thermophilic mode. HAc concentrations measured in thermophilic mode were nearly 3 times higher than for mesophilic conditions.

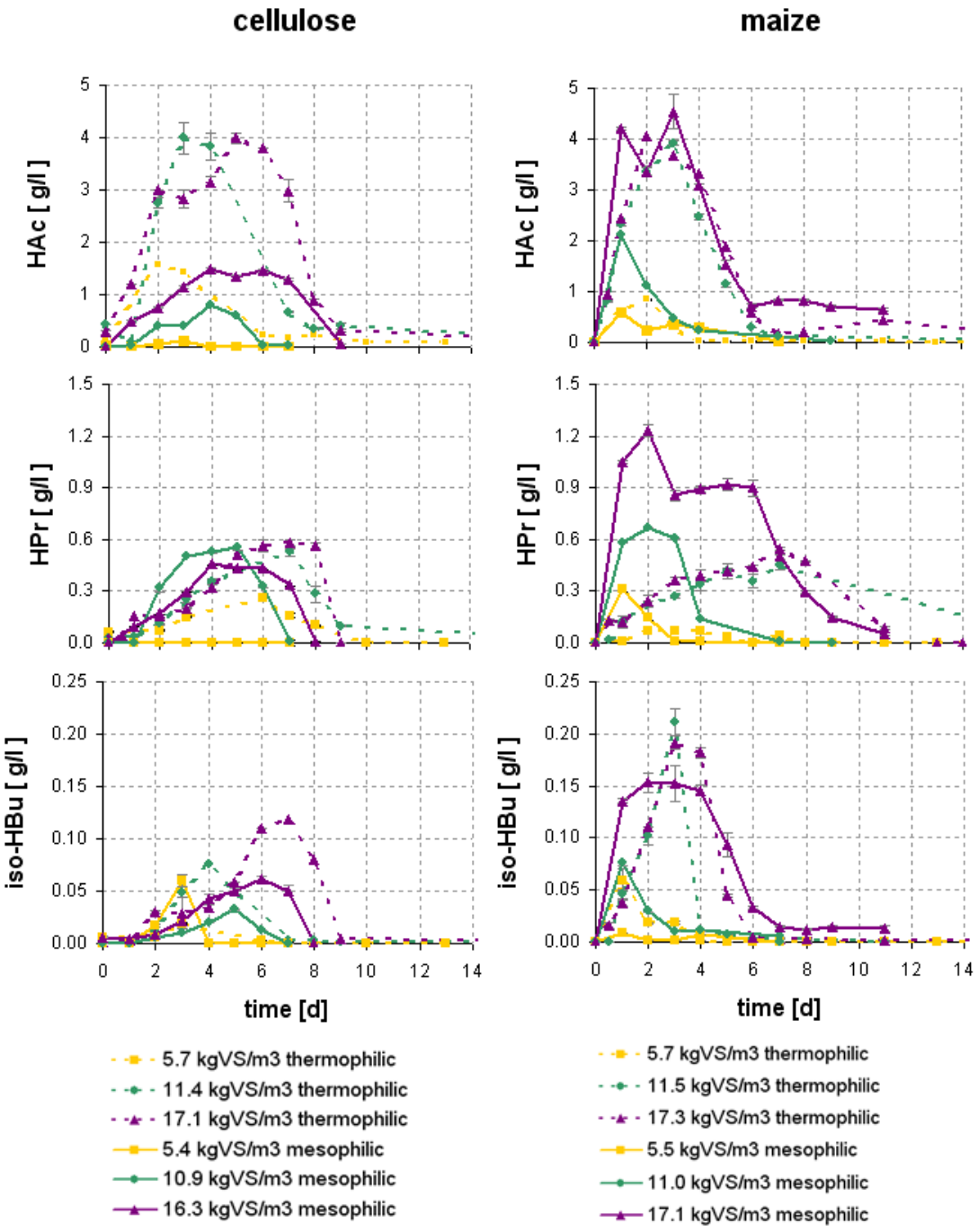

Fig. 4.8 Concentration changes of acetic (HAc), propionic (HPr) and iso-butyric acid (iso-HBu) measured for thermophilic and mesophilic digestion of cellulose and maize in batch mode

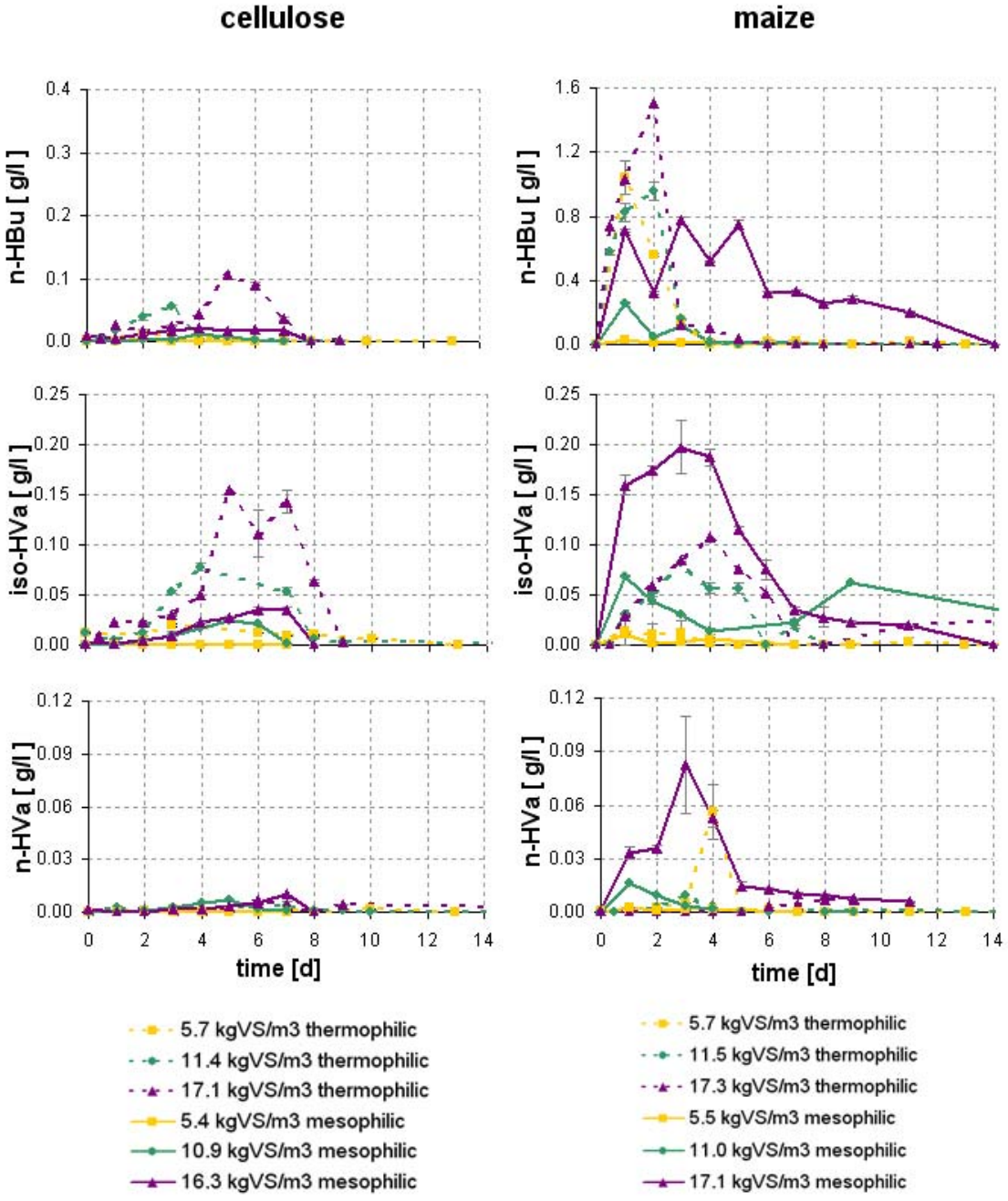

Fig. 4.9 Concentration changes of n-butyric (n-HBu), iso-valeric (iso-HVa) and n-valeric acid (n-HVa) measured for thermophilic and mesophilic digestion of cellulose and maize in batch mode

TVA and TIC trends

A typical example of time progress of TIC, TVA and TVA/TIC ratio is presented in Fig. 4.10, while a complete set of graphics for all experimental series is given in Attachment C.2 (Fig. C.5 and Fig. C.7). Independent of the applied substrate or

temperature conditions, the TVA maxima and TIC minima were measured between 1st and 3rd day of digestion although not always on the same day for one trial. The difference Δ between maxima and minima rose with the OLR increase. However for both TVA and TIC Δ was considerably lower in mesophilic tests. In all tests the TVA/TIC ratio ranged between 0.1 and 0.3 at the beginning and towards the end of the experiment. The higher the OLR was, the longer was the period of TVA/TIC higher than 0.4 independent of the temperature mode and substrate.

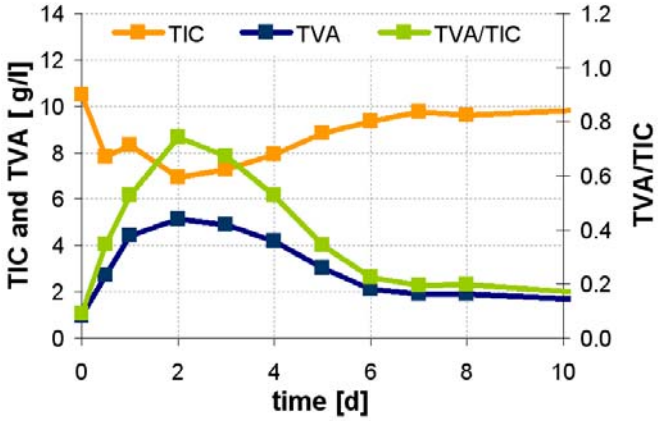

Fig. 4.10 A typical time sequence of titrated inorganic carbon (TIC), titrated volatile acids (TVA) and their ratio (TIC/TVA) presented for 17.3 kgVS/m³ maize in batch mode under thermophilic conditions

For maize TIC and TVA patterns for different temperature modes were similar for corresponding OLRs. TVA/TIC curves reached nearly similar maxima for comparable OLR at 38°C and 55°C. Only for 5.5 kgVS/m³ at mesophilic conditions both TVA/TIC limits of 0.3 and 0.4 were not exceeded.

The smallest value ranges for both TIC and TVA were measured for digestion of cellulose in mesophilic mode. Unlike for other tests, no pronounced increasing trend of ΔTVA and ΔTIC was observed for these experimental series, even though the carbonate buffer was much lower than in the other experiments. The maximum measured TVA/TIC ratio only slightly exceeded 0.4 for the highest OLR.

pH and ORP

An overview of pH and ORP trends is given in Tab. 4.6, while the detailed time progress of the parameters is shown in Fig. C.9 and Fig. C.10 (Attachment C.3). Both initial (7.60–8.60) and final (7.39–8.11) pH ranged within slight alkali conditions, except for 5.7 kgVS/m³ maize under thermophilic conditions. For maize the strongest pH drop was registered with one exception (11.5 kgVS/m³ thermophilic) directly on the 1st day of digestion, while for cellulose between the 2nd and the 5th day but always corresponding

Tab. 4.6 An overview of pH and ORP values measured for thermophilic and mesophilic digestion of maize and cellulose in batch mode

temperature mode	substrate	OLR [kgVS/m³]	pH			ORP [mV]		
			start value	min. (day)	final value	value range min.	max.*	Δ
mesophilic	cellulose	5.4	7.80	7.26 (3)	7.39	-322	-181	141
		10.9	7.60	7.23 (2)	7.40	-329	-262	67
		16.3	7.62	7.05 (5)	7.54	-332	-244	88
	maize	5.5	8.22	7.31 (1)	7.46	-380	-358	22
		11.0	8.27	7.03 (1)	7.35	-361	-340	21
		17.1	7.60	6.89 (1)	7.46	-386	-352	34
thermophilic	cellulose	5.7	8.12	7.13 (3)	8.04	-399	-343	56
		11.4	8.27	7.21 (2)	8.11	-	-	-
		17.1	8.43	7.21 (5)	7.96	-	-	-
	maize	5.7	7.70	6.32 (1)	6.98	-417	-332	85
		11.5	8.60	7.14 (3)	7.61	-417	-369	48
		17.3	7.92	7.18 (1)	7.84	-428	-369	59

* after reaching anaerobic conditions

for similar OLRs independent of the temperature mode. Independent of the OLR the complete pH regeneration was never attained at the end of the experiment but also no decrease of the final pH with the OLR raise was observed.

For all experimental series ORP ranged between -330 mV and -428 mV which correspond to the ideal conditions of acetogenesis and methanogenesis (s. Chapter 2.5.3). The exception to this was measured for mesophilic cellulose digestion, for which the rapid changes of ORP between days 0-2 reached even -181 mV regarded as typical for acidogenic and hydrolytic conditions. The final stable ORP value ranged always between -300 mV and -360 mV independent of the substrate and temperature mode. Similar to TVA and TIC, the narrowest pH and ORP range was measured for maize digested at mesophilic conditions.

4.2.3 Modeling of substrate degradation

In the first step all degradation curves were tried to be fitted with the same set of parameters. These turned out to be impossible. Consequently the kinetic parameters were adapted for each degradation curve separately. For Monod model the values of Ks and μ_{max} were adjusted, while x_0 and y were kept constant. For all cellulose batch experiments problems similar to those described in Chapter 4.1.3 were observed. Modeling of cellulose degradation with 1st order was possible only after excluding the initial 2 digestion days corresponding to 3-18% of the substrate (s. Tab. 4.7, Fig. D.1

and Fig. D.4 Attachment D). However, considering two lowest OLRs of cellulose, only 1-7% of cellulose was not fitted by the model, independent of the temperature mode.

The Monod model allowed a very good fit of the initial degradation stage of cellulose but with the chosen methodical approach (similar x_0 and y parameters for all curve sets as well as Ks and U_{max} ranging within certain limits) it could not follow the decrease of the degradation rate towards the end of the experiment (s. Fig. E.1 and Fig. E.3, Attachment E). This means that the degradation of even up to 37% of the substrate was not fitted by the model.

The degradation of maize with its higher structural complexity was better fitted with the 1st order model and could not be followed by the Monod model[13]. An example of 1st order and Monod fit for mesophilic digestion of maize is shown in Fig. 4.11, while the complete set of modeled curves is displayed in Fig. D.3, Fig. D.5, Fig. E.4 and Fig. E.5 (Attachment D and E).

Tab. 4.7 Summary of 1st order parameters obtained for mesophilic and thermophilic digestion of cellulose and maize in batch mode. Measured values are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1)

substrate	Tempe-rature	OLR [kgVS/m^3]	not modelled initial days	modelled fraction of S % of S	S_0 measured [gC]	S_0 modelled [gC]	k [d^{-1}]
cellulose	38°C	5.4	2	93	1.41	1.52	0.58
		10.9	2	95	2.89	3.20	0.49
		16.3	2	82	3.75	3.95	0.28
	55°C	5.7	1	97	1.56	1.55	0.34
		11.4	1	99	2.73	2.84	0.29
		17.1	2	90	4.32	4.73	0.25
maize	38°C	5.5	0	100	1.47	1.45	0.41
		11.0	0	100	2.90	2.85	0.39
		17.1	0	100	4.69	4.69	0.32
	55°C	5.7	0	100	1.50	1.51	0.36
		11.5	0	100	3.13	3.13	0.26
		17.3	0	100	4.59	4.65	0.24

[13] Monod Model was initially developed to describe the growth of homogenous bacterial culture on simple substrates and does not describe well the growth of homogenous cultures on homogenous substrates (TE BOEKORST ET AL.,1981). Monod equation also turned out to be useful in modelling of some steps of anaerobic digestion. However degradation of a complex substrate such as maize silage in one step (without intermediates) and without considering different substrate fractions (such as fats, proteins and carbohydrates) cannot be entirely followed.

Consequently only the 1st order model is discussed in the following chapter as it delivers important information about degradation performance for both substrates. The summary of the 1st order parameters obtained for digestion of maize and cellulose at thermophilic and mesophilic conditions is given in Tab. 4.7. For each experimental series independent of the substrate and operating temperature the fitted first order kinetic constants ranged between 0.24 and 0.58 and decreased with OLR increase. Except for cellulose at 38°C, the k range between the lowest and the highest OLR within a series did not exceed 0.12. For both substrates higher k values were obtained for respective OLRs at mesophilic conditions. In general cellulose degradation was faster than maize at 38°C for OLRs not exceeding 12 kgVS/m³, while at 55°C both maize and cellulose degradation proceeded at similar rates.

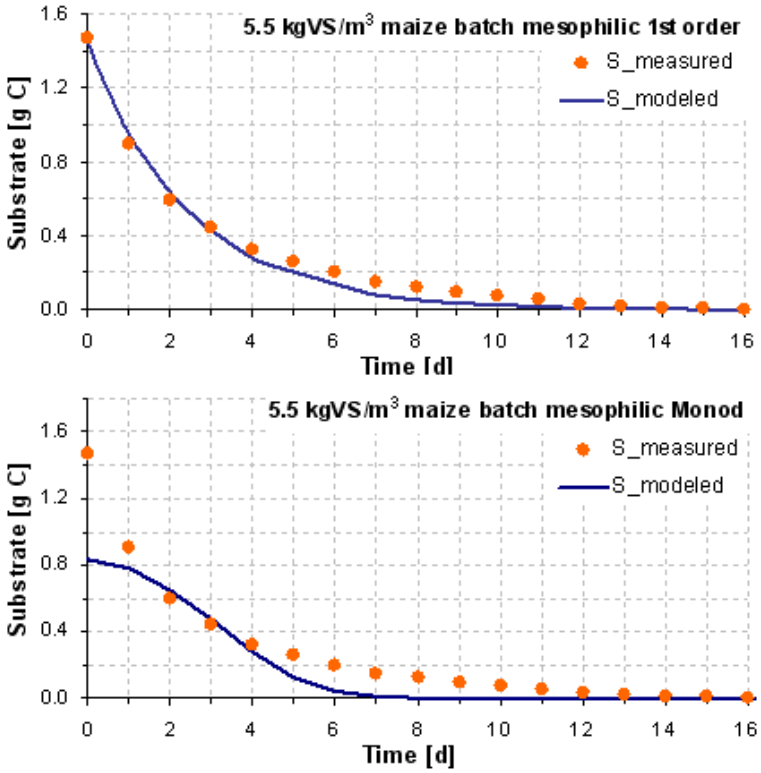

Fig. 4.11 Examples of modeled and measured maize degradation under mesophilic conditions in batch mode with Monod fit. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1).

4.3 Impact of operating mode on thermophilic degradation of maize silage

The following comparison is based on the results of 9 experimental series performed in different operating modes but for comparable OLRs. The subject of the study was the comparison of fermentation in batch, semi-batch and continuous mode for degradation of agricultural maize silage under thermophilic conditions.

4.3.1 Biogas production

Comparison of total biogas yield and quality for all operating modes

An overview of the experimental results in terms of biogas characteristics is given in Tab. 4.8. This comparison was possible for all three experimental modes: batch, semi-batch and continuous mode. Due to irregularities in the digestion performance[14] the following analysis excludes two test series: 11.7 kgVS/m³ in continuous mode and 5.7 kgVS/m³ in batch mode.

Tab. 4.8 Test parameters and summarized data for digestion of maize in different operating modes

parameter	unit	batch			semi-batch*			conti**		
OLR	kgVS/m³	5.7	11.4	17.1	5.9	11.7	17.6	4.1	5.9	11.7
total biogas yield	l_N/kgVS	(585) ± 5%	706 ± 1%	680 ± 1%	638 ± 10%	650 ± 6%	709 ± 8%	697 ± 1%	764 ± 9%	(170) ± 1%
% of max. biogas yield	%	87	106	102	95	97	106	104	114	25
% CH_4 in total biogas	%	54.8 ± 0.1%	57.2 ± 0.1%	55.4 ± 0.1%	54.9 ± 0.6%	54.4 ± 0.1%	54.4 ± 0.1%	53.6 ± 0.1%	52.4 ± 0.5%	22.8 ± 4.8%

* The reactors were charged every 3rd day with the given OLR

** The reactors were charged daily with the given OLR

In the experiments total biogas yield (Y_B) ranged between 95 and 114% of the maximum expected value, assuming 5% substrate loss for bacterial growth and 5% substrate leftovers in the effluents (s. Chapter 3.1). For semi-batch and continuous experiments total biogas yield increased with the raise of the OLR even exceeding the value regarded as maximal yield from the substrate[15]. The differences of CH_4 content in total biogas for different OLRs and operating modes did not exceed 5%. A slight but regular drop of CH_4

[14] The lowest sGP and CH_4 content were registered for 11.7 kgVS/m³ in continuous mode and were a result of digestion cease caused by acidosis within the reactor. A smaller sGP and lower CH_4 content were also measured for 5.7 kgVS/m³ in batch mode. This is considered as an effect of too low VS content and therefore smaller activity of the inoculum (s. Tab. B.2, Attachment A). Consequently both experimental series were not included in the following GP analysis in this chapter.

[15] calculated from the substrate composition

content in total biogas was observed with the increase of the loading frequency. The CH_4 content decreased from 55-57% for batch mode and 54-55% for semi-batch to 52-53% in continuous digestion.

Semi-batch digestion

As the reactors were fed every third day, the calculated daily OLRs were 2.0, 3.9 and 5.9 kgVS/(m³·d) respectively. The detailed changes of sGP calculated basing on the fed maize charge for the subsequent feedings are given in Fig. 4.12b. For all OLRs a lower sGP was measured for the 1st feeding period. In the tests with 5.9 kgVS/m³ and 11.7 kgVS/m³ the typical sGP was obtained already after the 2nd feeding period, while for 17.6 kgVS/m³ the sGP equalled the similar level for the 3rd feeding period. Further a general long-term reduction of sGP was registered for the highest OLR beginning with the feeding 7. The sGP did not diminish rapidly though a final decrease by 10-15% was

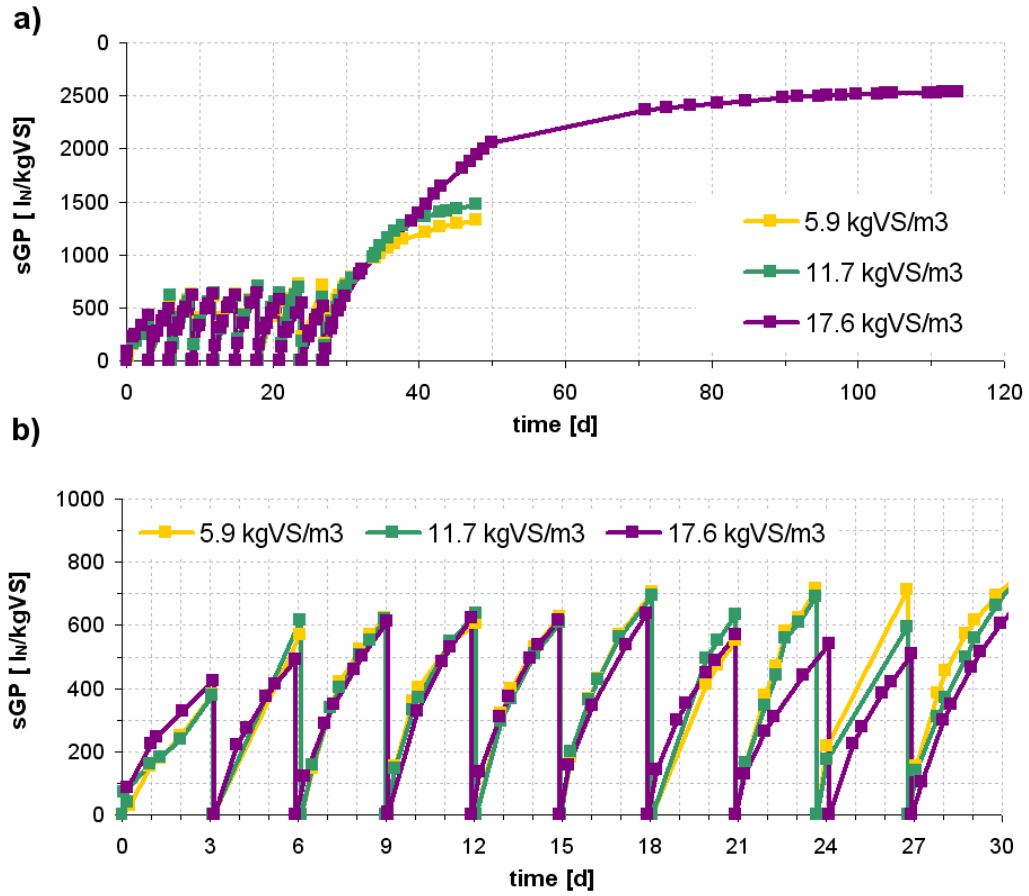

Fig. 4.12 Specific biogas production (sGP) calculated basing on the fed maize charge observed for semi-batch experiments (a) for the whole experimental period including the final gas production after the feeding stop, (b) only for the loading periods.

observed in comparison to feedings 4-6. Unlike for the other OLRs, sGP observed for the highest OLR never reached the level of the maximum expected GP of 669 l_N/kgVS (for details s. Chapter 3.1). Concerning only the feeding periods, the average substrate to biogas conversion amounted 87±10%, 84±6% and 75±10% respectively[16] for increasing OLRs. Additionally to the biogas production certain maize silage amount (up to 5%) has to be assumed as a substrate for bacterial growth and a further fraction (also up to 5%) is expected to be found in digestate (for details s. Chapter 3.1). The final sGP (after day 30) resulting from the substrate accumulation during semi-batch feeding is presented in Fig. 4.12a. For 5.9 kgVS/m³ and 11.7 kgVS/m³ the final sGP corresponds to the average substrate accumulation of 9±4% and 11±1% per feeding period respectively. The substrate accumulation observed for the highest OLR was more than 2 times higher in comparison to both lower OLRs; it reached the average of 27±4% per feeding period. However, for the highest OLR the accumulation was apparently stronger at the beginning of the test and after feeding 7 (s. Fig. 4.12a).

Continuous digestion

The time progression of sGP during feeding periods for all OLRs is presented in Fig. 4.13b. After the first feeding nearly the same sGP of ~200 l_N/kgVS was obtained independent of the OLR. These results are comparable with the sGP reached after the first day of digestion for the first feeding period in semi-batch (s. Fig. 4.12b). For both 4.1 kgVS/m³ and 5.9 kgVS/m³ sGP increased after 2nd feeding period and reached the stable level of 600-700 l_N/kgVS after the 3rd feed. The average substrate removal per feeding period[17] calculated for 4.1 kgVS/m³ and 5.9 kgVS/m³ amounted 83±3% and 91±9% respectively. The sGP observed after feeding period (final sGP) resulted from fermentation of the substrate accumulated during the whole digestion time (s. Fig. 4.13a). According to the final sGP the average substrate accumulation of 16±1% and 23±1% per feeding can be assumed for 4.1 kgVS/m³ and 5.9 kgVS/m³ respectively.

In the test with 11.7 kgVS/m³ a direct sGP decrease was observed already after feeding 2. The gradual sGP drop finished after feeding 6 with the ultimate cease of GP. In this test series the sGP began to drop even before its typical level for maize could be achieved (s. Fig. 4.13b). The maximum sGP reached was by 50% lower than the value measured in the two other OLRs. The GP cease supported by olfactometric analysis of reactor content and the observed very low total methane production was the direct

[16] calculated excluding feed 1

[17] calculated excluding feed 1-2

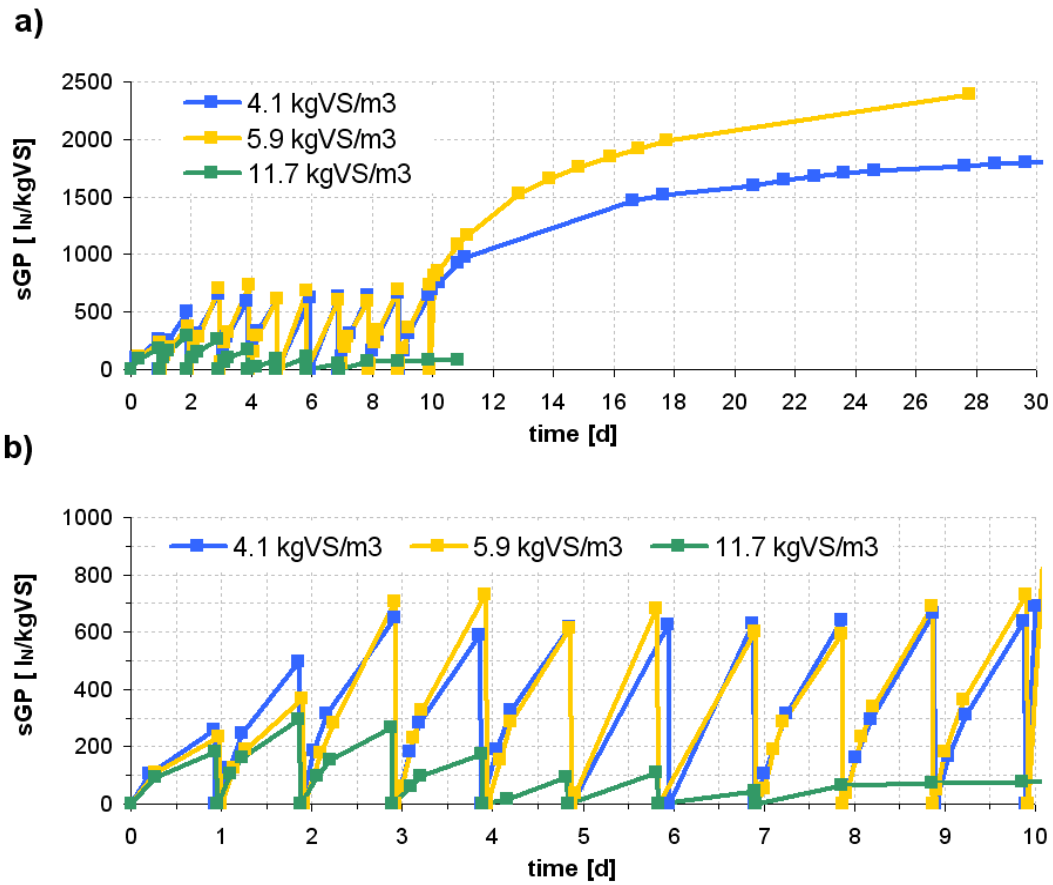

Fig. 4.13 Specific biogas production (sGP) calculated basing on the fed maize charge observed for continuous experiments (a) for the whole experimental period including the final gas production after the feeding stop, (b) only for the loading periods.

reason of the experiment brake for this OLR after feeding 6. For 11.7 kgVS/m³ only 23±3% of the total substrate was degraded before the GP ceased.

Comparison of trends in semi-batch and continuous mode

The maximum values of sGP obtained for each subsequent feeding in semi-batch and continuous mode are presented in Fig. 4.14. Considering two lower OLRs independent of the operation mode[18], the sGP for the feeding periods 3-10 ranged with few exceptions between 590 l_N/kgVS and 740 l_N/kgVS. Similar sGP was measured in batch tests even though the digestion proceeded over a longer time period. It can be clearly distinguished that the first sGP in semi-batch was almost double as high as in continuous mode. In the 2nd feeding sGP for semi-batch nearly reached the level observed in the following feeds while in continuous mode only the 3rd feeding produced the expected biogas volume. For 17.6 kgVS/m³ in semi-batch sGPs were within the

[18] 5.9 kgVS/m³ and 11.7 kgVS/m³in semi-batch as well as 4.1 kgVS/m³ and 5.9 kgVS/m³ in continuous mode

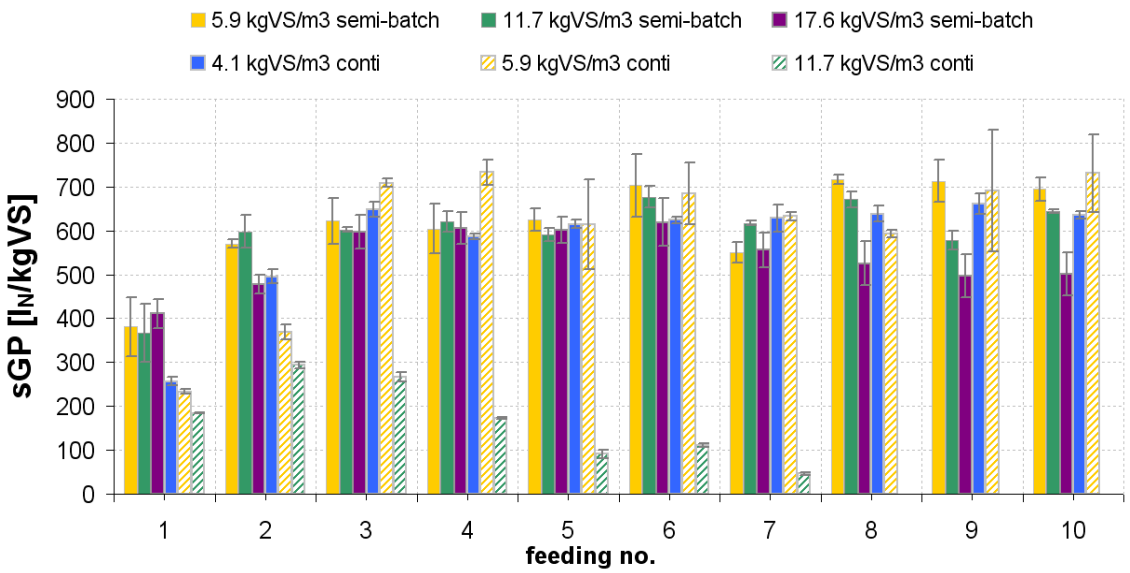

Fig. 4.14 Specific biogas production (sGP) calculated for separate feeding series in semi-batch and continuous experiments based on the fed substrate amount

typical range only for the feeds 3-6. For further feedings a gradual sGP decrease was observed. In continuous mode at 11.7 kgVS/m³ sGP never reached the sGP level typical for maize.

Although similar OLRs were investigated the direct comparison of experimental series was possible only by calculating daily OLRs. An overview of corresponding semi-batch and continuous series is given in Tab. 4.9. From this comparison it is clear that a strong increase of the degradation rate was measured with the raise of feeding frequency. The increase of the loading frequency between semi-batch and continuous mode (from every 3rd day to every day) resulted in tripling of the daily $sGPR_F$. Further the comparison between batch and semi-batch tests revealed that sGP similar to the one generated in semi-batch for 3 days was reached in batch mode within a period of 8-11 days only (s. Fig. 4.12b and Fig. C.3 in Attachment C.1).

Tab. 4.9 Comparison of the mean specific biogas production (sGPR) for semi-batch and continuous tests (calculated for feeding events beginning with the feed no. 3). The different operating modes are compared by the calculation of daily OLR for the semi-continuous tests.

semi-batch test			continuous test	
OLR [kgVS/m³]	daily OLR [kgVS/m³]	sGPR [l_N /(kgVS*d)]	OLR [kgVS/m³]	sGPR [l_N /(kgVS*d)]
11.7	3.9	208 ± 2%	4.1	630 ± 4%
17.6	5.9	188 ± 3%	5.9	674 ± 8%

4.3.2 Parameter changes within the reactor content

A comparison of VFA, TVA, and TIC was not possible for all experimental series. These parameters in semi-batch and continuous mode were registered, unlike in batch mode, just before every feeding. Therefore the in-between parameter changes comparable to the data for batch series and characterizing the digestion performance step by step were not recorded. The time evolution of TIC, TVA and TIC/TVA is presented in Fig. 4.15.

TVA and TIC trends in semi-batch and continuous mode

The most stable behavior was observed for 5.9 kgVS/m³ in both operating modes and for 11.7 kgVS/m³ in semi-batch. However a small increase of TVA and simultaneous decrease of TIC could be detected for these OLRs towards the end of the feeding period. For 4.1 kgVS/m³ in continuous mode a stable TVA value was reached after the 4th feeding, but a clear reduction of carbonate buffer was measured with each feeding. For both highest OLRs a regular decrease of TIC and accumulation of TVA was observed, although the effect was much stronger in continuous mode.

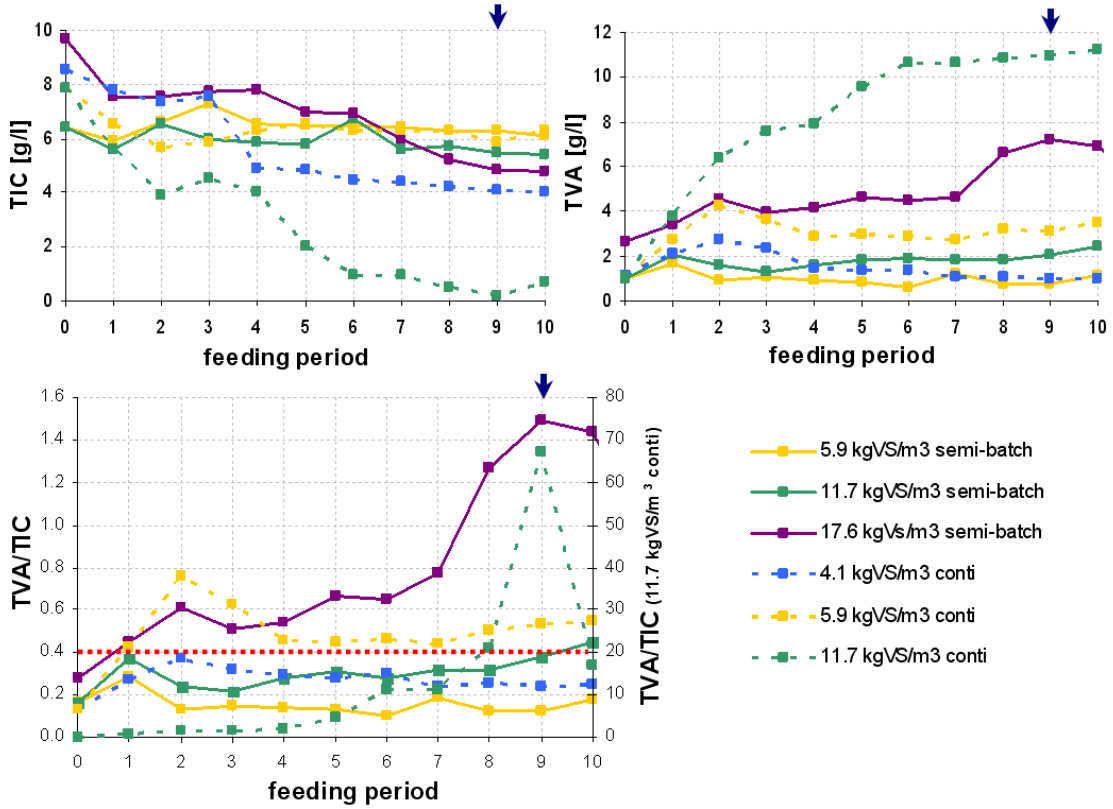

Fig. 4.15 The time progress of the titrated inorganic carbon (TIC), titrated volatile acids (TVA) and their ratio (TIC/TVA) measured just before every feeding and plotted for semi-batch and continuous thermophilic digestion of maize; blue arrow marks the last feeding event except for 11.7 kgVS/m³ in continuous mode, in which the feeding was stopped after day 5; red line marks the limit of uninhibited digestion.

The observed trends were shown even more clearly by the TIC/TVA ratio. The TVA/TIC inhibition mark (dotted red line in Fig. 4.15, right axis) was permanently exceeded for 5.9 kgVS/m³ in continuous mode. Although after initial maximum of 0.8 further values ranging only slightly above the 0.4 were registered. For 11.7 kgVS/m³ in semi-batch mode TVA/TIC values higher than 0.4 were recorded only after the 9th feeding period. A strong TVA/TIC raise reaching 1.5 towards the end of the feeding period was measured for 17.6 kgVS/m³ in semi-batch mode. However a really dramatic increase of the parameter value was observed for the highest OLR in continuous mode. The inhibition mark of 0.4 was exceeded already after the 1st feeding period. Directly after feeding stop (day 6) the TVA/TIV values of 10 were measured, while the further big value raise was registered with TVA/TIV approaching the maximum of 68 on day 9 and the final value of 17 on day 10. The details of TVA and TIC performance for two highest OLR in comparison to VFA performance are described in the subsequent subchapters “HBu inhibition” and “HPr inhibition”.

VFA trends in semi-batch and continuous mode

VFA values measured for semi-batch and continuous experiments are given in Fig. 4.16. For 5.9 kgVS/m³ and 11.7 kgVS/m³ in semi-batch and for 4.1 kgVS/m³ and 5.9 kgVS/m³ in continuous mode no extreme VFA trends were observed. HAc increased after initial 1-2 feedings but a subsequent value drop with the final values smaller than 1.5 g/l was recorded for each of 2 lower OLRs in both operating modes. In general HAc reached higher concentrations in continuous mode. The extreme HAc accumulation with acid concentration reaching nearly 5 g/l was measured for both highest OLRs. Initial accumulation of HPr was observed for all experimental series except for 5.9 kgVS/m³ in semi-batch mode. Subsequently HPr (i) was almost completely degraded (11.7 kgVS/m³ in semi-batch and 4.1 kgVS/m³ in continuous mode), (ii) stayed at a constant level (11.7 kgVS/m³ in semi-batch and 5.9 kgVS/m³ in continuous mode) or (iii) continued the raise trend (17.6 kgVS/m³ in semi-batch mode). The maximum HPr concentrations reached in semi-batch mode were 3 times higher than in continuous mode. The enhanced HAc values were accompanied by increased iso-HVa and variation of iso-HBu; however the concentration of 0.2 g/l was exceeded only in tests with the highest OLRs for both operating modes. Higher iso-HVa and iso-HBu concentrations were reached in semi-batch than in batch mode. A significant accumulation of n-HBu was observed for both highest OLRs independent of the operation mode. This process began earlier and was much more pronounced for continuous test. A light n-HVa increase was measured for the highest OLRs for both experimental modes.

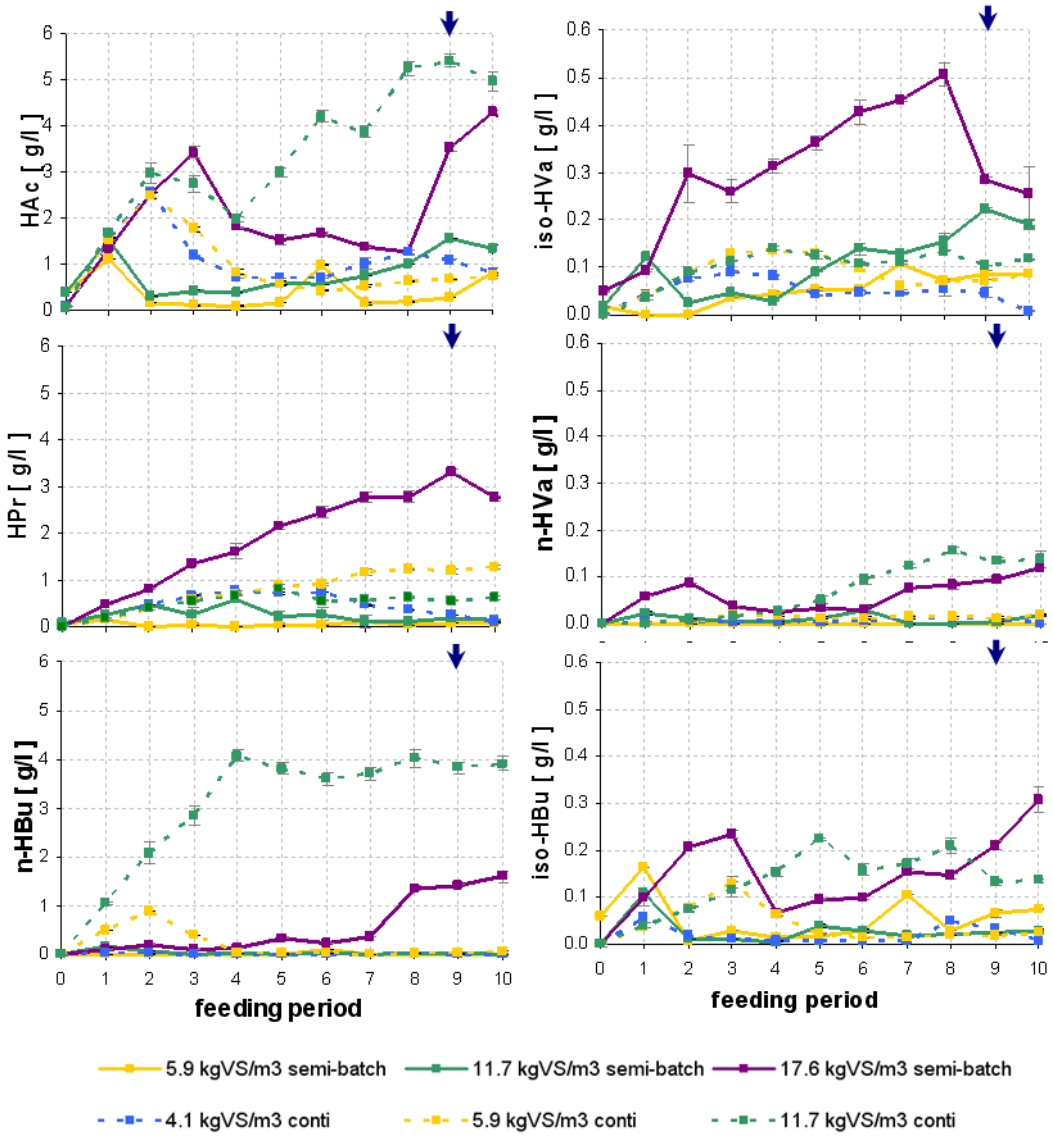

Fig. 4.16 Concentration changes of acetic (HAc), propionic (HPr), iso-butyric (iso-HBu), n-butyric (n-HBu) and iso-valeric (iso-HVa) and n-valeric acid (n-HVa) measured for semi-batch and continuous digestion of maize under thermophilic conditions; blue arrow marks the last feeding event except for 11.7 kgVS/m³ in continuous mode, in which the feeding was stopped after day 5.

The VFA values measured on the 3rd day of thermophilic maize digestion in batch (s. Fig. 4.8 and Fig. 4.9, Chapter 4.2.2) can be compared with the VFA recorded after the first feeding period in semi-batch mode (s. Fig. 4.16). For 5.7 kgVS/m³ in batch mode HAc concentration was comparable to the one measured in semi-batch. But for 11.5 kgVS/m³ and 17.3 kgVS/m³ even more than 2 times higher HAc concentrations were measured in batch than in semi-batch mode.

HPr/HAc trends in semi-batch and continuous mode

Changes of HPr/HAc ratio and OF the summarized VFA are presented in Fig. 4.17 and Fig. 4.18. These parameters if exceeded simultaneously are regarded as indicators of the instability in the digesting system (s. Chapter 2.6). According to HPr/HAc and summarized VFA trends, instable conditions could be confirmed only for 17.6 kgVS/m³ in semi batch mode. The series of 11.7 kgVS/m³ in continuous mode did not fulfill the criterion of HPr/HAc ratio exceeding 0.5 although the sum of VFA exceeded the mark of 10 g/l.

In all remaining experimental series the HPr/HAc instability mark (dotted red line in Fig. 4.17) was exceeded at least after one feed but the corresponding VFA value never

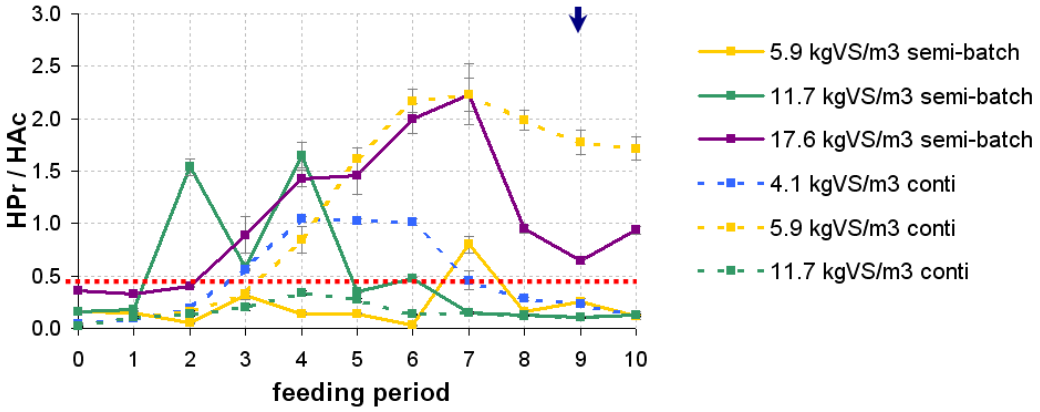

Fig. 4.17 Changes of propionic to acetic acid ratio (HPr/HAc) for semi-batch and continuous digestion of maize under thermophilic conditions; blue arrow marks the last feeding event except for 11.7 kgVS/m³ in continuous mode, in which the feeding was stopped after day 5.

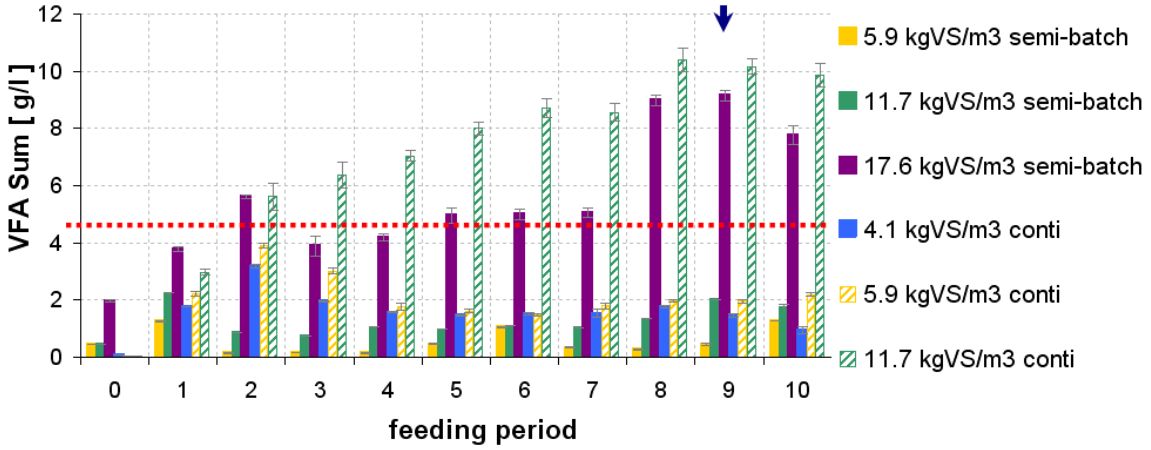

Fig. 4.18 Changes of the sum of volatile fatty acids (VFA) for semi-batch and continuous digestion of maize under thermophilic conditions; blue arrow marks the last feeding event except for 11.7 kgVS/m³ in continuous mode, in which the feeding was stopped after day 5.

exceeded 3.5 g/l. In semi-batch HPr/HAc increased sporadically at irregular intervals, while in continuous mode the enhanced values continued for few feeds (4.1 kgVS/m³) or grew systematically (5.9 kgVS/m³). The gradual increase of HPr/HAc for 5.9 kgVS/m³ in continuous mode was similar to the one observed for 17.6 kgVS/m³ in semi-batch mode.

ORP and pH trends in semi-batch and continuous mode

Independent of the operating mode the pH curve for each feeding proceeded on the same way with rapid value drop directly after feeding and the subsequent reincrease, while the ORP curves followed a reversed pattern (s. Fig. 4.19 and Fig. C.8, Attachment C.3). For 4.1 kgVS/m³ in continuous and 5.9 kgVS/m³ in both operating modes pH changes did not exceed the value of 0.5 and ORP changes were not greater

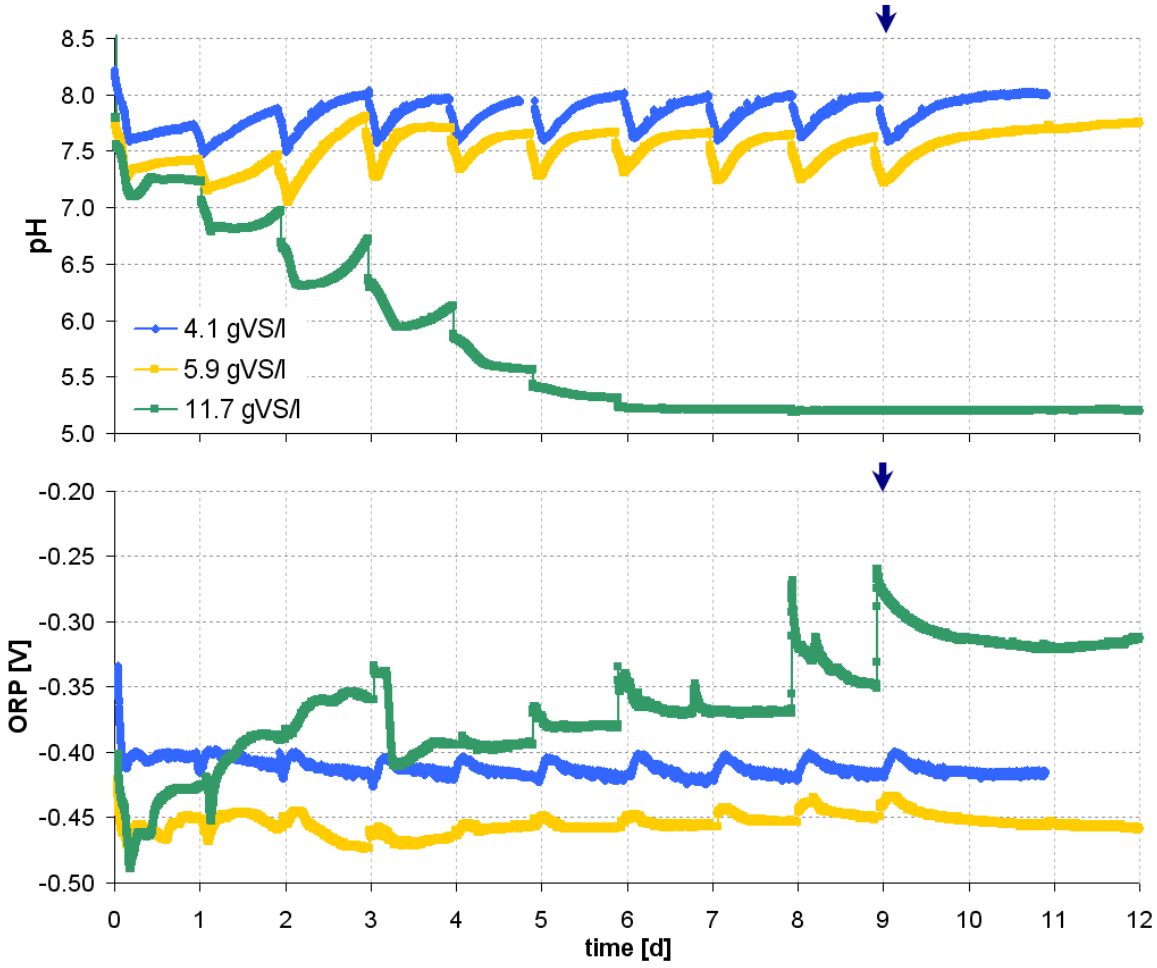

Fig. 4.19 Performance of pH and ORP during the continuous experiments with 4.1, 5.9 and 11.7 kgVS/m³; blue arrow marks the last feeding event except for 11.7 kgVS/m³ continuous, in which the last feeding was performed on day 5; further ORP peaks measured after day 5 were a result of the reactor being shaken due to maintenance of the water bath.

than 25 mV. In general ORP ranged between -0.500 V and -0.330 V, while pH values between 6.5 and 8.0 were measured. Both ORP and pH ranges changed for different OLRs and operating modes, even though the same registration system was applied.

For both 11.7 kgVS/m³ in continuous mode and 17.6 kgVS/m³ in semi-batch a regular increase or decrease of ORP and pH respectively was registered directly after the first digestion period and continued for the whole charging time. For 5.9 kgVS/m³ in continuous mode no vast changes of pH and ORP were observed. A slight pH drop and ORP increase trend was recorded during digestion of 11.7 kgVS/m³ in semi-batch.

HBu inhibition

For 11.7 kgVS/m³ in continuous mode a development of irreversible OLR-conditioned acidosis was observed. Both the extreme concentrations of TVA and the complete reduction of carbonate buffer occurred, even though the feeding was stopped already after day no. 5. Due to degradation of the entire TIC after day 5, the TVA/TIC increased 16 times reaching the maximum of 80 on day 9, despite almost constant concentration of TVA. HAc grew following a zigzag pattern and reached the final stable concentration of nearly 5 g/l. An almost linear increase of concentration was measured during 4-5 initial feedings for HPr, iso-HBu, n-HBu and iso-HVa or between 4th and 8th day of digestion for n-HVa. After the period of gradual increase, all VFA reached final stable values. HPr ranged between 0.5 and 0.8 g/l and did not present any serious accumulation trends. For n-HVa a stable value of ~0.13 g/l was obtained towards the end of the experiment, while both iso-HBu and iso-HVa ranged between 0.1 and 0.2 g/l. Only for n-HBu extremely high concentrations similar to HAc were attained. Although the sum of VFA reached the mark of 10 g/l, HPr/HAc did not exceed 0.5. This is due to the inhibition being caused by accumulation of n-HBu. Performance of the most relevant test parameter for 11.7 kgVS/m³ in continuous mode is shown in

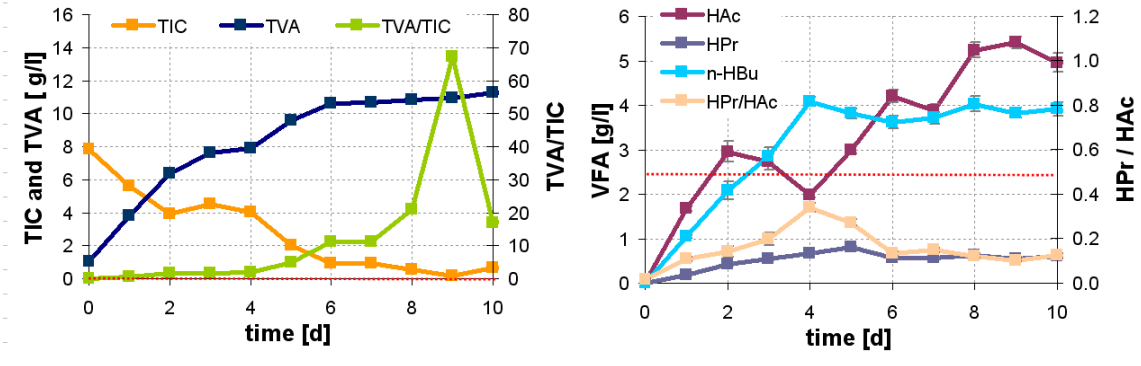

Fig. 4.20 Changes of titrated inorganic carbon (TIC), titrated volatile acids (TVA), their ratio (TVA/TIC), acetic acid (HAc), propionic acid (HPr), n-butyric acid (n-HBu) and propionic to acetic acid ratio (HPr/HAc) for continuous digestion of maize silage at 11.7kgVS/m³ under thermophilic conditions

Fig. 4.20. After the feeding was abandoned on day 6, no further GP or change of VFA concentration was registered. The reactor reached the complete and irreversible acidosis state.

HPr inhibition

Only in semi-batch mode at 17.6 kgVS/m^3 a reversible system inhibition was developed. During this experimental series a gradual TIC drop accompanied by TVA raise was observed. TVA/TIC exceeded the inhibitory mark already after the 1st feed but the vast increase of the parameter was measured after 8th feed. The performance of VFA differed from the one observed for the inhibition described in the continuous mode. HAc and iso-HBu rose directly after the first feeding reaching on 3rd day their local maxima of 3.4 g/l and 0.23 g/l respectively. The accumulated HAc and iso-HBu were partially degraded in the subsequent feeds, however after feed 7 a further increase of their concentrations was observed. HPr and iso-HVa accumulated gradually from the beginning of the experiment reaching extremely high maxima of 3.3 g/l and 0.5 g/l respectively between 8th and 9th feeding period. n-HBu concentrations did not exceed 0.36 g/l for the first 7 feedings but considerably increased by 1g/l after 8th feeding. The lowest concentration raise was measured for n-HVa. Its final concentration only slightly exceeded 0.1 g/l. The instability marks for both HAc/HPr and summarized VFA were simultaneously exceeded already after 2nd feeding period.

The accumulated VFA were completely degraded 10 days after the last feeding period except for n-HBu. On day 10, concentration of n-HBu was still reaching 1 g/l but the acid was completely degraded in the following 8 days. The VFA uptake was continuously accompanied by the GP and led to complete recovery of the reactor.

4.3.3 Modeling of substrate degradation

In the first step all degradation curves were tried to be fitted with the same set of parameters. These turned out to be impossible. Consequently the kinetic parameters were adapted for each degradation curve separately. A comparison of thermophilic maize silage degradation in batch mode calculated with the modified Chen & Hashimoto equation (s. Chapter 3.5.1) with the modelling results for both 1st order and Monod model is given in Fig. 4.21[19]. Independent of the temperature mode the degradation of

[19] The complete set of modeled curves and the kinetic parameters for both Monod and 1st order models is presented in Attachment D, E, and F.

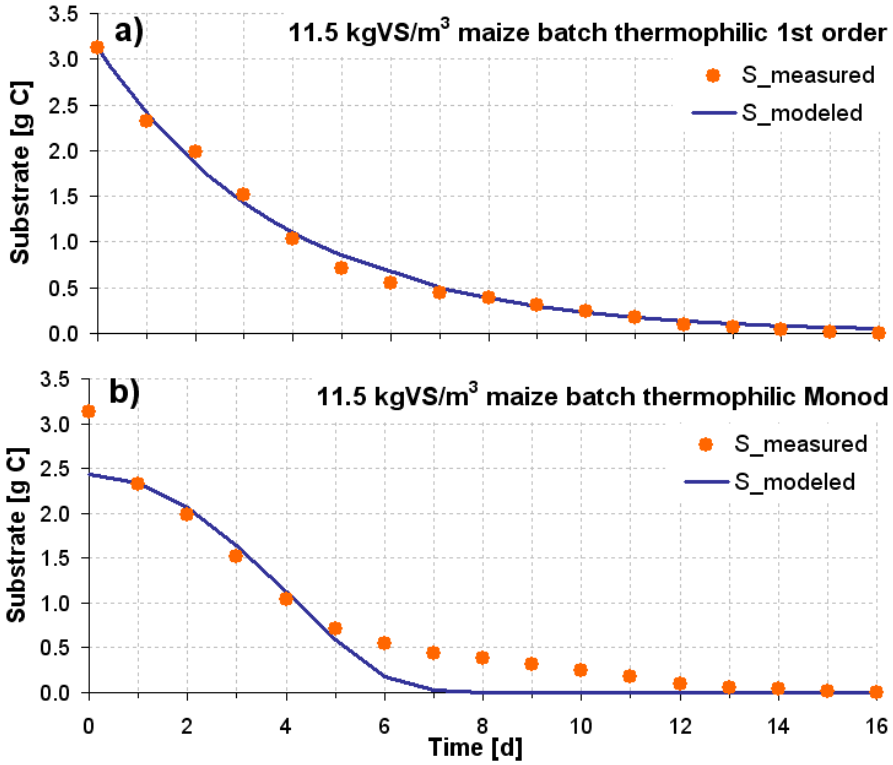

Fig. 4.21 An example of measured and modeled substrate degradation with (a) 1st order and (b) Monod model fit for thermophilic digestion of maize silage in batch mode. Measured points are calculated with Chen & Hashimoto equation from specific gas biogas production (s. Chapter 3.5.1).

maize silage could not be represented by the Monod model. Neither the final retarded substrate degradation nor the initial period of fast maize degradation could be predicted by the chosen model approach. For that reason the Monod fit was abandoned for semi-batch and continuous experiments with maize silage. A good fit of maize silage degradation was achieved with the 1st order model. An example of 1st order fit is presented in Fig. 4.21a. The summary of 1st order parameters for maize silage degradation in batch mode is given in Tab. 4.10.

Tab. 4.10 Summary of 1st order constants obtained for thermophilic digestion of maize silage in batch mode. Measured values are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1).

OLR [kgVS/m^3]	S_0 measured [gC]	S_0 modelled [gC]	k [d^{-1}]
5.7	1.50	1.51	0.36
11.5	3.13	3.13	0.26
17.3	4.59	4.65	0.24

In semi-batch and batch experiments each feed was modeled separately. The model set focused on the best fitting of the initial part of the curve mainly as a slight reduction of the digestion rate was observed in nearly all feeding periods towards the end of the feeding event. This effect was stronger in semi-batch than continuous mode. Further, certain feeding periods were excluded from modeling as not a sufficient number of GP data was available (s. Fig. 4.22). The summary of 1st order kinetic constants obtained for semi-batch and continuous digestion is given in Tab. 4.11. Independent of the operating mode 1st order kinetic constants followed the decreasing trend with the increase of OLR (for semi-batch and continuous mode k was compared for the same feeding periods). The comparison of semi-batch and continuous mode showed that for comparable feeding periods and OLRs higher k values were always obtained in continuous mode.

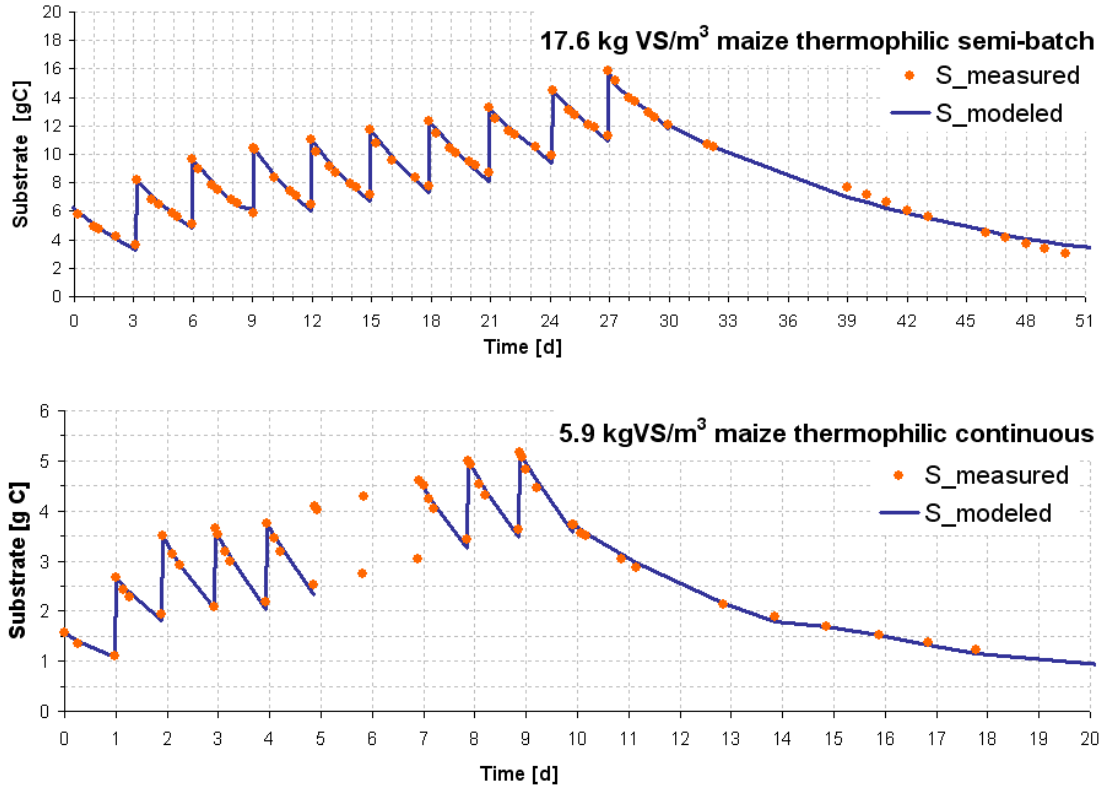

Fig. 4.22 Selected presentation of modeled and measured 1st order degradation of maize silage for corresponding daily OLRs in semi-batch and continuous mode[20]. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1). For the complete set of graphics for both semi-batch and continuous mode s. Fig. D.6 and Fig. D.7 in Attachment D.

[20] The loadings of both experimental series are comparable (for details s. Chapter 4.3.1and Tab. 4.9)

Tab. 4.11 Summary of first order constants obtained in semi-batch and continuous experiments for maize silage degradation

operating mode	OLR [kgVS/m³]	first order constants										
		k_1	k_2	k_3	k_4	k_5	k_6	k_7	k_8	k_9	k_{10}	k_{final}
semi-batch thermophilic	5.9	0.23	-	0.33	0.34	0.31	0.32	-	0.30	-	0.30	0.14
	11.7	0.23	-	0.31	0.31	0.28	0.29	-	0.26	-	0.21	0.14
	17.6	0.21	0.19	0.18	0.19	0.17	0.16	0.14	0.11	0.10	0.08	0.06
continuous thermophilic	4.1	0.64	0.76	0.81	0.67	0.69	-	-	0.51	0.47	0.46	0.14
	5.9	0.39	0.43	0.53	0.60	0.46	-	-	0.37	0.37	0.36	0.16
	11.7	0.30	0.35	0.20	0.10	0.03	0.03	0.01	-	-	-	0.002

The k_1 values of 0.21–0.23 in semi-batch mode were similar to the k obtained for the highest OLR in batch, while the maximal k value (k_4 = 0.34) fit in semi-batch for 5.9 kgVS/m³ was close to the k = 0.36 for 5.7 kgVS/m³ in batch mode.

In continuous mode k_1 decreased considerably with the increase of OLR. The 1st order kinetic constants obtained for the same feeding periods were 1.5–2 times higher for 5.9 kgVS/m³ in continuous mode than in semi-batch. The comparison of daily OLR showed that (i) for 4.1 kgVs/m³ in continuous mode and 11.7 kgVS/m³ (≈ daily 3.9 kgVS/m³) in semi-batch k_1–k_{10} values nearly doubled with the tripling of the feeding frequency, while (ii) for 5.9 kgVS/m³ in continuous mode and 17.6 kgVS/m³ (≈ daily 5.9 kgVS/m³) in semi-batch k_1 was double as high in continuous mode but the dominance increased with every further feeding period and reached the factor of 4.5 for k_{10} due to gradual k drop in semi-batch. For two lower OLRs in both semi-batch and continuous mode the very final stage of accumulated substrate degradation continued at the same k_{final} of 0.14–0.16.

Relative stable k values of 0.30–0.34 and 0.36–0.37 were obtained only for the lowest OLR in semi-batch and towards the end of the experiment for 5.9 kgVS/m³ in continuous mode respectively. These 1st order kinetic constants were similar to the value fitted for 5.7 kgVS/m³ in batch mode. For both 17.6 kgVS/m³ in semi-batch and 11.7 kgVS/m³ in continuous mode k values dropped nearly to 0, however in semi-batch it occurred only toward the end of the experiment while in continuous mode already after the 4th feeding.

5 Discussion

5.1 Influence of OLR on thermophilic digestion of cellulose in batch mode

During the experimental series a wide range of parameters was measured to recognize the coherences between them and evaluate their relevance for monitoring of anaerobic digestion. Batch experiments were charged only once and the digestion continued until the biogas production ceased. Consequently unlike in continuous mode the most important parameter defining the boundaries of inhibitory conditions was the substrate to inoculum ratio VS_s/VS_i= 0.5 (VDI, 2004). This was exceeded deliberately for 4 out of 6 test series (beginning with 17.1 kgVS/m³) to induce the OLR-suppressed digestion (s. Tab. 4.1, Chapter 4.1.1).

Comparison of inhibition indicators

Both pH and ORP measured during batch tests were within the range considered as typical for anaerobic digestion (methanogenesis) even for the highest OLRs (DEUBLEIN & STEINHAUSER, 2008; SCHOLWIN ET AL., 2009). The monitoring of pH and ORP changes allowed determining the period in which the majority of substrate was degraded. Neither absolute nor relative changes of both parameters uncovered any disturbances that could be regarded as endangering the digestion process.

The critical HPr/HAc ratio (HECHT ET AL., 2007; LEMMER, 2007) was not exceeded in any of the tests for the period of higher sGP. No significant changes in the ratio similar to (MARCHAIM & KRAUSE, 1993) were observed. The elevated HPr/HAc values towards the end of the test were a consequence of slower HPr degradation.

Elevated TVA/TIC ratio exceeding the 0.4 mark indicated digestion problems for all series excluding only the lowest OLR (CECCHI ET AL., 2003; TELSCHOW, 2007; LOSSIE & PÜTZ, 2008). However, this parameter is primarily used for continuously fed reactors, which are not being sampled as frequently as the batches. In biogas industry TVA/TIC does not characterize the dynamic of VFA creation and their degradation but represents more the accumulation trend frequently leading to digestion cease. The parameter turned out to be too sensitive for analysis of batch series.

Similar considerations refer to the C_4-C_5 VFA. Only for 5.7 kg VS/m³ concentrations of C_4-C_5 did not exceed 50 mg/l regarded as the upper limit for the non-inhibitory fermentation (SCHATTAUER & WEILAND, 2006). Nevertheless the trend of GP for 11.4 kg VS/m³ did not allow considering digestion process as imbalanced. This

observation is in accordance with AHRING ET AL. (1995), who also postulated that the diversity of anaerobic systems does not allow defining universal inhibitory VFA concentrations.

Response of analytical parameters to system changes

The observed synchronized changes of pH, ORP, and TVA/TIC-ratio were not in accordance with the observations of RIEGER & WEILAND (2006) for continuous fermentation of maize. Both pH and ORP reacted to the system changes at the same time as the TVA/TIC ratio. Consequently it can be assumed that either the incoherency of the parameter can be rather observed for continuous digestion or the effect was mainly induced by inhomogeneity of the reactor. Therefore in a homogenous and frequently sampled batch fermenter (or controlled with on-line system) changes of all three parameters can be used to detect altering reactor performance.

Digestion delay

The initial 1-2 days of experiment, in which almost no GP took place, are considered as a lag phase of the bacterial biocenosis and the time necessary for the hydrolytic substrate conversion. They were not expected to be observed as the inoculum was substrate adapted and temperature conditioned. The similar effect was also observed for cellulose digestion by different inoculum under mesophilic conditions but did not appear for digestion of maize (s. Chapter 4.2).

Instability of the system with OLR increase

In general, there was a difficulty in comparison of the obtained Monod parameters with the literature. Unlike to the other authors who considered the particular steps of anaerobic digestion, in this project the global substrate degradation was fitted. Consequently only the literature Monod parameters defined for acetogenesis and methanogenesis (s. Tab. E.1, Attachment E) were used for discussion of the results. For the purpose of comparison the calculated correlation factors of 0.4 between g C and g VS, and 1.3-1.45 between g COD and g VS (WICHERN ET AL., 2009) were applied. After recalculation of the literature values of K_S according to the above mentioned factors the experimental K_S turned out to be nearly two times higher than those of BEIERLEIN (2011), and KALFAS ET AL. (2006) but still within the wide range defined by VAVILIN ET AL. (2003) and GARCIA-HERAS (2003). According to the stoichiometry of the cellulose to VFA degradation, the carbon content of the substrate can be considered as double as high as the carbon amount found in VFA applied as a substrate in the literature models (BATSTONE AT AL., 2002; PIND ET AL., 2003, LAUKENMANN ET AL., 2010). Following this logic,

the fitted K_S values[21] are similar to the literature and within the expected range. The Monod maximal specific growth rate of bacteria (μ_{max}) obtained in the test for the lowest OLR was within the range defined by GARCIA-HERAS (2003) but much lower than the values reported in other publications (s. Tab. E.1, Attachment E) for degradation of VFA and HAc. The sensitivity of μ_{max} and the value decrease observed with the increase of OLR are linked to the bacterial growth being influenced by certain instability of the system. The sensitivity of μ_{max} resulting from ammonia conditioned inhibition of anaerobic digestion was reported by Kim et al. (2006) and Ko et al. (2006). However it might be as well a sign of inhibition caused by other factors such as the high concentration of substrate, process intermediates (LCFA, VFA, lactate, etc.) or lack of necessary nutrients or trace elements.

Neither the concentration of lactate nor of ammonia was measured during the tests. Therefore a possible inhibition of hydrolysis caused by elevated lactate concentrations similar to the observations of LÜ ET AL. (2008), MOLDES ET AL. (2001) and IYVER & LEE (1999) cannot be excluded especially for the higher OLRs. The ammonia inhibition effect is less probable as the increased ammonia concentrations are always accompanied by elevated pH values (JEWELL ET AL., 1993), which were not observed in the tests. Further the applied substrate (cellulose) did not deliver nitrogen in any form. The concentration of nutrients and trace elements was not measured in the tests. Since only 6 batches in a sequence were performed, they would hardly lead to the nutrients and trace elements deficiency in the inoculum. However it cannot be directly excluded that the thermophilic inoculum applied in the experiments[22] indicated a shortage of trace elements already before the experiments.

The problem of instable μ_{max} could be removed if a proper inhibitory term would be integrated into the specific growth rate equation. This, however, is only possible if an inhibitory factor is identified.

Rate limiting step of the fermentation process

Due to extremely high initial loadings it is possible that the rate limiting step varied during the digestion for different OLRs.

The fitted k values for 2 initial OLRs (regarded as a good fit) ranged between 0.29 d^{-1} and 0.34 d^{-1}, which was in accordance with the values (0.04-1 d^{-1}) reported in the

[21] double as high as in the literature for VFA degradation

[22] as it was retrieved from a fermenter charged only with energy crops

literature for the hydrolysis of carbohydrate-based substrates (for a detailed review s. Tab. D.1, Attachment D). Similar kinetic constants were obtained by BEIERLEIN (2011)[23] but also given by KALFAS ET AL. (2006) and suggested in ADM 1 (BATSTONE ET AL., 2002) for hydrolytic step. However, in the literature only the substrate disintegration and hydrolytic steps are modeled basing on the 1st order equation. Consequently no comparable k values can be found for further digestion steps.

The increased sGPR accompanied by relatively low HAc concentrations during digestion of the lowest OLR of cellulose suggest the acetogenesis as a rate limiting step.

For the enhanced OLRs the decreasing trend of the 1st order kinetic constants, prevailing lower CH_4 content, suppressed GPRs and instable μ_{max} in Monod model were observed, even though for each of the series the expected final biogas yield was obtained. All the parameters, which are mentioned, combined with nearly constant maximum of HAc indicate the instability of the fermentation which resulted in suppressed methanogenesis[24].

At 34.3 kg VS/m³ (the highest OLR), a strongly reduced HAc maximum was measured (s. Fig. 4.3, Chapter 4.1.2). Consequently both suppressed methanogenesis and acetogenesis can be assumed.

Such change of a rate limiting step in anaerobic digestion would not be contradictory to the literature. Among scientists there is a general agreement on considering hydrolysis as rate-limiting step (NOIKE ET AL., 1985; TOMEI ET AL., 2008, VAVILIN ET AL., 1996). However VAVILIN ET AL. (2008) emphasizes that the rate-limiting step may alter for higher OLRs depending on the bioavailability of the substrate. Also SEYFRIED ET AL. (1994) reports on cellulose degradation at higher OLRs, being limited by acetogenesis.

[23] modeling the same data set but based on methane production (according to the original Chen & Hashimoto (1978) equation)

[24] For these series methanogenesis is presumed as a rate limiting step

5.2 Substrate and temperature influence on digestion in batch mode

Influence of the inoculum characteristics on anaerobic digestion

The low degradation activity for thermophilic digestion of maize at 5.7 kgVS/m³ can be attributed to poor initial inoculum conditions. Similar to the influence reported in the literature (HASHIMOTO, 1989; CHEN & HASHIMOTO, 1996; VDI 2004) a very low concentration of VS caused a range of serious parameter changes including lower total GP, sGPR, lower pH and TIC range but higher ORP range and increased TVA/TIC ratio as well as a changed sGPR pattern. Only the CH_4 content in total biogas remained uninfluenced. KAYHANIAN & RICH (2005) suggested that the characteristics of the inoculum have smaller impact on the digestion than the availability of some nutrients. Since the test with maize at the smallest OLR of 5.7 kgVS/m³ was the only one behaving in a different way, the deficiency of nutrients as a reason of low degradation activity could be excluded for this trial.

Excess biogas production

For certain maize experiments (usually for higher OLRs) independent whether at 38°C or 55°C higher biogas yields were obtained (102-106%) than maximum expected value. This can be caused by a few independent factors.

For batch trials the natural biodiversity of ensiled maize harvest is primarily considered as responsible for the observed effect. The calculation of maximum biogas yield bases on the results of Van Soest and Weende analysis for a sample of maize silage. Since the silage is produced by chopping the complete plant mass (inclusive leaves, stalks and corn-cobs), its natural inhomogeneity is very high (s. Chapter 3.1). Each change of maize composition comparing to the sample analysed by Van Soest and Weende method may cause changes in the maximum expected biogas yield, which cannot be quantified for such experiment. Therefore with the increase of the substrate volume being introduced into system, higher uncertainty of maximum expected biogas yield can be assumed. For that reason a development of substrate homogenization method e.g. by designing a model substrate similar to the composition of the maize plant (POBEHEIM, 2011) or by drying and milling (RICHARDS ET AL., 1991; JEWELL ET AL., 1993) to improve the final representativity of the results would be advisable.

Another possible source of excess biogas production for higher OLRs might have been an enhanced bacterial activity due to higher total substrate concentration. This is however less probable since the raise of OLRs did not always result in corresponding increase of biogas yield but it occurred in a more random way.

The explanation postulating higher substrate degradation level and/or lower uptake of substrate for bacterial growth than 10% assumed following the carbon balance from the literature (GREPMEIER, 2002; VDI, 2004) is more probable for semi-batch and continuous operating modes with repeated feeding (for details s. Chapter 5.3)[25].

Gas quality

In general higher CH_4 content in biogas was measured for maize than cellulose. However for corresponding OLRs in 3 out of 6 comparisons the difference amounted only 1%, while for the other 3 cases CH_4 content was higher by 3 - 5 %. This is not surprising as the ingredients that could increase the CH_4 content in biogas (VDI, 2004) represent only 10% of maize silage (3% fat and 7% proteins). For both substrates in 4 out of 6 cases for the comparable OLRs a CH_4 content decreased by 2-4% was measured under thermophilic conditions in comparison to mesophilic ones. Such trend was expected due to lower solubility of CO_2 in digestate at elevated temperatures (DEUBLEIN & STEINHAUSER, 2008) and was also observed by LIEBENEINER (2010).

Biogas yield and degradation rate

No influence of the temperature mode on the total biogas yield was observed. Although the VS quantity in the thermophilic inoculum was usually higher, the highest biogas yield was not corresponding to the highest VS content.

This might be a consequence of an inaccurate VS determination or interpretation. As the VS determination method includes drying at 105°C, a partial volatilization of inoculum VS cannot be excluded. In the experiments the corrections of VS content of maize according to WEIßBACH & KUHLA (1995) were performed (s. Chapter3.1). But no correction of the inoculum VS content was possible as the necessary conversion factors are unknown. Further via this method all organic mass present in the reactor was estimated. The method however does not give insight into activity of the bacteria. Consequently a not quantifiable part of VS is made out by the dead microorganisms.

The similar total biogas yields were measured independent of the temperature mode, which is in agreement with other investigations (HASHIMOTO ET AL., 1981; ANGELIDAKI & AHRING, 1994). Unlike in the literature (BAADER ET AL., 1978; KALTWASSER, 1980; WELLINGER ET AL., 1991; KALTSCHMITT ET AL., 1993; DEUBLEIN & STEINHAUSER, 2008; CAVINATO ET AL., 2010; LIEBENEINER, 2010) and contradictory to the biogas praxis, higher sGPRs were observed for mesophilic conversions. The faster degradation of both

[25] This assumes also that the bacterial decay rate was constantly at the low level.

substrates in mesophilic mode could also be confirmed by the results of the 1st order model. An explanation for that could be any kind of inhibition within the thermophilic inoculum, which would systematically reduce the sGPR for all thermophilic tests. The important inhibiting factors to be considered are the excess of ammonia and the shortage of trace elements. Both parameters were not controlled in the experiments. Since the inoculum used for thermophilic digestion was retrieved from continuous reactor operated only with energy crops it cannot be excluded that the micro nutrients were deficient already before the beginning of the batch series causing slower bacterial growth and substrate conversion for all batches under thermophilic conditions. The elevated ammonia concentration in the inoculum is regarded as less probable due to the origin of the inoculum and not elevated pH values (JEWELL ET AL., 1993).

Substrate pre-treatment

Unlike for maize silage in all experimental series with cellulose[26] in batch mode independent of the digestion temperature or OLR an identical lag phase was observed. This digestion delay was caused by the necessary period of setting hydrolytic conditions. Contrary to cellulose maize silage was used in the ensilaged form. This means that the hydrolysis of the substrate was more advanced already at the time of the reactor feeding. Some part of maize was already converted to easier biodegradable lactic acid or acetic acid. Consequently no time delay in gas production was observed.

Degree of substrate degradation

A higher fraction of maize silage was degraded in comparison to cellulose independent of the temperature mode and OLRs. Despite higher substrate complexity and lower (55°C) or comparable (38°C) VS content of the inoculum over 92% of maize silage[27] was degraded during the tests[28]. Unlike to the maize degradation in the experiments with microcrystalline cellulose only around 77-89% of substrate was decomposed. As for a one-component homogenous substance the degradation level was fairly low. An insufficient bacteria adaption to the cellulose digestion could not explain this performance as also further tests at higher OLRs indicated similar substrate degradation level (GOLKOWSKA & GREGER, 2010) although the already adapted inoculum was used. As the inocula from different sources where applied for cellulose digestion depending on

[26] also in the thermophilic series with cellulose at OLR higher than 17.1 kgVS/m³ described in Chapter 4.1 and by GOLKOWSKA & GREGER (2010)

[27] except for thermophilic maize digestion at 5.7 kgVS/m³

[28] Biodegradability of the substrate was calculated basing on the results of Van Soest and Weende analysis for maize silage samples. The ash and lignin content were not regarded as degradable.

the operating temperature (s. Chapter 3.2), the lower degradation grade was either not attributed to the characteristics of the inoculum or both inocula did not fulfill the conditions required for optimum substrate degradation.

According to the literature both micro and macro nutrients are particularly important for regulation of the degradation velocity and bacterial activity in thermophilic mode (SAI RAM ET AL., 2000; KAYHANIAN & RICH, 2005; HINKEN ET AL., 2008; LEBUHN ET AL., 2008; POBEHEIM ET AL., 2010 & 2011; DEMIREL & SCHERER, 2011). Since the nutrients were not analyzed in the experiment, their deficiency cannot be excluded as a potential source of suppressed substrate degradation. It would be possible and even more probable for cellulose digestion as the substrate itself consists of C, O and H only and no further nutrients input was delivered by the substrate. Further, due to both the inoculum origin and higher micro element demand, the shortage of nutrients would be expected more for thermophilic than mesophilic inoculum. Comparing the substrate degradation level within this context it can be noticed, that the lowest substrate degradation level of 77-83% was indeed measured for thermophilic digestion of cellulose.

sGPR pattern

A typical substrate linked degradation pattern could be observed. Maize was degraded much faster than cellulose in the first stage of the degradation (t_{50}), while for the final digestion (t_{90}) period much slower degradation rates were registered. This corresponds to the more complex composition of maize. The maxima of sGPR for corresponding OLRs were always measured for maize independent of the temperature mode. Different ingredients of maize degraded with different rates causing both prolongation of total degradation time and multiple maxima[29]. The first and highest sGPR maximum was a result of degradation of easily accessible substrates such as non-fibrous carbohydrates, proteins and fats, while the last and smallest peak was linked to decomposition of not easily degradable cellulose or hemicellulose (NOIKE ET AL., 1985; JUNG, 1997, GREPMEIER, 2002).

According to the test results for thermophilic temperatures in a biogas fermenter charged daily with maize some hard degradable maize fractions would scarcely be digested if too short hydraulic retention time would be applied. Such conditions would mainly favour the digestion of easily degradable maize fractions continuously delivered with subsequent feeds.

[29] The trend was not that much pronounced for mesophilic conditions, which is regarded as a proof of more stable degradation

Comparing the results of thermophilic and mesophilic maize digestion in batch mode for the first 3 days a higher substrate uptake by 25% could be distinguished for mesophilic conditions. Therefore according to these results a continuous digestion of maize in mesophilic mode should result in higher sGP and better substrate humification. However the faster mesophilic conversion of maize silage in the initial digestion period is contradictory to the literature. It may be explained by a possible inoculum linked inhibition of fermentation in all batch experiments (for detailed explanation s. subchapter "Biogas yield and degradation rate").

Reaction pathways

For all substrates and temperature modes ORP stayed in the range typical for anaerobic degradation (EHRING, 1985; UTEC, 2003; KARPENSTEIN-MACHAN, 2005) independent of the temperature mode or the OLR applied. However a difference in the ORP range was observed between cellulose and maize silage digestion. Mesophilic cellulose[30] was mainly digested within the range higher that -300mV, while degradation of maize silage primarily occurred between -350mV and -500mV. Studies with anaerobic cultures specified on hydrogen production revealed that the ORP drop from -350mV to -550mV was observed in the days of experiments identified as a period of the highest hydrogen production (KATAOKA ET AL., 1997; LIN ET AL., 2008). This could mean that the hydrogenotrophic methanogens were active mainly during digestion of maize silage independent whether under mesophilic or thermophilic conditions.

As after hydrolysis both cellulose and a greater part of maize[31] is being converted into monosaccharides, mainly HAc, HPr and HBu were expected as the intermediates in the subsequent digestion paths (DENAC ET AL., 1988; BATSTONE ET AL., 2002; PIND ET AL., 2003).

Cellulose degradation showed VFA concentrations, which were close to the ranges considered as typical for undisturbed anaerobic digestion (MAWSON ET AL., 1991; WU ET AL. 1993; WANG ET AL., 1999; and PIND ET AL., 2003). Only HAc for two higher OLRs in thermophilic mode reached an untypically high concentration range. Peaks of HAc, being a direct HPr degradation product, were linked to elevated HPr concentrations. However according to VFA trends a simultaneous conversion of monosaccharides to HAc and HPr (similar to BATSTONE ET AL., 2002) can be assumed. Both HBu and HVa were present at

[30] Due to the partial unavailability of the equipment ORP could only be recorded for 2 out of 6 experiments run with cellulose under thermophilic conditions. Especially the absence of the ORP values for 11.4-17.1 kgVS/m³ made the comparison with other temperature and substrate series impossible.

[31] except for 7% of fats and 3% of proteins

very low concentrations only sporadically exceeding 150mg/l. Their presence might also be considered as an effect of HPr and HAc backreactions (PIND ET AL., 2003).

In general, higher concentrations of C_3-C_5 VFA were measured for maize silage. Unlike for cellulose, the VFA trends varied considerably for different OLRs and temperature modes. For the experiments with maize a considerable increase of the n-HBu concentration reaching nearly the level typical for HAc or HPr was observed. This trend was more pronounced for thermophilic trials and is similar to those reported by BATSTONE ET AL. (2002) and PIND ET AL. (2003). The concentrations of both iso-HBu and of n-HBu were increased simultaneous to the enhanced HAc concentration which is a direct product of HBu degradation (AHRING & WESTERMANN, 1987; THOLOZAN ET AL., 1988; STIEB & SCHINK 1989; MATTHIES & SCHINK, 1992; WU ET AL., 1993; WANG ET AL.,1999). According to previous studies of ZINDER ET AL. (1984), THOLOZAN ET AL. (1988), STIEB & SCHINK (1989), AGULIAR ET AL. (1990) and MATTHIE & SCHINK, (1992), iso-HBu serves as equilibrium storage of n-HBu so that the isomerization form is irrelevant for further degradation to HAc. A prevailing enhanced concentration of HAc for thermophilic series despite still low HPr might be either a sign of HAc being produced directly from monomers or confirms the observation of ÖZTÜRK (1991) that HAc produced from HBu is not directly degraded to CH_4 like it happens for HAc coming from other intermediates. The conversion of maize monomers into HAc via iso-HBu and n-HBu with such high n-HBu concentrations was not reported in the literature and by now mainly the HPr pathway similar to the observations for cellulose was assumed.

Enhanced HPr followed subsequent to elevated HBu and HAc for thermophilic maize digestion and the highest OLR in mesophilic mode. This is regarded as a proof of hydrogen[32] and HAc-conditioned inhibition of HPr degraders (MAWSON ET AL., 1991; ÖZTÜRK, 1991; WU ET AL. 1993; ANGELIDAKI & AHRING, 1995; MÖSCHE & JÖRDENING, 1999; WANG ET AL., 1999). The final slow degradation of HPr was in accordance with literature characterizing HPr as the last VFA to stabilize due to its slow degradation rate (WIEGANT ET AL., 1986; PIND ET AL. 2003). HBu inhibition by HAc higher than 1.5 g/l (AHRING & WESTERMANN, 1987, 1988) was not observed for maize in thermophilic mode however cannot be excluded for the highest OLR of maize in mesophilic mode.

The presence of HBu as degradation intermediate during maize digestion implied a subsequent high activity of HBu degraders producing high H_2 concentrations

[32] Increased hydrogen concentration is reported for the activity period of HBu to HAc degraders (ÖZTÜRK, 1991; BATSTONE ET AL., 2003)

(ÖZTÜRK, 1991). Therefore registered lower ORP values, typical for hydrogen production, only confirm the assumed reductive conditions.

The observed differences in digestion pathways confirm the latest findings of NETTMANN ET AL. (2008), KRAKAT ET AL. (2010) and LAUKENMANN ET AL. (2010) reporting about new reaction paths in anaerobic digestion[33]. Both substrate and temperature conditions promote the substrate degradation via different pathways[34] more than inoculum or OLR.

Relevance of pH, ORP, TVA and TIC for batch mode

In general pH, ORP, TIC, and TVA did not show any considerable trend changes for different substrates or OLRs. The TIC and TVA ranges[35] increased with the OLR raise independent of the substrate used. However for all four parameters under mesophilic conditions narrower ranges were measured than in thermophilic mode.

Both TVA and TIC showed similar trends (corresponding maxima/minima and increase/decrease periods) for the same OLRs independent whether cellulose or maize was used although the differences in sGPR patterns were observed.

All four parameters seem to be less important in characterising balanced batch digestion as the observation of GP. They deliver more information for digestion in semi-batch or continuous operating mode.

Optimum OLR for batch mode

The sGP of maize fermentation increased identically during the period of the most intensive GP independent of applied organic loading and the temperature (s. Fig. C.3, Attachment C.1). In contrast to that, the steepness of the sGP slope for fermentation of cellulose decreased for the highest OLRs in both temperature modes. This might by a sign of overloading for cellulose degradation at the highest OLRs.

The comparison of kinetic constants from 1st order fitting for both maize and cellulose shows a decline of k values with OLR increase independent of temperature mode. This suggests that the lowest OLRs had the optimal digestive conditions within investigated maize and cellulose trials. However it cannot be excluded that the OLRs lower than those investigated in the experiment would deliver more stable results.

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[33] Before it was assumed that the degradation paths are similar independent of the substrate, temperature mode and the source of the inoculum and that the methane is mainly produced via HAc (BUSWELL & SOLLO, 1948; STADTMAN & BARKER, 1949; JERIS & MCCARTY, 1965; SMITH & MAH, 1966; ROEDIGER ET AL., 1990).

[34] with HBu as intermediate or hydrogenotrophic methanogenesis

[35] calculated as difference between maximum and minimum value of TIC and TVA

On the contrary, for both substrates and temperature modes the lowest biogas yield was attributed to the lowest OLR. The analysis of sGP trends together with the comparison of sGPR and VFA data revealed that both maize and cellulose at the OLR of 11-12kgVS/m^3 could be optimally degraded by the bacterial biocenosis in batch mode independent of the digestion temperature. This discrepancy between the trends of kinetic constants and the other parameters can be explained by the model, which approximates only certain mechanisms of anaerobic digestion. In this case the conclusion based on the directly measured data seems to be more valid.

Optimum digestion temperature

All analyzed parameters[36] registered only minor and rather smooth trend changes for mesophilic experiments, even though higher sGPR were measured for the initial days of fermentation. This observation was even more explicit for cellulose than for mesophilic maize digestion and is in accordance with the literature (WELLINGER ET AL., 1991). For both digestion of cellulose and maize at 38°C much lower HAc concentrations were measured than at 55°C. This is due to the higher activity of methanogenic cultures while degrading acetate under mesophilic conditions (VAN DEN BERG, 1977).

Under thermophilic conditions either the time delay of the sGPR with increasing OLRs was observed for cellulose or the multiple degradation peaks and consequently strongly varying sGPR was measured for maize. In contrast to that, mesophilic conditions seem to support a more stable degradation.

Rate limiting step

For all investigated batch series the raise of sGPR and sGP with the increase of OLR in the initial digestion phase (first 3 days of digestion) was only observed in mesophilic cellulose experiments (s. Fig. C.3, Attachment C.1). The subsequent reduction of the substrate degradation rate for the highest OLR and the increase of sGPR for the lowest OLR reset the parameter dependency similar to those observed for other batch experiments. As the same inoculum was applied for the whole experimental series the enhanced initial sGPR for the highest OLR could be a sign of the optimal inoculum adaption to increased cellulose loadings under mesophilic conditions.

However another explanation related to the changes of reaction rate for different digestion steps seems to be more plausible. Comparing the HAc and sGPR patterns

[36] except for VFA at the highest OLR of maize

(s. Fig. 4.8, Chapter 4.2.2 and Fig. C.3, Attachment C.1), for maize and cellulose[37] degradation mainly methanogenesis seemed to be conditioning the degradation rate[38]. For mesophilic degradation of cellulose the rate limiting step changed during digestion dependant on the OLR. In the initial degradation period the hydrolysis seemed to set the reaction rate, consequently the highest substrate amounts were degraded with the highest rate. However after 2nd day of digestion the acetogens or methanogens did not manage to keep the high substrate conversion rate and the intermediate degradation proceeded faster for the lower OLRs.

[37] excluding the 1-day digestion delay for thermophilic cellulose degradation caused by the lag phase (s. Chapter 4.1.3)

[38] for all lowest OLRs and for all series of cellulose in mesophilic mode it could be methanogenesis or acidogenesis

5.3 Impact of operating mode on thermophilic degradation of maize silage

Inoculum performance

For comparable degradation periods[39] in batch, semi-batch and continuous mode (s. Fig. 4.12b, Fig. 4.13b and Fig. C.3, Attachment C.1), the attained biogas yields were similar. This shows the reproducibility of the bacterial biocenosis performance in these test series under similar conditions and was in accordance with the literature (STEWART ET AL., 1984).

Although the inoculum was not adapted to the gradually increasing OLRs (like in the biogas industry) but directly confronted with the high OLR, the full culture adaption was reached already after 1st or 2nd feed depending on the operating mode[40].

Influence of operating mode on substrate degradation

The sGPs obtained for the feeding periods in semi-batch and continuous mode (s. Fig. 4.14, Chapter 4.3.1) were nearly equal[41]. All observed differences were within the range of 10%, which could be considered as an effect of natural biological diversity (HELLFRICH & ÖCHSNER, 2003; HEUWINKNEL ET AL., 2009; MEBNER ET AL., 2009).

With increase of the feeding frequency and the related reduction of retention time from semi-batch to continuous mode an increase of the calculated daily sGPR was observed (s.Tab. 4.9). This is similar to the dependency between retention time and sGPR reported by SCHATTAUER & WEILAND (2006). Comparing the periods of optimal sGP in continuous and semi-batch mode, the anaerobic biocenosis managed to degrade nearly the same substrate amount within a three times shorter period. Such high and fast adaption ability to the increased feeding frequency and higher daily OLRs was not reported elsewhere. The increase of bacterial productivity with shortening of retention time was also confirmed by the 1st order model, even though the model suggested the increase of the degradation rate by a factor of 2 only. This can be seen as a consequence of the fitting curves in semi-batch focusing mainly on the biogas production for the initial 2 days of the feeding period. The increase of anaerobic digestion rate with decrease of retention time means that the lowest possible retention time limit (SCHATTAUER & WEILAND,

[39] comparison of first 3 days of batch experiments with initial feeding in semi-batch as well as the 1st day of anaerobic digestion in semi-batch and the initial feeding in continuous mode

[40] This means that the full adaption was reached after 3 days in semi-batch and 2 days in continuous mode.

[41] excluding the period of initial load adaption and the highest investigated OLR in both operating modes

2006) was not exceeded for these OLRs. Similar to results of ZAUNER & KÜNTZEL (1986), TRNOVEC & BRITZ (1998) and RINCON ET AL., (2008), for the highest OLRs in both semi-batch and continuous mode reduction of sGP was observed. For these both OLRs the retention time was too short to enable the degradation of such high substrate loads.

According to the residual biogas production around 10% of biomass per feeding was accumulated during digestion of 11.7 kgVS/m³ in semi-batch. For the comparable experiment in continuous mode (4.1 kgVS/m³) already 16% of substrate per feed was accumulatined, which represents an increase by 50% in comparison to semi-batch mode. The enhanced substrate accumulation does not automatically mean an overloading of the reactor. In continuous mode it is an effect of longer bacterial adaption (2-3 feeding periods) in the initial phase of continuous digestion. The biomass accumulated within this period could be digested only after the end of the feeding.

The correlation between shortening of retention time and reduction of CH_4 content in biogas could be observed. The maximum difference between batch and continuous mode did not exceed 5%, which is however a non-negligible quantity for biogas production plants. Therefore in case of using the results of substrate batch degradability studies to calculate the efficiency of biogas plants, a correction has to be done on the expected methane yield.

Influence of OLR on substrate degradation

The higher OLR, the higher total specific biogas yield was achieved. One of the factors, which could have caused this effect, might be the difference in carbon balance to that assumed in the thesis (s. Chapter 3.1). Similar to VDI (2004) and GREPMEIER (2002) 10% of the substrate was considered as lost in form of substrate for bacterial growth or carbon leftovers in effluent. However according to GREPMEIER (2002) the carbon loss due to each of the factors mentioned may vary between 1% and 5%. Therefore it is possible that for higher OLRs and regular feeding less residual carbon was present in the effluent or under optimal conditions the substrate uptake for bacterial growth was smaller. This factor probably overlapped with the high natural inhomogeneity of maize silage (for details s. Chapter 5.2).

Optimal and inhibited OLR for continuous (semi-batch) digestion

4.0 kgVS/m³ is regarded in practice as the OLR limit for undisturbed continuous digestion of energy crops without additives in CSTR mode (JUCKENACK, 2005; LINKE & MÄHNERT, 2005; REINHOLD, 2005; HUBER ET AL., 2007; GERSTL, 2008; ZOSEL ET AL., 2008; SCHOLWIN ET AL., 2009).

The TIC, TVA, VFA, pH and ORP trends confirmed no irregularities for 5.9 kgVS/m³ in semi-batch mode (~2.0 kgVS/m³ daily), which was in accordance with OLR limits mentioned in the literature.

According to the literature all 3 experiments in continuous mode and 2 semi-batch series (comparing daily OLR) can be assumed as overloaded. However, for 4.1 kgVS/m³ in continuous mode (~4.1 kgVS/m³ daily) no indication of inhibition but a small decrease of TIC was observed. For 5.9 kgVS/m³ in continuous mode (~5.9 kgVS/m³ daily) partially even higher daily sGPs were measured than for 4.1 kgVS/m³, although some parameters (HPr, HPr/HAc and ORP) increased gradually. The irregularities for similar parameters but also for TVA/TIC, HAc and pH were observed for 11.7 kgVS/m³ in semi-batch mode (~3.9 kgVS/m³ daily) but only towards the end of the experimental series. The same parameters confirmed unquestionably the inhibitory changes for 17.6 kgVS/m³ in semi-batch mode (~5.9 kgVS/m³ daily) after 7th feeding period (28 day) and for 11.7 kgVs/m³ in continuous mode (~11.7 kgVS/m³ daily) already after 4th day of operation, even though two different inhibitory mechanisms (HBu and HPr inhibition) were discovered (s. Chapter 4.3.2).

Due to the rapid decline of k values, the results of 1st order fit confirmed the inhibition of the digestion at 17.6 kgVS/m³ in semi-batch (~5.9 kgVS/m³ daily) and 11.7 kgVS/m³ in continuous mode (~11.7 kgVS/m³ daily). For uninhibited series each increase of OLR independent of the operating mode resulted in a decrease of the first order coefficients. This suggests that the anaerobic digestion at the lowest OLR continued always with the highest rate and for each of the operating modes tested the lowest OLR can be assumed as the most optimal to obtain the fastest conversion[42].

According to kinetic coefficients the optimal digestion was achieved for 5.9 kgVS/m³ in both continuous (~5.9 kgVS/m³ daily) and semi-batch mode (~2.0 kgVS/m³ daily), even though in semi-batch this state was attained already for the third feeding period (k_3) while in continuous mode only for the eighth one (k_8). A steady digestion pattern can also be presumed for the lowest continuous OLR beginning with k_9 but the feeding stop after the 10th charge did not allow the verification of this presumption. A longer experimental series would be required to confirm the findings. For that purpose however unlike to the applied methodology real chemostat[43] conditions are necessary.

[42] It cannot be excluded that a lower not investigated OLR would be more optimal

[43] In chemostat culture each reactor loading is accompanied by removal of respective amount of digestate. This was not the case in the semi-batch and continuous series, in which only sporadic small amounts of effluent for analytical purposes were taken out of the reactor.

Inhibition indicator: HPr/HAc and the sum of VFA

The analysis of HPr/HAc ratio together with VFA reveals the following correlations:

- only for 17.1 kgVS/m³ in semi-batch a simultaneous HPr/HAc and VFA increase was recognized;
- a considerable accumulation of HPr caused a significant increase of HPr/HAc ratio for both 4.1 kgVS/m³ and 5.9 kgVS/m³ in continuous mode although no clearly disturbed digestion was detected with help of other parameters;
- unlike reported by HECHT ET AL. (2007) and LEMMER (2007) the HPr/HAc ratio did not exceed the inhibition limit of 0.3 in case of extremely suppressed digestion observed for 11.7 kgVS/m³ in continuous mode even though the parallel considerable raise of VFA sum was measured.

The application of HPr/HAc as indicator of inhibition during anaerobic digestion includes the presumption, that in case of digestion disturbance the HAc-conditioned suppression of HPr degradation takes place (FISCHER ET AL., 1981; CHEN & DAY, 1986; MAWSON ET AL., 1991; ÖZTÜRK, 1991; MÖSCHE & JÖRDENING, 1999; WANG ET AL., 1999; PIND ET AL., 2003). Consequently faster HPr than HAc accumulation and the increase of HPr/HAc ratio should be observed (BOONE & XUN, 1987; BOONE & BRYANT, 1980; KASPAR & WUHRMANN, 1987; THOLOZAN ET AL., 1988; GORRIS ET AL., 1989). Similar acids performance was observed for cellulose and maize degradation in batch, semi-batch and even continuous mode for two lower OLRs. However in all those cases increase of HPr/HAc ratio was scarcely accompanied by VFA higher than 3500 mg/l and no inhibition was reported also according to other parameters. Only in case of undoubtedly suppressed digestion for 11.7 kg VS/m³ in continuous mode the immense increase of HAc was accompanied by not that strongly elevated HPr values. Such VFA performance was not reported in the literature in case of overloaded continuous digestion so far. The HPr/HAc ratio was not confirmed as useful in detecting digestion disturbances. Due to more complex VFA interactions than assumed in the hitherto publications not excluding backreactions and isomerisation (WU ET AL., 1993; PIND ET AL., 2003), the VFA performance in a considerably disturbed biogas reactor might be unpredictable and irreproducible.

Inhibition indicator: C_4-C_5 VFA

The 50 mg/l regarded as the upper limit for the non-inhibitory fermentation (SCHATTAUER & WEILAND, 2006) were exceeded in all experiments, even for the lowest OLR, for which no other irregularities were observed. Therefore it would be advisable to set

higher limits of undisturbed anaerobic digestion for iso- and n-HBu as well as iso- and n-HVA at the level of 100 or 150 mg/l for non-batch tests. Further, this limit should be related only to elevated VFA concentrations lasting longer than 3 days and not to the short-termed increase of C_4-C_5 VFA concentrations.

On the other hand setting of universal C_4-C_5 VFA limits seems to be impossible as according to the latest studies (KRAKAT ET AL., 2010; NETTMANN ET AL., 2008) there might be considerable differences in anaerobic digestion mechanisms between various temperature modes, substrates and operating modes so that the different degradation paths should be considered (LAUKENMANN ET AL., 2010). By now it was assumed that under inhibitory conditions HAc-conditioned suppression of HPr degradation takes place (MAWSON ET AL., 1991; ÖZTÜRK, 1991; MÖSCHE & JÖRDENING, 1999; WANG ET AL., 1999; PIND ET AL., 2003). But the results of this study show a significant accumulation of n-HBu instead of HPr for the observed irreversible inhibition (s. Chapter 4.3.2).

Inhibition indicator: TVA/TIC

The TVA/TIC limits regarded as defining the unsuppressed anaerobic digestion (CECCHI ET AL., 2003; TELSCHOW, 2007; LOSSIE & PÜTZ, 2008) were exceeded considerably for the highest OLR in semi-batch and both higher OLRs in continuous mode. Although for 5.9 kgVS/m³ in continuous mode only slight increase of HPr and ORP were registered, a TVA/TIC curve was progressing permanently above the defined upper inhibitory limit. As the series was stopped after 10th feeding the applicability of TVA/TIC as an indicator of early stage of inhibition for a permanently light overloaded system could not be validated.

Response of analytical parameters to the system changes

Similar to RIEGER & WEILAND (2006) in continuous mode and different to GOLKOWSKA & GREGER (2010) in batch mode the development of instable conditions in semi-batch were announced in the first place by TIC drop and ORP increase followed by TVA and TVA/TIC raise and subsequent slower pH drop. In continuous experiment, though, TIC, TVA, pH and ORP reacted at the same time to the inhibition in the reactor (11.7 kgVS/m³). Nevertheless for 5.9 kgVS/m³ in continuous mode a gradual HPr accumulation was permanently accompanied by increased TIC/TVA, followed by a raised HPr/HAc ratio after 4 days and by enhanced ORP after 7 days only. The differences in the response time of different parameters reveal the whole complexity and differences between inhibitory mechanisms depending on operating mode and OLR. Consequently only one parameter giving the fastest response in case of all inhibitory fermenters cannot be defined.

Rate limiting step

The hydrolytic rate constants (s. Attachment D) given in the literature for carbohydrate based substrates were in most cases smaller than the first order rate coefficients k of 0.21-0.81 d^{-1} calculated for the maize experiments. Similar values for substrate degradation were given by GARCIA-HERAS (2003), KALFAS ET AL. (2006) and suggested in ADM 1 (BATSTONE ET AL., 2002). The obtained values lie in the range considered as characterizing substrate disintegration step. In particular the k values fitted for the uninhibited continuous digestion of maize are much higher than expected for hydrolysis of carbohydrates. Basing on this observation it can be assumed that in this case the substrate disintegration determines the rate of anaerobic digestion for maize.

5.4 Modeling approach

The problems with the data fitting especially for the higher OLRs of maize and/or cellulose with the Monod model were partially linked to certain modeling methodology chosen in the study. If x_0 and y had not been set constant and K_s and μ_{max} not kept strictly within defied limits a much better curve fitting could have been achieved with Monod model. This, however, means that the chosen parameters would not have been typical for the methanogenic/acetogenic biocenosis as the inhibition linked to the substrate excess would have been integrated into the parameter values. Since such approach was not tested it is also unclear whether such curve fitting would have been possible for all the experimental series with comparable parameters (x_0, y, K_s, μ_{max}). Also a more global approach in sensitivity analysis could deliver information for the choice of different parameter values for the Monod model.

In the approach chosen in the study the inapplicability of the Monod model for higher OLRs delivered an indication of substrate excess inhibition. For such approach the inhibition could be integrated into the model equation by implementing a separate inhibition term.

6 Conclusions

Recovery of the system after single enhanced loads

Even extremely high but single charges of cellulose under thermophilic conditions did not lead to the collapse of the system or to acidosis. The increase of OLR resulted in exceeded degradation time only. The results show a great flexibility of the inoculum in terms of fast adaption to high loadings of cellulose if only single charges are applied.

Assuming that similar adaption is possible also for maize silage, this observation is of a great importance for thermophilic operated biogas plants. It shows that a single mistake in reactor charging for thermophilic reactors regarded as very sensible can be simply corrected by the prolongation of the digestion time if extremely high substrate charge was introduced into fermenter.

The parameter changes after each feeding for both maize silage and cellulose in mesophilic mode were less pronounced than in thermophilic mode. Consequently it can be assumed that under mesophilic conditions even a few increased OLRs introduced by mistake into an industrial biogas reactor would not cause serious digestion problems.

Inhibitory indicators in batch mode

The comparisons of different parameters in batch experiments revealed that all of them allowed following changes within the fermenter and responded simultaneously. The different parameters regarded as explicit inhibition indicators showed contradictory results. For some of them at higher OLRs the inhibitory marks were exceeded (VS_s/VS_i; TVA/TIC, C_4-C_5 VFA) while for the others not (total biogas yield, ORP, pH, HPr/HAc ratio together with the sum of VFA). The parameter reaction and the flexible recovery of the reactor balance even for the enhanced OLRs shows the low applicability of any inhibition indicators in batch mode. This is even valid for thermophilic systems tending to strong reactions to any change of conditions.

Degradation of cellulosic material

During degradation of cellulosic material a 1-2 day delay linked to the adaption phase of bacterial biocenosis and slowly starting hydrolysis has to be considered. This delay would probably be less pronounced in case of regular feeding. During digestion the hydrolysis determined the digestion rate but only in its very early stage. In the subsequent digestion phase acetogenesis was the rate limiting step for the lowest OLR. For the elevated OLR the degradation rate was affected mainly by methanogenesis while

at the highest OLR both acetogenesis and methanogenesis were influencing the rate of digestion.

Further much lower conversion grades were reached for cellulose than for maize. The effect was even strongly pronounced for thermophilic conditions. It is very probable that this trend is a consequence of shortage of some nutrients necessary for the optimal digestion. However, this assumption cannot be confirmed by any results as the elementary composition of the inoculum was not investigated during the studies.

Influence of VS content on biogas yield

In the experiments the negative influence of too low VS content of inoculum on the total biogas yield could be shown. On the other hand the highest biogas yields were not always linked to the highest VS content of inoculum. The parameter turned out to be to some extent unreliable in predicting the activity of biocenosis as it includes the hard degradable substrate residuals and dead microorganisms. Both fractions elevate the VS content of the inoculum, which suggests higher bacterial activity than can actually be expected from the existing biocenosis.

System specific changes of biogas yield

Despite strictly controlled experimental procedure higher than expected biogas production was observed for some tests with maize silage independent of the operating mode. The main factor assumed as generator of this effect is the strong natural inhomogeneity of maize silage, while further influencing factors might be: changing activity of bacterial biocenosis, variable substrate demand for bacterial growth as well as different amount of substrate residues in the effluent. All four factors cannot be directly influenced and calculated/measured by an operator of a biogas plant; however they may have an enormous impact on the gas production.

Optimal temperature of digestion

From the comparison of experiments in batch mode for maize and cellulose more stable digestion took place under mesophilic conditions. No differences in biogas yield could be observed for different temperature modes, while methane content in biogas was slightly higher for mesophilic experiments. The observed faster substrate conversion rate for mesophilic trials was contradictory to the literature and is believed to be a sign of a systematic nutrients shortage in all thermophilic batches originating from the inoculum.

Degradation pathways for different substrates

The degradation kinetics differed depending on the substrate composition and complexity. The experiments conducted on cellulose and maize silage revealed different fermentation mechanisms. Cellulose was degraded mainly via propionic acid to acetic acid and finally to methane. Maize, however, was converted in the first step to butyric acid and than via propionic and acetic acid as intermediates to methane.

Adaption of inoculum

Batch series with cellulose at extremely high OLRs as well as semi-batch and continuous digestion results show a great adaption ability of the bacterial biocenosis from the inoculum.

An extremely high adaption of anaerobic biocenosis to elevated OLRs or increased feeding frequency under thermophilic conditions could be observed in both semi-batch and continuous mode. This information is important for biogas plants operators. The result show that a stable biogas reactor operated only with maize silage without additives in thermophilic mode at OLR of 4 kgVS/m^3 (daily) can endure not only a single enhanced load but for even 10-day-period of the OLR increased by 50%.

Methane content in biogas

The expected methane fraction in biogas can decrease between batch and continuous digestion mode. According to the results presented in this thesis, the methane volume correction of 5% resulting form different operating modes is necessary for calculation of biogas yields for agricultural biogas plants basing on the substrate degradability tests in batch.

Optimum OLR

In batch mode substrate load of 11-12gVS/m^3 resulted in the most optimal digestion independent of the applied substrate and temperature mode. For semi-batch the most optimal degradation of maize silage was observed for 5.9 kgVS/m^3 (daily 2.0 kgVS/m^3), while for continuous digestion the optimum daily OLR ranged between 4.1 kgVS/m^3 and 5.9 kgVS/m^3 (daily 4.1-5.9 kgVS/m^3). However, a long-term study, including comparison of additional lower and higher OLRs, would be necessary to confirm this observation and precise the optimal OLR. Consequently the question of possible raise of OLR limit for energy crops digestion still remains open.

Inhibition mechanisms

Two inhibition mechanisms could be shown in the study: a reversible and irreversible one. For highest OLR in semi-batch mode a reversible inhibition with gradual increase of propionic acid was observed. However the accumulated propionic acid was degraded after the feeding stop. Another type of inhibition with low propionic acid values but extremely high n-butyric acid concentrations was registered for the highest OLR in continuous mode. In this experiment no VFA were degraded after the feeding stop. Such VFA trend was not reported by now in the literature for maize digestion.

Facing different inhibition mechanisms, not all parameters regarded as indicators of inhibition could detect its occurrence. Some of them indicated inhibition for the OLRs for which the anaerobic digestion continued in an undisturbed way.

Comparison of all parameters controlling anaerobic digestion reveals that in case of energy crops the limits describing uninhibited conditions for sludge or manure can turn out to be unreliable. Another question is, whether setting of such limits is generally reasonable. It seems to be more advisable to observe the trends and react to the prevailing parameter changes than focus on comparing the parameter results to the defined anaerobic digestion limits.

The discovered different inhibition mechanisms as well as incomplete reliability of the parameters regarded as inhibition indicators show the huge research potential in the field of anaerobic digestion with the focus on energy crops.

7 Perspectives

The research project, the results of which are presented in this thesis, was divided in two stages: basic methodology development and the laboratory experiments. Both steps turned out to be extremely time intensive and were additionally limited by a very strict 4-year time frame. This automatically reduced the number of experiments possible to conduct. The continuation of the study with maize silage in several experimental series at 38°C in semi-batch and continuous mode would allow completing the existing results and enabling a more comprehensive comparative analysis of mesophilic and thermophilic conditions and different operating modes under mesophilic regime.

Also different energy crops are of a great interest for the scientists. Maize silage has been the most commonly used and researched energy crop for anaerobic digestion process by now. However, the application focus is expected to shift from maize to grass silage in the future. The substrate diversification trend has been supported by national and regional European environmental policy development. The high but still unused energy potential of greenery cuttings (e.g. from landscape conservation, verges and buffer stripes), makes them very appealing for public and private stakeholders. This, in consequence, creates a high demand for specific knowledge on grass silage digestion and opens new research possibilities.

Fast technological progress especially in microbiological techniques gives scientists nowadays powerful tools for characterizing anaerobic digestion systems in the way that was not possible few years ago. Nevertheless, still not much research was done by implementing more advanced tools (e.g. fluorescent bacteria labeling or DNA sequencing methods) to characterize the development of microbial dynamics during energy crops digestion. The latest studies reveal also several methodological research gaps, which need to be closed, e.g.:

- development of sampling and pre-treatment methodology for measurements of COD in a biogas fermenter or digestate, as the existing methodology overtaken from waste water treatment does not give reproducible results;
- adjusting of van Soest and Weende methodology for the analysis of non-forage substances such as samples coming from biogas reactor or digestate;
- improving the reproducibility of NIRS for silage characterization and if possible NIRS application in analysis of biogas reactor and digestate samples;

These methodology developments would enable more advanced characterization of subsequent steps of energy crops digestion including analysis of residual substrate fractions in the reactor content.

Last but not least, the advanced modelling of energy crops digestion especially with the help of ADM1 creates a great challenge for scientists and is the subject of current investigation. There were only a few successful attempts reported in this field by now.

Some of the above mentioned aspects are also in the focus of the Process Engineering Unit at the University of Luxemburg. The collected results are expected to give more information about grass silage digestion, characterize subsequent degradation steps and microbial dynamics as well as be applied as a basis for modelling of energy crops digestion with ADM 1.

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A Attachment Bonus Systems for Biogas Plants

Tab. A.1 Comparison of the existing bonus systems for biogas plants in Luxemburg and Germany (RGD, 2008; EEG, 2009)

	Luxemburg	Germany	
First legal act	1994	1991	
Last law act	RGD, 2008	EEG, 2009	
Basic bonus in cent/kWh			
0 - 150 kW	15	11.67	
150 - 300 kW	14	9.18	
300 - 500 kW	13	9.18	
500 - 2500 kW	12	8.25	
2500 - 5000 kW	-	8.25	
5000 - 20000 kW	-	7.79*	
Further conditions:			
	bonus declining with the year of putting into operation	bonus declining with the operation year	
	up to 50% of investments can be refounded	some aditional boni possible:	- combined heat and power generation - max. 3 cent /kWh
			- use of energy crops - max. 6 cent /kWh
			- innovative technology (e.g for dry fermentation technology) - max. 2 cent /kWh
			- manure use - max. 4 cent/kWh
			- use of landscape conservation plant waste - max. 2 cent/kWh

* bonus can be applied only if combined heat and power generation takes place

B Attachment Material and Methods

B.1 Substrate

Tab. B.1 Composition of maize silages used in the tests the data including corrections of VFA (s. Chapter 3.1)

Component		DM_N	VS_N	DM_K	VS_K	Crude ash	Crude protein	Crude fat	NFC	NDF	ADF	Lignin/ ADL	Hemicel lulose	Cellu lose	VFA
Unit		% FM		% FM		% DS									
Maize	MZ I	33.9	32.6	33.9	32.6	4.1	6.7	3.2	43.2	40.1	26.3	3.5	13.8	22.8	2.7
	MZ II	33.4	32.2	33.4	32.2	3.3	7.3	2.8	49.4	34.4	22.3	3.5	12.0	18.8	2.8

B.2 Inoculum

Tab. B.2 History of the inoculum used in the tests

Temp.	Date		Substarte	OLR [kgVS/m³]	Mode	DS inoc. [%FM]	VS inoc. [%DS]	VS inoc. [%FM]
	Start	End						
thermophil	01.07.08	14.07.08	cellulose	5.7	batch	4.5	60	2.71
	14.07.08	04.08.08	cellulose	11.4	batch	4.5	54	2.43
	04.08.08	26.08.08	cellulose	17.1	batch	4.5	54	2.43
	22.09.08	13.10.08	cellulose	22.9	batch	2.8	55	1.54
	13.10.08	12.11.08	cellulose	28.6	batch	2.3	46	1.06
	24.11.08	15.12.08	cellulose	5.7	batch	2.1	55	1.16
	10.12.08	05.01.09	cellulose	22.9	batch	1.9	54	1.02
	16.02.09	23.03.09	cellulose	34.3	batch	2.6	61	1.58
	16.03.09	03.04.09	maize	5.7	batch	1.0	59	0.59
	04.06.09	27.07.09	maize	5.9 x 10	semi-batch	2.0	58	1.2
	04.06.09	27.07.09	maize	11.7 x 10	semi-batch	2.0	58	1.2
	27.07.09	14.08.09	maize	11.5	batch	3.2	56	1.8
	27.07.09	14.08.09	maize	17.3	batch	3.2	56	1.8
	20.10.09	15.12.09	maize	17.6 x 10	semi-batch	3.4	54	1.8
	22.02.10	21.03.10	maize	5.9 x 10	conti	3.4	55	1.9
	22.02.10	21.03.10	maize	11.7 x 10	conti	3.4	55	1.9
	22.03.10	12.04.10	maize	4.1 x 10	conti	3.9	58	2.3
mesophil	16.11.09	15.12.09	maize	5.7	batch	2.1	54	1.11
	16.01.09	05.02.10	maize	5.5	batch	3.7	53.9	2
	16.01.09	05.02.10	maize	11.0	batch	3.7	53.9	2
	08.02.10	26.02.10	maize	17.1	batch	3.1	57.3	1.75
	19.04.10	20.05.10	cellulose	5.4	batch	3.0	59.3	1.8
	03.05.10	16.05.10	cellulose	10.9	batch	3.0	59.3	1.8
	17.05.10	07.06.10	cellulose	16.3	batch	3.0	59.3	1.8

C Attachment Results

C.1 GP results

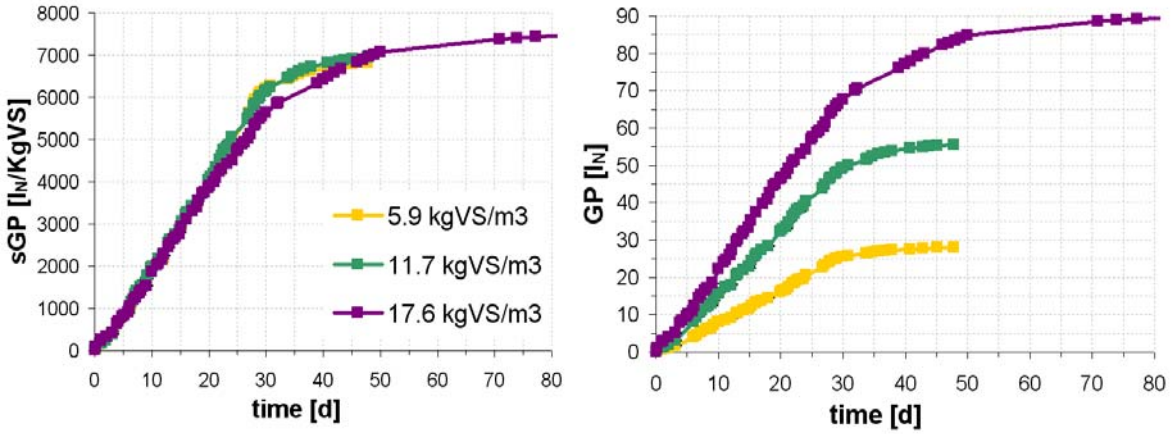


Fig. C.1 Biogas production observed during semi-batch fermentation of maize in thermophilic mode

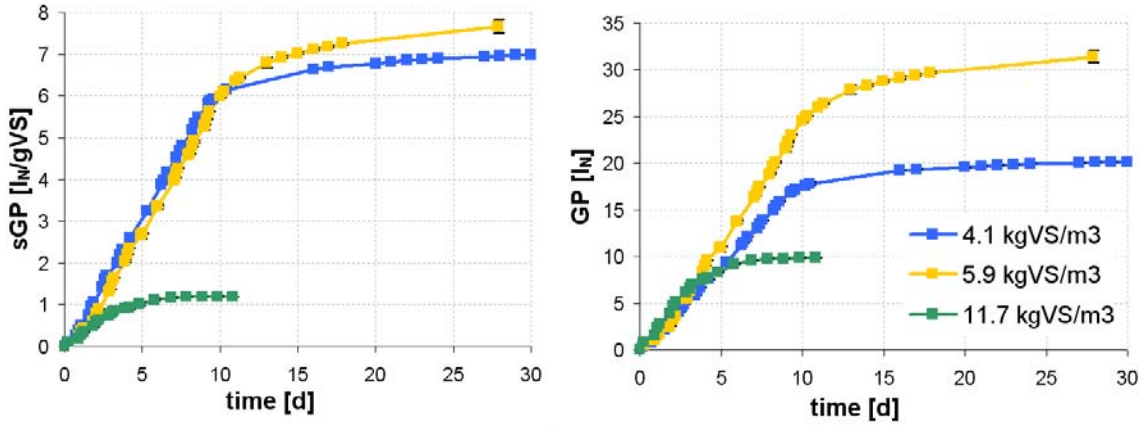


Fig. C.2 Biogas production observed during continuous fermentation of maize in thermophilic mode

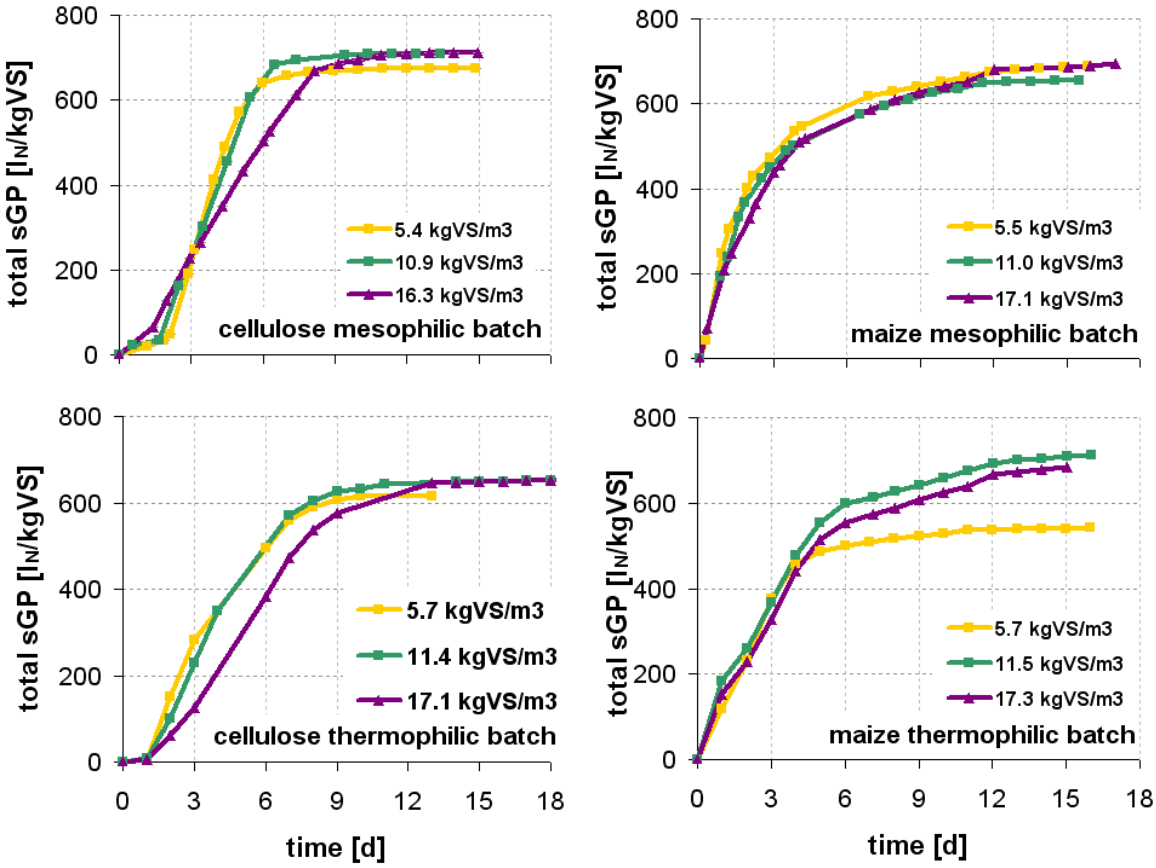


Fig. C.3 Biogas production observed during batch fermentation of maize and cellulose under mesophilic and thermophilic conditions. For all investigated batch series the raise of sGP with the increase in OLR for the initial digestion phase (first 3 days of digestion) was only observed in mesophilic cellulose experiments.

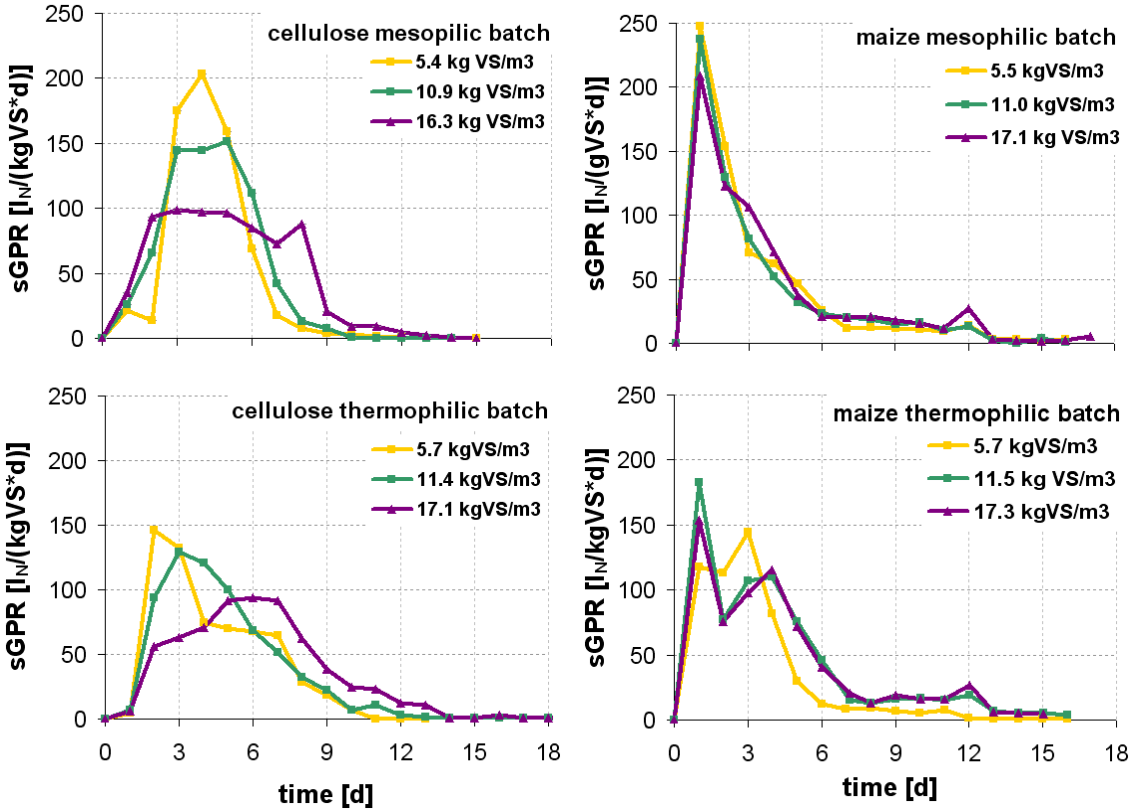


Fig. C.4 Specific biogas production rate (sGPR) measured during batch fermentation of maize and cellulose under mesophilic and thermophilic condition. For all investigated batch series the raise of sGPR with the increase in OLR for the initial digestion phase (first 3 days of digestion) was only observed in mesophilic cellulose experiments.

C.2 TIC & TVA

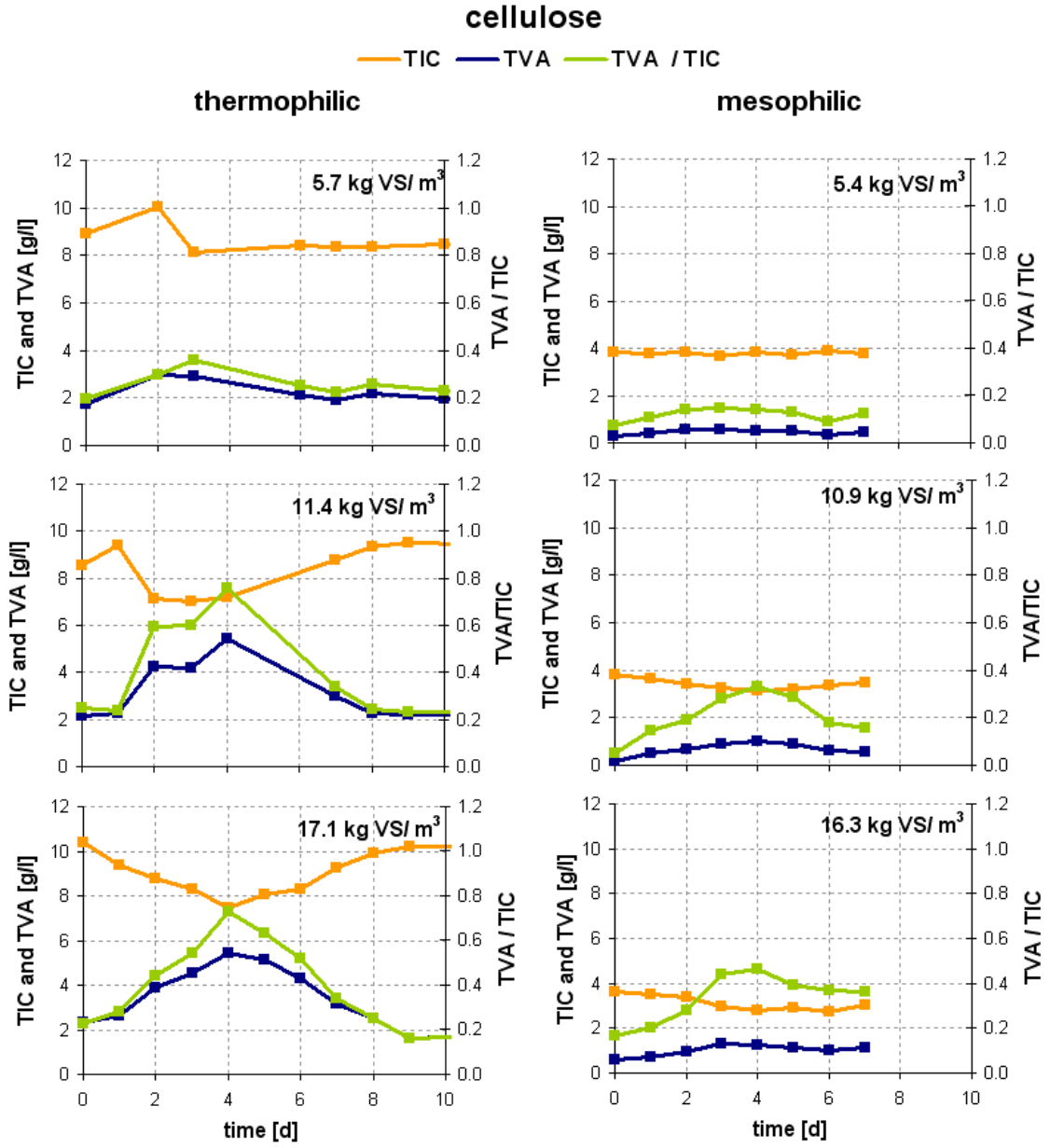


Fig. C.5 The time progress of the titrated inorganic carbon (TIC) and titrated volatile acids (TVA) as well as their ratio (TVA/TIC) plotted for cellulose batches under thermophilic and mesophilic conditions

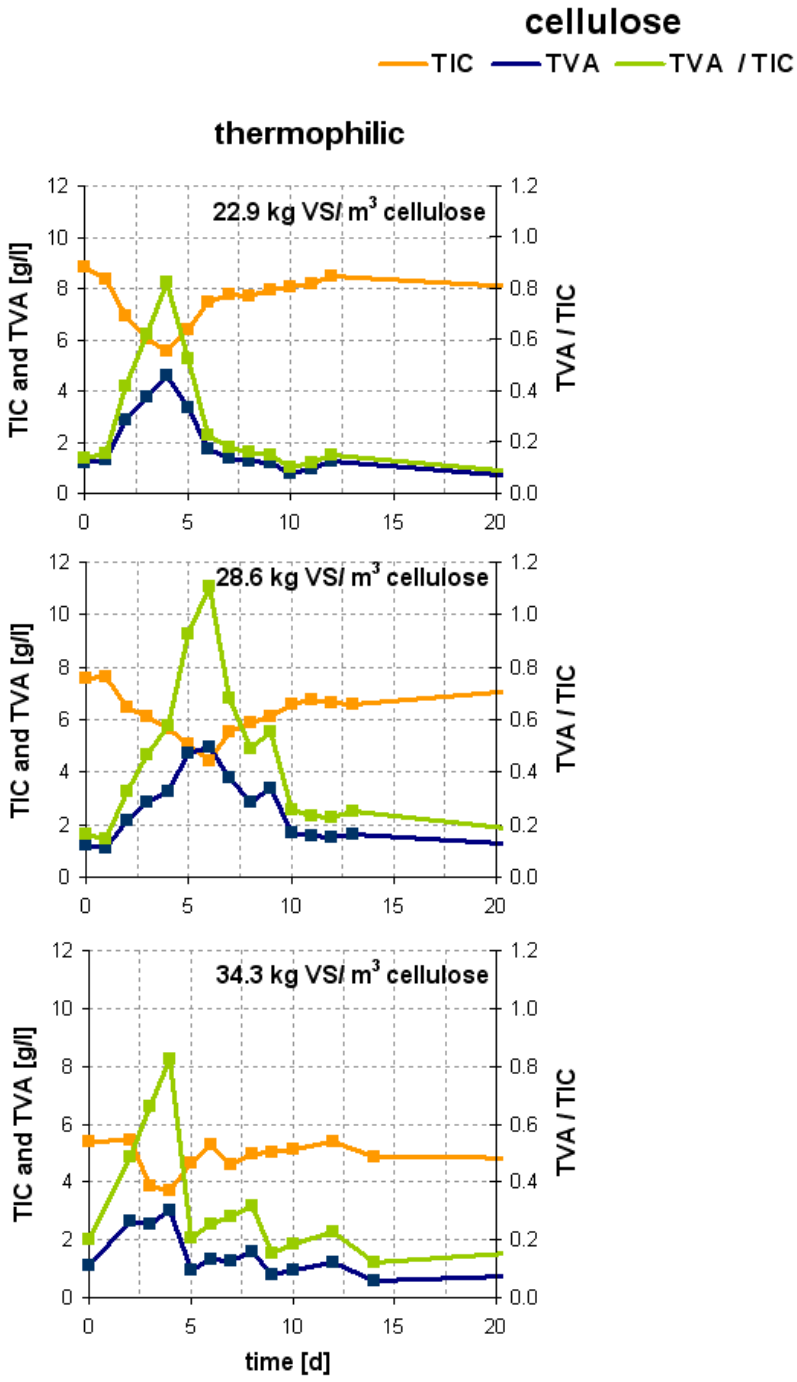


Fig. C.6 The time progress of the titrated inorganic carbon (TIC) and titrated volatile acids (TVA) as well as their ratio (TIC/TVA) plotted for cellulose batches under thermophilic conditions

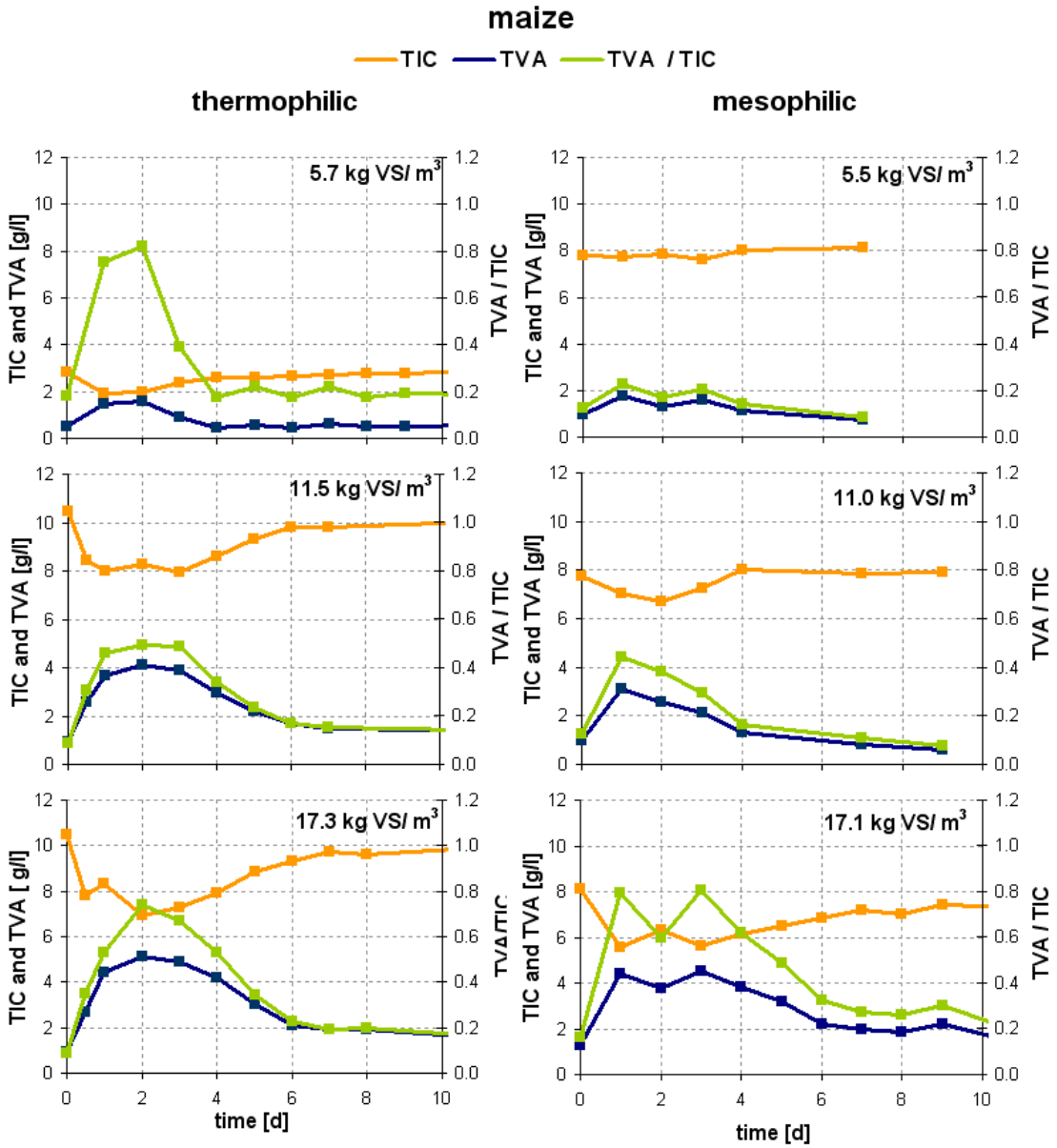


Fig. C.7 The time progress of the titrated inorganic carbon (TIC) and titrated volatile acids (TVA) as well as their ratio (TIC/TVA) plotted for maize batches under thermophilic and mesophilic conditions

C.3 ORP and pH

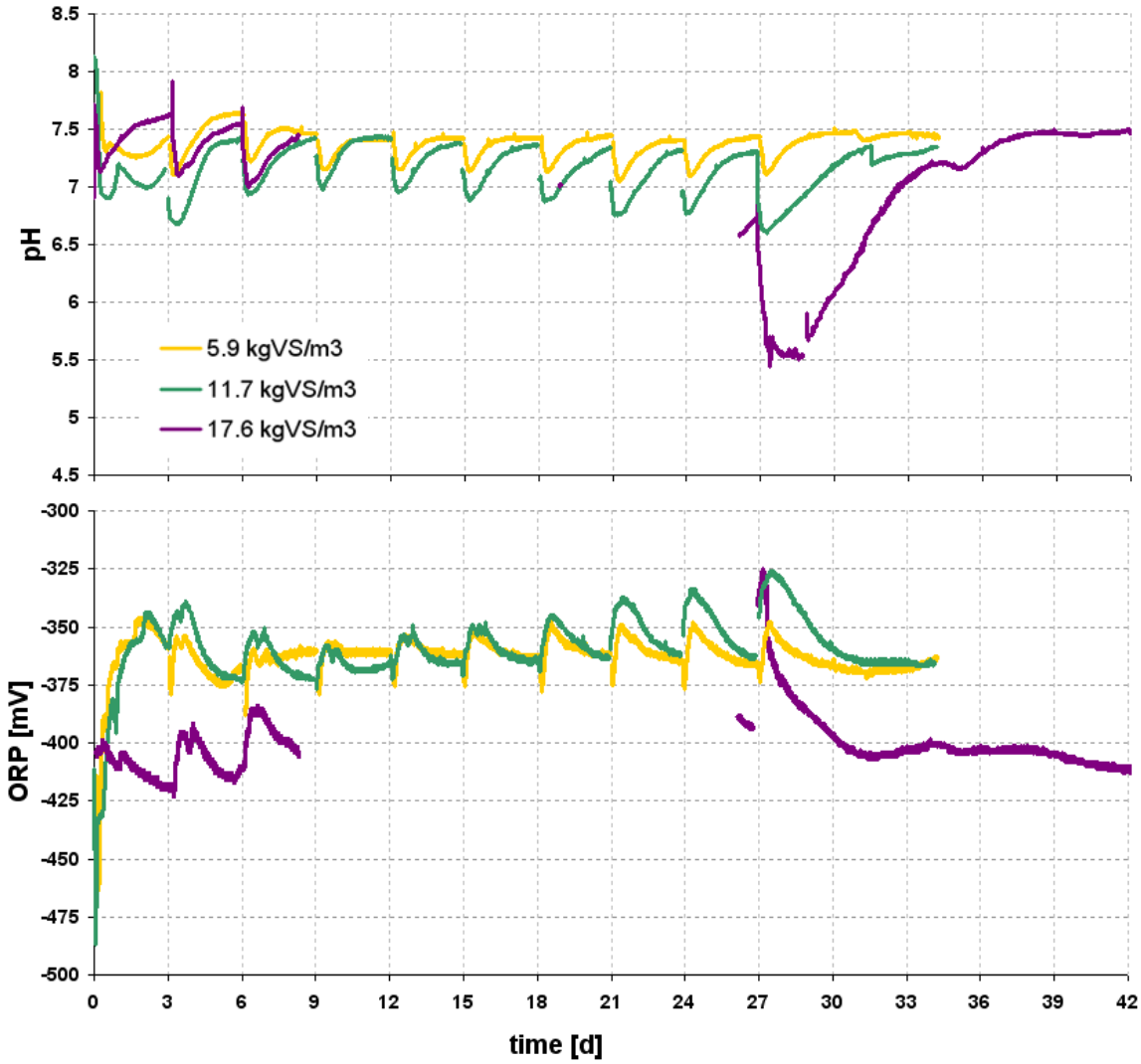


Fig. C.8 Performance of pH and ORP during the semi-batch experiments with maize at 5.9, 11.7 and 17.6 kg VS/m³ under thermophilic conditions (due to the system error the on-line data for 17.1 kg VS/m³ are only partially available)

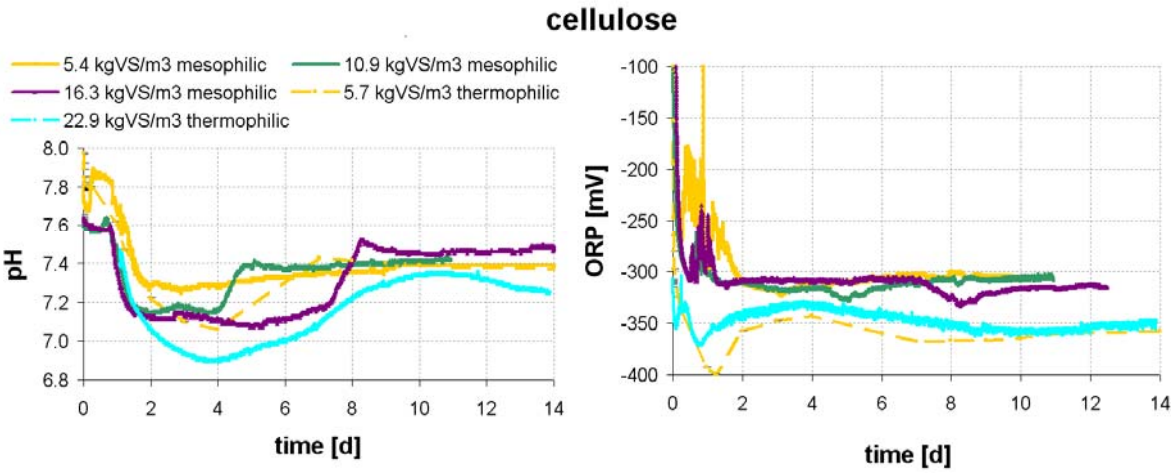


Fig. C.9 The time progress of redox potential and pH for cellulose batches under mesophilic and thermophilic conditions

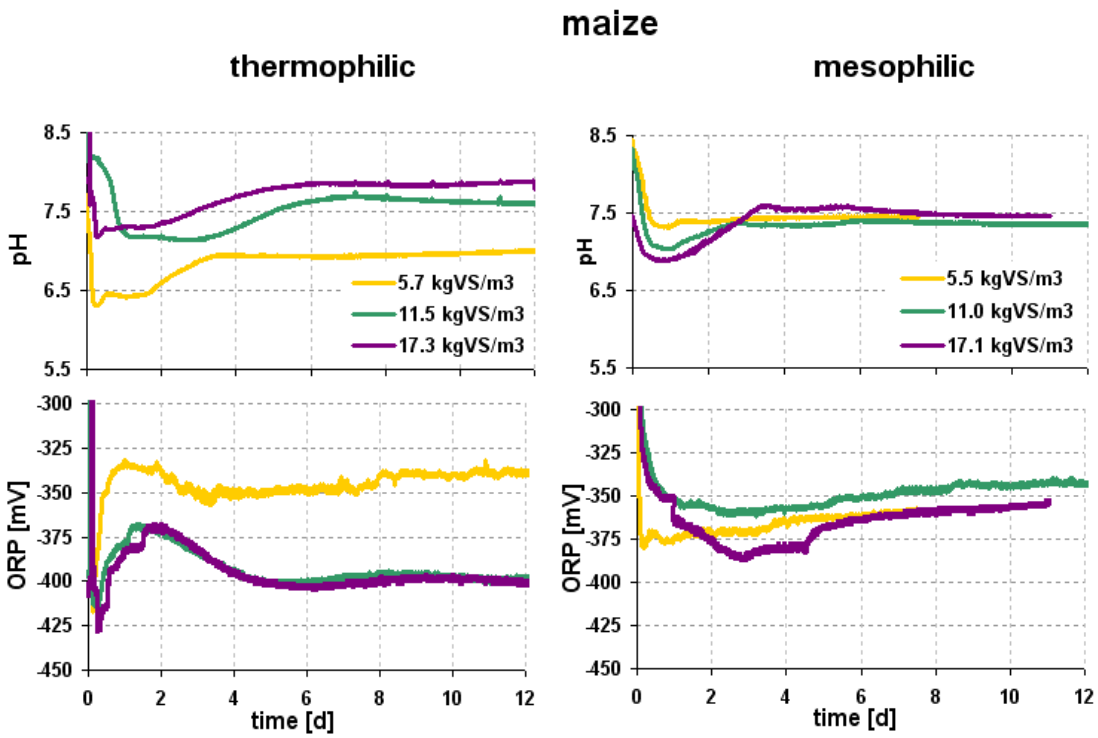


Fig. C.10 The time progress of ORP and pH for maize batches under mesophilic and thermophilic conditions

D Attachment – Modeling 1st order

Tab. D.1Overview of literature 1st order kinetic parameters for carbohydrates, organic waste or energy crops

Substrate	k_{dis} [d^{-1}]	k_H [d^{-1}]	Reference
cellulose	0.11 - 0.37	-	Beierlein, 2011
cellulose	-	0.15	Batstone at el., 2002
food waste	0.41	-	Batstone at el., 2002
solid waste	-	0.11 - 0.17	Sosnowski et al., 2007
carbohydrates	0.4 - 1.0	0.25 - 10	Batstone at el., 2002
cellulose	-	0.04 - 0.13	Gujer & Zender, 1983
grass silage	1.0	-	Wichern et al., 2009
cellulosic material	-	0.012 - 0.020	Qu et al., 2009
carbohydrates	-	0.025 - 0.2	Christ et al., 2000
carbohydrates	-	0.5 - 2	Garcia-Heras, 2003
carbohydrates	-	0.35	Kalfas et al., 2006

Tab. D.2 Summary of first order model parameters obtained for batch experiments. Measured values are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1).

substrate	Tempe-rature	OLR [kgVS/m^3]	not modelled		S_0 measured [gC]	S_0 modelled [gC]	k [d^{-1}]
			initial days	% of S			
cellulose	38°C	5.4	2	7	1.41	1.52	0.58
		10.9	2	5	2.89	3.20	0.49
		16.3	2	18	3.75	3.95	0.28
	55°C	5.7	1	3	1.56	1.55	0.34
		11.4	1	1	2.73	2.84	0.29
		17.1	2	10	4.32	4.73	0.25
		22.9	3	17	5.33	5.49	0.20
		28.6	4	17	6.62	6.93	0.12
		34.3	4	19	7.75	8.23	0.14
maize	38°C	5.5	0	-	1.47	1.45	0.41
		11.0	0	-	2.90	2.85	0.39
		17.1	0	-	4.69	4.69	0.32
	55°C	5.7	0	-	1.50	1.51	0.36
		11.5	0	-	3.13	3.13	0.26
		17.3	0	-	4.59	4.65	0.24

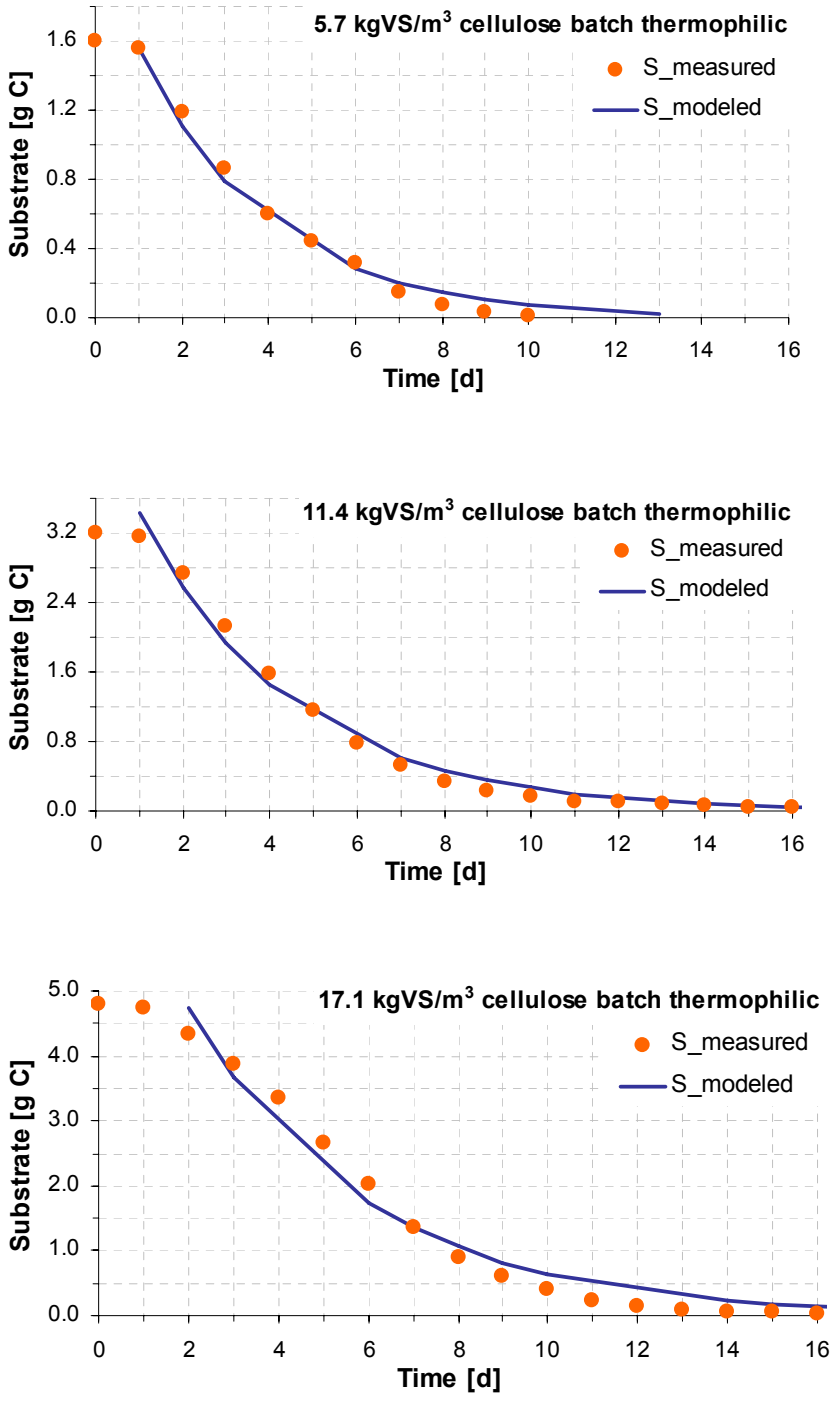


Fig. D.1 Substrate degradation measured and modeled with 1st order equation for thermophilic digestion of cellulose in batch mode. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1).

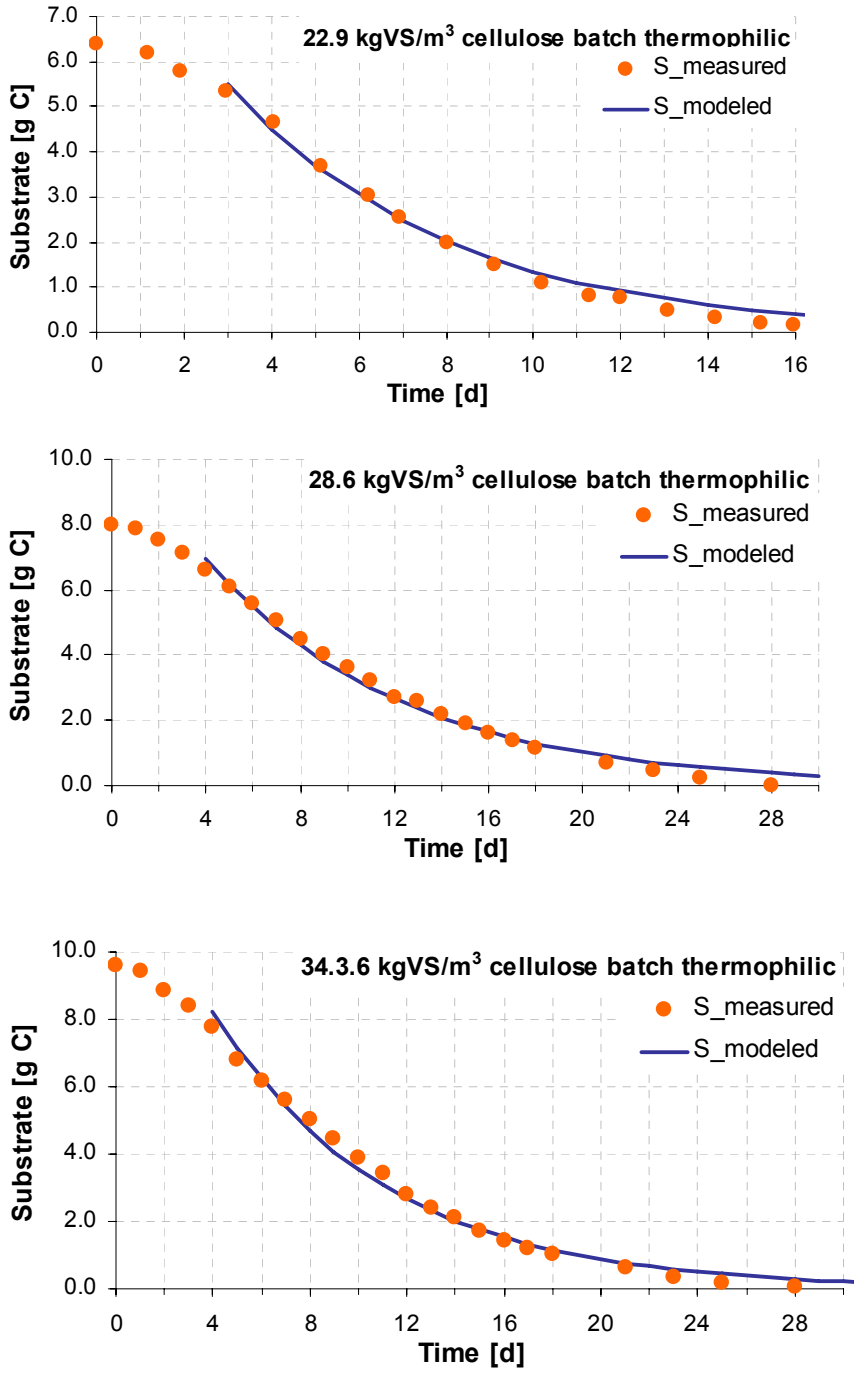


Fig. D.2 Substrate degradation measured and modeled with 1st order equation for thermophilic digestion of cellulose in batch mode. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1).

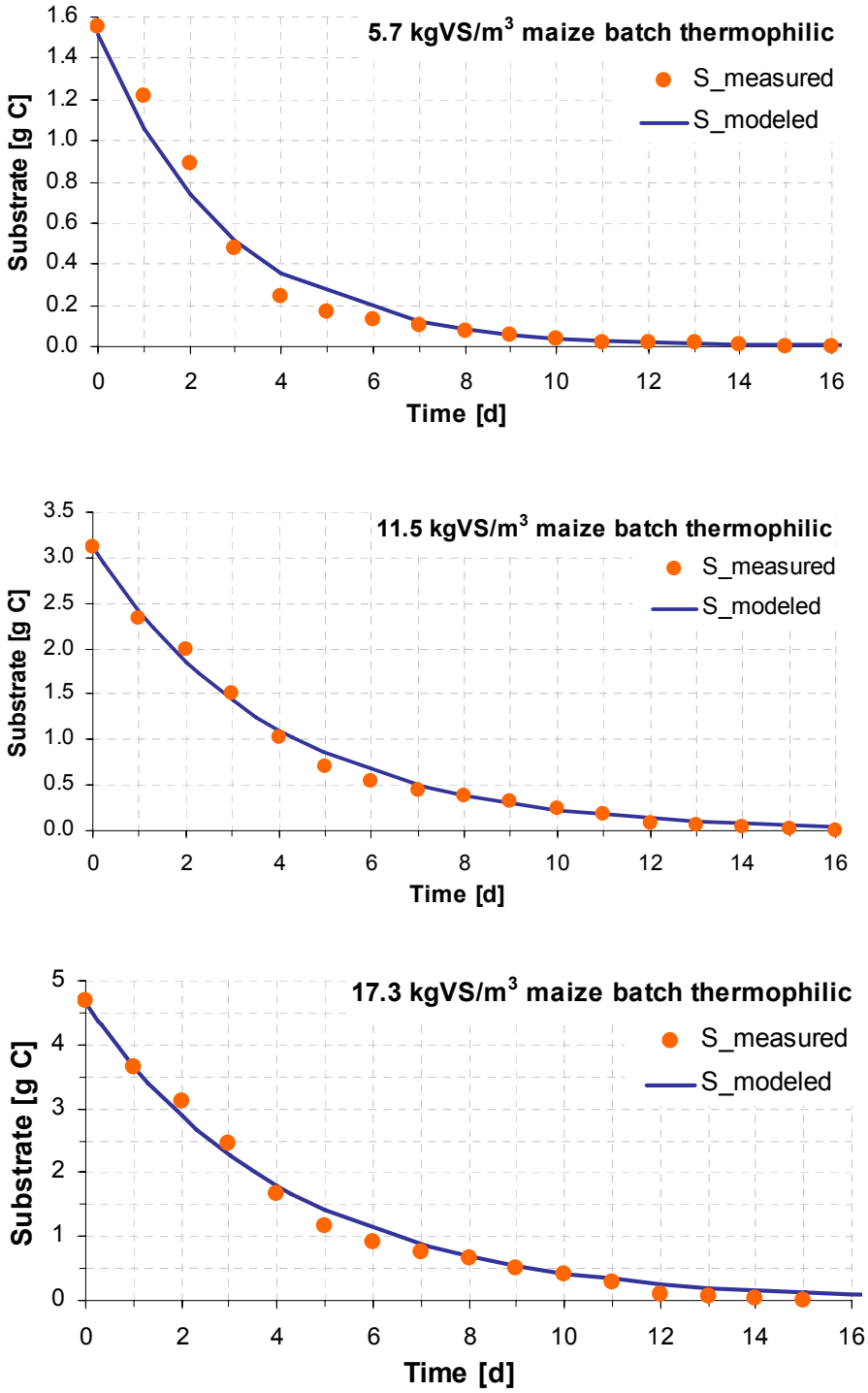


Fig. D.3 Substrate degradation measured and modeled with 1st order equation for thermophilic digestion of maize silage in batch mode. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1).

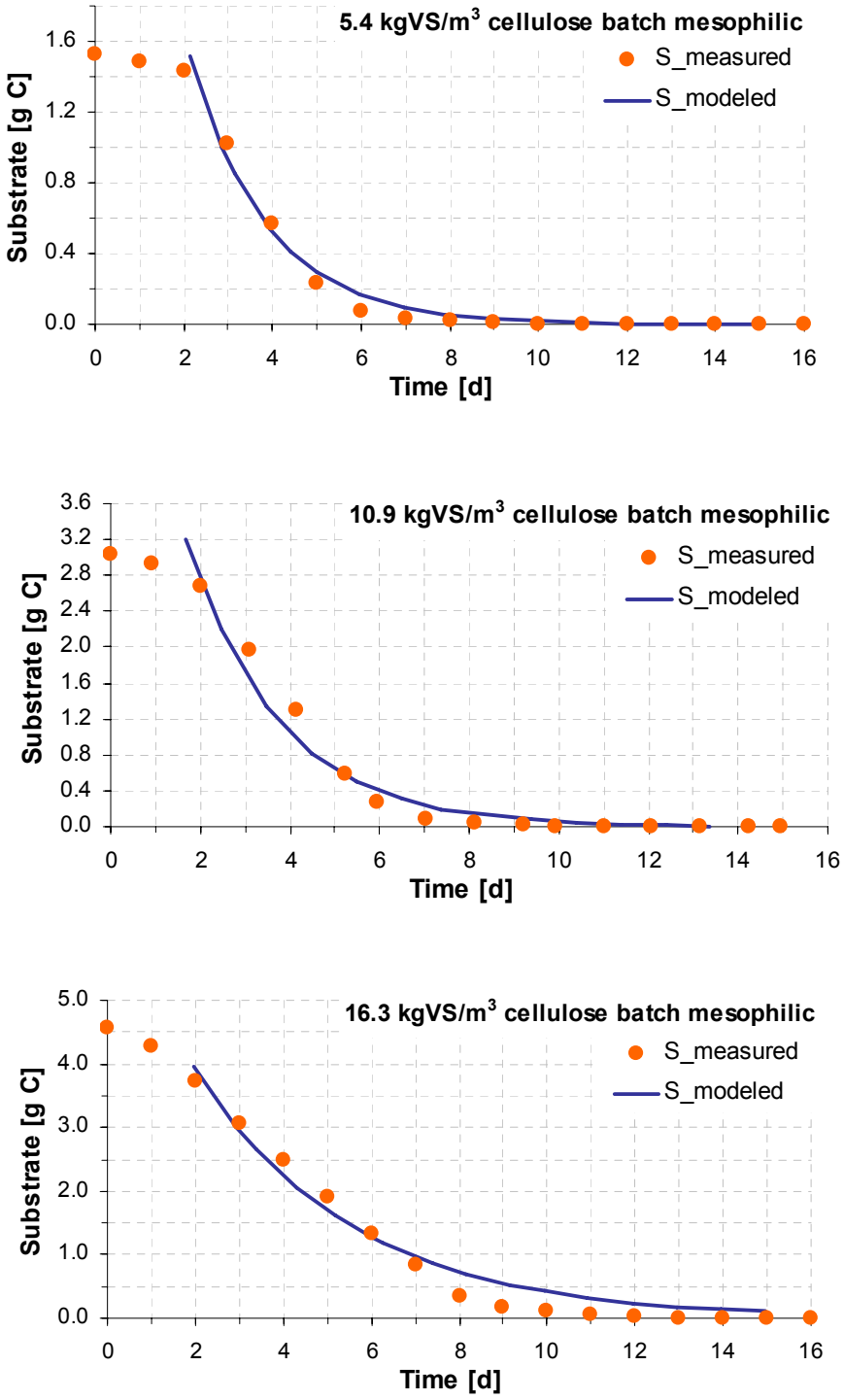


Fig. D.4 Substrate degradation measured and modeled with 1st order equation for mesophilic digestion of cellulose in batch mode. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1).

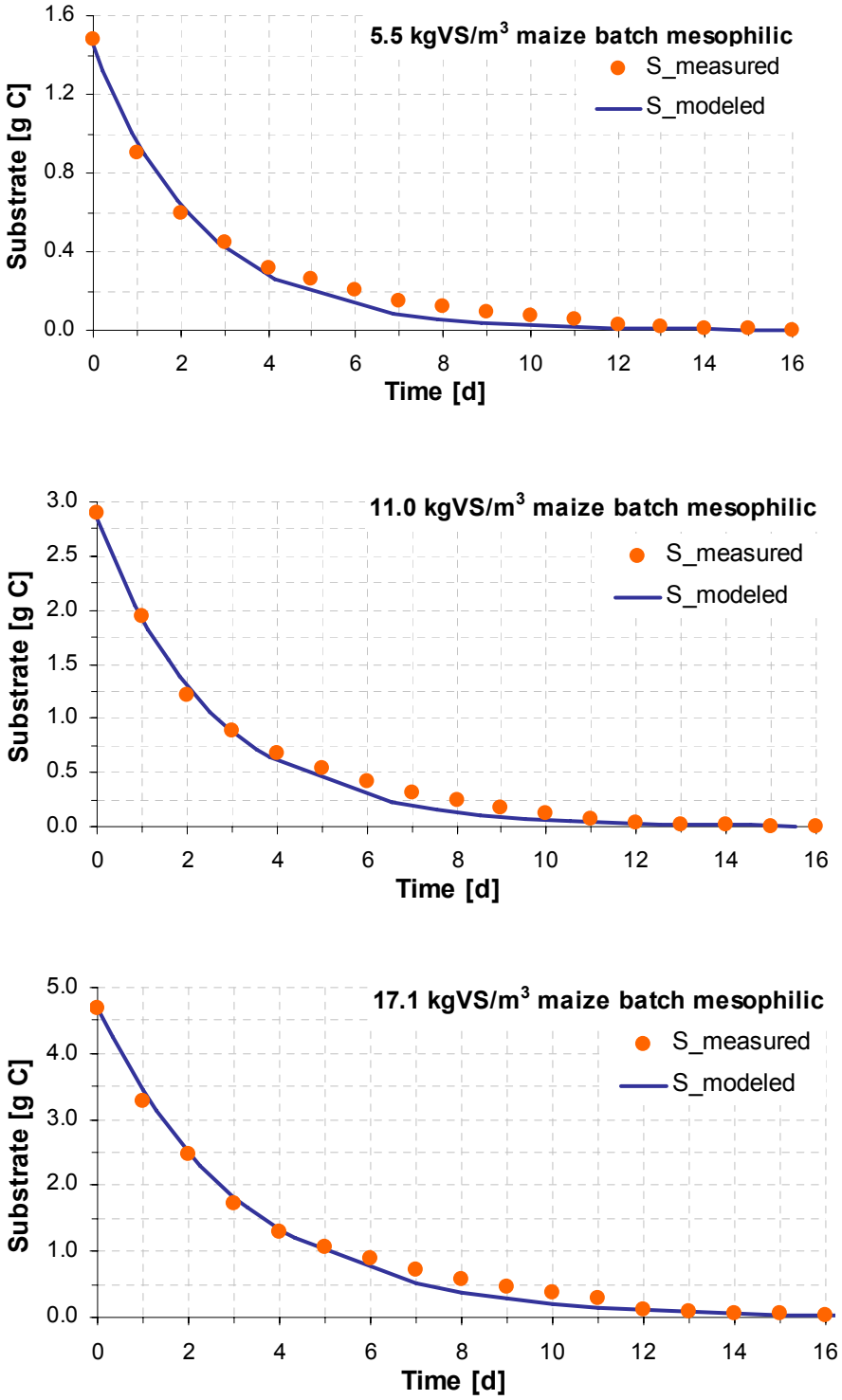


Fig. D.5 Substrate degradation measured and modeled with 1st order equation for mesophilic digestion of maize silage in batch mode. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1).

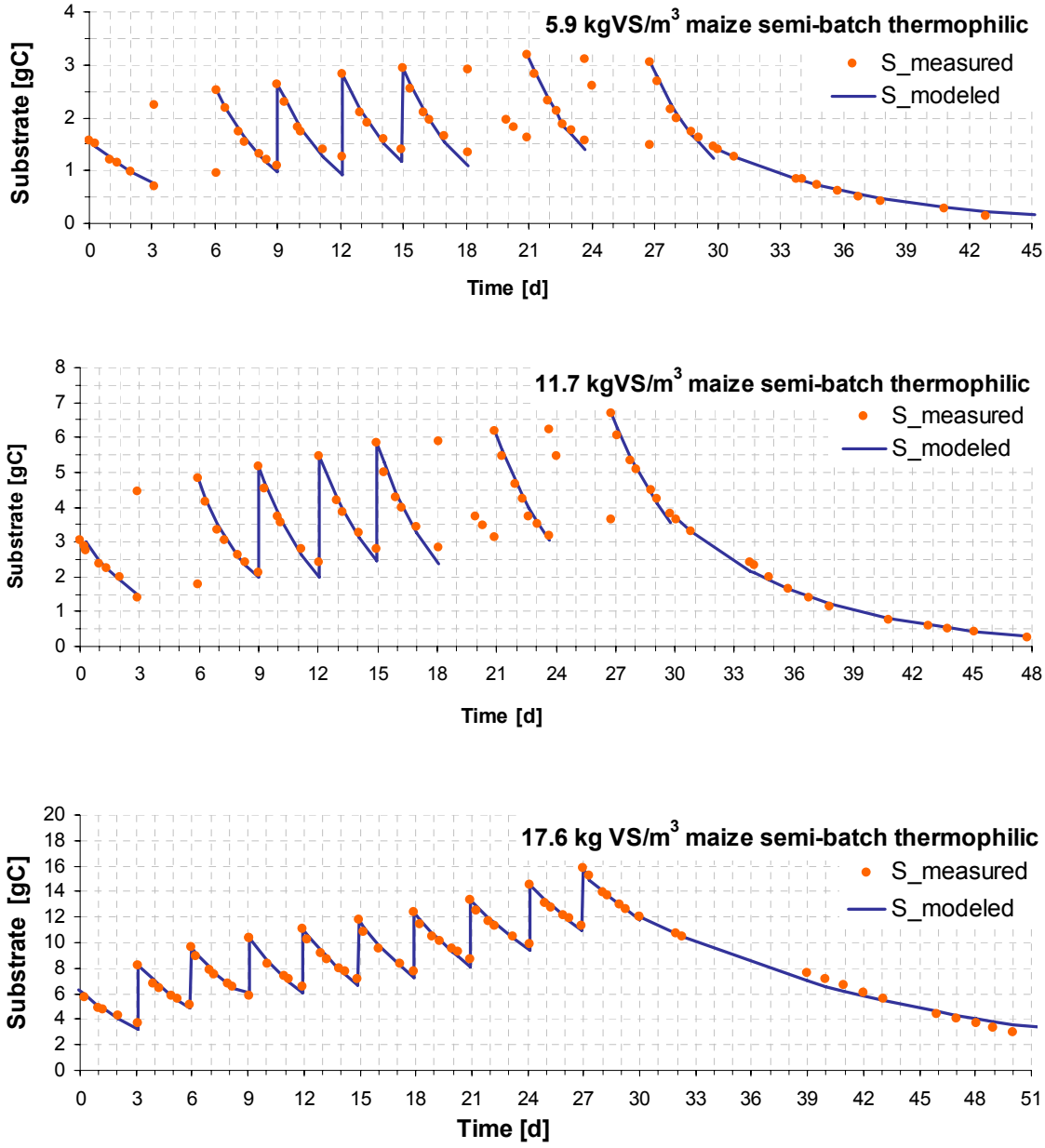


Fig. D.6 Substrate degradation measured and modeled with 1st order equation for thermophilic digestion of maize silage in semi-batch mode. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1).

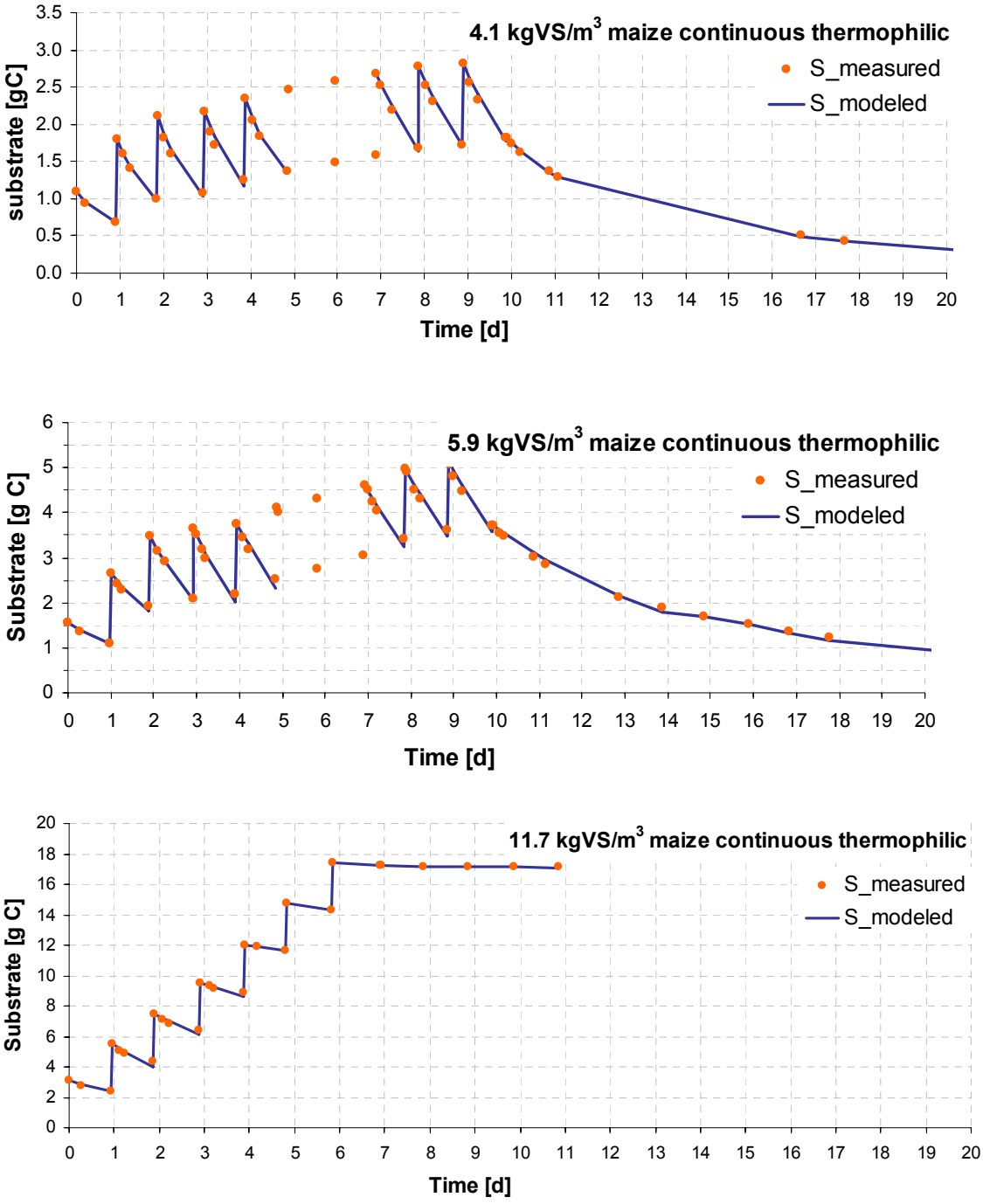


Fig. D.7 Substrate degradation measured and modeled with 1st order equation for thermophilic digestion of maize silage in continuous mode. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1).

E Attachment – Modeling Monod

Tab. E.1 Overview of literature Monod kinetic parameters for energy crops and organic waste

K_S	K_m	U_{max}	y	Substrate	Temp.	Degrad. Step	Units	Reference
0.46 - 0.74	3.15 - 8.60	0.3 - 0.41	0.048 - 0.059	cellulose	55°C	VFA	gC	Beierlein, 2011
0.01 - 0.20	11 - 54	0.50 - 2.70	0.050 - 0.080	cellulosic material	35°C / 55°C	VFA	gVS	Qu et al., 2009
0.15	8	0.30	0.038	grass silage	38°C	HAc	gVS	Wichern et al., 2009
1.20	2.70	0.32	0.120	domestic refuse (Barlaz et al. 1989)	41°C	VFA	gVS	Vavilin et al., 2003
0.12	12*	0.60*	0.05*	solid waste	55°C	HAc	gVS	Angelidaki et al., 1998
0.65	9	0.342*	0.038*	olive mill solid waste	37°C	HAc	gCOD	Boubaker & Ridha, 2008
7.40 - 13.4	1.24 - 15	0.63 - 0.68	0.04 - 0.05	organic waste	38°C	VFA	gC	Sosnowski et al., 2007
0.025	6.7	0.4	0.06	animal waste	25°C	HAc	gVS	Hill & Barth, 1977
0.31	10	-	-	olive pulp	35°C	VFA	gCOD	Kalfas et al., 2006
0.63	-	-	-	olive pulp	55°C	VFA	gCOD	Kalfas et al., 2006
0.1 - 4	5 - 20	0.3 - 1.3	0.02 - 0.07	-	35°C	VFA	gCOD	Garcia-Heras, 2003
0.05 - 0.6	2 - 7	0.1 - 0.4	0.02 - 0.05	-	35°C	HAc	gCOD	Garcia-Heras, 2003
0.015	7.5	0.6	0.08	organic waste	36°C	HAc	gVS	Kiley et al., 1997

Tab. E.2 Summary of the Monod kinetic parameters obtained for the batch experiments. Measured values are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1). For all fits the initial microbial concentration (x_0) of 0.001 g C/l and microbial yield coefficient (y) of 0.05 g C/g C were applied.

substrate	temp. mode	OLR [kgVS/m^3]	S_0 measured	good fit for initial		Monod constants			
				days	% of S	S_0	Umax	Km	Ks
maize	55°C	5.7	1.6	1-4	63	1.41	0.22	4.41	0.33
		11.5	3.1	1-5	52	2.43	0.09	1.84	0.33
		17.3	4.7	1-5	53	3.80	0.08	1.61	0.33
	38°C	5.5	1.5	2-4	40.0	0.83	0.16	3.15	0.33
		11.0	2.9	1-2	25.0	2.42	0.37	7.40	0.33
		17.1	4.7	1-3	33.0	3.60	0.16	3.16	0.33
cellulose	55°C	5.7	1.6	0-4	63	1.63	0.14	2.75	0.49
		11.4	3.2	0-5	64	3.23	0.08	1.52	0.41
		17.1	4.8	0-8	82	4.80	0.04	0.80	0.41
		22.9	6.4	0-7	60	6.20	0.03	0.66	0.42
		28.6	8.0	0-6	44	8.00	0.02	0.36	0.41
		34.3	9.6	0-7	47	9.60	0.02	0.38	0.43
	38°C	5.4	1.5	-	100	1.56	0.10	2.00	0.20
		10.9	3.1	-	100	3.08	0.09	1.71	0.33
		16.3	4.6	0-6	71	4.40	0.06	1.27	0.33

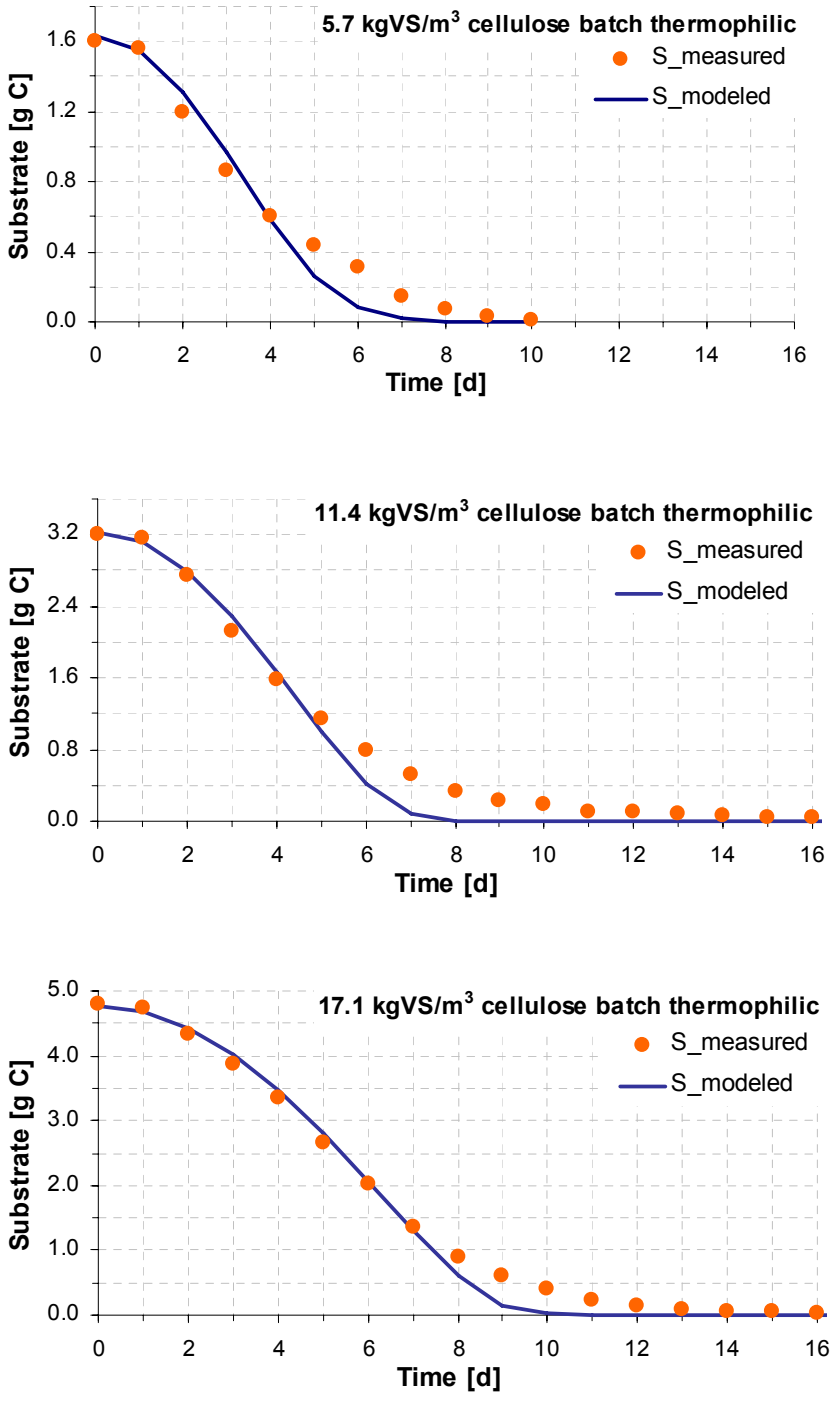


Fig. E.1 Substrate degradation measured and modeled with Monod equation for thermophilic digestion of cellulose in batch mode. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1).

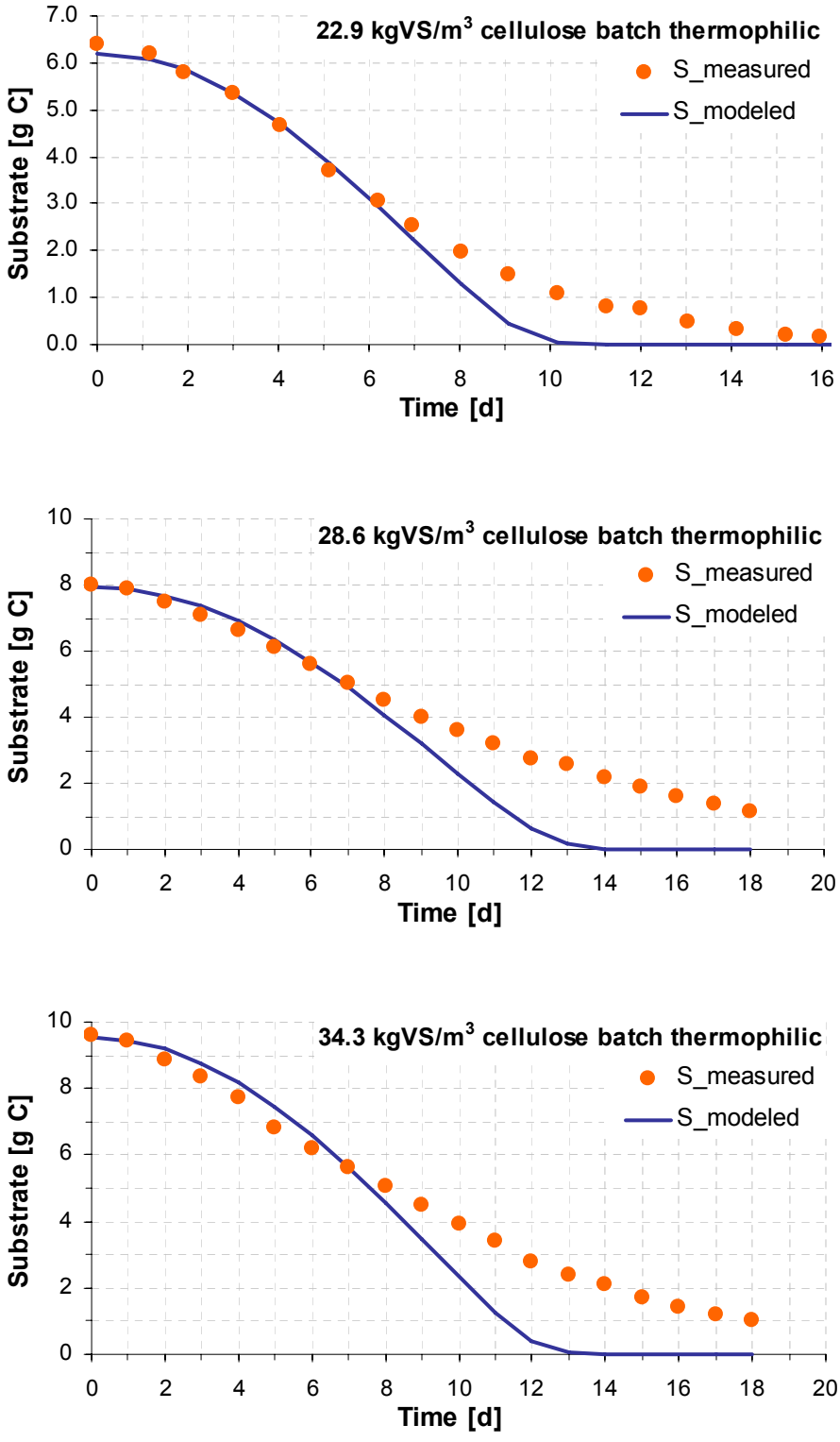


Fig. E.2 Substrate degradation measured and modeled with Monod equation for thermophilic digestion of cellulose in batch mode. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1).

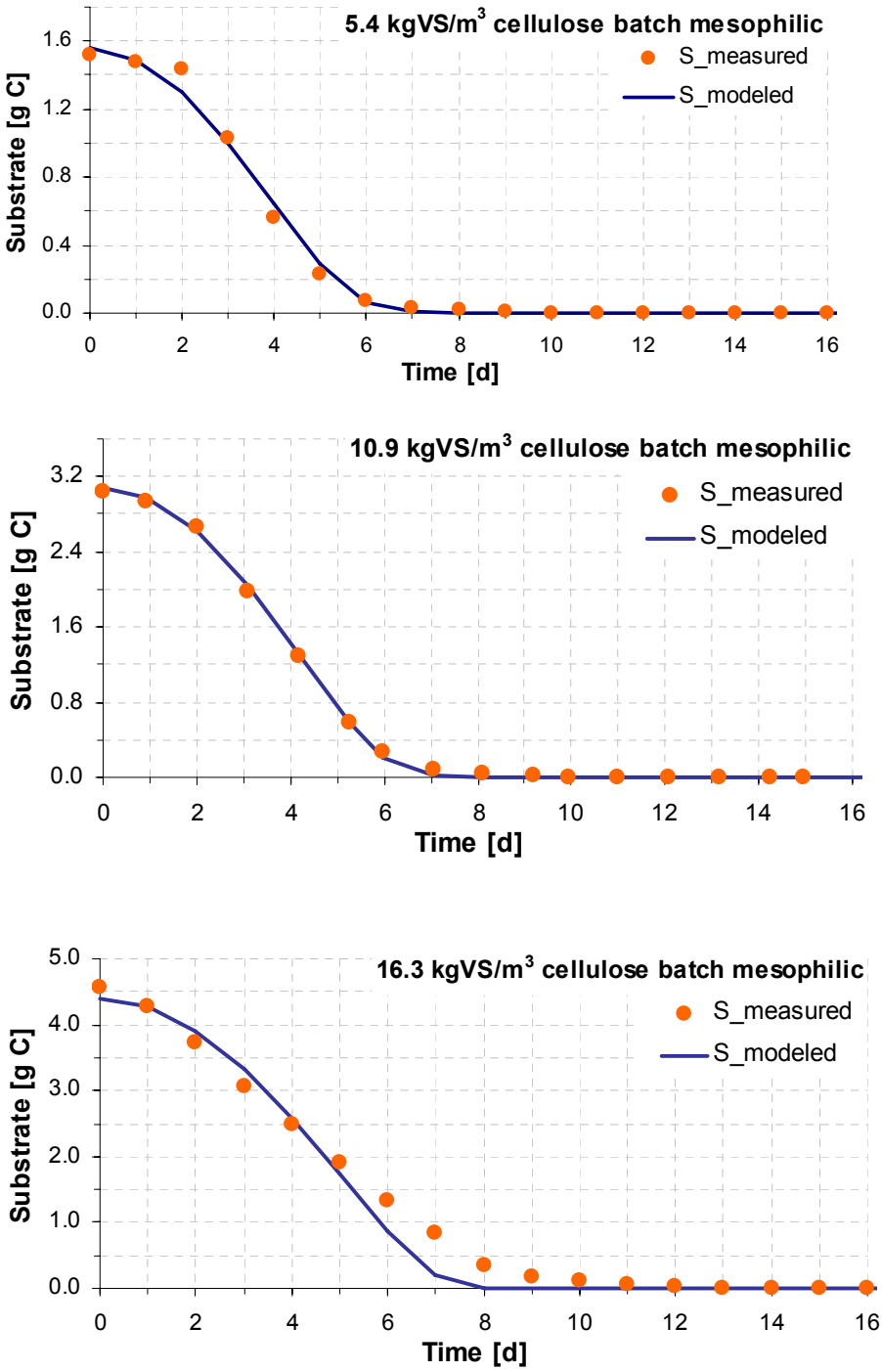


Fig. E.3 Substrate degradation measured and modeled with Monod equation for mesophilic digestion of cellulose in batch mode. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1)

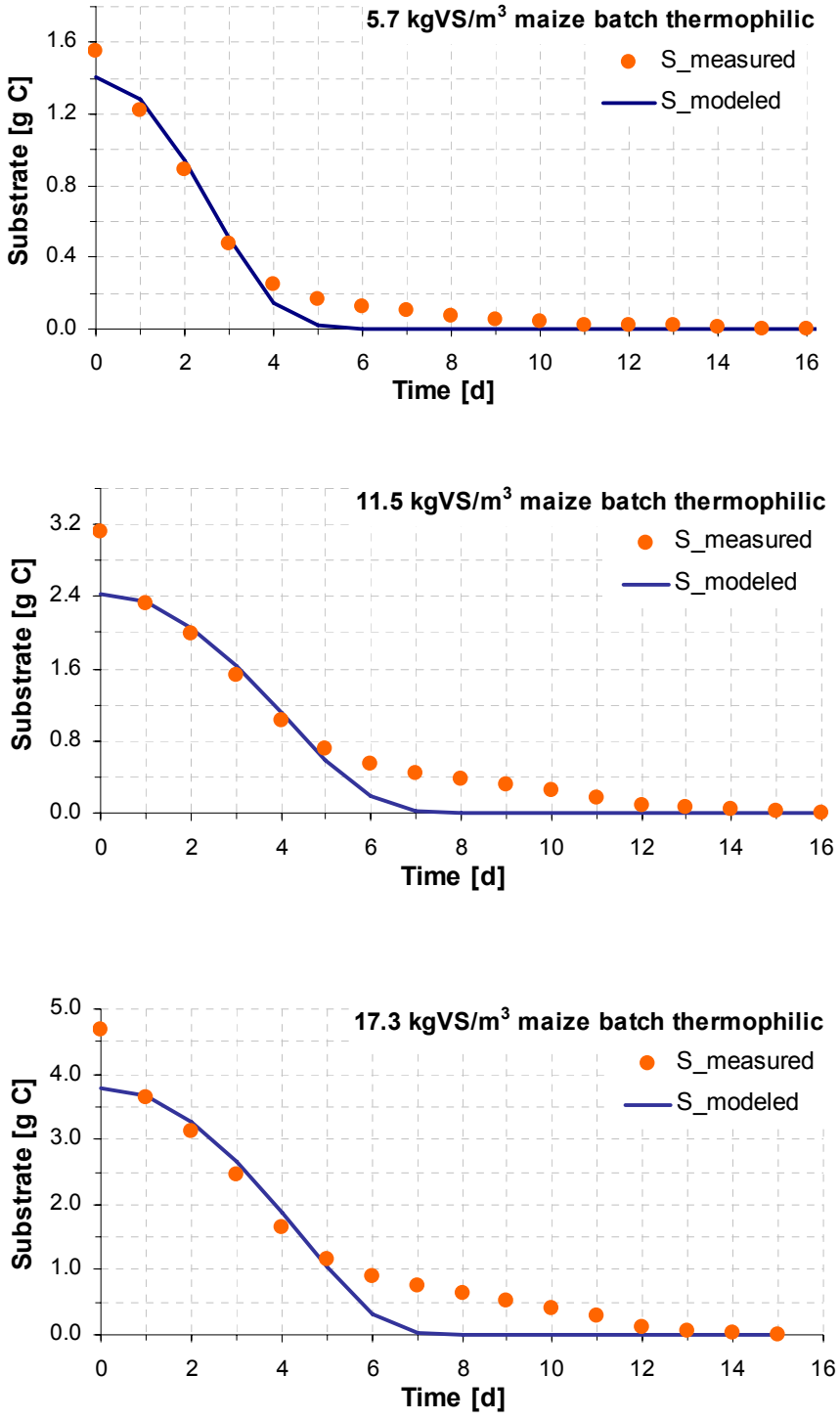


Fig. E.4 Substrate degradation measured and modeled with Monod equation for thermophilic digestion of maize silage in batch mode. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1)

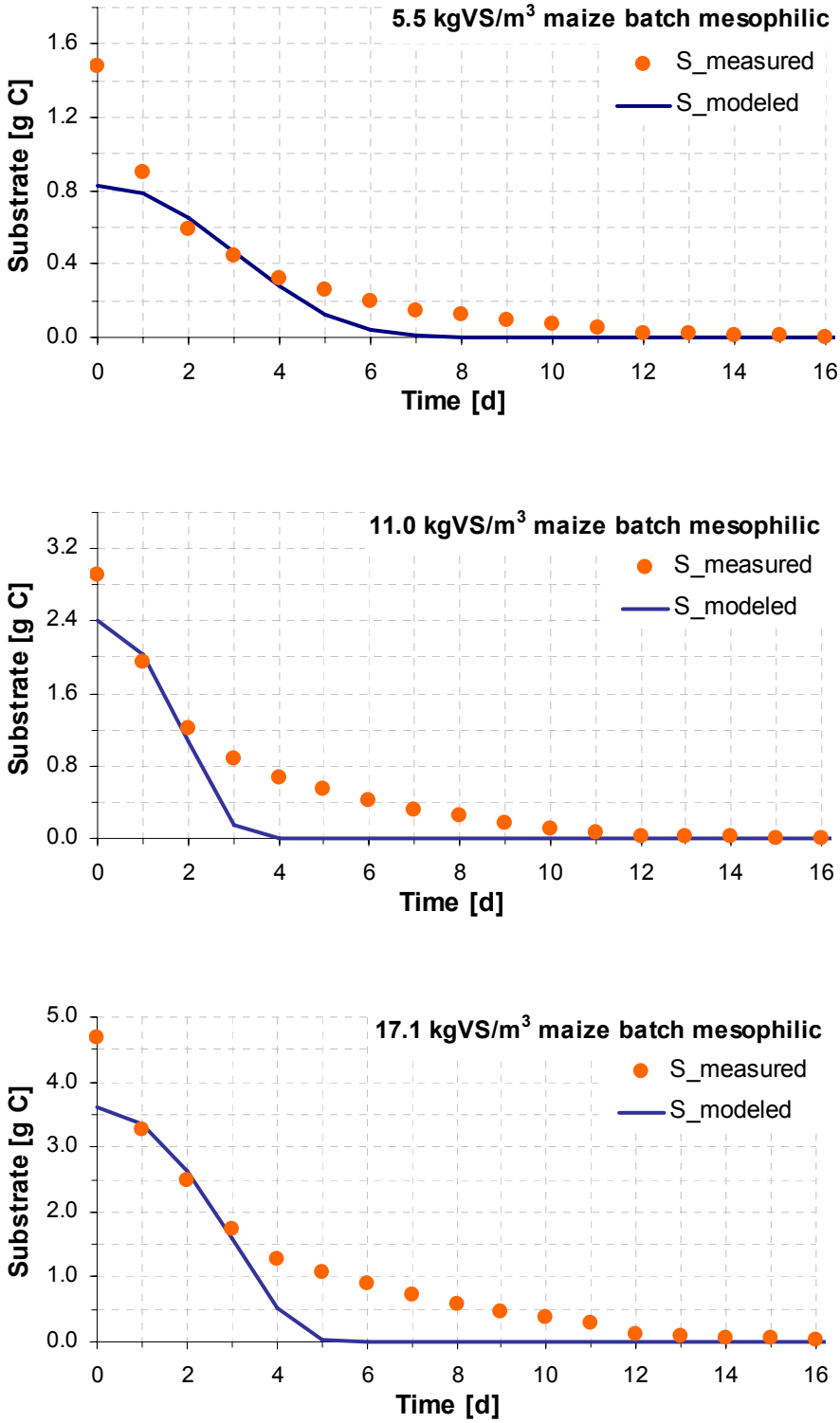


Fig. E.5 Substrate degradation measured and modeled with Monod equation for mesophilic digestion of maize silage in batch mode. Measured points are calculated with Chen & Hashimoto equation from specific biogas production (s. Chapter 3.5.1)

F Attachment – Sensitivity analysis for Monod model

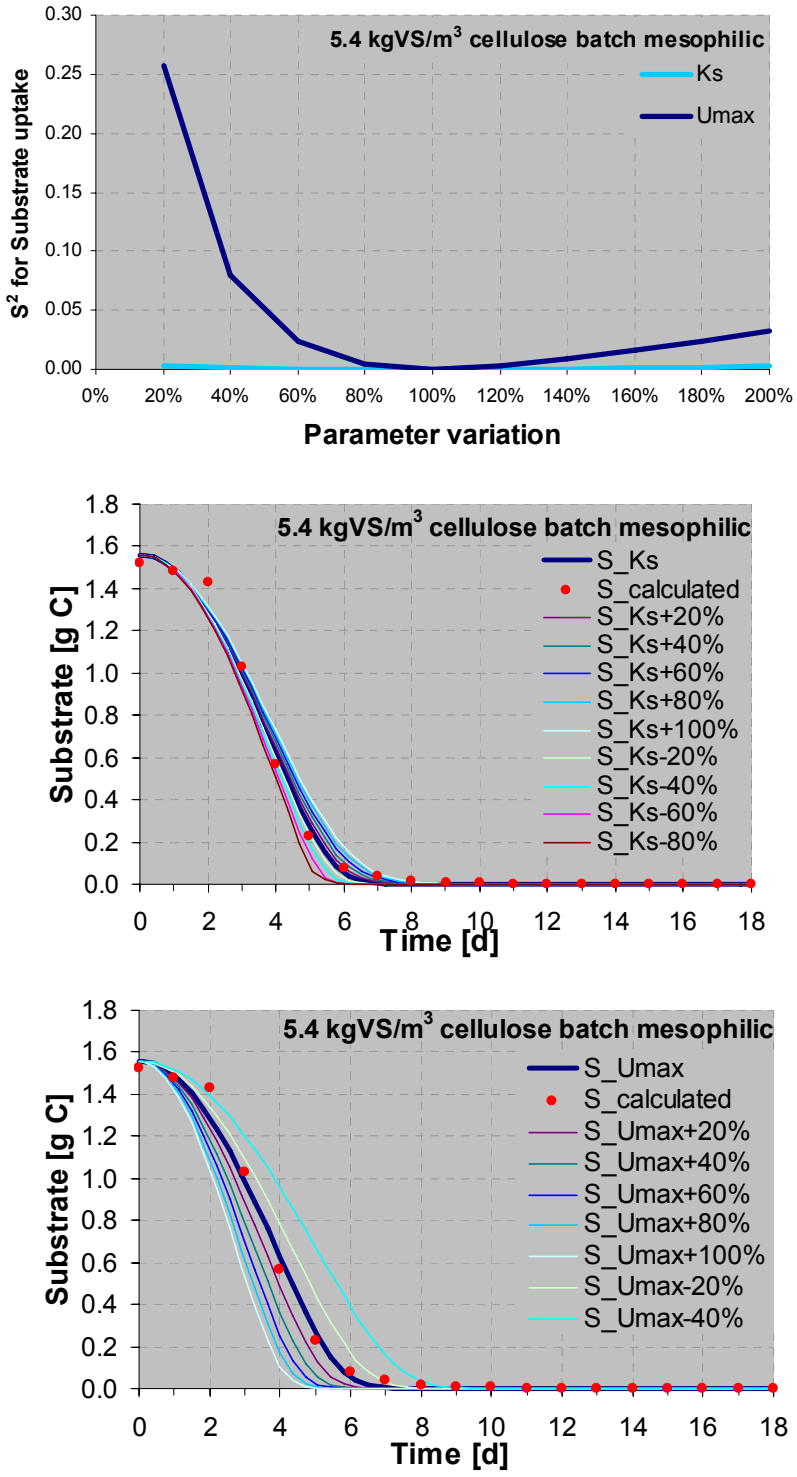
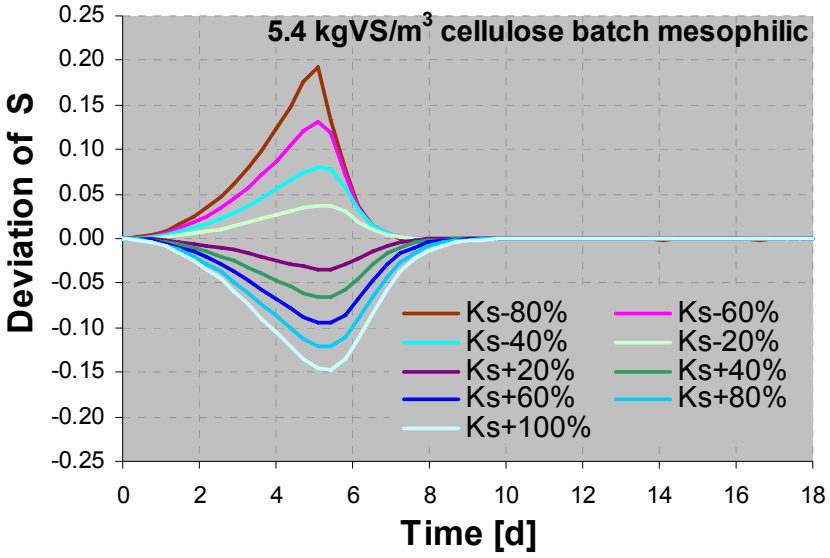


Fig. F.1 Sensitivity analysis for Monod fit 5.4 kgVS/m³ cellulose in mesophilic batch mode



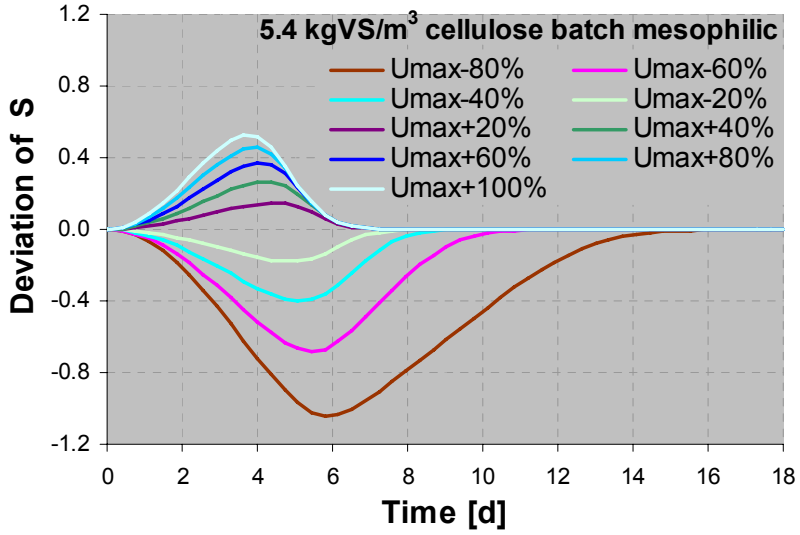
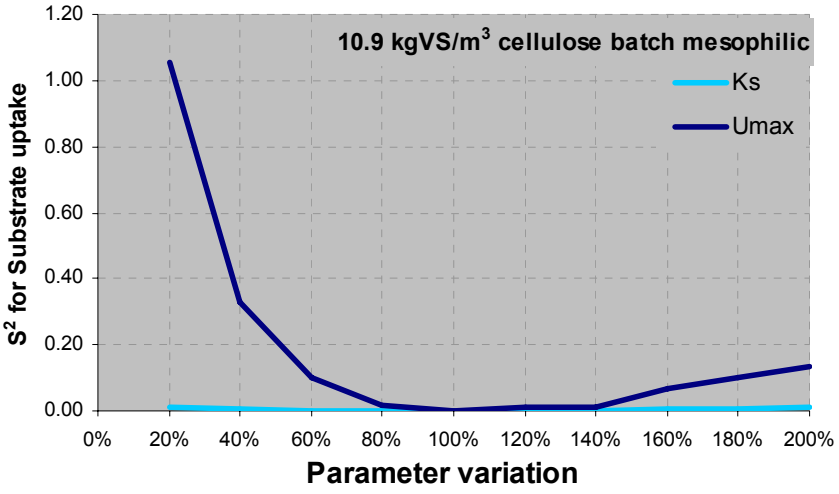
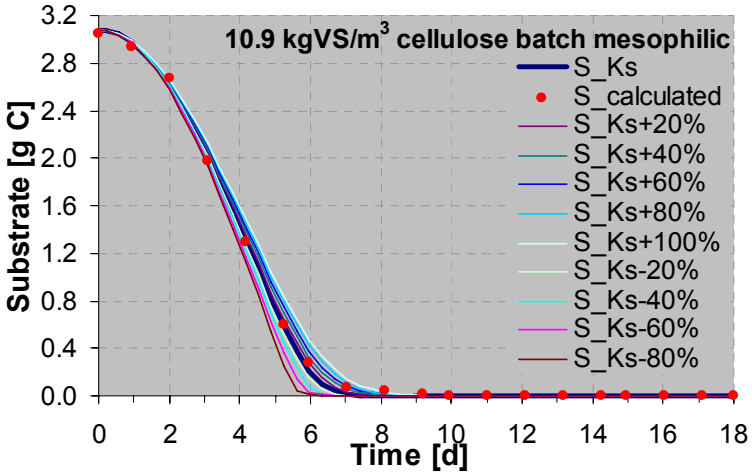


Fig. F.2 Sensitivity analysis for Monod fit 5.4 kgVS/m³ cellulose in mesophilic batch mode (continued)





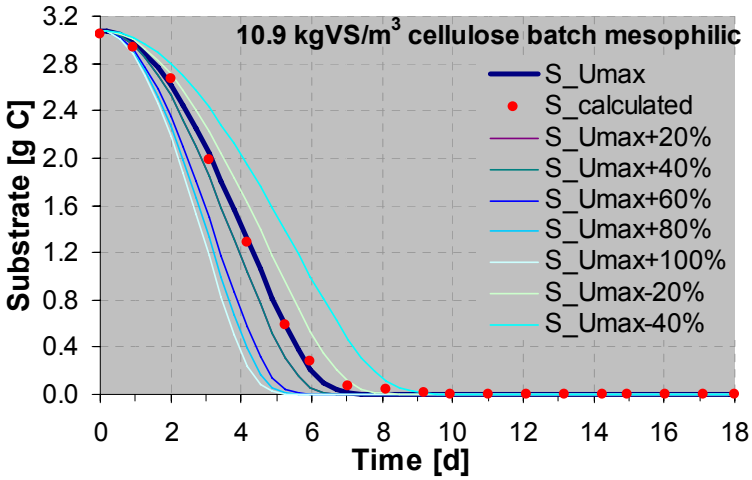
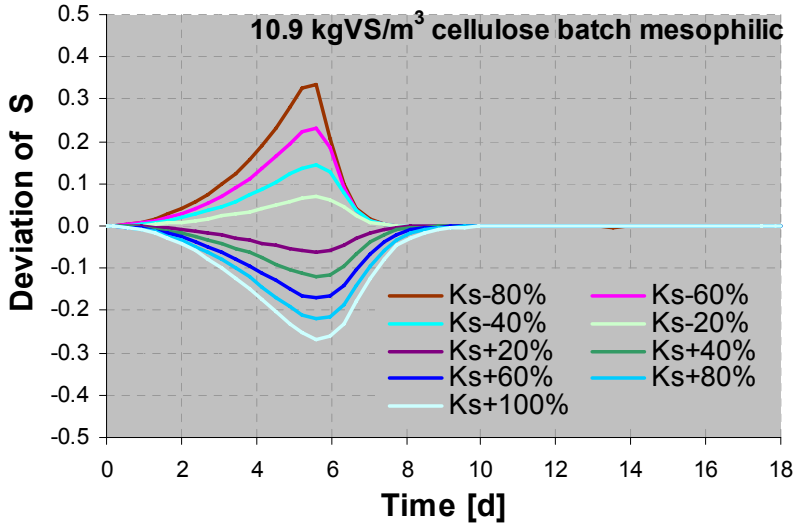


Fig. F.3 Sensitivity analysis for Monod fit 10.9 kgVS/m³ cellulose in mesophilic batch mode



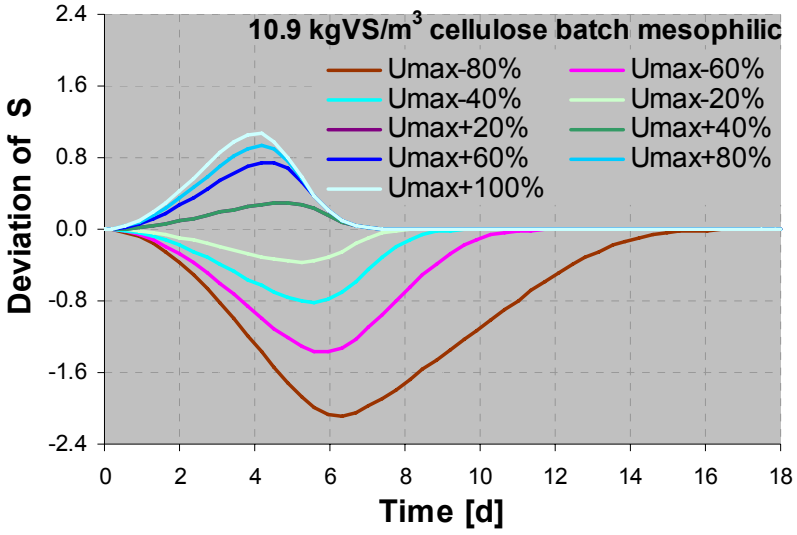
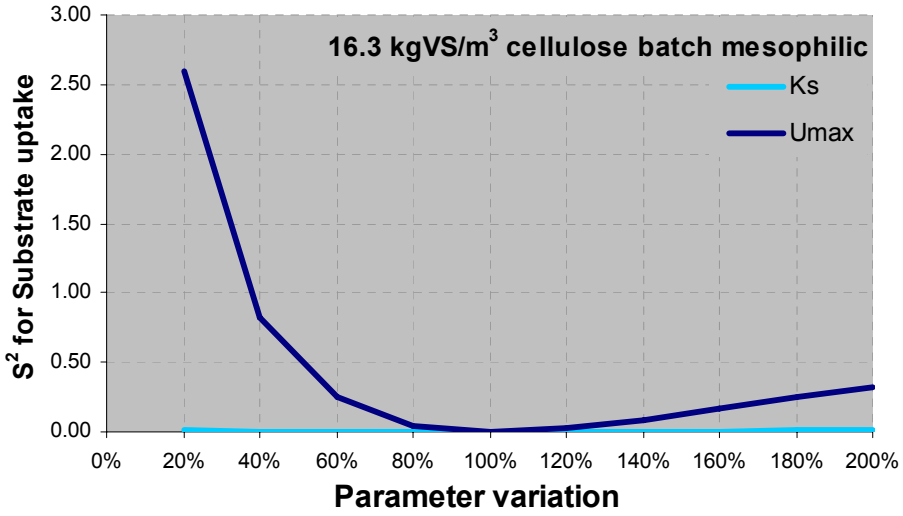
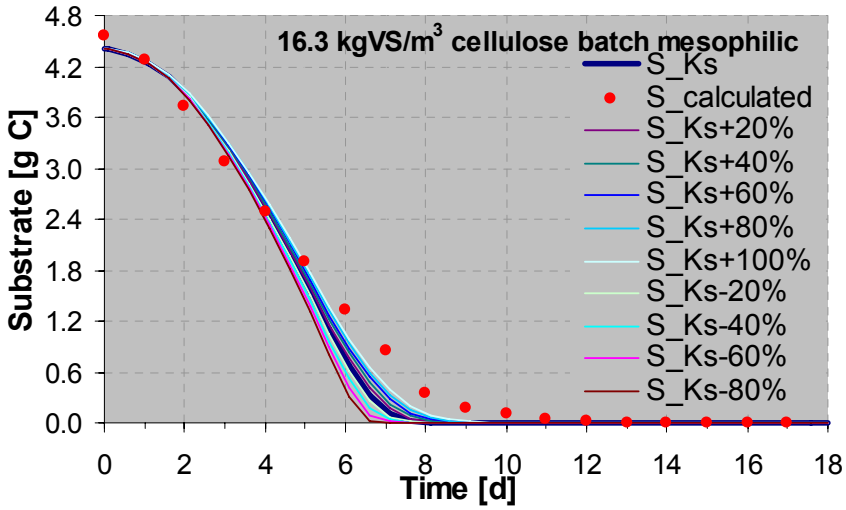


Fig. F.4 Sensitivity analysis for Monod fit 10.9 kgVS/m³ cellulose in mesophilic batch mode (continued)





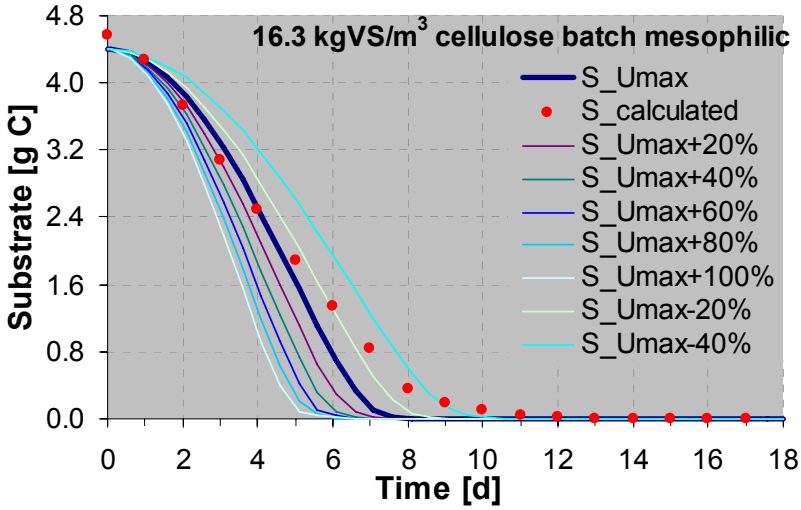


Fig. F.5 Sensitivity analysis for Monod fit 16.3 kgVS/m³ cellulose in mesophilic batch mode

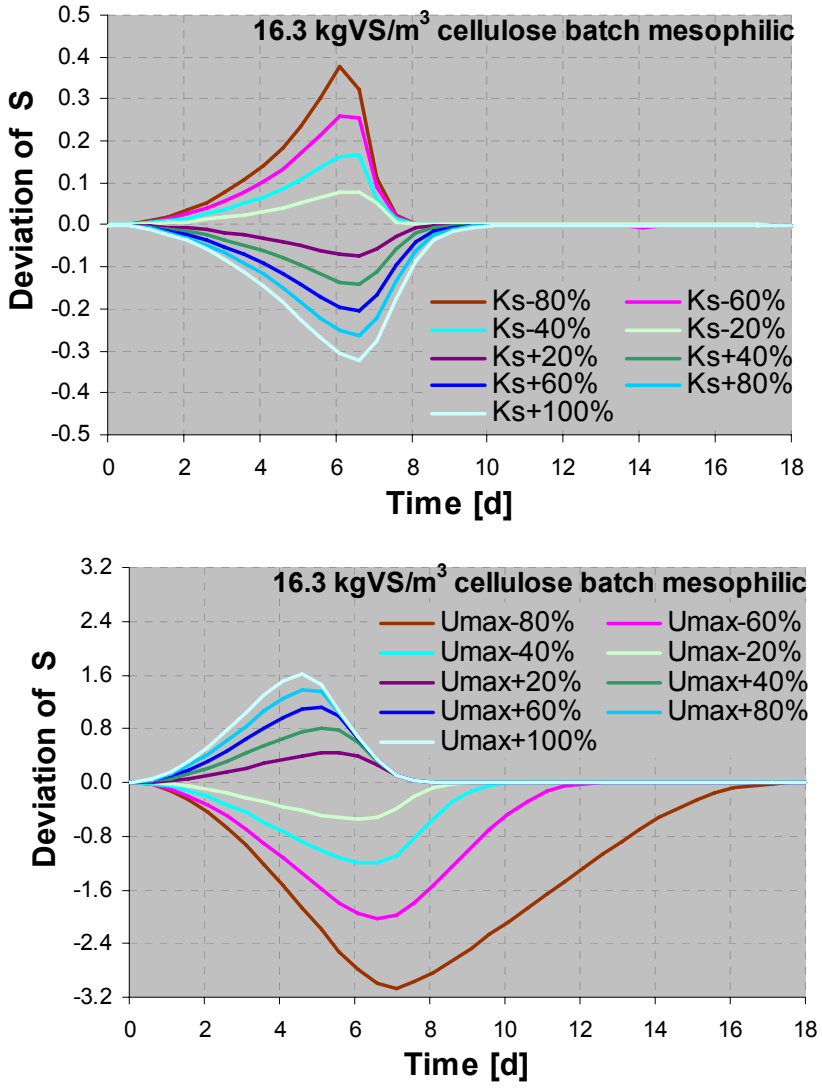
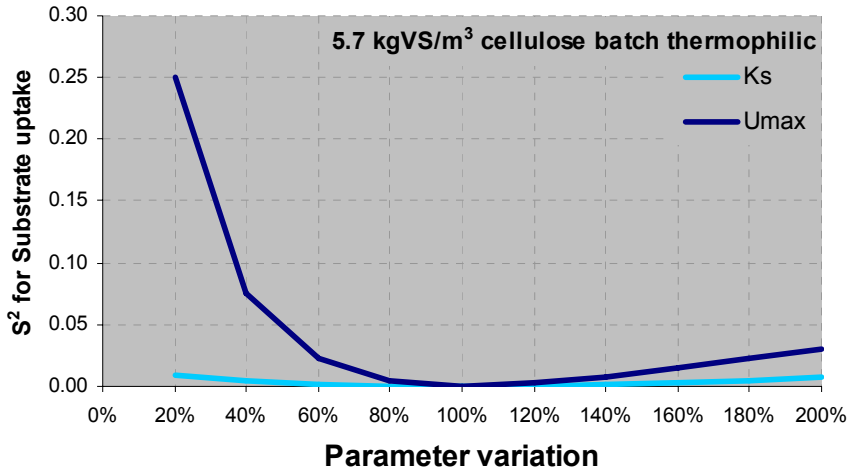
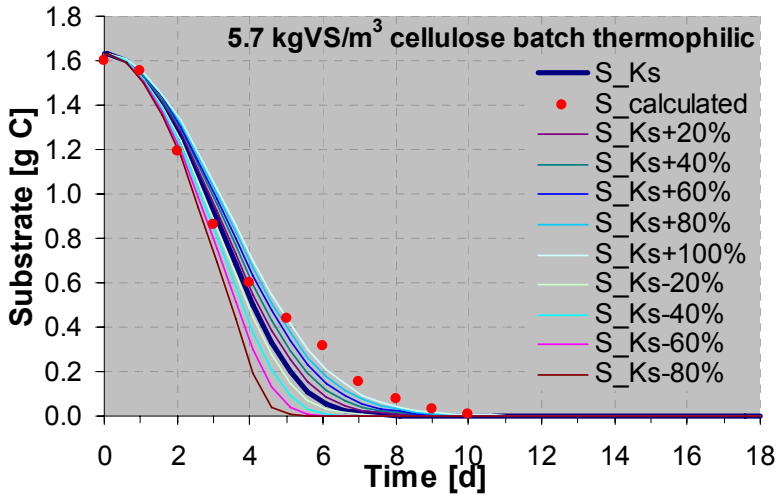


Fig. F.6 Sensitivity analysis for Monod fit 16.3 kgVS/m³ cellulose in mesophilic batch mode (continued)





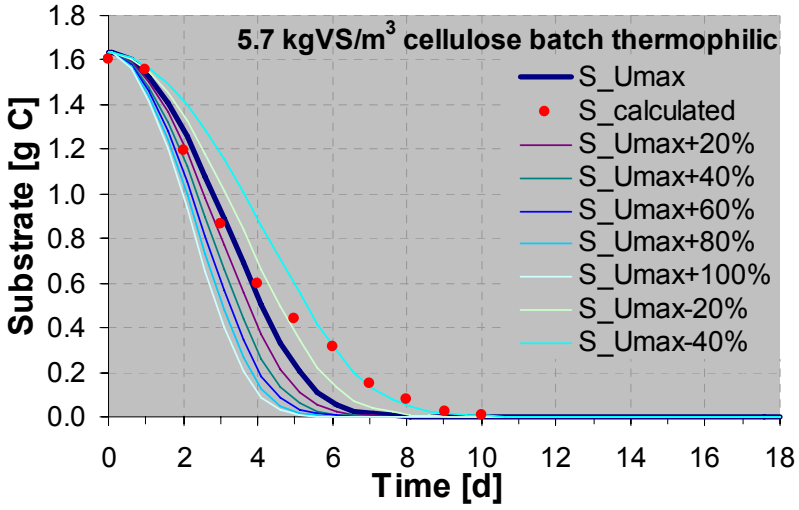


Fig. F.7 Sensitivity analysis for Monod fit 5.7 kgVS/m³ cellulose in thermophilic batch mode

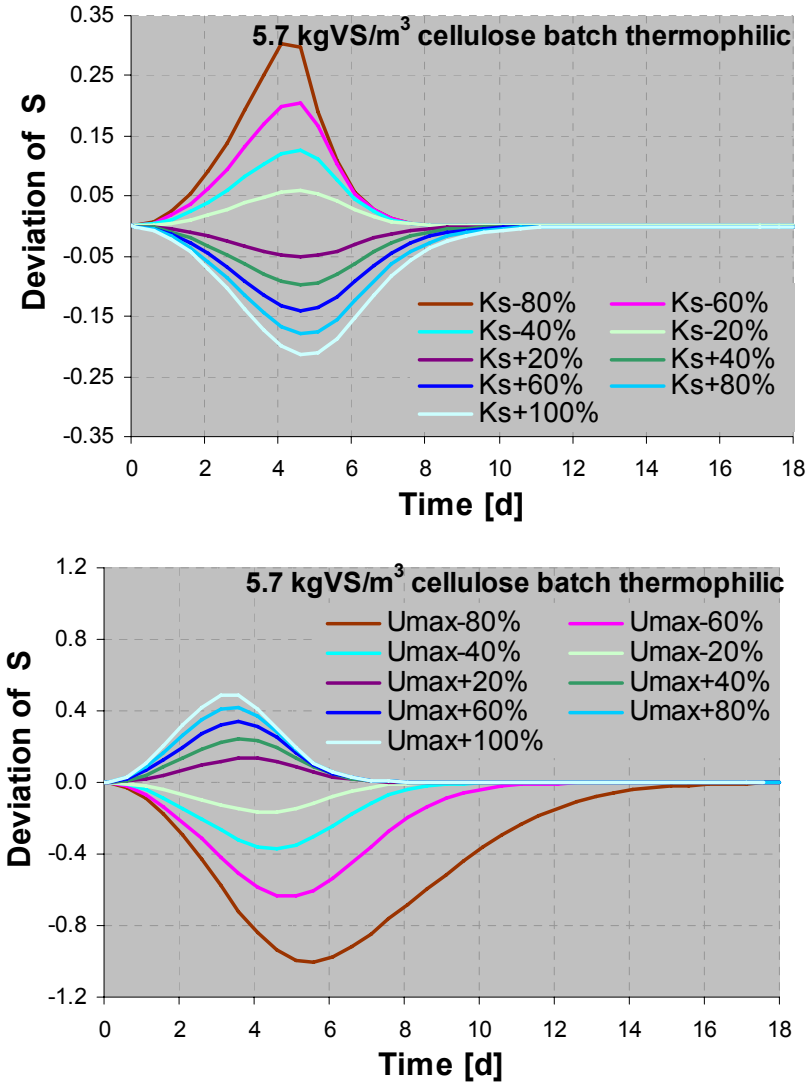
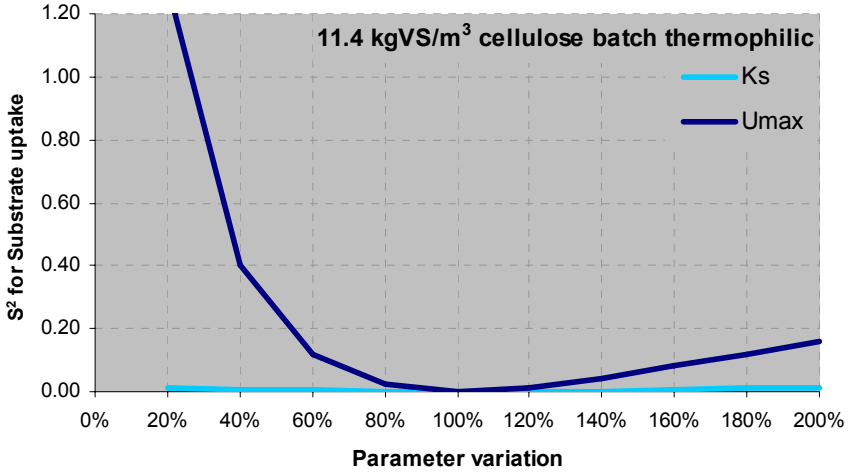
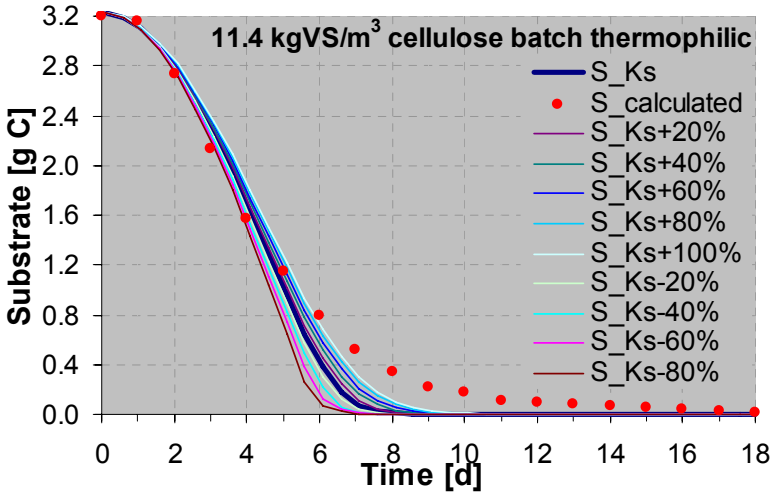


Fig. F.8 Sensitivity analysis for Monod fit 5.7 kgVS/m3 cellulose in thermophilic batch mode (continued)





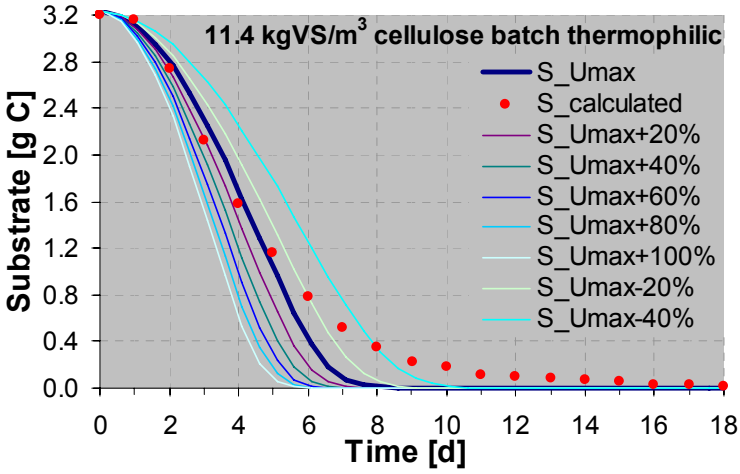


Fig. F.9 Sensitivity analysis for Monod fit 11.4 kgVS/m³ cellulose in thermophilic batch mode

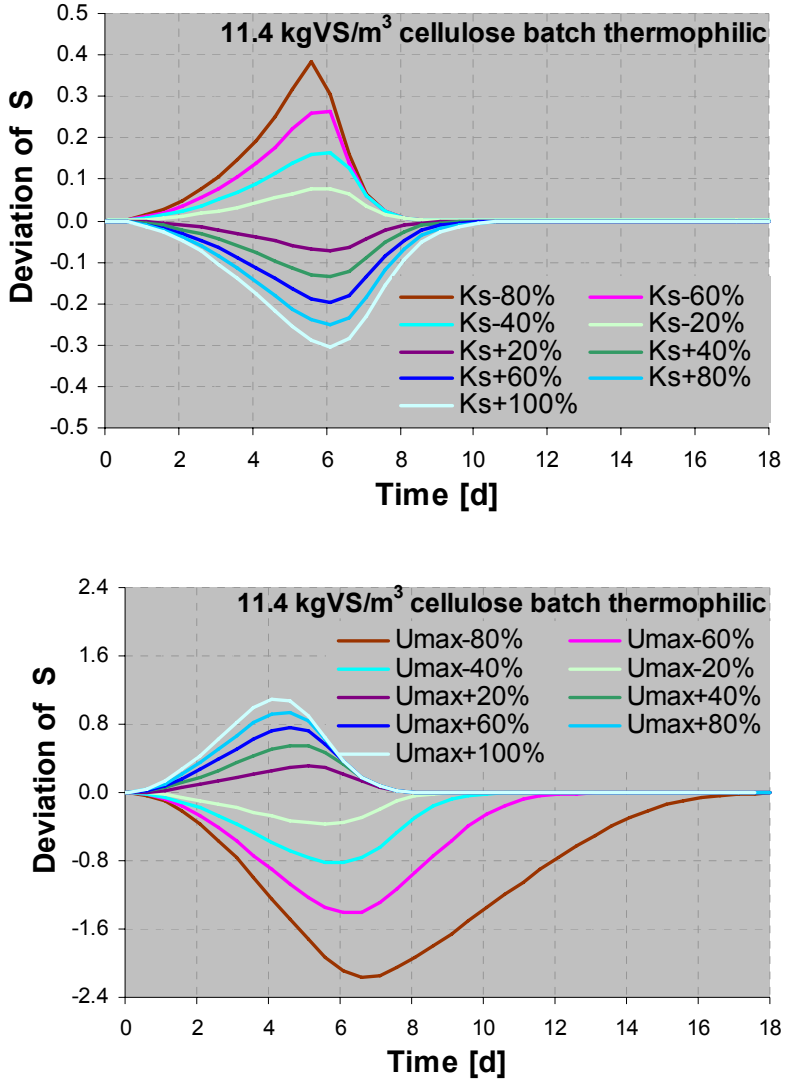
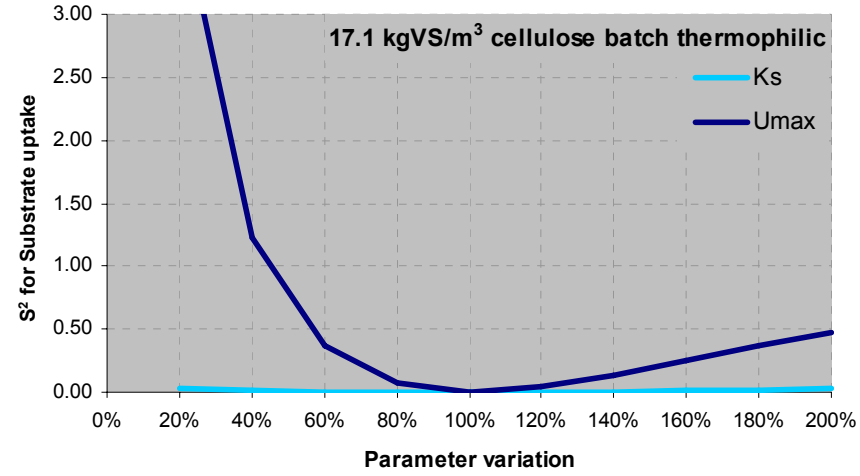
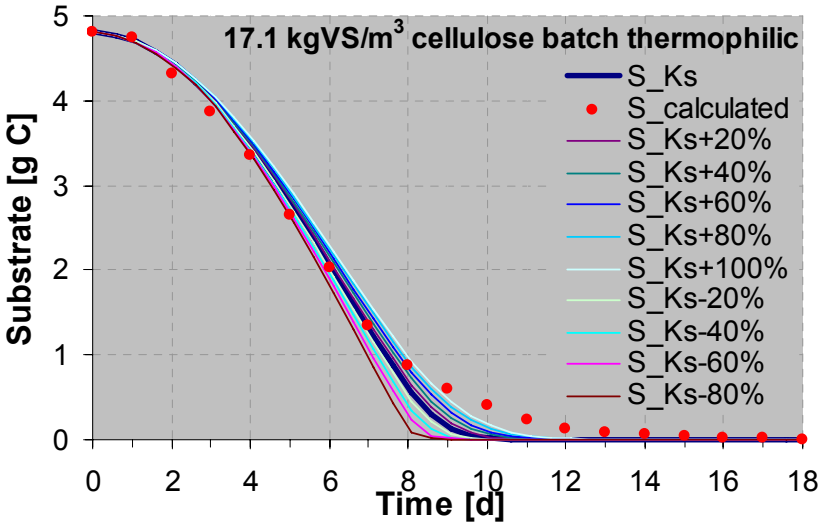


Fig. F.10 Sensitivity analysis for Monod fit 11.4 kgVS/m³ cellulose in thermophilic batch mode (continued)





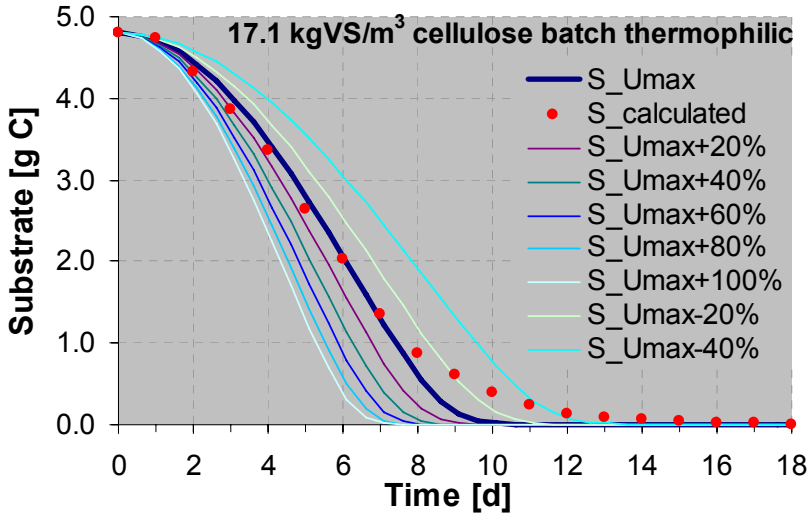


Fig. F.11 Sensitivity analysis for Monod fit 17.1 kgVS/m³ cellulose in thermophilic batch mode

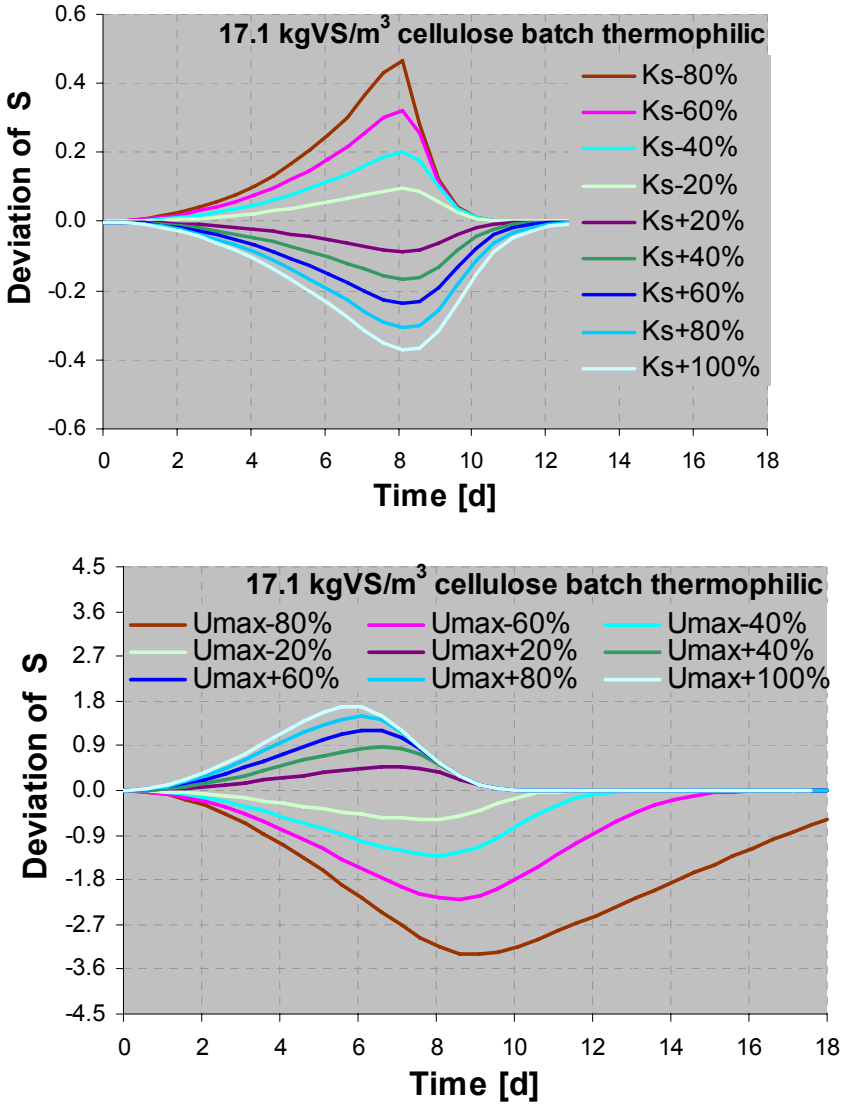
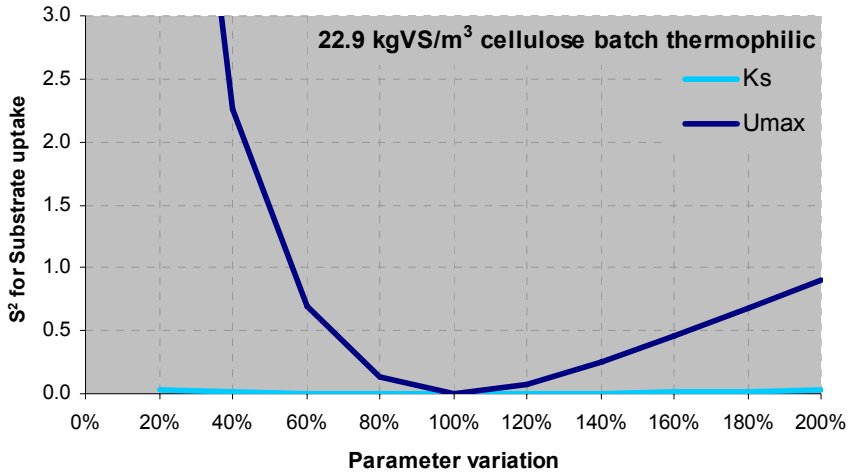
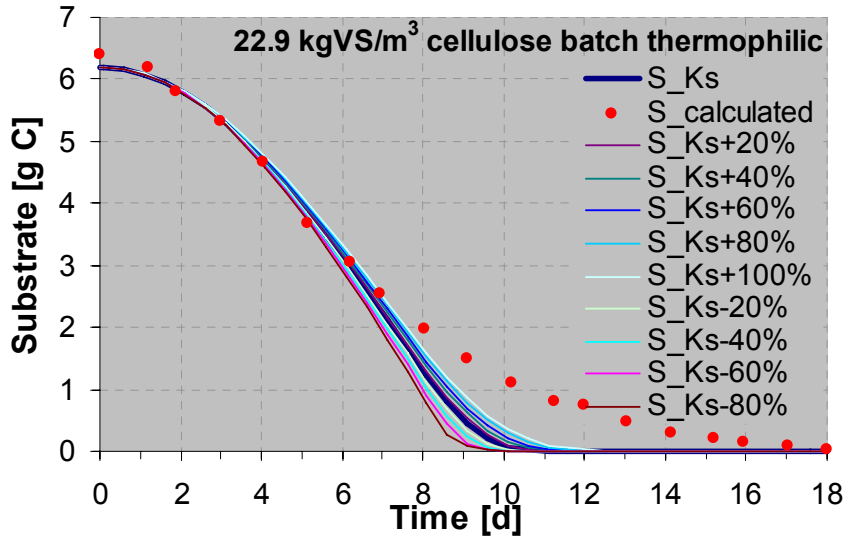


Fig. F.12 Sensitivity analysis for Monod fit 17.1 kgVS/m³ cellulose in thermophilic batch mode (continued)





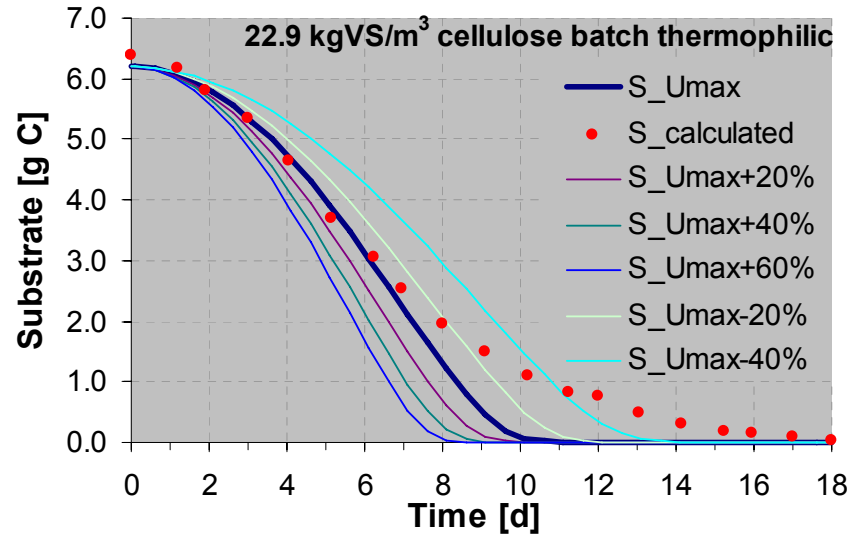


Fig. F.13 Sensitivity analysis for Monod fit 22.9 kgVS/m³ cellulose in thermophilic batch mode

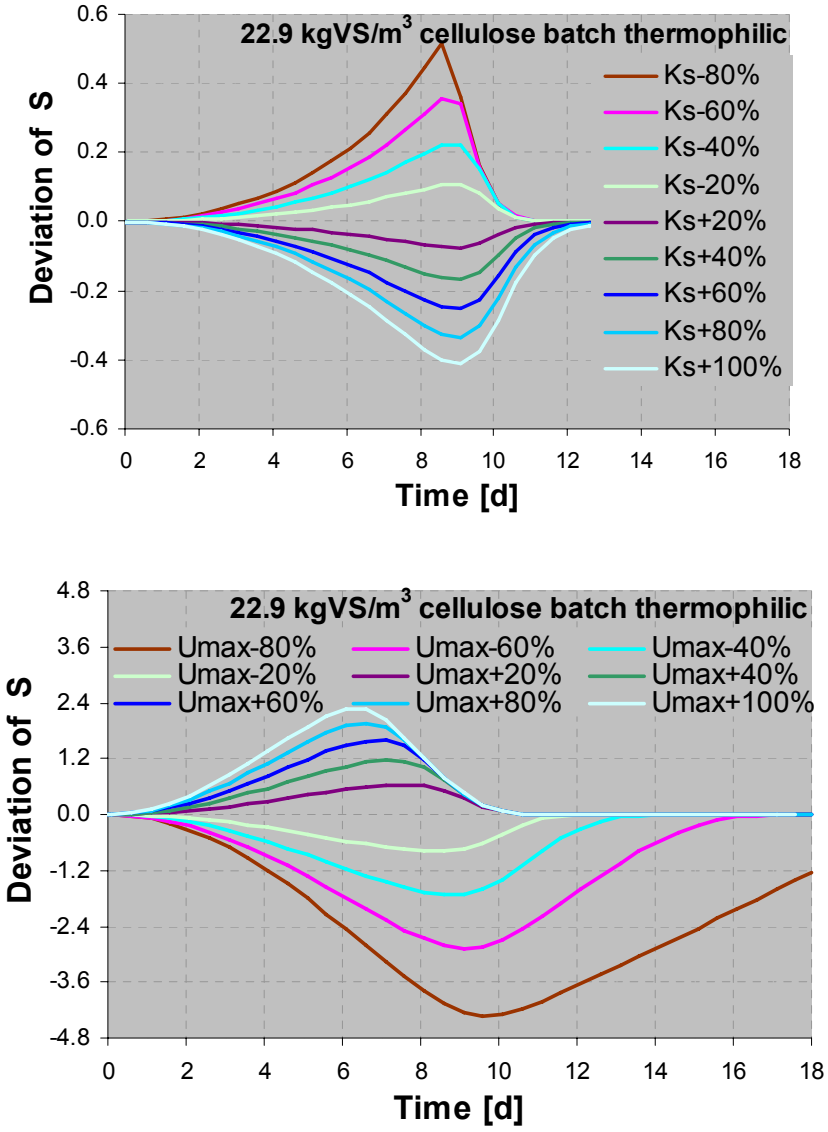


Fig. F.14 Sensitivity analysis for Monod fit 22.9 kgVS/m³ cellulose in thermophilic batch mode (continued)

G Report on performance of pH and ORP on-line electrodes

The information about performance and life-span of the on-line pH and ORP electrodes in a biogas fermenter was additionally collected during the study.

WTW on-line registering system used in the tests was developed for heavy loaded waste water and maximal temperature of 60°C (WTW, 2007). For that reason the electrodes were strictly observed during the test to collect the information about the performance and the life-span of the sensors in the biogas reactor environment under conditions approaching the application limits. Both pH and ORP sensors were used in the reactors at 38°C and 55°C (which is close to the upper operating limit). Every 4th to 6th week the electrodes were taken out, cleaned and calibrated.

Monitoring of the long-term performance of both electrodes revealed that under investigated conditions no signal drift was detectable after one month in use under thermophilic conditions.

Fig. G.1 ORP electrodes – after 3 months in biogas reactor and a new one before use; red marked the critical diaphragma opening – a place of microbial growth

After 4-6 measuring periods of 1 month a drift of the registered value by 10 to 20 mV was noted. A microbial growth was observed around the diaphragm openings[44]. A complete removal of the microbial colonies from diaphragms during maintenance was not possible as the bacterial growth continued also inside the electrode glass. The white polymer electrolyte filling of the electrodes darkened from test to test, which indicates slow diffusion of the reactor content over the diaphragm openings. Despite colour change directly after the first test, both electrodes still delivered reliable results.

[44] two diaphragm openings on each electrode are regarded as critical for the measurements accuracy

However after each regular cleaning of pH electrode, calibration problems occurred. This caused the out-of-use periods lasting for several days. In most cases the recalibration was possible after restoring the electrodes for some days in KCl solution. The electrode malfunction was observed after 5-6 months of use. Changes on the on-line electrodes after multiple application are shown in Fig. G.1.

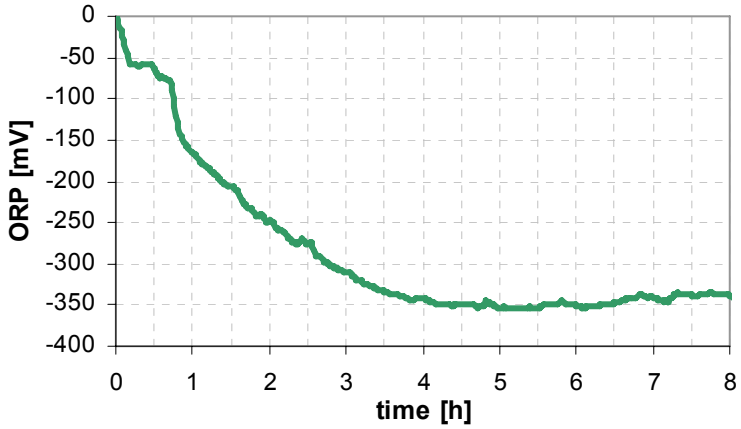


Fig. G.2 Changes of ORP directly after the test start caused by the presence of dissolved oxygen (22.9 kgVS/m3 cellulose, thermophilic)

Effects of initially dissolved oxygen on the ORP signal were observed in the fermenters during the studies. The decrease of ORP directly after start in a thermophilic test with 22.9 kgVS/m^3 cellulose is presented in Fig. G.2. The stable ORP value (typical for methanogenesis) was achieved only after first 3 hours of run. However, the setup of anaerobic conditions was quicker for thermophilic than mesophilic conditions which was in accordance with the literature (DEUBLEIN & STEINHAUSER, 2008). Similar sensitivity of ORP electrode to oxygen was already mentioned by PLOOG ET AL. (1996) and UTEC (2003). Both authors report that contact with oxygen strongly increased the measured values.

Both electrodes performed very well under extreme conditions (heavy loaded waste water and the operating temperature of 55°C). The experiments showed a high measuring accuracy despite sporadic calibration only. Also the electrode life-span of 6 months in such environment under thermophilic conditions and even higher life-spans for mesophilic experiments can be regarded as a very good result.

H Report on development of VFA purification method

A pure sample for VFA GC injections can be obtained if steam distillation method (DIN, 1999) is applied to extract the VFA acids from contaminated reactor content. However the procedure is highly time-consuming and delivers approx. 10 times diluted VFA values, which in many cases fall beyond the GC detection limits. For this reason a faster method similar to KITTELMANN ET AL. (1983), PECHER (1989) and PIND ET AL. (2003) was developed. The reactor samples were centrifuged at 12 000g (MiniSpin, Eppendorf). The supernatant was acidified with an acid reagent in the proportion 9:1 and passed through a nylon 0.45-µm-pore-size filter (Rotilabo, Carl Roth). The implementation of phosphoric acid in the acid reagent at concentrations similar to PECHER (1989) led to variations in the post-treatment sample purity for different samples despite similar treatment (s. Fig. H.1). pH control revealed different values in both samples. After increasing the volume of H_3PO_4 in acid reagent by 100% the post-treatment purity of all samples increased considerably.

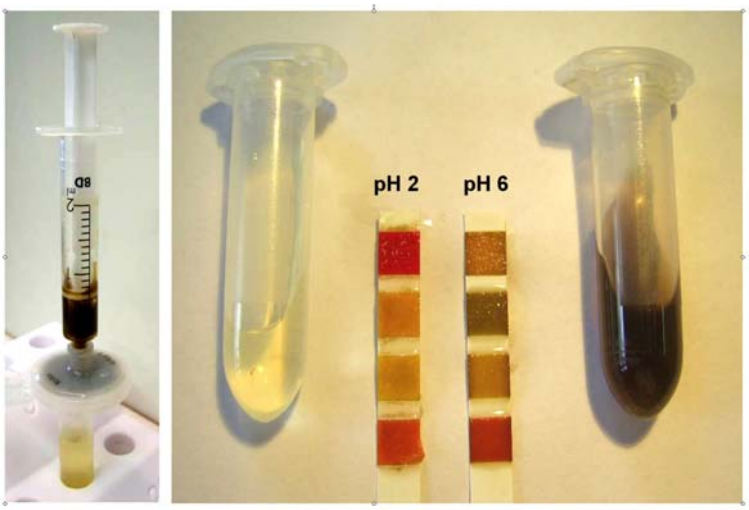


Fig. H.1 Sample filtration for GC analysis (left), two samples after treatment – varying purity due to different pH

This effect can be explained by the dependence of humic acids solubility on the pH value. Unlike to PECHER (1989), who analysed landfill samples, the TIC value for samples originating from biogas reactors is much higher. Therefore the pH drop to 2.0 necessary to fall all the humic acids out of the solution (IHSS, 2007) could be achieved only after doubling the applied concentration of phosphoric acid. The 4-methylpentane acid was applied in the reagent as a standard, which gave a direct possibility to compare the quality of each single measurement with the others.