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Modelling and Control of the Anaerobic Digestion of Energy Crops

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Kurzfassung

Unter anaerober Vergärung versteht man die Umsetzung von organischem Material zu anorganischem Material und methanhältigem Gas unter Ausschluss von Sauerstoff. Diese Technik ist eine der ältesten in der Abwasserreinigung eingesetzten Methoden (Feng *et al.*, 2006). Zwar wird die Anaerobtechnik auch heute zumeist in diesem Bereich eingesetzt, doch gewinnen Biogasanlagen zur Energiegewinnung immer mehr an Bedeutung. Die meisten der neuren Biogasanlagen sind sogenannte landwirtschaftliche Biogasanlagen in denen neben Gülle, auch Energiepflanzen vergärt werden, insbesondere Mais (-silage).

Verschiedenste Probleme verringern jedoch die Effizienz dieser Anlagen, wie niedrige Methankonzentration im Biogas, Selbsterwärmung der Anlagen oder Geruchsprobleme.

Daher ist eine Optimierung des Prozesses notwendig. Diese Optimierung kann viele verschiedene Gesichtspunkte umfassen: neue Reaktorformen, gezielte Auswahl von Substraten oder verbesserte Steuerung des Prozesses. Ziel dieser Arbeit nun ist die Optimierung des Prozesses durch Modellierung und Steuerung der anaeroben Vergärung von Energiepflanzen.

Basis jeder guten Steuerung ist nicht nur ein ausgereiftes Messsystem, sondern auch ein umfangreiches Wissen über den Prozess selbst. Um einen guten Einblick in den Biogasprozess zu bekommen, sind Modelle ein gutes Hilfsmittel. In dieser Arbeit wurde das Anaerobic Digestion Model No.1 (ADM1) (Batstone *et al*., 2002) herangezogen.

Das ADM1 ist ein mathematisches Model das den Biogasprozess beschreibt und von der IWA Task group for Mathematical Modeling of Anaerobic Digestion Processes entwickelt wurde.

Da das Modell sehr allgemein ist, wurde es für die Modellierung der anaeroben Vergärung von Energiepflanzen leicht adaptiert. Aufgrund der Komplexität des Modells befasst sich ein großer Teil der Arbeit mit der Kalibrierung des Models. Dabei wurde unter anderen eine Methode entwickelt mit denen die Anfangsbedingungen abgeschätzt werden können ohne aufwendige Messungen.

Das modifizierte Model wurde schließlich in das sogenannte Virtual Laboratory (VL) implementiert – ein Softwaretrainingstool, dass den Benutzer Einblick in den Biogasprozess geben soll.

Weiters wurde ein Steuerelement – ein Decision Support Tool (DSS) - auf Fuzzy Logik basierend weiterentwickelt. Um verschiedene Tools miteinander vergleichen zu können, wurde eine auf Composite Programming und dem ADM1 basierend Methode verwendet.

Abstract

Anaerobic digestion (AD) of wastewater, organic wastes and biomass results in the formation of a useful end-product (biogas). Biogas is primarily composed of methane and carbon dioxide and can be processed to electrical energy and heat. Thanks to special government subsidies, there has been a veritable boom in biogas-plant technology in Austria, in recent times. Most of these biogas plants are agricultural biogas plants and use energy crops, especially maize (silage), as a renewable and sustainable substrate.

In order to integrate the production of energy from biogas into the existing energy infrastructure, the AD of biomass has to optimised, not only to make the process economically attractive, but also to solve certain problems e.g. low methane values in the biogas (approximately 50%), self-heating in the plants and the odours from fermentation.

This optimization of the biogas process can be achieved by technical improvements (e.g. by identifying new process designs, that overcome process instabilities when dealing with high-cellulose substrate), by testing reactor designs and operating modes and by classifying and ranking crops regarding their energy production. Objective of this study is the optimization of the process by enhanced process control.

There are different possibilities of controlling the anaerobic digestion process, for example PID control, Fuzzy logic based control, neural network based control or control based on linear and non-linear models. In this case, the advanced control is achieved using a Decision Support System (DSS) based on Fuzzy Logic.

Yet the implementation of an effective control tool, apart from the advanced monitoring of the plant, requires a good knowledge of the AD process itself. This knowledge of the biogas process can be obtained using process models. In this study the Anaerobic Digestion Model No.1 (Batstone *et al*, 2002) was used.

The ADM1 is mathematical model describing the biogas process and was developed by the IWA Task group for Mathematical Modeling of Anaerobic Digestion Processes (Batstone *et al*, 2002) and first presented at the 9th IWA Anaerobic Digestion Conference in Belgium in 2001.

As the ADM1 is not fully suitable for modelling the anaerobic digestion of energy crops the model had to be slightly adapted. A large part of the study deals with the calibration of the model, due to its complexity.

The adapted version of the model was then used as the basis for the so-called “Virtual Laboratory” (VL) – a software training tool, which provides users with the possibility of gaining more insight into the biogas process.

Abbrevations

AD	Anaerobic Digestion
ADM1	Anaerobic Digestion Model No.1
ADMML	Anaerobic Digestion Model based on Marsili-Libelli
ASM1	Activated Sludge Model No.1
ATP	Adenosintriphosphate
ATV	Abwassertechnische Vereinigung
BMP	Biochemical Methane Potential
BSA	Bovine Serum Albumin
CA	Crude Ash
CF	Crude Fibre
C_i	Carbon Content of Component i
CL	Crude Lipid
COD	Chemical Oxygen Demand
CP	Crude Protein
CSTR	Continuous Stirred Tank Reactor
DSS	Decision Support System
d_x	index of agreement
FID	Flame Ionisation Detection
FL	Fuzzy Logic
FTIR - ATR	Fourier Transform Infrared – Attenuated Total Reflection
GA	Genetic Algorithm
GC	Gas Chromatograph
GP	Gas Production
GPS	Whole Plant Maize Silage
HMI	Human Machine Interface
HRT	Hydraulic Retention Time
IAE	Integral Absolute Error
$I_{Inhibitor,Process}$	Inhibition Function
IWA	International Water Association
$K_{a,Acid}$	Acid-Base Equilibrium
k_{dec}	First Order Decay Rate
K_H	Henry's Law Coefficient
k_La	Gas-Liquid Transfer Coefficient
$k_{m,i}$	Monod Maximum Specific Uptake Rate
$k_{Process}$	First Order Rate
$K_{S,i}$	Half Saturation Value
LCFA	Long Chain Fatty Acids
LJ	Luus-Jaakola
Maize	Maize Silage

MAX	Maximum
MC	Methane Content
MIN	Minimum
MRE	Maximum Relative Errors
NfE	Nitrogen free Extract
N_i	Nitrogen Content of Component i
NN	Neural Networks
ODE	Ordinary Differential Equation
OF	Objective Function
OLR	Organic Loading Rate
ORP	Oxidation Reduction Potential
PF	Plug Flow Reactor
PID	Partial Integral Derivate Control
R	Gas Law Constant
R_{Mean}	Ratio of Means
RMSE	Root Mean Square Errors
RO	Reverse Osmose
SA	Simulated Annealing
SAE	Sum of Absolute Errors
SESP	Search Space
S_i	Soluble Component i
SR	Sulphate Reduction
SRB	Sulphate Reduction Bacteria
SRT	Sludge Retention Time
SS	Suspended Solids/Dry Matter
SSE	Sum of Squared Errors
STD	Standard Deviation
Sunflower	Sunflower Press Residues
TAN	Total Ammonia Nitrogen
TC	Total Carbon
TCD	Thermal Conductivity Detection
TKN	Total Kjieldahl Nitrogen
TN	Total Nitrogen
TOC	Total Organic Carbon
UASB	Upflow anaerobic Sludge Reactor
UV	Ultraviolet
VAR	Variance
VFA	Volatile Fatty Acids
VL	Virtual Laboratory
VSS	Volatile Suspended Solids
WWTP	Waste Water Treatment Plant

X_i	Particulate Component i
Y_i	Yield of Biomass on i

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1 Introduction

1.1 Principles of Anaerobic Digestion

Anaerobic digestion (AD) is one of the longest used processes for wastewater treatment and sludge stabilization (Feng *et al*. 2006). AD uses biological processes in the absence of oxygen with methane and carbon dioxide as end-products and is characterised by the harmonized work of different micro-organisms (Bischofsberger *et al*. 2005). The natural habitats of such a mixed bacteria consortium are swamps, bogs, the sludge layer of seas and rivers and the rumen of ruminants (Braun 1982).

The attractive features of the anaerobic digestion process are its ability to degrade recalcitrant (persistent) and xenobiotic compounds, the reduction of chlorinated organic toxicity levels, the biodegradation of aerobic non-bio-degradables, the reduction of malodours and numbers of pathogens, the elimination of off-gas air pollution, the lower sludge handling and disposal costs compared to aerobic processes and the lower volatile content of sludge. Further attractive features are the production of methane, the improved dewaterability of the sludge and its suitability for high concentrated industrial wastewater (Speece 1996; Gerardi 2003). Residues from the biogas process can be used as fertilizer or soil conditioner (Gallert and Winter 2002; Amon *et al*. 2007).

The conversion of organic substances by micro-organisms is normally from high-molecular to low-molecular substances whereby energy is released (Gerardi 2003; Bischofsberger *et al*. 2005). During this degradation two types of nourishment are gained: carbon and energy (Gerardi 2003). The carbon is used for the synthesis of cellular material (Gerardi 2003). The energy obtained is used for the formation of Adenosintriphosphate (ATP) and heat (Braun 1982; Bischofsberger *et al*. 2005). A big difference between aerobic conversion and anaerobic digestion is the energy output (Bischofsberger *et al*. 2005). Only 2 molecules ATP can be gained through the anaerobic conversion of glucose, whereas during the aerobic process 38 molecules ATP are formed (Bischofsberger *et al*. 2005). This also results in low growth rates of anaerobic bacteria and thus generally in a lower production of biomass compared to aerobic processes (Bischofsberger *et al*. 2005). The biomass formed is about 3 to 6 % of the degraded carbon (Kämpfer and Weißenfels 2001), which means that > 90 % of the degradable Chemical Oxygen Demand (COD) is converted to biogas (Speece 1996).

In the AD process a wide range of substrates, for example energy crops, animal manures and organic wastes can be used (Amon *et al*. 2007). The composition of the substrate and the environmental conditions cause the development of a specific mixed bacteria population (Braun 1982; Bischofsberger *et al*. 2005).

The production of energy from biomass is a pertinent topic in Austria at the moment. Due to the need to reach ambitious political aims, for example the Kyoto Protocol or the green electricity law (Ökostromgesetz 2002 (BGBI.I Nr. 149/2002)), renewable energy technologies were effectively promoted in Austria. This resulted in a boom in the construction of new plants (Lindorfer 2007). By 2005 231 plants were already in operation (Laaber 2007). Most of the plants are now using energy crops especially maize for the production of biogas.

Nevertheless, these plants often face severe problems, such as a low methane values in the biogas (approximately 50 %). Yet, a high biogas yield is required for the process to be financially viable (Amon *et al*. 2007). Other problems are the self-heating of the plants (Lindorfer 2007), unstable processes, long start-up times, slow kinetics at low temperatures and the odours from fermentation (Speece 1996). These problems mean that plants operate most of time very ineffectively. To make the biogas process more economically attractive, the process has to be optimized. There is a potential for optimisation in almost every process step – beginning with the cultivation of the crops, the digestion itself and finally the utilisation of the resulting gas and digestate (Lindorfer 2007).

One possibility to overcome the problems in the bioconversion process is an advanced control of the process. There are different possibilities for controlling the anaerobic digestion (AD) process, for example PID control, Fuzzy logic based control, neural network based control or control based on linear and non-linear models (Steyer *et al*. 2005). However, the implementation of an effective control tool requires a good knowledge of the AD process. Process models are a good possibility to gain more insight into the biogas process. Yet, a model is always a simplification and an abstraction of the reality (Strik 2004).

Models can be classified in different ways: based on the structural form (mechanistic, empirical, grey-box), on the nature of the process inputs and outputs (deterministic, uncertainty, stochiastic), on the temporal dependency (static and dynamic) and on the form of mathematical functions (functional, neuronal network and qualitative models) used (Olsson and Newell 1999).

Due to the complexity of the AD process, the construction of a descriptive model is difficult (Wilcox *et al*., 1995) and not normally possible without any assumptions and simplifications.

Generally the structure and complexity of a model depends on the purpose of the model (Strik 2004) - depending on the specific case intermediates can be included or excluded in the model (Feng *et al*. 2006).

All functional (here mostly referred to as mathematical) AD models are based on the Monod definition of the relationship between the growth rate and the limiting substrate

concentration (Equation (1)). This term is then extended for different influencing variables such as the pH, inhibitions, etc. (Braun 1982).

$$\mu = \mu_{\max} * \frac{S}{K_S + S} \quad \textbf{(1)}$$

where:
μ growth rate [d^{-1}]
μ_{max} maximum growth rate [d^{-1}]
S concentration of the used Substrate [$g.l^{-1}$]
K_s half-saturation constant [$g.l^{-1}$]

The first mathematical models of the AD process emerged in the 1960s (Angelidaki *et al*. 1998). In the meantime a lot of different anaerobic digestion models have been developed, such as the model from Jain *et al*. (1991), Angelidaki and co-workers (1993), Vavilin (2000) or Siegrist (2002).

In 2002 a new model was developed by the IWA Task group for Mathematical Modelling of Anaerobic Digestion Processes – the Anaerobic Digestion Model No.1. It purports to be the first really general model of anaerobic digestion, combining the knowledge of different experts in this field (Batstone *et al*. 2002). As set of ordinary differential equations (ODE) the ADM1 consists of 32 dynamic state concentrations and 19 biochemical process rates, 6 acid-base rates and 3 gas liquid transfers (Blumensaat and Keller 2005).

The model describes the AD process in several subsequent steps, according to the common understanding of the process (Figure 1.1.1), such as: the disintegration of complex particulates to carbohydrates, proteins and lipids, followed by the hydrolysis to monosaccharides, amino acids and long chain fatty acids (LCFA). Furthermore the degradation of sugars and amino acids to volatile fatty acids (VFAs), hydrogen and carbon dioxide by acidogens; the acetogenesis from LCFAs and VFAs to acetate and the methanogenesis from acetate and hydrogen to methane (Speece 1996; Batstone *et al*. 2002). Further specified physico-chemical processes which are included are acid-base reactions and liquid gas transfer processes (Batstone *et al*. 2002). The ADM1 works on a COD basis (Blumensaat and Keller 2005) and is one of the most complex AD Models (Feng *et al*. 2006).

The primary aim of the publication on the ADM1 is the augmented model application for full-scale plants, further developments on process optimization and control and a common basis for further model development (Batstone *et al*. 2005).

The advantages of the model compared to other mechanistic AD models is that it uses a unified nomenclature and kinetics (Batstone *et al*. 2002).

The disadvantages of the ADM1 are, like using, calibrating or constructing any other model, that there is a need to understand the process. Moreover there is a lack of information in some areas: the analysis and validation of biological parameters (different feeds and reactor designs), the effects of inhibitory compounds and changes in the

kinetics for different temperature ranges (psychrophilic, mesophilic, thermophilic) (Batstone *et al*. 2002). Furthermore a detailed substrate definition and analysis is necessary (Kleerebezem and Van Loosdrecht 2004).

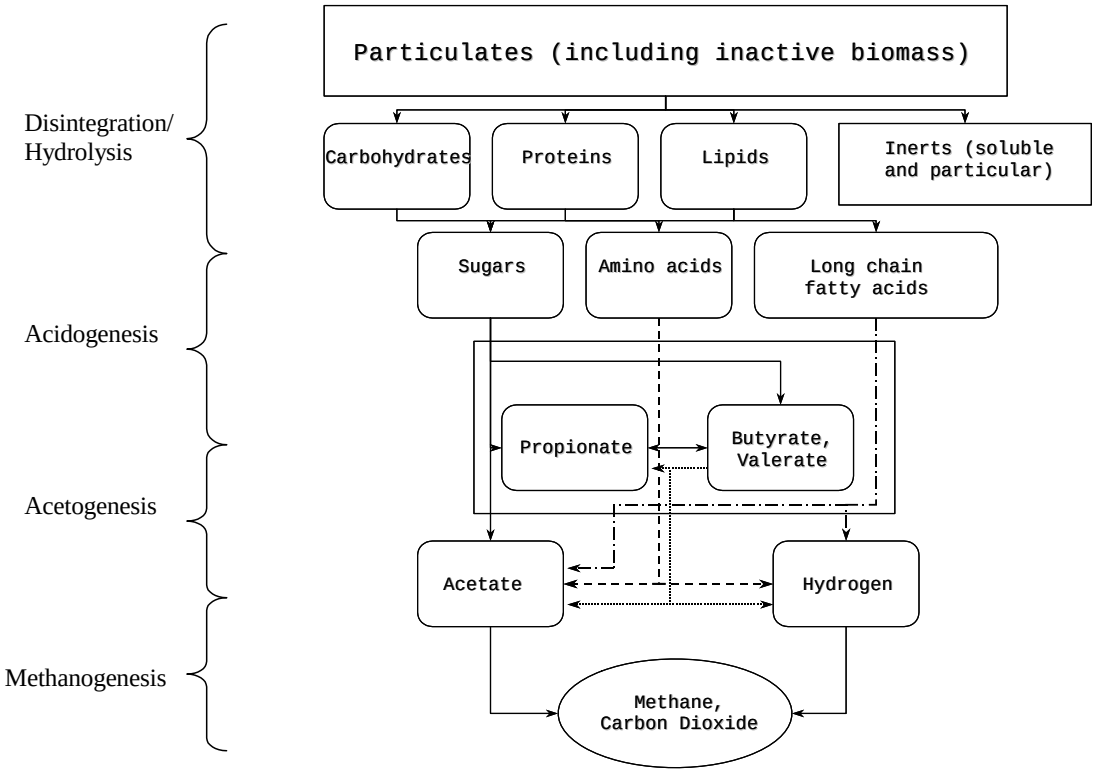

Figure 1.1.1: Processes of the anaerobic model, according to (Batstone *et al*. 2002)

1.1.1 Hydrolysis

Hydrolysis is the first step in the AD process and means the degradation of particulates (compounds) and the addition of water (Gerardi 2003); it is brought by extra-cellular enzymes and is rather a slow process (Henze and Harremoes 1983). According to different authors (Pavlostathis and Gossett 1985; Kämpfer and Weißenfels 2001; Gerardi 2003; Bischofsberger *et al*. 2005) the limiting steps in AD are those related to the conversion of substrate into a soluble form and the formation of methane from acetate and propionate. The IWA task-group came to a similar conclusion in their description of the Anaerobic Digestion Model No.1 (Batstone *et al*. 2002).

The conceptual model used in the ADM1 for the hydrolysis process is that the micro-organisms attach themselves to the particulates, produce extra-cellular enzymes and benefit then from the soluble products formed (Batstone *et al*. 2002).

Hydrolysis now can be described as a surface-related hydrolysis kinetics (Vavilin *et al*. 1996; Batstone *et al*. 2000; Sanders *et al*. 2000) or Contois kinetic (Vavilin *et al*. 1996; Batstone *et al*. 2002). Contois models use one single parameter to delineate the saturation of the biomass and the substrate (Vavilin *et al*. 2004). The IWA Task group (Batstone *et al*. 2002) suggest that Contois kinetic be used if the biomass to substrate ratio is low enough to be rate limiting.

Normally however, the hydrolysis of complex substrates is described based on first order reactions (Equation (2) to (5)) (Batstone *et al*. 2002; Rosen and Jeppsson 2002), for reasons of simplification and the reduction of constant assumptions (Elmitwalli *et al*. 2006).

$$\rho_1 = k_{dis} * X_c \quad \textbf{(2)}$$

$$\rho_2 = k_{hyd,ch} * X_{ch} \quad \textbf{(3)}$$

$$\rho_3 = k_{hyd,pr} * X_{pr} \quad \textbf{(4)}$$

$$\rho_4 = k_{hyd,li} * X_{li} \quad \textbf{(5)}$$

where:

ρ_i process rate of the specific substrate (c – complex particulates, ch – carbohydrates, pr – proteins, li - lipids) [$kg_{COD}.m^{-3}d^{-1}$]
k_{dis} disintegration rate [d^{-1}]
$k_{hyd,i}$ hydrolysis rate [d^{-1}]
X_i concentration of the substrate [$kg_{COD}.m^{-3}$]

The new aspect of the ADM1 is the inclusion of the disintegration step in the model (Blumensaat and Keller 2005). The disintegration step was included in order to consider the degradation of material with “lumped” characteristics, while the hydrolysis step describes the degradation of “well defined” substrates (Batstone *et al*. 2002). The disintegration step and the hydrolysis of carbohydrates, proteins and lipids together describe the extra-cellular solubilisation step (Batstone *et al*. 2002). Whereby the disintegration is mainly a non-biological process, including the lysis, non-enzymatic decay, phase separation and physical breakdown (Batstone *et al*. 2002). This first step produces carbohydrates, proteins, lipids, and soluble and particulate inerts (Batstone *et al*. 2002). The hydrolysis step produces monosaccharides, amino acids and long chain fatty acids (Batstone *et al*. 2002).

Table 1.1.1: Examples for the disintegration rates of different substrates

Substrate	Disintegration rate [d^{-1}]	Reference
Grass	$3.5*10^{-2}$ – $2.66*10^{-1}$	Veeken and Hamelers 1999
Leaves	$6.8*10^{-2}$ – $3.86*10^{-1}$	Veeken and Hamelers 1999
Nettle	$3*10^{-2}$ – $7.6*10^{-2}$	Lehtomäki et al. 2005
Newsprints	$5.6*10^{-2}$ / $5.68*10^{-2}$	Jokela et al. 2005/Vavilin et al. 2004
Orange peelings	$1.45*10^{-1}$ – $4.74*10^{-1}$	Veeken and Hamelers 1999
Red clover	$2.8*10^{-2}$ – $3.9*10^{-2}$	Lehtomäki et al. 2005

The hydrolysis rate is influenced by pH, type of substrate, temperature and age of the sludge - where the speed of the hydrolysis depends on the concentration and availability

of the substrate, the biomass concentration and the mixing of the reactor (Gerardi 2003; Bischofsberger *et al*. 2005).

The substrate is clearly one of the most influential factors. For example sugars and Hemicellulose can be easily hydrolysed, whereas cellulose or starch is much slower to hydrolyse (Bischofsberger *et al*. 2005). The hydrolysis of proteins is very complex and hydrolysis rates are normally slower than for carbohydrates or fats (Bischofsberger *et al*. 2005)

Because the hydrolysis rate is influenced by a multiplicity of factors, values found in the literature are divers (Table 1.1.1 and Table 1.1.2).

Table 1.1.2: Range of the hydrolysis rates for carbohydrates, proteins and lipids

Substrate	Hydrolysis rate [d^{-1}]	Reference
Carbohydrates	$2.5*10^{-2}$ – $1.06*10^{2}$	Angelidaki et al. 1998; Christ et al. 2000; Miron et al. 2000; Batstone et al. 2002; Rosen and Jeppsson 2002; Feng et al. 2006
Proteins	$9.6*10^{-3}$ – $1*10^{1}$	Angelidaki et al. 1998; Christ et al. 2000; Miron et al. 2000; Tzouvaras 2001; Batstone et al. 2002; Rosen and Jeppsson 2002; Feng et al. 2006
Lipids	$5*10^{-3}$ – $1*10^{1}$	Miron et al. 2000; Tzouvaras 2001; Batstone et al. 2002; Rosen and Jeppsson 2002; Feng et al. 2006

In their Scientific and Technical Report No. 13 (Batstone *et al*. 2002) the IWA Task Group for Mathematical Modelling of AD Processes suggests for the disintegration rate values of 0.4 d^{-1}, 0.5 d^{-1} and 1 d^{-1} for the mesophilic high rate digestion, the mesophilic digestion of solids and the thermophilic digestion of solids, respectively. For the hydrolysis rate of carbohydrates, proteins and lipids values of 0.25 d^{-1}, 0.2 d^{-1} and 0.1 d^{-1} for the high-rate mesophilic digestion is given (Batstone *et al*. 2002). For the mesophilic and thermophilic digestion of solids a uniform hydrolysis rate of 10 d^{-1} is suggested (Batstone *et al*. 2002).

A further process which, in the ADM1, is strongly linked to the disintegration and hydrolysis step is the decay of biomass, as this process produces particulate organic matter (Blumensaat and Keller 2005). The decay process for all groups of bacteria is described as a first order process, as well (Equation ((6) to (12)) (Batstone *et al*. 2002; Rosen and Jeppsson 2002):

$$\rho_{13} = k_{dec,Xsu} * X_{su} \quad \textbf{(6)}$$

$$\rho_{14} = k_{dec,Xaa} * X_{aa} \quad \textbf{(7)}$$

$$\rho_{15} = k_{dec,Xfa} * X_{fa} \quad \textbf{(8)}$$

$$\rho_{16} = k_{dec,Xc4} * X_{c4} \quad \textbf{(9)}$$

$$\rho_{17} = k_{dec,Xpro} * X_{pro} \quad \textbf{(10)}$$

$$\rho_{18} = k_{dec,Xac} * X_{ac} \quad \textbf{(11)}$$

$$\rho_{19} = k_{dec,Xh2} * X_{h2} \quad \textbf{(12)}$$

where:

ρ_i process rate of the specific substrate (su – sugar, aa – amino acids, fa –long chain fatty acids, c^4 – butyric + valeric acid, pro – propionic acid, ac – acetic acid, h2 – hydrogen) [$kg_{COD}.m^{-3}d^{-1}$]

$k_{dec,i}$ decay rate [d^{-1}]

X_i concentration of the substrate [$kg_{COD}.m^{-3}$]

Similarly to the hydrolysis rates, the values for decay rates found in the literature are rather divers (Table 1.1.3).

Table 1.1.3: Examples for the decay rates of acidogenic, acetogenic and methanogenic bacteria from literature[1]

Bacteria group	Decay rate (Range) [d^{-1}]	Reference
Sugar Fermenter	$1.00*10^{-2}$ – 3.20	see Reference[1]
Amino Acids Fermenter	$1.00*10^{-1}$ – 6.10	see Reference[2]
Fatty acid using bacteria	$1.00*10^{-2}$ – $2.00*10^{-1}$	see Reference[3]
Valerate using bacteria	$1.00*10^{-2}$ – $3.00*10^{-2}$	see Reference[4]
Butyrate using bacteria	$9.60*10^{-3}$ – $3.00*10^{-1}$	see Reference[5]
Propionate using bacteria	$9.60*10^{-3}$ – $2.00*10^{-3}$	see Reference[6]
Aceticlastic Methanogens	$3.12*10^{-3}$ – $4.32*10^{-1}$	see Reference[7]
Hydrogen degrader	$9.00*10^{-3}$ – 1.20	see Reference[8]

[1] [1] Angelidaki *et al*., 1998; Batstone *et al*., 2000; Tzouvaras, 2001; Batstone *et al*., 2002

[2] Angelidaki *et al*., 1998; Batstone *et al*., 2000; Tzouvaras, 2001; Batstone *et al*., 2002

[3] Angelidaki *et al*., 1998; Batstone *et al*., 2000; Tzouvaras, 2001; Batstone *et al*., 2002

[4] Angelidaki *et al*., 1998; Batstone *et al*., 2000; Batstone *et al*., 2002; Jeong *et al*., 2005

[5] Costello *et al*., 1991; Ryhiner *et al*., 1993; Kalyuzhni, 1996; Angelidaki *et al*., 1998; Batstone *et al*., 2000; Batstone *et al*., 2002; Fedorovich *et al*., 2003; Jeong *et al*., 2005; Feng *et al*., 2006; Kalfas *et al*., 2006

[6] Costello *et al*., 1991; Ryhiner *et al*., 1993; Kus and Wiesmann, 1994; Maillacheruvu *et al*., 1996; Angelidaki *et al*., 1998; Batstone *et al*., 2000; Batstone *et al*., 2002; Seok and Komisar, 2002; Fedorovich *et al*., 2003; Blumensaat and Keller, 2004; Kalfas *et al*., 2006; Aceves-Lara et.al, 2005; Jeong *et al*., 2005; Feng *et al*., 2006

[7] Costello *et al*., 1991; Ryhiner *et al*., 1993; Kiely *et al*., 1996; Kalyuzhni, 1996; Marsili-Libelli and Beni, 1994; Angelidaki *et al*., 1998; Kaspar and Wuhrmann, 1978; Kus and Wiesmann, 1994; Maillacheruvu *et al*., 1996; Batstone *et al*., 2000; Bernard *et al*., 2001; Tzouvaras, 2001; Batstone *et al*., 2002; Seok and Komisar, 2002; Fedorovich *et al*., 2003; Blumensaat and Keller, 2004; Aceves-Lara et.al, 2005; Jeong *et al*., 2005; Feng *et al*., 2006; Kalfas *et al*., 2006

[8] Costello *et al*., 1991; Ryhiner *et al*., 1993; Kalyuzhni, 1996; Maillacheruvu *et al*., 1996; Batstone *et al*., 2000; Batstone *et al*., 2002; Fedorovich *et al*., 2003; Blumensaat and Keller, 2004; Jeong *et al*., 2005; Rosen and Jeppsson, 2006

In the ADM1 report (Batstone *et al*. 2002) decay rates of 0.02 d^{-1} in the mesophilic range and 0.04 d^{-1} in the thermophilic temperature range are suggested for all bacteria groups.

1.1.2 Acidogenesis

Acidogenesis is defined as a microbial process producing anaerobic acids without any additional electron acceptor or –donator (Bischofsberger *et al*. 2005). Characteristic for the acidogenesis is that COD reduction is very low (Bischofsberger *et al*. 2005).
The products of this step are organic acids, H_2 and CO_2, other short chain acids or alcohols (Bischofsberger *et al*. 2005). The products depend on the substrate, the environmental conditions in the reactor and the organic loading rate and hence resulting partial pressure of the hydrogen and pH (Bischofsberger *et al*. 2005).
Glucose is used as model monomer in the ADM1 for the acidogenesis from monosaccharides to acetate, propionate and butyrate (Batstone *et al*. 2002) (Equation (13) to (15)):

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad \textbf{(13)}$$

$$3C_6H_{12}O_6 \rightarrow 4CH_3CH_2COOH + 2CH_3COOH + 2CO_2 + 2H_2O \quad \textbf{(14)}$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad \textbf{(15)}$$

There are two main pathways for the amino acid fermentation: the Stickland oxidation-reduction paired fermentation and the oxidation of a single amino acid (Batstone *et al*. 2002). The Stickland reaction has different characteristics: such as that different amino acids can act as electron donor and as electron acceptor. The electron donor loses one carbon atom to CO_2 and forms a carboxylic acid with one carbon atom shorter than the original amino acid and the electron acceptor forms a carboxylic acid with the same amount of carbon atoms as the original amino acid (Batstone *et al*. 2002). The oxidation reaction above all occurs with low hydrogen and formate concentrations under thermophilic conditions (Batstone *et al*. 2002).
Further intermediate products which are formed during acidogenesis are, for example, lactate and ethanol. These processes are not described more exactly here as these processes are not included in the ADM1 (Batstone *et al*. 2002).
Ethanol is preferentially produced to acetate at low pH (Batstone *et al*. 2002), which leads to an under-prediction of organic acids and pH at low pH values (Batstone *et al*. 2002). Lactate is a key intermediate, but is under normal conditions relatively fast degraded (Batstone *et al*. 2002).

Batstone and co-workers (2002) consider valerate, butyrate, propionate and acetate as products in their model.

In the model the acidogenesis is described in the form of the Monod equation (Batstone *et al*. 2002; Rosen and Jeppsson 2002): (Equation (16) to (17)) describe the process rates for the acidogenesis from monosaccharides and amino acids:

$$\rho_5 = k_{m,su} * \frac{S_{su}}{K_{S,sz} + S_{su}} * X_{su} * I_5 \quad \textbf{(16)}$$

$$\rho_6 = k_{m,aa} * \frac{S_{aa}}{K_{S,aa} + S_{aa}} * X_{aa} * I_6 \quad \textbf{(17)}$$

where:

ρ_i process rate of the specific substrate [$kg_{COD}.m^{-3}d^{-1}$]
$k_{m,i}$ maximum specific uptake rate [$kg_{COD}.kg_{COD}^{-1}d^{-1}$]
S_i concentration of the substrate [$kg_{COD}.m^{-3}$]
$K_{S,i}$ half saturation constant [$kg_{COD}.m^{-3}$]
X_i concentration of the sugar degraders [$kg_{COD}.m^{-3}$]
I_i Inhibition factor

Table 1.1.4 shows different values for the substrate uptake rate, the maximum half-saturation rate and the yield of acidogenic bacteria found in literature.

Table 1.1.4: Examples for the substrate uptake rate, maximum half-saturation rate and yield of acidogenic bacteria (mean values)[2]

Substrate	Substrate uptake rate [$kg_{COD}.kg_{COD}.d^{-1}$]	Half Saturation constant [$kg_{COD}.m^{-3}$]	Yield [$kg_{CODX}.kg_{CODS}^{-1}$]	Reference
Amino Acids	$6.47*10^{1} \pm 3.95*10^{1}$	$5.57 \pm 1.29*10^{2}$	$1.09*10^{-1} \pm 5.00*10^{-2}$	see Reference[1]
Sugar	$5.00*10^{2} \pm 1.43*10^{3}$	$8.55 \pm 3.07*10^{1}$	$1.25*10^{-1} \pm 6.69*10^{-2}$	see Reference[2]

Batstone and co-workers (2002) proposed a k_m, K_S and Y for sugar degraders of 30 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$, 0.5 $kg_{COD}.m^{-3}$, 0.1 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$ and for amino acid using bacteria of 50 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$, 0.3 $kg_{COD}.m^{-3}$. 0.08 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$ for the digestion of solids in the mesophilic temperature range and 70 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$, 1 $kg_{COD}.m^{-3}$, 0.10 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$ and 70 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$, 0.3 $kg_{COD}.m^{-3}$, 0.08 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$ respectively in the thermophilic temperature range.

The half-saturation constant K_s normally depends on the specific micro-organisms and on the substrate used (Braun 1982).

1.1.3 Acetogenesis

Acetogenesis, also referred to as anaerobic oxidation, is the conversion of carboxylic acids or organic acids into acetate, CO_2 and H_2O (Bischofsberger *et al*. 2005) using an external electron acceptor (Batstone *et al*. 2002). The conversion of all fatty acids occurs by β-oxidation (Bischofsberger *et al*. 2005).

[2] [1] Angelidaki *et al*., 1998; Batstone *et al*., 2000; Tzouvaras, 2001; Batstone *et al*., 2002

[2] Costello *et al*., 1991;Kalyuzhni, 1996; Angelidaki *et al*., 1998; Batstone *et al*., 2000; Tzouvaras, 2001; Batstone *et al*., 2002; Jeong *et al*., 2005

The most important acetogenic reactions can only proceed if the partial pressure of hydrogen is less than 10^{-4} atm (Speece 1996; Kämpfer and Weißenfels 2001; Bischofsberger *et al*. 2005). The acetogenic bacteria live in a symbiosis with the methanogenic bacteria, which use the hydrogen in their metabolism and thus the partial pressure of hydrogen in the system is kept low. (Braun 1982; Gerardi 2003; Bischofsberger *et al*. 2005). If hydrogen accumulates and the hydrogen pressure increases the activity of acetate-forming bacteria decreases and finally stops completely (Braun 1982; Gerardi 2003), which leads to an accumulation of acids in the reactor, which in turn results in an inhibition of the methanogenic bacteria (Braun 1982; Speece 1996). The regulative effect of the methane-forming bacteria on the electron flux creates the thermodynamic prerequisites for the oxidation of alcohols and fatty acids (Braun 1982).

For the hydrogen regulation a non-competitive inhibition function was used in the ADM1 (Batstone *et al*. 2002) (Equation (18)):

$$I = \frac{1}{1 + S_i / K_i} \quad \textbf{(18)}$$

where:

I	Inhibition factor
S_i	concentration of inhibitor i [$kg_{COD}.m^{-3}$]
K_i	inhibtion parameter [$kg_{COD}.m^{-3}$]

Reactions for fatty acid oxidising micro-organism are for example (Braun 1982; Batstone *et al*. 2002) (Equation ((19) to (21))[3] the oxidation of valerate, butyrate and propionate:

$$CH_3CH_2CH_2CH_2COOH + 2H_2O \rightarrow CH_3CH_2COOH + CH_3COOH + 2H_2 \quad \textbf{(19)}$$

$$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 \quad K\,\Delta G^0 = 41.66\,kJ \quad \textbf{(20)}$$

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 \quad K\,\Delta G^0 = 81.85\,kJ \quad \textbf{(21)}$$

Since the degradation of the long chain fatty acids (LCFA) is also an oxidation reaction with an external acceptor, it is as well included in this section (Batstone *et al*. 2002). Altogether three acetogenic bacteria groups are included in the ADM1, considering LCFA, valerate and butyrate and propionate as substrates (Batstone *et al*. 2002).

The following process rates are found for the acetogenesis in the ADM1 (Batstone *et al*. 2002; Rosen and Jeppsson 2002) (Equation ((22) to (25))):

$$\rho_7 = k_{m,fa} * \frac{S_{fa}}{K_{S,fa} + S_{fa}} * X_{fa} * I_7 \quad \textbf{(22)}$$

[3] ΔG^0 free energy release for standard conditions

$$\rho_8 = k_{m,c4} * \frac{S_{va}}{K_{S,c4} + S_{va}} * X_{c4} * \frac{S_{va}}{S_{bu} + S_{va} + 1^{-6}} * I_8 \quad \textbf{(23)}$$

$$\rho_9 = k_{m,c4} * \frac{S_{bu}}{K_{S,c4} + S_{bu}} * X_{c4} * \frac{S_{bu}}{S_{bu} + S_{va} + 1^{-6}} * I_9 \quad \textbf{(24)}$$

$$\rho_{10} = k_{m,pr} * \frac{S_{pro}}{K_{S,pro} + S_{pro}} * X_{pro} * I_{10} \quad \textbf{(25)}$$

where:
ρ_i = process rate of the specific substrate (va – valeric acid, bu – butyric acid) [$kg_{COD}.m^{-3}d^{-1}$]
$k_{m,i}$ = maximum specific uptake rate [$kg_{COD}.kg_{COD}^{-1}d^{-1}$]
S_i = concentration of the substrate [$kg_{COD}.m^{-3}$]
$K_{S,i}$ = half saturation constant [$kg_{COD}.m^{-3}$]
X_i = concentration of the sugar degraders [$kg_{COD}.m^{-3}$]
I_i = Inhibition factor

Table 1.1.5 shows different values for the substrate uptake rate, the maximum half-saturation rate and the yield of acetogenic bacteria taken from literature.

Table 1.1.5: Examples for the substrate uptake rate, maximum half-saturation rate and yield of acetogenic bacteria[4]

Substrate	Substrate uptake rate [$kg_{COD}.kg_{COD}.d^{-1}$]	Half Saturation constant [$kg_{COD}.m^{-3}$]	Yield [$kg_{CODX}.kg_{CODS}^{-1}$]	Reference
LCFA	$5.19*10^{1} \pm 1.08*10^{*2}$	1.90 ± 2.65	$5.33*10^{-2} \pm 3.81*10^{-2}$	see Reference[1]
Valerate	$2.30*10^{1} \pm 9.60$	1.06 ± 1.93	$4.87*10^{-2} \pm 2.15*10^{-2}$	see Reference[2]
Butyrate	$2.71*10^{1} \pm 2.72*10^{1}$	$5.64 \pm 2.25*10^{1}$	$5.67*10^{-2} \pm 2.39*10^{-2}$	see Reference[3]
Propionate	$2.39*10^{1} \pm 3.13*10^{1}$	$5.22 \pm 2.23*10^{1}$	$6.05*10^{-2} \pm 3.95*10^{-2}$	see Reference[4]

For the acetogenic bacteria in the ADM1 (Batstone *et al*. 2002) values of 6 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$,0.4 $kg_{COD}.m^{-3}$, 0.06 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$ for the oxidation of LCFA, 20 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$, 0.2 $kg_{COD}.m^{-3}$, 0.06 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$ for valerate and butyrate using bacteria. 13 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$, 0.1 $kg_{COD}.m^{-3}$, 0.04 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$ for propionate using bacteria for the k_m, K_S and Y, for the fermentation of solids in the mesophilic temperature range are suggested and values of 10 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$, 0.4 $kg_{COD}.m^{-3}$,

[4] [1] Angelidaki *et al*., 1998; Batstone *et al*., 2000; Tzouvaras, 2001; Batstone *et al*., 2002

[2] Angelidaki *et al*., 1998; Batstone *et al*., 2000; Batstone *et al*., 2002; Jeong *et al*., 2005

[3] Costello *et al*., 1991; Ryhiner *et al*., 1993; Kalyuzhni, 1996; Angelidaki *et al*., 1998; Batstone *et al*., 2000; Batstone *et al*., 2002; Fedorovich *et al*., 2003; Jeong *et al*., 2005; Feng *et al*., 2006; Kalfas *et al*., 2006

[4] Costello *et al*., 1991; Ryhiner *et al*., 1993; Kus and Wiesmann, 1994; Maillacheruvu *et al*., 1996; Angelidaki *et al*., 1998; Batstone *et al*., 2000; Batstone *et al*., 2002; Seok and Komisar, 2002; Fedorovich *et al*., 2003; Blumensaat and Keller, 2004; Aceves-Lara et.al, 2005; Jeong *et al*., 2005; Feng *et al*., 2006; Kalfas *et al*., 2006

0.06 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$; 30 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$, 0.4 $kg_{COD}.m^{-3}$, 0.06 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$ and 20 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$, 0.3 $kg_{COD}.m^{-3}$, 0.05 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$ respectively for the thermophilic range.

1.1.4 Methanogenesis

Methanogenic bacteria are archea-bacteria and one of the oldest life-forms on earth and they are strictly anaerobic (Braun 1982; Gerardi 2003; Bischofsberger *et al*. 2005). These bacteria are a morphologically divers group (Gerardi 2003). About 50 species of methanogenic bacteria are known (Gerardi 2003), which can be distinguished in three groups: *Methanobacteriales, Methanococcales* and *Methanomicrobiales* (Braun 1982; Gerardi 2003; Bischofsberger *et al*. 2005). Due to the unique chemical structure of their cell wall (the polar lipid-layer consists of Phytanyl- and Biphytanyl-Glycerine instead of saponifable ester (Braun 1982)) these bacteria are very sensitive to the toxicity of fatty acids (Gerardi 2003). In general methanogenic bacteria are very sensitive to changes in pH, temperature and alkalinity (Gerardi 2003). Yet, they contribute to the stabilisation of pH in the reactor, as they remove acetate from the system (Braun 1982).

Methane can be formed from H_2 and CO_2, formic acid, acetic acid, methanol and methylene during following reactions (Braun 1982; Speece 1996; Le Mer and Roger 2001; Bischofsberger *et al*. 2005) (Equation ((26) to (29))):

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \text{ K } \Delta G^0 = -136\,kJ \quad \textbf{(26)}$$

$$4HCOO^- + H_2O + H^+ \rightarrow CH_4 + 3HCO_3^- \text{ K } \Delta G^0 = -130\,kJ \quad \textbf{(27)}$$

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \text{ K } \Delta G^0 = -30\,kJ \quad \textbf{(28)}$$

$$4CH_3OH \rightarrow 3CH_4 + HCO_3^- + H^+ + H_2O \text{ K } \Delta G^0 = -314\,kJ \quad \textbf{(29)}$$

Thus the methane-forming bacteria can be classified according to the substrate used: hydrogenotrophic methanogens, aceticlastic methanogens and methylotrophic methanogens (Gerardi 2003).

Nearly all methanogenic bacteria can convert H_2 and CO_2, whereas just few can convert methanol or acetate – for example *Methanosarcina barkeri*, *Methanosarcina mazei* or *Methanosaeta* (Bischofsberger *et al*. 2005). Hydrogen can be seen as the universal substrate for all methanogenic bacteria (Gerardi 2003; Bischofsberger *et al*. 2005). *Methanosarcina* and *Methanosaeta* are normally found together in anaerobic systems, whereby *Methanosaeta* is predominate at low acetate levels and *Methanosarcina* at high acetate levels (Speece 1996). In order to simplify the model only one aceticlastic methanogenic bacteria group is included (Batstone *et al*. 2002).

Acetate using bacteria have 2 to 4 times lower growth rates than bacteria, which convert hydrogen (Bischofsberger *et al*. 2005), as the conversion of acetate to methane produces little energy (Le Mer and Roger 2001). Generally the reproductive time for

methanogenic bacteria lies between 3 to 50 days, which leads to the long retention times, required in anaerobic reactors (Gerardi 2003).

Even though the hydrolytic methanogenesis is more advantageous than acetogenic methanogenesis, 70 % of the methane is formed from acetate (Braun 1982; Speece 1996; Le Mer and Roger 2001; Gerardi 2003; Bischofsberger *et al*. 2005). This has its origin in the limitation of substrates in the natural surroundings (Bischofsberger *et al*. 2005).

The process rates for the hydrogen-utilising and acetate-utilising methanogens are given by (Batstone *et al*. 2002; Rosen and Jeppsson 2002) (Equation ((30) to (31))):

$$\rho_{11} = k_{m,ac} * \frac{S_{ac}}{K_{S,ac} + S_{ac}} * X_{ac} * I_{11} \quad \textbf{(30)}$$

$$\rho_{12} = k_{m,h2} * \frac{S_{h2}}{K_{S,h2} + S_{h2}} * X_{h2} * I_{12} \quad \textbf{(31)}$$

where:
ρ_i = process rate of the specific substrate [$kg_{COD}.m^{-3}d^{-1}$]
$k_{m,i}$ = maximum specific uptake rate [$kg_{COD}.kg_{COD}^{-1}d^{-1}$]
S_i = concentration of the substrate [$kg_{COD}.m^{-3}$]
$K_{S,i}$ = half saturation constant [$kg_{COD}.m^{-3}$]
X_i = concentration of the sugar degraders [$kg_{COD}.m^{-3}$]
I_i = Inhibition factor

Values for the substrate uptake rate, the maximum half-saturation rate and the yield of methanogenic bacteria are found in Table 1.1.6.

Table 1.1.6: Examples for the substrate uptake rate, maximum half-saturation rate and yield of methanogenic bacteria[5]

Substrate	Substrate uptake rate [$kg_{COD}.kg_{COD}.d^{-1}$]	Half Saturation constant [$kg_{COD}.m^{-3}$]	Yield [$kg_{CODX}.kg_{CODS}^{-1}$]	Reference
Acetate	$1.19*10^{1} \pm 1.19*10^{1}$	$3.90 \pm 2.38*10^{1}$	$1.73*10^{-1} \pm 5.60*10^{-1}$	see Reference[1]
Hydrogen	$4.13*10^{1} \pm 4.88*10^{1}$	$3.49*10^{-3} \pm 1.33*10^{-2}$	$4.12*10^{0} \pm 7.60$	see Reference[2]

Batstone *et al*. (2002) proposed a substrate uptake rate of 8 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$, a maximum half-saturation rate of 0.15 $kg_{COD}.m^{-3}$ and a yield of 0.05 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$ in the mesophilic range and an uptake rate of 16 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$, a half saturation rate of

[5] [1] Kaspar and Wuhrmann, 1978; Costello *et al*., 1991; Ryhiner *et al*., 1993; Kus and Wiesmann, 1994; Marsili-Libelli and Beni, 1994; Kalyuzhni, 1996; Kiely *et al*., 1996; Maillacheruvu *et al*., 1996; Angelidaki *et al*., 1998; Batstone *et al*., 2000; Bernard *et al*., 2001; Tzouvaras, 2001; Batstone *et al*., 2002; Seok and Komisar, 2002; Blumensaat and Keller, 2004; Fedorovich *et al*., 2003; Aceves-Lara et.al, 2005; Jeong *et al*., 2005; Feng *et al*., 2006; Kalfas *et al*., 2006

[2] Costello *et al*., 1991; Ryhiner *et al*., 1993; Kalyuzhni, 1996; Maillacheruvu *et al*., 1996; Batstone *et al*., 2000; Batstone *et al*., 2002; Fedorovich *et al*., 2003; Blumensaat and Keller, 2004; Jeong *et al*., 2005

0.3 $kg_{COD}.m^{-3}$ and a yield of 0.05 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$ in the thermophilic range for aceticlastic methanogens and values of 35 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$, $7*10^{-6}$ $kg_{COD}.m^{-3}$ and 0.06 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$ for k_m, K_S and Y respectively and values for hydrogen using bacteria for the mesophilic digestion of solids and of 35 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$, $5*10^{-5}$ $kg_{COD}.m^{-3}$ and 0.06 $kg_{COD}.kg_{COD}^{-1}.d^{-1}$ for the thermophilic digestion of solids respectively.

1.1.5 Biogas

Biogas is one of the products formed during the AD process, and consists of CO_2 and CH_4, H_2S, H_2O and some traces of other substances depending on the composition of the substrate (Kämpfer and Weißenfels 2001; Bischofsberger *et al*. 2005), whereby CH_4 and CO_2 are the main components (Gerardi 2003) (Table 1.1.7).

The amount and composition of the biogas formed depends on the amount and composition and the degradability of the substrate, the influence of toxic substances, the process technique and the operation of the plant (Gallert and Winter 2002; Gerardi 2003; Bischofsberger *et al*. 2005).

The CO_2 formed is balanced with the liquid, thus a higher CO_2 content in the biogas leads also to a higher concentration of carbonic acid (Bischofsberger *et al*. 2005). Therefore the CO_2 content in the gas is a good indicator for the performance of the plant (Bischofsberger *et al*. 2005).

Table 1.1.7: Composition of Biogas (Bischofsberger *et al*. 2005)

Substance	Range [%]
Methane CH_4	60 - 70
Carbon Dioxide CO_2	30 – 40
Hydrogen Sulphate H_2S	0 – 0.7
Nitrogen N_2	0 – 0.2
Hydrogen H_2	0 – 0.2
Oxygen O_2	traces

The theoretical amount of biogas can be calculated from the composition of the substrate - of carbohydrates, lipids and proteins in the substrate (Table 1.1.8) (Bischofsberger *et al*. 2005).

Table 1.1.8: Substance specific gas production (Bischofsberger *et al*. 2005)

Substance	Biogas production $[m^3 \cdot kg^{-1}]$	Methane content [%]	Methane production $[m^3 \cdot kg^{-1}]$
Carbohydrates	0.79	50	0.4
Lipids	1.27	68	0.86
Proteins	0.7	71	0.5

For complex substrates the Buswell equation can be used (Equation (32)) (Braun 1982; Speece 1996).

$$C_nH_xO_y + \left(n - \frac{x}{4} - \frac{y}{2}\right) * H_2O \rightarrow \left(\frac{n}{2} - \frac{x}{8} + \frac{y}{4}\right) * CO_2 + \left(\frac{x}{8} + \frac{n}{2} - \frac{y}{4}\right) * CH_4 \quad \textbf{(32)}$$

Table 1.1.9: BMP values for different substrates (composed by C. Pabon, University Wageningen)[6]

Substrate	BMP [$m^3_{CH4}.kg^{-1}_{VSS}$]	Reference
Barley	0.46 ± 0.11	Heiermann, M et al., 2002
Fruit and vegetable wastes mixture	0.23 ± 0.15	Gunaseelan, V.N., 1997
Grass Hay	0.21 ± 0.05	Lehtomaki, A et al., 2004
Jerusalem artichoke	0.31 ± 0.01	Zubr, J., 1986
Maize	0.41 ± 0.06	Neureiter,M. et al., 2005
Rye	0.41 ± 0.08	Heiermann, M et al., 2002
Triticale	0.48 ± 0.09	Heiermann, M et al., 2002
Wheat	0.23 ± 0.04	Sharma, S. et al., 1988

In order to achieve a more realistic estimation of the gas yield the biochemical methane potential (BMP) is determined in batch tests. The practical gas yield is always lower than the theoretical one calculated (Braun 1982). This batch test determines the suitability of the substrate for AD as well as the non-biodegradable fraction (Braun 1982; Speece 1996) (Table 1.1.9). The advantages of the BMP is that the test realistically measures the anaerobic biodegradability and requires a minimal laboratory set-up (Speece 1996).

In the ADM1 only CH_4, CO_2 and H_2 are considered as components (Batstone *et al*. 2002). Whereby the liquid-gas transfer is expressed using Henry's law. The gas transfer

[6] Gunaseelan, V.N., 1997, Anaerobic digestion of biomass for methane production: a review, Biomass and Bioenergy, 13 (1/2):83-114

Heiermann, M. Plöchl, M. Linke, B. Schelle, H., 2002, Preliminary evaluation of some cereals as energy crops for biogas production, World Renewable Energy Congress VII

Lehtomaki, A. Viinikainen, T.A. Ronkainen, O.M. Alen, R. Rintala, J.A., 2004, Effect of pretreatments on methane production potential of energy crops and crop residues, Proceedings Anaerobic Digestion Conference

Neureiter,M. Teixeira Pereira dos Santos, J. Perez Lopez, C. Pichler, H. Kirchmayr, R and Braun, R., 2005, Effect of silage preparation on methane yields of whole crop maize silages, ADSW 2005 Conference Proceedings Vol 1.

Sharma, S. Mishra, I.M, Sharma, M.P, Saini, J.S, 1988, Effect of particle size on biogas generation from biomass residues, Biomass, 17 (251-263)

Zubr, J., 1986, Methanogenic fermentation of fresh and ensiled plant materials, Biomass, 11 (159-171)

rates is therefore described in the model as (Batstone *et al*. 2002; Rosen and Jeppsson 2002) (Equation (33) to (35)):

$$\rho_{T,8} = k_L a\left(S_{h2} - 16K_{H,H_2} * p_{gas,H_2}\right) \quad \textbf{(33)}$$

$$\rho_{T,9} = k_L a\left(S_{ch4} - 64K_{H,CH_4} * p_{gas,CH_4}\right) \quad \textbf{(34)}$$

$$\rho_{T,10} = k_L a\left(S_{co2} - 16K_{H,CO_2} * p_{gas,CO_2}\right) \quad \textbf{(35)}$$

where:

ρ_i gas transfer rate [$kg_{COD}.m^{-3}d^{-1}$]
k_La overall mass transfer coefficient multiplied by the specific area [d^{-1}]
S_i concentration of i [$kg_{COD}.m^{-3}$]
$K_{H,i}$ Henry coefficient [$M.bar^{-1}$]
$p_{gas,i}$ partial pressure of component i [bar]

The k_La value is a parameter that concentrates multiple phenomena – where the k_L corresponds with an absorption coefficient and the *a* can be seen as an area/volume ratio (Olsson and Newell 1999). K_La values depend on the temperature, mixing and liquid properties and are normally rather divers, in order to simplify the ADM1 to simplify the model one value (k_La = 200 d^{-1} (Rosen and Jeppsson 2002)) is given for all three gas components (CH_4, CO_2 and H_2) (Batstone *et al*. 2002).

For the Henry constant Rosen and Jeppson (2002) proposed a value of 0.0271 $M.bar^{-1}$ for CO_2, 0.00116 $M.bar^{-1}$ for CH_4 and $7.38*10^{-4}$ $M.bar^{-1}$ for H_2 at 35 °C for the ADM1.

In industrial plants the biogas has to be dried and the sulphur needs to be removed, due to the vapour and the H_2S. The biogas is dried by cooling the gas to ambient temperature and collecting the condensate (Bischofsberger *et al*. 2005). This condensate is normally very corrosive, due to its low pH and the hydrogen sulphate also leads to odour emissions (Bischofsberger *et al*. 2005). The sulphur can be removed through bacterial oxidation by *Thiobacillus* bacteria, by iron ore, by a scrubber with sodium hydroxide or by air injection (3 – 5 Vol.-% of the produced biogas) (Bischofsberger *et al*. 2005). The gas contains a lot of sludge particles, which can be removed using gravel filters together with the condensate (Bischofsberger *et al*. 2005).

A further problem is the appearance of siloxanes in the gas – as they oxidise and accumulate in crystal form in the gas engines and result in an augmented abrasion (Bischofsberger *et al*. 2005).

Attention also has to be given to the CO_2 content in the gas. Normally the biogas is lighter than air, but if the CO_2 content increases then the gas can became heavier than air and can accumulate on the soil (Bischofsberger *et al*. 2005).

It has further to be considered that due to the ability of methane to absorb infrared radiation, it is 20 to 30 times more effective greenhouse gas than carbon dioxide ((Blake

and Rowland, 1988)[7] cited in (Le Mer and Roger 2001)). Moreover methane is very reactive ((Cicerone and Oremland, 1988)[8] cited in (Le Mer and Roger 2001)). 70 % of the methane emissions are from anthropic sources e.g. rice fields, domesticated livestock and the other 30 % are from natural resources for example from wetland soils (Le Mer and Roger 2001).
In order to effectively control of the emission an improved recovery of methane from anaerobic treatment systems is absolutely necessary (Mulder 2001). Additionally CH_4 and N_2O emissions are reduced if the storage tank is covered (Edelmann *et al*. 2005)

1.1.6 Environmental Condition and Inhibitors

The environmental conditions have a influence on the process stability, biogas yield and bacteria consortium.

Temperature

All biochemical reactions depend on the temperature – an increase in temperature normally leads to an increase in the metabolic activity (Braun 1982; Gerardi 2003; Bischofsberger *et al*. 2005). However, the temperature can influence the biochemical reaction in other ways, as well: An increase in the temperature can lead to a decrease in the reaction rate, too, if the temperature is above the optimum; it can cause a decrease or shift in yields and an increase in K_s (Speece 1996), as well as an increase in the death rate (Batstone *et al*. 2002). The temperature dependence of different groups of bacteria and the effect on the disintegration and hydrolysis can be described with the Arrhenius equation ((Equation (36)) (Braun 1982; Speece 1996)) (Batstone *et al*. 2002). The effect of temperature on the thermodynamic of reactions can be delineated with the Van't Hoff equation (Equation (37) (Speece 1996; Vavilin *et al*. 1997)) (Batstone *et al*. 2002).
The higher the temperature the lower the change in the speed of the reaction is, whereas in low temperature ranges small changes in temperature can already double the speed of reaction (Braun 1982).

$$k_2 = k_1 * e^{\frac{E_A}{R}\left(\frac{T_2 - T_1}{T_2 - T_1}\right)} \quad \textbf{(36)}$$

where:
k_i velocity constants [d^{-1}]
R gas law constant [$J.mol^{-1}.K^{-1}$]
E_A activation energy [$J.mol^{-1}$]
T_i temperature [K]

[7] Blake D.R., Rowland F.S., 1988, Continuing worldwide increase in tropospheric methane, 1978 to 1987, Science, 239

[8] Cicerone, R.J., Oremland, R.S., 1988, Biochemical aspects of atmospheric methane, Global Biochem. Cycles 2, 299-327

$$K_2 = K_1 * e^{\frac{\Delta H^0}{R}(T_2 - T_1)} \quad \textbf{(37)}$$

where:

K_i equilibrium coefficient [M]
T_i temperature [K]
R gas law constant [J.mol^{-1}.K^{-1}]
ΔH^0 heat of reaction at standard temperature and pressure [J.mol^{-1}]

It is common approach, that micro-organisms have an optimum temperature range, whereby one can distinguish between psychrophilic (< 20 °C), mesophilic (20 - 40 °C) and thermophilic (> 40 °C) (Bischofsberger *et al*. 2005).

However, Lindorfer (2007) showed that there is no general temperature optima for anaerobic digestion – the specific optima depend on different parameters specific to the particular plant, such as reactor design, composition of substrate, hygiene aspects, and viscosity of the sludge. In Austria the plants are normally operated between 35 – 55 °C, whereby it is not possible to make a definite classification depending on the different temperature ranges (Laaber 2007). Digestion in the psychrophilic temperature range is not often used in Europe due to slow degradation and the high amount of space required (Bischofsberger *et al*. 2005).

Normally the temperature chosen is a compromise between the optima of the bacteria consortium, as high growth rates and the energy balances of the reactor system (Braun 1982).

In the thermophilic temperature range, higher degradation grades and a better hygenisation can be reached (Bischofsberger *et al*. 2005), and the viscosity of the sludge is lower (Laaber 2007). The disadvantage of the thermophilic operation is the increased instability due to the fact that the system responds faster to perturbations as a result of the higher reaction rates, the increase of free ammonia and the decrease in solubility of carbon dioxide, which leads to worse carbonate-buffer systems (Laaber 2007) and energy balance.

pH

The stable operation of a biogas plant is strongly related to the optimal pH range, as the enzyme activity of the bacteria very much depends on the pH (Braun 1982; Bischofsberger *et al*. 2005).

Normally the optimum for the overall AD process is a pH of 6.8 to 7.2 (Gerardi 2003). The methanogenic bacteria have stricter pH requirements than acidogenic bacteria (Frostell 1985). Methane production normally stops if the pH is lower than 6 (Gerardi 2003). Speece (Speece 1996), however, states a broader pH range, from 6.5 to 8.2.

While, with industrial plants using energy crops as substrate, generally a pH in the range of 7.5 – 8 can be observed, plants using refuses as input substrate normally operate at a higher pH range (Laaber 2007).

A pH value outside the optimum value results in a normally reversible inhibition, which affects the overall cell kinetics and functions (especially growth) (Batstone *et al*. 2002). In the ADM1 two empirical pH inhibition functions are used (not both in one model): one if both high and low pH inhibition occurs (Equation (38)) and another one if only low pH inhibition occurs (Equation (39)) (Batstone *et al*. 2002):

$$I = \frac{1 + 2 * 10^{0.5(pH_{LL} - pH_{UL})}}{1 + 10^{(pH - pH_{UL})} + 10^{(pH_{LL} - pH)}} \quad \textbf{(38)}$$

$$I = \begin{cases} e^{\left(-3\left(\frac{pH - pH_{UL}}{pH_{UL} - pH_{LL}}\right)^2\right)} \Big|_{pH < pH_{LL}} \\ I = 1 \Big|_{pH > pH_{UL}} \end{cases} \quad \textbf{(39)}$$

where:

I	Inhibition factor
pH_{LL}	lower limit of pH Inhibition
pH_{UL}	upper limit of pH Inhibition

Inhibition by pH is always a combination of different effects: at low pH values the inhibition occurs due to the disruption of the homeostasis and increased acid concentration. At high pH values transport limitations result in an inhibition of the process (Batstone *et al*. 2002). In the ADM1 pH inhibition is included for all intracellular processes (acetogenesis, acidogenesis and methanogenesis) (Batstone *et al*. 2002). Inhibition of hydrolysis by pH was not included in the model (Batstone *et al*. 2002).

Alkalinity

Alkalinity prevents rapid changes in the pH, as it serves as buffer (Braun 1982; Gerardi 2003). In other words the alkalinity is the capacity to neutralise acids without a significant pH change (Olsson and Newell 1999). In a systems with a low buffer capacity the organic acids formed have a bigger impact on the pH (Bischofsberger *et al*. 2005). In order to maintain a stable pH, high alkalinity levels are necessary (Gerardi 2003). The composition of the influent substrate directly influences the alkalinity in the reactor, e.g. proteinaceous rich feed increases the alkalinity due to release of ammonia (Gerardi 2003).

An alkalinity below the normal operating level is an indicator for operational failure (Gerardi 2003)

In first place, alkalinity is present in form of bicarbonates, which are in equilibrium with carbon dioxide (Braun 1982; Gerardi 2003) (Equation (40)):

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-} \quad \textbf{(40)}$$

If the buffer capacity is too low, then “neutralisation” substances have to be added, such as potash, sodium hydroxide or lime (Bischofsberger *et al*. 2005).

Oxidation-reduction Potential (ORP)

The ORP is a measurement of the relative amount of oxidised oxygen (NO_3^-, SO_4^{2-}, NH_4^+,…) (Gerardi 2003).
At an ORP of between +50 and –50 mV denitrification occurs (anoxic conditions) (Gerardi 2003). If the ORP decreases to lower than –50 mV sulphate reduction begins and for ORP values of lower than –100 mV mixed-acid fermentation occurs (Gerardi 2003). If the ORP drops to values of lower than –300 mV anaerobic degradation and methane production takes place (Braun 1982; Gerardi 2003).
Due to the absorption of oxygen, the formation of metabolites (Acetate, Hydrogen,…) and the activity of co-factors (NADH, $FADH_2$,…) a change in the ORP occurs (Braun 1982).
The ORP in the reactor can be reduced by adding reduced substances such as cysteine, thioglkolate dithionate or ascorbate (Braun 1982).

Substrate

The composition of the substrate has a big influence on the environmental conditions in the reactor (Bischofsberger *et al*. 2005).
The growth rate and metabolism of all bacteria depend on the available nutrients, trace elements and vitamins (Kim *et al*. 2002; Bischofsberger *et al*. 2005).
Due to the low growth rates of the bacteria the nutrient requirement is low (Gerardi 2003; Bischofsberger *et al*. 2005) especially compared to aerobic bacteria (Gerardi 2003).
Essential nutrients are nitrogen, phosphorus and sulphur (Gerardi 2003; Bischofsberger *et al*. 2005). A limitation of essential nutrients leads to a decrease or complete stop of metabolic activities (Bischofsberger *et al*. 2005). Therefore the composition of the substrate is essential to attain high degradation rates (Bischofsberger *et al*. 2005).
The dry matter of bacteria consists on average of 50 % carbon, 11 % nitrogen, 2 % phosphorus and 1 % sulphur (Bischofsberger *et al*. 2005). Thus the COD:N:P:S – ratio should be 800:5:1:1 (Bischofsberger *et al*. 2005). Other sources (Speece 1996; Gerardi 2003) give a COD:N:P ratio of 1000:7:1 for high-strength loads and 350:7:1 for low loadings. This ratio also indicates that in anaerobic digestion only a small amount of nitrogen and phosphorus can be eliminated (Bischofsberger *et al*. 2005).
Essential trace elements (Table 1.1.10) are nickel, cobalt, molybdenum, iron, selenium, wolfram, zinc, copper and manganese (Braun 1982; Gerardi 2003; Bischofsberger *et al*. 2005). An absence of trace elements leads to a limitation of growth, but too high concentration, for example of heavy metals, can also result in an inhibition (Bischofsberger *et al*. 2005).

Table 1.1.10: Requirements of trace elements based on the COD degradation (Bischofsberger *et al*. 2005)

Trace element	Demand [$mg.kg_{COD\text{-}Degradated}^{-1}$]
Fe	100 – 2000
Ni	5 – 300
Co	5 – 200
Mo	1 – 4
Se	2 – 4
Wo	2 – 8

In the ADM1 a secondary substrate inhibition function is included in all cases of substrate uptake (Batstone *et al*. 2002) (Equation (41)):

$$I = \frac{1}{1 + K_i / S_i} \quad \textbf{(41)}$$

where:
I Inhibition factor
S_i concentration of inhibitor i [$kg_{COD}.m^{-3}$]
K_i inhibtion parameter [$kg_{COD}.m^{-3}$]

In the model a competitive substrate uptake function is included for butyrate and valerate using bacteria. As in the model these substrates are used by the same bacteria group (Batstone *et al*. 2002) (Equation (42)):

$$I = \frac{1}{1 + S_i / S} \quad \textbf{(42)}$$

where:
I Inhibition factor
S_i concentration of inhibitor i [$kg_{COD}.m^{-3}$]
S substrate for process [$kg_{COD}.m^{-3}$]

The organic loading rate (OLR) is used as a parameter for the amount of feed. This is defined as the amount of organic dry matter or chemical oxygen demand per day and reactor volume. The disadvantage is that the OLR gives no information about the biodegradability of the substrate used (Laaber 2007).

During start up of the reactor the organic load has to be increased by increments, starting with about one tenth of the normal loading rate (Braun 1982; Bischofsberger *et al*. 2005).

An increase of the OLR to increase the productivity is always limited on the one hand by mechanical problems (pumpability of the substrate, mixing of the reactors) and on the other hand by biological problems such as high levels of fatty acids, ammonia and H_2S (Braun 1982).

Toxicity

Inhibition means a reversible change in the kinetic parameters of the bacteria and toxicity results in an increased decay of the bacteria (Bischofsberger *et al*. 2005).
Inhibition can be competitive or non-competitive. In the case of a non-competitive inhibition of the bacteria the maximal possible growth rate decreases and therefore also the capacity for the substrate uptake (Bischofsberger *et al*. 2005). An important fact, however, is that micro-organisms can adapt to toxic substances (Henze and Harremoes 1983; Speece 1996).
Indicators for toxicity (disappearance of hydrogen and methane, decrease in alkalinity and pH, and increase in VFAs) can occur either suddenly or gradually depending on the type of toxicity and concentration (Gerardi 2003).
Competitive inhibition is mostly caused by substances that are mistaken as a substrate by the bacteria, but which cannot be degraded (Bischofsberger *et al*. 2005).
Characteristic for all types of inhibition is that the decay rate does not decrease (Bischofsberger *et al*. 2005), which can lead, if the inhibition is not stopped, to an extinction of the bacteria population and to a complete break down of the process.
The most frequent cause for operational problems is the substrate inhibition of aceticlastic methanogenic bacteria (Bischofsberger *et al*. 2005).

To reduce the complexity of the model only a few inhibition mechanisms are implemented in the model (Blumensaat and Keller 2005), apart from the above described inhibition function for hydrogen inhibition, pH inhibition, substrate competition for C_4 and the secondary substrate inhibition function, only one non-competitive inhibition function for free ammonia is included in the ADM1.

Ammonium and Ammonia

High protein concentrations can lead to high ammonia concentrations and therefore cause process inhibition and failure (Flotats *et al*. 2006), as ammonia is released during the degradation of amino acids (Gerardi 2003).
The free non-dissociated ammonia is the most toxic compound, and inhibits from 100 – 200 $g_N.l^{-1}$ (Henze and Harremoes 1983). Total ammonia and ammonium concentrations can be tolerated up to 5000 to 8000 $mg_N.l^{-1}$ if the reactor pH is low enough (Henze and Harremoes 1983). The toxicity not only depends on the total concentration, but also on the rate of formation and thus on the adaptation time (Braun 1982).
Ammonia and ammonium are in equilibrium and therefore their relative concentrations depends on the pH and temperature (Braun 1982; Gerardi 2003). The main part however is found as ammonium in the reactor (Braun 1982). An increase in the ammonium also leads to an increase in the pH (Gerardi 2003). The augmented pH results in heightened

ammonia concentration, which results in an inhibition of the methanogenic bacteria and results in a rise in the concentration of organic acids in the reactor, which in turn drops the pH and thus decreases the ammonium concentration (Angelidaki *et al*. 1993; Gerardi 2003; Bischofsberger *et al*. 2005). In this manner the ammonium can stabilise the pH in the reactor (Angelidaki *et al*. 1993; Bischofsberger *et al*. 2005). So inhibition due to ammonia does not initiate a self-increasing process, but results in a lower COD reduction and smell nuisances (Bischofsberger *et al*. 2005). A further problem caused by ammonia is the production of foam and scum (Gerardi 2003).

In the ADM1 the inhibition by free ammonia is modelled with a non-competive inhibition function similarly to the hydrogen inhibition (see Equation (18)) (Batstone *et al*. 2002). The relationship between the ammonium, ammonia and pH is included in the acid-base rates (Batstone *et al*. 2002; Rosen and Jeppsson 2002) (Equation (43)):

$$\rho_{A,11} = k_{A,B_{vIN}} \left(S_{nh3} \left(K_{a,IN} + S_{H^+} \right) - K_{a,IN} S_{IN} \right) \quad \textbf{(43)}$$

where:

$\rho_{A,i}$ acid-base rate [$kg_{COD}.m^{-3}.d^{-1}$]
$k_{A,Bi}$ acid base kinetic parameter [$M^{-1}.d^{-1}$]
$K_{a,Ii}$ acid-base equilibrium constant [M]
S_i concentration of soluble i [$kg_{COD}.m^{-3}$]

Biochemically bound oxygen such as in nitrate or nitrite can cause inhibitions in the AD process (Bischofsberger *et al*. 2005). The denitrification process ((Equation (44)) (Braun 1982)) can have a significant impact on the whole AD process: it can channel electron equivalents away from methanogenesis and results in the decrease of methane production and an increased CO_2 content in the biogas (Braun 1982), competition with other microbial bacteria and an inhibition of methanogenesis by nitrogen oxides (Batstone *et al*. 2002), due to an increased redox potential and therefore the inhibition of the methanogens (Bischofsberger *et al*. 2005). This process was not included in the ADM1, as it is too complex (Batstone *et al*. 2002).

$$NO_3^- + H^+ + 4H_2 \rightarrow NH_4^+ + OH^- + 2H_2O \quad \textbf{(44)}$$

Sulphate

Sulphate reducing bacteria (SRB) compete with the methanogenic bacteria for the same substrate, as the SRB need the hydrogen for the conversion of sulphate (Braun 1982; Gerardi 2003; Bischofsberger *et al*. 2005) (Equation (45) to (46)):

$$SO_4^{2-} + 4H_2 \leftrightarrow H_2S + 2H_2O + 2OH^- \quad \textbf{(45)}$$

$$SO_4^{2-} + CH_3COOH \leftrightarrow H_2S + 2HCO_3^- \quad \textbf{(46)}$$

Whereby the SRB are energetically advantageous compared to the methanogens (Bischofsberger *et al*. 2005).

Moreover the H_2S in the sludge causes an inhibition of the metabolic activity of the bacteria (Gerardi 2003). Inhibition caused by H_2S increases at low pH (<6.5) (Henze and Harremoes 1983). This is a very dangerous factor: As an inhibition caused by sulphide can result in a self-augmenting process which finally ends in the total breakdown of the biogas production (Bischofsberger *et al*. 2005). An inhibition of the methanogenic bacteria leads to a rise of organic acids, subsequently a drop in the pH and therefore a further increase in the H_2S in the reactor (Bischofsberger *et al*. 2005).
One has to count with the first inhibition effects at a H_2S concentration of 30 $mg.l^{-1}$. A H_2S content of more than 10 % in the biogas the acetate production is disrupted (Bischofsberger *et al*. 2005).
An elimination of the H_2S is not only important for successful operation of the AD process, but also necessary to prevent corrosion problems in the plant and minimize SO_2 emissions during combustion of the biogas (Bischofsberger *et al*. 2005).
Inhibition by H_2S can be solved by adding iron salts or iron dusts to initiate precipitation of the sulphide or an increase of the pH (Henze and Harremoes 1983; Gerardi 2003; Bischofsberger *et al*. 2005). Oxygen is also used in industrial plants to remove H_2S from the biogas, where the sulphur precipitates as elemental sulphur according to the reactions (Bischofsberger *et al*. 2005) (Equation (47)):

$$2H_2S + O_2 \leftrightarrow 2H_2O + S_2 \quad \textbf{(47)}$$

Additionally an increase in the temperature (thermophilic temperature range) leads to a reduction of the H_2S inhibition as the solubility of H_2S decreases with higher temperatures (Bischofsberger *et al*. 2005). A further possibility is an external H_2S scrubber and circulation of the biogas (Bischofsberger *et al*. 2005).
But the sulphate reduction has positive effects, such as an increase of the pH in the reactor, due to formation of OH^- ions and as well as the decrease in the hydrogen partial pressure (Bischofsberger *et al*. 2005). Moreover the sulphide ions serve as an important precipitant for heavy metals (Braun 1982; Bischofsberger *et al*. 2005) and as nutrient supplement for the bacteria.

Due to its complexity the sulphate reduction process is excluded from the ADM1, but this makes the model incapable of modelling systems with a certain amount of sulphide (Batstone *et al*. 2002).

Organic acids

The concentration of organic acids in the reactor should be rather low (< 200 $mg.l^{-1}$) for a stable process (Henze and Harremoes 1983; Bischofsberger *et al*. 2005). If the production of acids is higher than the degradation performance the methanogens are inhibited (Bischofsberger *et al*. 2005). In general acetic acid is less toxic than propionic acid (Henze and Harremoes 1983). During an inhibition of aceticlastic methanogens an

increase of the propionic acid can always be observed (Angelidaki *et al*. 1993; Gerardi 2003; Bischofsberger *et al*. 2005). Increased values of propionic acid may therefore indicate difficulties in any of the metabolic steps of anaerobic treatment (Speece 1996). High levels of organic acids have less effects if the reactor is adapted to them (Braun 1982).

Elevated volatile fatty acids (VFA) concentrations in the reactor can, for example, be caused by trace metal limitation, toxicity, kinetic overload, mass transfer limitation, hydraulic short circuiting, nitrogen and phosphorus limitation and thermodynamic impairment of propionate conversion due to evaluated H_2 concentrations (Speece 1996).

A high amount of organic acids also cause a decrease in the pH, which leads to an augmented inhibition of the methanogenic bacteria (Rosen and Jeppsson 2002; Gerardi 2003; Bischofsberger *et al*. 2005). At lower pH values the acids can more easily diffuse into the bacteria cell and lead to shift in the pH in the cell (Braun 1982). An increase of the VFA and decrease of the pH is a major limiting factor (Vavilin *et al*. 2004).

As inhibition by weak acids and bases is strongly pH dependant, the following acid-base rates are included in the ADM1 (Batstone *et al*. 2002; Rosen and Jeppsson 2002) (Equation ((48) to (52))):

$$\rho_{A,4} = k_{A,B_{va}}\left(S_{va^-}\left(K_{a,va} + S_{H^+}\right) - K_{a,va}S_{va}\right) \quad \textbf{(48)}$$

$$\rho_{A,5} = k_{A,B_{bu}}\left(S_{bu^-}\left(K_{a,bu} + S_{H^+}\right) - K_{a,bu}S_{bu}\right) \quad \textbf{(49)}$$

$$\rho_{A,6} = k_{A,B_{pro}}\left(S_{pro^-}\left(K_{a,pro} + S_{H^+}\right) - K_{a,pro}S_{pro}\right) \quad \textbf{(50)}$$

$$\rho_{A,7} = k_{A,B_{ac}}\left(S_{ac^-}\left(K_{a,ac} + S_{H^+}\right) - K_{a,ac}S_{ac}\right) \quad \textbf{(51)}$$

$$\rho_{A,10} = k_{A,Bco2}\left(S_{hco3^-}\left(K_{a,co2} + S_{H^+}\right) - K_{a,co2}S_{IC}\right) \quad \textbf{(52)}$$

where:

$\rho_{A,i}$ acid-base rate [$kg_{COD}.m^{-3}.d^{-1}$]
$k_{A,Bi}$ acid base kinetic parameter [$M^{-1}.d^{-1}$]
$K_{a,Ii}$ acid-base equilibrium constant [M]
S_i concentration of soluble i [$kg_{COD}.m^{-3}$]

Long chain fatty acids (LCFA) are a product of the lipids in the substrate and can already be inhibitory at low concentrations (Angelidaki and Ahring 1992; Batstone *et al*. 2002). Heavy inhibitions by LCFAs are mostly irreversible, but the system can adapt to high LCFA concentrations (Batstone *et al*. 2002). The inhibition effect of the LCFAs is their adsorption on the cell wall (Batstone *et al*. 2002). Whereby it is mostly aceticlastic methanogens that are affected (Batstone *et al*. 2002).

The effect of organic acid inhibition is largely included in the empirical pH function (Batstone *et al*. 2002). Due to its potential complexity the inhibition of LCFAs is not included in the ADM1 (Rosen and Jeppsson 2002).

Other inhibitory substances were not included in the ADM1, either:

Oxygen

Anaerobes can be divided into two groups: facultative (oxygen-tolerant) and strictly anaerobic (oxygen-intolerant) species (Braun 1982; Gerardi 2003). Facultative anaerobic bacteria, for example most of the acidogenic bacteria, grow in presence of oxygen (Gerardi 2003; Bischofsberger *et al*. 2005). Whereas for strictly anaerobic bacteria a small amount of oxygen already means an inhibition of the metabolism (Braun 1982; Bischofsberger *et al*. 2005).
Depending on the ORP buffer capacity of the mixed population the bacteria consortium tolerates variable amounts of oxygen (Braun 1982).

Heavy metals

Heavy metals are not strictly toxic. On the contrary, a lot of heavy metals are essential for the bacteria in low concentrations, such as copper, nickel, chrome, cadmium or zinc. It is only at higher concentrations (Table 1.1.11) that all heavy metals lead to an inhibition and toxicity (Bischofsberger *et al*. 2005).
Only soluble heavy metals can inhibit the process (Braun 1982). Using customary substrates no inhibition by heavy metals can be expected, as the sulphate content of the substrate itself is high enough to remove all heavy metals (Braun 1982).

Table 1.1.11: Inhibitory concentration of different harmful substances (Bischofsberger *et al*. 2005)[9]

Hevy metal	Inhibtion [mg.l^{-1}]	Toxicity [mg.l^{-1}]	Inhibtion [mg.l^{-1}]	Toxicity [mg.l^{-1}]	Inhibtion [mg.l^{-1}]	Toxicity [mg.l^{-1}]
Cu	150-250	300	40-250	170-300	40-250	170-300
Cd	-	-	150-600	-	-	20-600
Zn	150	250	250-400	250-600	150-400	250-600
Ni	100-300	500	10-300	130-500	10-300	30-1000
Pb	-	-	340	340	300-340	340
Cr III	100-300	500	120-300	260-500	120-300	200-500
Cr VI	100	200	100-110	200-220	100-110	200-420
Reference	Köhler, 1996		Scherber u. Steiner, 1982		Konzeli-Katsiri u. Kartsona, 1986	

[9] Köhler, H., 1996, Schadenswirkungen auf den Schlammfaulprozess durch stagnierend und toxisch wirkende Stoffe, Wasser-Luft und Betrieb, 6, 388-395
Konzeli-Katsiri, A., Kartsonas, N., 1986, Inhibtion of Anaerobic Digestion by Heavy Metals, Anaerobic Digestion of Sewage Sludge and Organic Agriculture Wastes, Edited by: Bruce, A.M., Konzeli-Katsiri, A., Newman, P.J., Elsivier Applied Science Publishers, London, New York, 104-119
Scherber, K., Steiner, A., 1982, Zur Toxizität von Schwermetallen bei der biologischen Abwasserreinigung, Münchner Beiträge zur Abwasser-, Fischerei- und Flussbiologie, Bd 34, 191-207

Other Substances

Other substances, for example halogenated hydrocarbons, cyanide, antibiotics, disinfectant or biocides, can cause inhibition or toxic effects (Braun 1982; Bischofsberger *et al*. 2005).

1.2 Types of Digesters and Operation

A lot of different process types are used for agricultures biogas plants – one can distinguish between dry and wet processes (Laaber 2007) and further between discontinuous and continuous processes (Bischofsberger *et al*. 2005). There are no typical plant types for a specific range of applications, each plant is planned individually (Laaber 2007).

1.2.1 Continuous Stirred Tank Reactor (CSTR)

Most plants are continuous stirred tank reactors (Bischofsberger *et al*. 2005; Laaber 2007). These cylindrical reactors have lower acquisition costs than other reactor forms (Bischofsberger *et al*. 2005).

CSTRs can be operated both in single phase or two phase operation. In single phase operation the acid formation and methane production takes place in the same reactor (Speece 1996). However, one possibility to increase the efficiency of the process is to separate the AD process into two phases, one acid forming phase and one methane forming phase (Speece 1996), where optimal conditions for the bacteria groups are provided (Blumensaat and Keller 2005). Optimal pH range are from 4 – 6.5 for the acid formation step and 6.5 to 8.2 for the methane formation (Speece 1996). The disadvantages of such a separation are higher investment costs (Blumensaat and Keller 2005).

The implementation of the ADM1 in the CSTR is shown in the Scientific and Technical report No. 13 (Batstone *et al*. 2002). For each state component the following mass balance is stated (Batstone *et al*. 2002) (Equation (53)). The derivation of the dynamic process model is based on the conservation of mass and energy (Olsson and Newell 1999).

$$\frac{dS_{liq,i}}{dt} = \frac{qS_{in,i}}{V_{liq}} - \frac{qS_{liq,i}}{V_{liq}} + \sum_{j=1-19} \rho_j \nu_{i,j} \qquad \textbf{(53)}$$

where:

$S_{liq,i}$ concentration of soluble i [$k_{COD}.m^{-3}$]
$S_{in,i}$ input concentration of soluble i [$k_{COD}.m^{-3}$]
$\nu_{l,j}$ rate coefficients for component i on process j [$kg_{COD}.m^{-3}$]
ρ_j process rate j [$kg_{COD}.m^{-3}.d^{-1}$]
t time [d]
V_{liq} liquid Volume [m^3]

For the solids in the reactor the subsequent mass balance is given by (Batstone *et al*. 2002) (Equation (54)):

$$\frac{dX_{liq,i}}{dt} = \frac{qX_{in,i}}{V_{liq}} - \frac{qX_{liq,i}}{V_{liq}} + \sum_{j=1-19} \rho_j \nu_{i,j} \quad \textbf{(54)}$$

where:
$X_{liq,i}$ concentration particulate i in the reactor [$k_{COD}.m^{-3}$]
$X_{in,I}$ input concentration of the particulate i [$k_{COD}.m^{-3}$]
$\nu_{I,j}$ rate coefficients for component i on process j [$kg_{COD}.m^{-3}$]
ρ_j process rate j [$kg_{COD}.m^{-3}.d^{-1}$]
t time [d]
V_{liq} liquid Volume [m^3]

For the gas phase the mass balance can be written similarly to the liquid phase (Batstone *et al*. 2002) (Equation (55)):

$$\frac{dS_{gas,i}}{dt} = -\frac{q_{gas}S_{gas,i}}{V_{gas}} + \rho_{T,i}\frac{V_{liq}}{V_{gas}} \quad \textbf{(55)}$$

where:
$S_{gas,i}$ gaseous concentration of i [$k_{COD}.m^{-3}$]
q_{gas} flow rate gas [$m^3.d^{-1}$]
V_{gas} gaseous Volume [m^3]
V_{liq} Volume of liquid phase [m^3]
$\rho_{T,I}$ gas transfer rate [$kg_{COD}.m^{-3}.d^{-1}$]
t time [d]

1.2.2 Plug Flow Reactor (PF)

These are horizontal reactors where substrate flows through (Braun 1982); the diameter/length ratio is 1:5 to 1:10. They are mostly equipped with a lengthwise paddle agitator (Bischofsberger *et al*. 2005). The substrate flows through the reactor as a plug, which is ideally not mixed (Braun 1982).

The advantages of this reactor type are that normally high degradation rates and a better hygenisation can be attained (Bischofsberger *et al*. 2005). Furthermore these reactor can accommodate toxic sludges more efficiently than CSTRs (Speece 1996).

The disadvantages are the limited reactor size (max 500 m^3), otherwise complex and expensive agitators are needed, the high amount of space required and the awkward surface to volume ratio (Bischofsberger *et al*. 2005). Less than 20 % of new plants are erected in this form (Bischofsberger *et al*. 2005)

1.2.3 Other reactor types

Special reactor types are employed in the anaerobic treatment of waste water treatment, as here the retention of the biomass has a big influence on the efficiency (Bischofsberger *et al*. 2005). Different retention techniques exist, such as external retention by gravity (settling tanks or flotation), integrated retention (UASB), biofilm reactors (packed bed, fluidised bed) or retention by membranes (micro filtration, ultra filtration) (Bischofsberger *et al*. 2005).

1.3 Monitoring and Control

Monitoring and control can prevent a minor operational problem becoming a major catastrophy and leading to a complete breakdown of the AD process (Speece 1996).
Process control of AD is mostly difficult due to the relationship between different parameters which influence the process (Gerardi 2003). This is true for most biochemical processes and is a result of the sensitivity of the bacteria and the impossibility of influencing the internal environment of the cells (Yamuna Rani and Ramachandra Rao 1999). The main difficulty controlling fermenters are their non-linear behaviour, the scarcity of accurate process models, unpredictable process parameters, as well as hardly available reliable biosensors (Yamuna Rani and Ramachandra Rao 1999).
Optimising control deals with the problem of changing the operating conditions in such a manner that the AD process is brought to its optimum (Yamuna Rani and Ramachandra Rao 1999). Advanced monitoring and control system can lead to an increased methane content (MC), augmented biogas yields, higher loading rates, smaller reactor volumes and prevent reactor overload.
Any control system of any process consists of four components – the process, the measurement, the decision-making and the implementation, whereby the key component is the process itself (Olsson and Newell 1999).

1.3.1 Monitoring Parameters

Typical parameters for monitoring full scale plants are alkalinity, gas production (GP), biogas composition, dissolved hydrogen, VFAs, pH and volatile suspended solids (VSS). Furthermore the Chemical Oxygen Demand (COD) and especially the COD reduction, the alkalinity or buffering capacity, ORP, CO and the hydrogen content.
Biogas and methane production are not really accurate indicators as changes in the methane content can not only be connected to reactor failure but also be due to feed changes (Gerardi 2003). However a combination of methane production and alkalinity as indicators has much more significance (Gerardi 2003).
A further parameter, which can be used for monitoring, is the volatile acid-to-alkalinity ratio; here a value of 0.1 to 0.2 is acceptable, whereas a ratio >0.5 indicates a reactor failure (Gerardi 2003). Another possible control parameter is the ratio of propionate to acetate – this parameter indicates a failure caused by organic overload (Speece 1996).
While the pH can only serve as an indicator for what already has happened in the reactor, like methane content in the biogas, augmented VFA concentrations or the ratio of VFA to alkalinity (Speece 1996); alkalinity indicates what is happening at the moment (Gerardi 2003). The potential for using hydrogen as an indicator of digester viability has been shown for example by Hager (Hager 2001), but the feasibility of H_2 as control parameter for complex substrates is unclear (Sterling Jr. *et al*. 2001). However, using H_2

as control parameter has some limitations. H_2 levels in steady-state operation depend on the type of reactor and the energetic content of the substrate (Speece 1996).
Due to metabolic activity the ORP alters all the time, but only in a specific area and can therefore be used for monitoring the process (Braun 1982). Coupled with other parameters ORP is a useful parameter (Speece 1996).

1.3.2 Control strategies

A controller has to be designed in such a manner that the complete system (controller itself and the system controlled) is stable (Kremling and Saez-Rodriguez 2007). Features that lead to industrial acceptance of a controller are primarily the simplicity of its design and the corresponding robustness of control ((Benson and Perkins, 1997)[10] cited in (Mwembeshi *et al*. 2004)). One control philosophy is, that if a system can modelled, then it can also be controlled (Mwembeshi *et al*. 2004).

PID

The Partial-Integral-Derivate (PID) is the most common algorithm in use (Olsson and Newell 1999). In over 90 % of industrial process control systems a PID-type controller is used ((Benson and Perkins, 1997)[10] cited in (Mwembeshi *et al*. 2004))
A PID controller is a three part control mechanism, which combines proportional, integral, and derivative control actions (Olsson and Newell 1999).
This kind of control should be used if a low amount of data is available, no model is valid and little knowledge exists about the plant (Steyer *et al*. 2005). Applications of a PID controller are normally limited to single input, single output strategies and to linear cases (Steyer *et al*. 2005).
Thus, this kind of controllers is not adequate for AD control – as the process itself is too complex. Moreover PID controllers show a bad performance for the AD process, but this control strategy has the advantage that it is not very complex (Liu 2005).

Mechanistic Models

Improved control is mostly based on modelling and simulation (Olsson and Newell 1999). A model based predictive control uses different kinds of models to forecast the future development of the process (Pannocchia 2003), considering threshold values and stability of the process. A key feature of the model used is its ability to predict the behaviour of the plant (Olsson and Newell 1999). Therefore a model based control consists of two parts: prediction and control (Olsson and Newell 1999).

[10] Benson, R.Perkins, J., 1997, The Future of Process Control – A UK perpective. In Proceedings of the 5th international conference on chemical process control (vol. 93, p. 192). AIChE symposium series.

The complexity of a model used for control purposes is determined by two factors – the impact of control actions and its verifiability (Olsson and Newell 1999). A further factor is the number of parameters needed for the model.
The advantage of model based controller is that compared to a model free controller simpler sensors can be used, but the disadvantage is that knowledge of the input concentrations and advanced mathematical calculations are required (Steyer *et al*. 2005).
For use in a control system the ADM1 is far too complex, as it is rarely fully calibrated and validated (Steyer *et al*. 2005). Furthermore the mathematical behaviour can be very complex (Steyer *et al*. 2005). Thus for control purposes a more simplified model should be used (Steyer *et al*. 2005).

Neuronal Networks

An alternative to deterministic models are black box models, for example neuronal networks (NN) or expert systems, such as fuzzy logic and hybrid systems - Neuro-fuzzy combinations.
NNs consist of an assembly of simple processing elements (neurones) combined in a network by a set of weights (Nauck *et al*. 1994). NNs are inspired by biological nervous systems (Zani 2001) and can be seen as a simplified view of the structure of the brain (Olsson and Newell 1999). NNs are especially applicable for modelling non-linear systems (Strik 2004). This kind of control strategy is defined by the structure of the network, the value of the weights and the mode of operation (Strik *et al*. 2004).
In the most common networks the neurones are arranged in layers, with an input layer, several hidden layers and an output layer (Zani 2001; Strik 2004). The input layer consists of one neuron for each model input and distributes the input value to all neurons in the next layer (Olsson and Newell 1999). In at least one hidden layer, the neurone takes input values, weights them, sums them up and adds a bias which finally results in the argument of an output function, the so-called transfer function (Olsson and Newell 1999; Zani 2001). The output layer also gives a weighted sum, but the transformation is normally much simpler than in the hidden layers (Olsson and Newell 1999). The input and output of the NN can be a vector or a scalar (Zani 2001).
If the number of layers is too low the ability for modelling the process is limited, conversely, too many layers would result in too much freedom for the weights to adjust (Linko *et al*. 1997). Training of the NN changes the weights of the network.
The advantages provided by the NNs are that is a self-learning system (through mathematical functions). They learn from examples and exhibit some capability for generalisation beyond the training data (Zani 2001). Further less know-how and expert knowledge are necessary compared to Fuzzy Logic based control tools (see beneath). However, a big disadvantage is that a lot of data is needed for training the models,

moreover complete data sets are required and for an adequate training the data should have an high range of margin.

Fuzzy Logic

A further possibility is a Fuzzy Logic (FL) based control. The use of this type of controller makes sense if there is no analytical model of the plant or process available, the model is too complicated to be used in a controller or the control goals are not precisely defined (Olsson and Newell 1999).

FL was originally developed by Lofti Zadeh (Sproule *et al*. 2002). FL uses "Fuzzy" numbers, not crisp numbers, in the form of membership functions, representing degrees of truthfulness at the interval of [0 1], whereby a membership value is not a probability, but rather a possibility (Olsson and Newell 1999). Fuzzy numbers are uncertain numbers, where some values can be defined as more possible then others (Bogardi 2004).

A fuzzy set is a class having members with a degree of membership – the distinction between membership or non-membership is gradual and not abrupt (Olsson and Newell 1999).

The control strategy itself is based on control rules, mostly in form of descriptive terms. Such rules have the form: "*IF <conditional clause> THEN <action clause>*" (Olsson and Newell 1999).

Normally, a FL based control consists of the fuzzyfication of an input, the application of the interference rules and the subsequent defuzzyfication of the output (Figure 1.3.1). Defuzzification is the translation of the membership function into a crisp control signal (Olsson and Newell 1999). There are different techniques for defuzzfification, one possibility is to use the "centre of area" (Olsson and Newell 1999).

This kind of control is also called an expert system (≠ a model!), as this control tool needs process-specific experience and knowledge. The characteristics of an expert system are that it works only in a specified area, it is transparent and also diffuse, as normally knowledge is only available in fragments ((Görz, 1993)[11] cited in (Nauck *et al*. 1994))

Compared to the model based control tools, however, for a reduced amount of data measured, only a short observation period (2-3 month) is necessary. Moreover, it is possible to minimise the parameters required and no information about substrate is necessary. Further there is no need for a model, no knowledge of the input calculation and only simple calculations are required (Steyer *et al*. 2005). A disadvantage is that this kind of control is difficult to evaluate as it uses descriptive terms (Zani 2001).

[11] Görz, G., Hrsg., 1993, Einführung in die künstliche Intelligenz, Addison-Wesley, Bonn

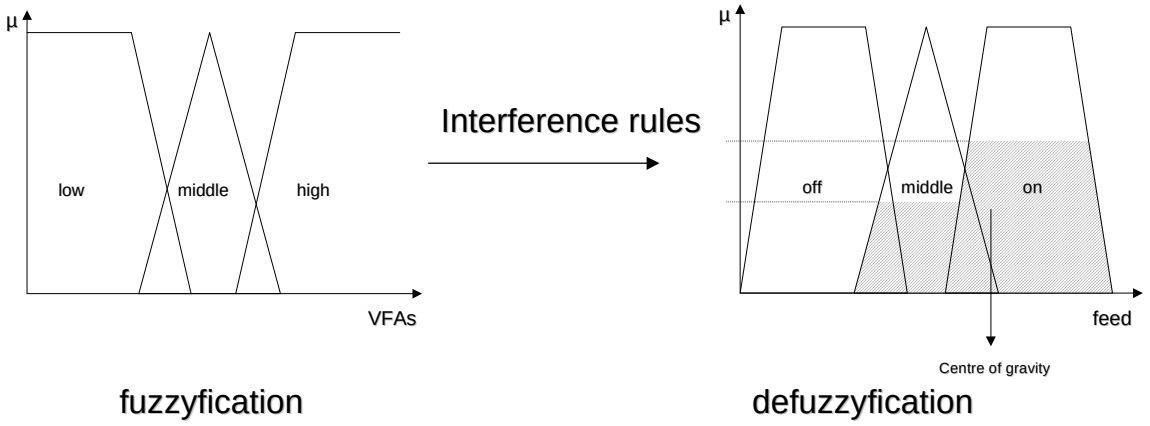

Figure 1.3.1: Principles of Fuzzy Logic

It has been reported that control tools without an implemented model, such as FL based tools, show very good results (Domnanovich *et al*. 2004).

"*The difference between a fuzzy controller and classical controller is not the resulting behaviour is achieved, but the way this behaviour us achieved*" (Olsson and Newell 1999).

2 Objectives

The biological conversion of crops and agro-wastes without oxygen (anaerobic digestion) can be used to produce a sustainable fuel, by converting the formed biogas via gas engines yields electrical energy and heat. However industrial biogas plants often have to face severe problems: Low methane values in the biogas (approximately 50%) and low overall biogas production, instable process conditions, reactor overload and process failure, low efficiency and high operational costs, self-heating in the plants and odour problems, which often results in a low economical viability. To make the biogas process more economically attractive the process has to be optimised.

The main goal of the so-called CROPGEN project (Renewable energy from crops and agro-wastes) is the integration of energy crops as an economically attractive energy source into the existing energy infrastructure. The integration as sustainable fuel source will be achieved by the optimisation of the biogas process. This optimisation achieved by new reactor designs, the determination of methane yields, the optimisation of storage and pre-treatment and identification of optimal processes using a Decision Support Systems (DSS).

The project started in March 2004 and ran until June 2007. Several project partners both from industry and universities worked on the project from all over Europe.

The study was accomplished within the framework of the CROPGEN project and encompasses the modelling of the AD process using energy crops as substrate and the development of a DSS for optimisation. The main task to prove was that the AD model used can predict the digestion of energy crops accurately enough.

The majority of the AD models was developed for AD processes using wastewater, sewage sludge or manure as substrate, but hardly any model developments or applications for AD processes using energy crops as substrate exist in literature.

Thus one objective of this study is the extension and adaptation of an existing AD model for the use of energy crops as substrate, here the ADM1 (Batstone *et al*. 2002) was used as a basis for further development.

A further goal was the development of a “Virtual Laboratory” (VL) for data processing and the simulation of the AD process. The adjusted model is foreseen to be implemented in this VL. The VL should primarily help to provide a better understanding of the AD process.

Moreover an existing Decision Support System based on Fuzzy Logic was further developed to assist in operational control for optimisation. The DSS is used to identify process-control strategies in order to yield an optimised biogas production and high methane content.

3 Material and Methods

3.1 Reactor Set-up

To gain data for model calibration and validation four 25 l (total volume) lab-scale anaerobic completely stirred tank reactors (CSTR) are operated. Figure 3.1.1 shows the schema of the CSTRs used in the laboratory experiments.

The reactors are constructed according ATV (Abwassertechnische Vereinigung, 1996)[12] and are completely made of glass (supplier: Prohaska) with a removable head and several connections for measurement devices, feed inlet, gas outlet and sludge outlet.

The digesters are equipped with online measurement devices for temperature, methane content and biogas production rate. The reactors are stirred with both a magnetic stirrer (IKA; Model Maxi MR digital) with a stirring speed of about 100 rpm and a peristaltic pump (Verder; Model CR-240; Pump head H5; Pumping tube Verderprene 6.4x2.4 mm, respectively Verder; Model CR70; Pump Head H5; Pumping tube Verderprene 6.4x2.4) to pump the sludge trough an external circulation loop. In this external loop a sampling point is suited. A height-adjustable U-tube is used to keep the liquid level constant and served as safety outlet in case of excess pressure as well.

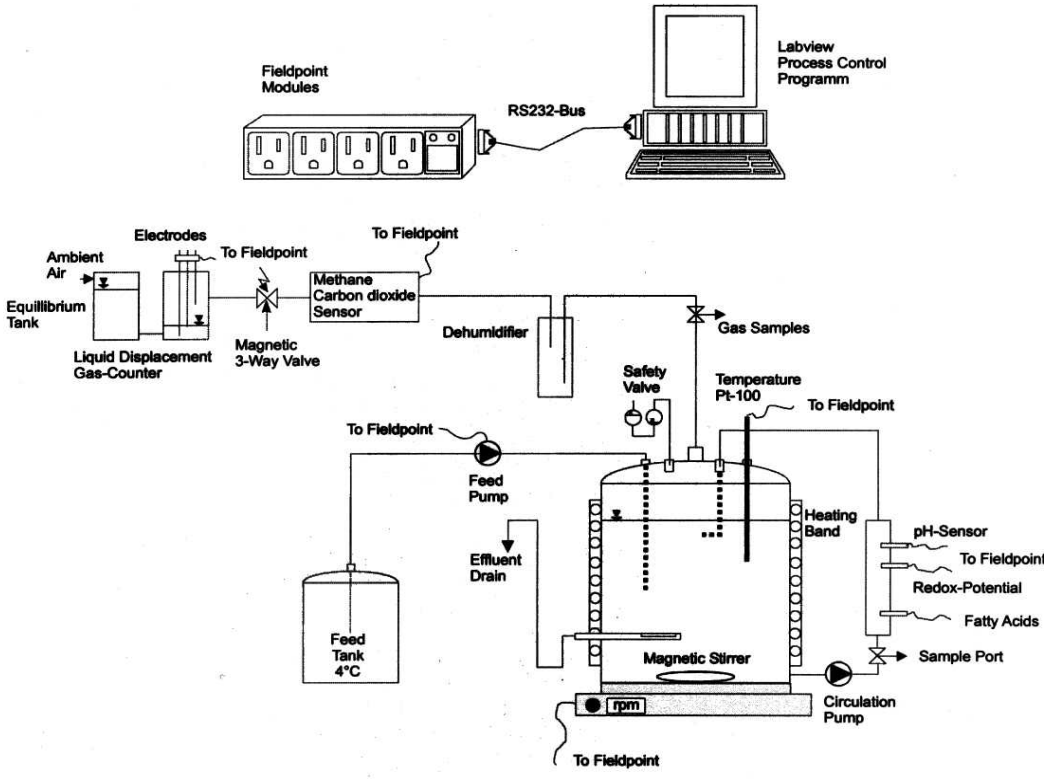

Figure 3.1.1: Schematic of the CSTRs used in the laboratory experiments (Hager 2001)

The operating temperature for two reactors is set at 35 °C (mesophilic) and for the other

[12] Abwassertechnische Vereinigung (ATV), 1996, Handbuch Klärschlamm, 4. Aufl., Ernst & Söhne Verlag

two reactors at 60°C (thermophilic). The temperature is maintained using a heating band, with a fluctuation of ±1 °C around the set-point and is controlled by the reactor control system.

The feed is stored at 4 °C in a refrigerator and is pumped 5 to 10 times a day in the reactor by a peristaltic pump (Verder; Model CR-240; Pump head H5; Pumping tube Verderprene 6.4x2.4 mm, respectively Verder; Model CR70; Pump Head H5; Pumping tube Verderprene 6.4x2.4) as well controlled by the reactor control system.

The biogas vented from an outlet in the bottom of the reactor and first reached a dehumidifier, a gravel-packed filter (particle size 10-15 mm), to remove water from condensation from the gas. Afterwards the hydrogen sulphide is scrubbed from the biogas by passing through a saturated $CuSO_4$ solution, where an heavy-soluble precipitate is formed, to prevent corrosion problems in the down-stream measuring devices. Subsequently the methane content of the biogas is determined by an infrared gas sensor. Finally the gas production rate is measured by a gas meter using a liquid displacement technique.

To prevent uncontrollable increase of the pressure in the reactor, due to clogging in the gas tube a U-tube filled with water is placed on the top of the reactor.

3.2 Control Program

To operate and control the lab-scale reactor system automatically and for on-line data acquisition a process software written in LABVIEW® 6.5 (National Instruments, Austria) in combination with so called “Field Points” was used. The Field Points allow an I/O system for monitoring and control to interface with the measurement probes, as well as operate the pumps and the heating band.

The reactor control program is designed to control the reactor temperature, the feed and mixing of the reactor. The temperature is controlled by a Pt-100 measurement signal, a temperature set-point and an on/off operation of the heating band. The feed control determines the feeding times a day, the volume of each charge and the feed rate. The mixing control allows either interval or continuous mixing of the reactor.

Values from the on-line measurements (methane content, gas production and temperature) as well as values calculated by the control program (loading rate, retention time and gas yield) are written and stored automatically every 24 h in Excel (Microsoft) files.

3.3 Measurements

3.3.1 On-line measurements

Gas sensors

The methane content is measured with an MonoGas-Sensor GS 10 ZP/HC, range 0 - 100 Vol% methane (Sensor Devices Company), which is positioned in-line. The sensor provides a linear analogue direct current output signal within a range of 4-20 mA according to the Vol% of methane measured. The signals are connected to an AI Field Point module. The biogas consists nearly up to 100 % of CO_2 and CH_4. Thus the CO_2 content is calculated as the difference between 100 % and the methane content.
The infrared sensors work according to a 2-array-process (measurement and reference channel), which helps to compensate for different influencing factors.

Gas counter

The biogas production is determined by an electrode gas meter that is build at the institute.
The measurement principle of the gas counter is based on the water displacement until a set-point. The counter is made up of two bottles (1-Liter Schott bottle) filled with water. In the first bottle three high-grade steel-electrodes (Kobold, electrodes Teflon coated, yard goods), which are set at different depths in the bottle, where the deepest electrode is the reference electrode. An electrode relay monitors when the upper respectively the lower water levels in the bottle are reached, as defined by the upper and middle electrodes. Until the lower water level in the first bottle is not reached, the biogas flows via a magnet valve (Bürkert; 3/2 way valve Typ 330-T-03; material 0-FF-VA-GM82-024) into this bottle. If the lower level is reached the relay closes a circuit and the magnet valve switches and the entrance into the bottle is closed and the water level increases again and a new measurement cycle starts.
Each time a gas count occurs, a 10 mA signal is produced and sent to an AI Field Point module. By measuring the time between two counts the gas production can be calculated.

Temperature

Temperature is measured by a Pt-100 element (Testo; PT100 Typ 04; 4-conductor; diameter 3 mm; NL 500 mm) connected to a RTD Field Point module, which is directly installed in the sludge. The RTD module is specified for measurement Pt-100 signals.

3.3.2 Off-line measurements

Additionally to the on-line parameters measured, the following parameters are measured off-line for the characterisation of the reactor content and the substrates.

pH

The pH should be measured from a freshly taken sample by avoiding long contact with air and without loss of CO_2 (Bischofsberger *et al*. 2005). Therefore the pH had to be measured within a maximum of 15 min after sampling by a standard pH electrode (WTW electrode) after a calibration of the electrode with a pH 7 and pH 4 buffer solutions.

Volatile Fatty Acids (VFA)

VFAs are measured by FTIR/ATR (Spectrum One, Perkin Elmer) using a ZnSe crystal. Whereby acetic acid, propionic acid and a total VFA concentration are measured.

The measurement by FTIR is rather simple, the samples are centrifuged (10 min at 10000 rpm) and the supernatant is spread over the crystal. Each sample was measured twice.

The Spectrum One had the following settings:

Spectral Range	4000 – 650 cm^{-1}
Spectral Resolution	1.0 cm^{-1}
Detector	DTGS
IR-Source	Mid-IR-ICE (Infrared ceramic emitter)
Sensor	45° ZnSe HATR
Software	Spektrum 3.0.1, Perkin Elmer
Scans per minute	20
Scan-Speed	0.2 $cm.s^{-1}$
Apodisation	strong
Phase Correction	Magnitude
Resolution	4 cm^{-1}

As a reference measurement the organic acid concentration (as acetic acid) is also determined using a cuvette-test (LCK 365). For this measurement the sample (double-test) is centrifuged twice, to ensure that no particles are in the supernatant, which could influence the measurement.

Firstly the heating block (Dr. Lange; Model LT 1W) is preheated to 100 °C. 0.4 ml of solution A and 0.4 ml of the centrifuged sample are pipetted into the test cuvette and put into the heating block for 10 min. After the cuvette cooled down 0.4 ml of solution B, 0.4 ml of solution C and finally 2 ml of solution D are added. After 3 min the sample is measured with the corresponding photometer (Dr. Lange; Photometer LKT) at 497 nm.

Suspended Solids (SS)/Volatile Suspended Solids (VSS)

The Volatile Suspended Solids are a measure for the amount of organic matter in the sample. Suspended Solids (SS) and Volatile Suspended solids (VSS) are measured according to DEV H1 (DEV 1997).

About 10 g of a sludge sample is weighted in tarred pots (double determination) and dried at 105 °C until the weight stabilised (drying time about 24 h) for the determination of the dry matter (SS). After cooling and subsequent weighting of the sample, the sample is annealed at 550 °C (at least 2 h), again cooled and weighted. The SS and the VSS are then calculated as (Equation (56) and (57)):

$$SS = \frac{m_d - m_p}{m_o}\left[kg.kg^{-1}\right] \quad \textbf{(56)}$$

where:
m_d mass of the dried sample
m_p mass of the pot
m_o mass of the original sample

$$VSS = \frac{m_d - m_a}{m_o}\left[kg.kg^{-1}\right] \quad \textbf{(57)}$$

where:
m_a mass of the annealed sample
m_d mass of the dried sample
m_p mass of the pot

Chemical Oxygen Demand (COD)

The Chemical Oxygen Demand (COD) is a measure for the amount of the oxygen required for the chemical oxidation of organic matter to CO_2 and water and is defined as the amount of oxygen, which is equivalent to the mass of potassium-dichromate that reacts with oxidisable substances in the sample.
The determination of the COD is in accordance with the DEV S9 (DEV 1997), by a quick test (Dr. Lange; cuvette-test LCK 114; effective range: 150-1000 $mg.l^{-1}$).
Here for about 1 – 3 g of homogenized sludge weighted in a 25 ml graduated flask and diluted with RO water. 2 ml of the solution are pipetted into the test cuvette (double determination) and heated at 148 °C in a heating block (Dr. Lange; Model LT 1W) for 2 h. After cooling the cuvette, the sample is measured with the corresponding photometer (Dr. Lange; Photometer LKT) at 605 nm.

Total Organic Content (TOC)

TOC is determined by a TOC analyser (Shimadzu, TOC-500 + ASI-502 (Automatic Sample Injector)), Mode: TC, Stroke: 10; Repeats: 3).
For sample preparation the sludge sample is centrifuged and if necessary diluted. 0.5 ml phosphoric acid are added to about 4 ml of sample in a glass tube and subsequently put in an ultrasonic bath (Branson 2210) for 45 min to remove inorganic carbon, in order to shorten analysing time as then TC=TOC.
As standard the following solution was prepared: 2.125g potassium-hydrogen-phthalate (Merck, $KHC_8H_4O_4$) per litre for 1000 ppm - diluted to concentrations of 200 ppm and 400 ppm as standard solution and to 300 ppm as control solution with RO-water.
After sample preparation the samples are put together with the standard and the control solution in the TOC analyser and the measuring cycle is started.

Methane Content (CH_4)/Carbon Dioxide Content (CO_2)

As reference measurement the CH_4 and CO_2 content in the biogas are determined by GC (GC HP 5890 Series II) analysis, as well. The interpretation is done by the GC corresponding software HP GC ChemStation.

Detectors: FID (Flame Ionization Detection)
TCD (Thermal Conductivity Detection)
Gases: air + hydrogen (fuel gases)
nitrogen (carrier gas)
Package: HP-Plot Q
Length 30 m
I.D. 0.53 mm
Film 40 µm
Temp -60 °C to 270 °C (290 °C)
Temperature: Gradient 30 °C per min
Start temperature 50° C
End temperature 230° C

For calibration a two-point calibration is used: (1) 50 % CH_4 (Rest CO_2) (Linde Gas) and (2) natural gas (97.38 % CH_4).

The sample (about 20 ml) is taken directly from the reactor via a gas tight (Hamilton, 50 ml) and injected into the reactor by an automatic valve. Immediately afterwards the analysis using the above described method is started.

Carbohydrates (CH)

Carbohydrate concentration is identified using the anthrone method originally developed by Dreywood (Dreywood 1946) using “anthrone”-solution as a test reagent with glucose as standard and RO water as reference.

For the “anthrone”-solution 0.2 g anthrone ($C_{14}H_{10}O$, VWR) are dissolved in 100 ml concentrated sulphuric acid (H_2SO_4).

First and foremost a calibration curve is generated, with a standard solution of 10 $mg.l^{-1}$, 40 $mg.l^{-1}$, 80 $mg.l^{-1}$, 120 $mg.l^{-1}$, 150 $mg.l^{-1}$ glucose using a photometer (NovaspecII Rapid, λ = 540 nm). Subsequently the sample is centrifuged and diluted about 10 fold. To 1 ml of diluted sample 2 ml of “anthrone”-solution is added (triple-testing + blanc (RO water)), vortexed, and put into a boiling water bath for 10 min. After cooling with cold water and a 30 min waiting period the sample is measured by the photometer at 540 nm and analysed using the calibration curve.

Proteins (PR)

For the determination of proteins, two different methods are used for analysis: Protein determination via the Bradford method (Biorad Protein assay) and the Lowry method (Biorad DC Protein assay). Both methods were used together for comparison only at the beginning of the experiments.

The Bradford method is a dye-binding method. Here, the proteins must have a macromolecular structure, which can lead to an underestimation. (Raunkjaer *et al*. 1994).

Lowry *et al*. (1951) described a method where the proteins are determined by the Folin-Ciocalteau reagent. In this case the peptide bonds react with copper in an alkaline solution followed by the reduction of Folin-reagent by the copper-protein complex.

Raunkjaer *et al*. (1994) states that, for wastewater the Bradford method gives an underestimation of the total proteins, whereas the Lowry method gives an overestimation.
For both tests BSA (Bovine Serum Albumin, protease free, VWR) is used as standard and RO water as reference. A calibration curve with concentrations of 0.2 $g.l^{-1}$, 0.5 $g.l^{-1}$, 0.7 $g.l^{-1}$, 1 $g.l^{-1}$, 1.5 $g.l^{-1}$ is plotted at the beginning.
In preparation the sample is centrifuged (10000 rpm for 10 min) and if necessary diluted. The Bradford reagent (Dye Reagent Concentrate, BioRad Protein Assay, (containing Phosphoric acid and Methanol), BioRad) is diluted before the application: the reagent is mixed with RO-water with a volumetric ratio of 1:4 and the diluted solution is subsequently filtered through a Whatman filter No.1.
For the Bradford test 5 ml of Bradford reagent is added to 0.1 ml of sample/standard/reference and vortexed. After a waiting time of 5 min the sample is measured with a photometer at a wavelength of 595 nm.
Using the Lowry test method 0.1 ml sample/standard/reference 0.5 ml of reagent A (alkaline copper tartate solution + reagent S, D_c Protein Assay, BioRad) vortexed. Thereafter 4 ml of reagent B (dilute Folin reagent, D_c Protein Assay, BioRad) are added and spun in a vortex. After a waiting period of 15 min the sample is measured with a photometer at 165 nm.
Each sample is done in triplicate for each of both methods.

Total Nitrogen (TN)

The total nitrogen in feed samples is determined using Dr. Lange cuvette test LCK 338.
The feed sample (double determination) is homogenised by shaking thoroughly. An adequate amount of the feed sample is pipetted gravimetrically into a 100 ml volumetric flask and the volumetric flask is filled to the label with RO-water and shaked.
0.2 ml of the diluted feed sample is put into a reaction vessel and 2.3 ml of reagent A and one pill of B are added. The cuvette is shaken thoroughly and heated up in the heating block (Dr. Lange LT 1W) for 20 min at a temperature of 148 °C.
After the cuvette has cooled down to room temperature 1 pill of C is added and the cuvette is vortexed again. Subsequently 0.5 ml of the sample and afterwards 0.2 ml of solution D is slowly added and the reaction vessel is vortexed again. After a waiting period of 15 min the sample is measured by the Dr. Lange photometer (Dr. Lange Lasa 50) at a wavelength of 345 nm.

Phosphate

Phosphate (ortho) in feed samples is measured by Dr. Lange cuvette test LCK 348 in duplicate.
Before taking the sample the feed has to be homogenised by shaking thoroughly. An adequate amount of the feed sample is put gravimetrically into a 250 ml volumetric and the flask is filled up to the label with RO-water and shaken.

For each sample, the test cuvette is shaken and vortexed. 0.5 ml of the diluted feed sample is added to each cuvette. The foil from the cap of the cuvette is removed and the cuvette is closed again by screwing down the cap with the corrugation on the upper side. The cuvette is shaken and put into the heating block (Dr. Lange LT 1W) for 20 min at a temperature of 148 °C.
After the cuvette has cooled to room temperature, 0.2 ml of reagent B is added and the cuvette is immediately closed using a grey cap (C) and shaken. Finally the sample cuvette is insert into the photometer (Dr. Lange Lasa 50, λ = 890 nm).

Ammonium

Ammonium and ammonia are measured using a gas sensitive single-rod measuring cell (WTW, NH 500/2) and a pH/mV-digital meter (WTW, 1 mV resolution).
The sludge is centrifuged at 10000 rpm for 10 min for sample preparation.
The single-rod measuring cell has to be calibrated at least daily. The lowest value that can be measured with the NH 500/2 is 1 $mg_{NH4}.l^{-1}$.
First a calibration curve is plotted with concentrations of 125 $mg.l^{-1}$, 1500 $mg.l^{-1}$ and 2670 $mg.l^{-1}$.
16 ml of the standard solution is put into a beaker (first the lowest concentration) and stirred. About 5 drops of 10 N NaOH are added and the ammonium content of the solution measured.
For the analysis of the sample (double determination) 15 ml RO water, 1 ml of the centrifuged sample and 5 drops of 10 N NaOH are put into a beaker and stirred. The ammonium concentration is then measured with the sensor.
The calculation of NH_3 and NH_4^+ is done following the specifications of Kayhanian (Kayhanian, 1999)[13].

Sulphate

Sulphate is determined with the Dr. Lange cuvette test LCK 153.
The sample is homogenised by Ultra-Turrax (20 seconds, stage 6-7) and stirred by vortex (15 seconds, stage maximum). An adequate amount of the homogenised and stirred anaerobic sludge is weighted into a 100 ml flask and 50 ml of 0.3 M HCl added.
The flask is boiled in a water bath for 60 minutes. Subsequently 20 ml of cold RO-water is filled into 10 ml volumetric flask. This solution is then filtered through filter paper ("Weißband") into another 100 ml flask. The second flask is filled up to the mark – this solution is used for the further determination of sulphate.
Two cuvettes (Dr. Lange LCK 153) are prepared for each sample (double determination) and an empty, dry cuvette for reference measurement (each sample needs its own reference!).

[13] Kayhanian, M., 1999, Ammonia inhibition in high-solids biogasification: An overview and practical solutions, *Environmental Technology* 20 (4), 355-365

5 ml of the sample solution is filled into each cuvette and for the reference 5 + 1.8 ml of RO-water are filled into the cuvette for the blank. 1 spoon (included in the test package) of BaCl is added to each cuvette, except the reference cuvette, and the cuvettes are shaken for 2 min. The sample is measured immediately afterwards by the photometer (Dr. Lange Lasa 50).

Alkalinity

Alkalinity is a measure for the buffer capacity of the sludge, as it is the ability to react in an aqueous solution with H^+ ions. The alkalinity is obtained by measuring the amount of acid in mmol necessary to attain a pH of 4.5 (Total alkalinity) (DIN 1996) (Bischofsberger *et al*. 2005).

The total alkalinity is determined by titration with a 0.1 M HCl solution to an end-point at pH 4.5.

The actual HCl concentration is estimated by the titration of 25 ml 0.025 M Na_2CO_3 (V1) and 75 ml RO-H_2O with 0.1 M HCl titrated to pH 4.5 (V2) as well as the titration of 100 ml RO-H_2O again with 0.1 M HCl (V3) to pH 4.5 for reference. The actual HCl concentration is then given as (Equation (58)) (according to DEV C23 (DEV 1997)):

$$c(HCl)\left[mol.l^{-1}\right] = \frac{m_{Na_2CO_3} V_1}{53 * (V_2 - V_3)} \quad \textbf{(58)}$$

The alkalinity of the sample is then determined by titrating 100 ml of sludge (V4) with the 0.1 M HCl solution to an end-point at pH 4.5 (V6). The alkalinity of the sample is then calculated according to equation (59) (according DEV C23 (DEV 1997)):

$$A_T[mmol.l^{-1}] = \frac{c(HCl)[mol.l^{-1}] * V_6[ml]}{V_4[ml]} \quad \textbf{(59)}$$

3.4 Substrate

3.4.1 Feed and Feed preparation

Three different substrates are used in single crop experiments: Maize silage (only corn, “Maize”), whole crop corn silage (“GPS”) and sunflower press residues (“Sunflower”).

The substrates are frozen at –20 °C for storage. For usage they are dried, milled with a food processor and finally sieved (<1 mm) (to prevent clogging).

The sieved substrates are mixed with RO water (in concentrations from 64.2 $g_{Substrate}.l^{-1}$ to 288.4 $g_{Substrate}.l^{-1}$ (for all substrates)) and buffered with 10 M NaOH (pH 7.73 (on average)). The final feed mixture is then stored at 4 °C.

Table 3.4.1: Trace compounds

Trace compounds	[mg.l^{-1}]/[ml.l^{-1}]	Trace compounds	[mg.l^{-1}]/[ml.l^{-1}]
$FeCl_2 \cdot 4H_2O$	1000	$Na_2SeO_3 \cdot 5H_2O$	82
ZnCl	25	EDTA	500
$MnCl_2 \cdot 4H_2O$	250	$AlCl_3 \cdot 6H_2O$	45
$CuCl_2 \cdot 2H_2O$	19	Resazurine	250
$(NH_4)_6Mo_7O_{24} \cdot 7H_2O$	25	HCl 37%	1
$CoCl_2 \cdot 6H_2O$	1000	H_3BO_3	25
$NiCl_2 \cdot 6H_2O$	71		

At first no additional trace materials were used, but after problems with high fatty acid concentrations a mixture of different trace materials (Table 3.4.1) was added to the maize silage (8 ml mixture of trace compounds to 5 l of feed mixture). The same trace materials mixture is also added to the whole plant silage and the sunflower residues.
Feeding is done automatically and controlled by the reactor control system. The total amount of feed is split up into 5 to 10 smaller charges, which are distributed evenly throughout the day. The total amount of feed per day is limited by the hydraulic retention time, which should not be lower than 20 days. Whereby the organic loading rate is thus limited by the consistency of the feed, as the substrate concentration of the feed mixtures is limited due to pumping and clogging problems. Higher loading rate were gained by manually feeding directly into the reactor.

3.4.2 Characteristics of the feed

For the extensive characterisation of the feed, required for the ADM1, and in order to estimate the OLR, the pH, the COD, the SS and VSS, the total nitrogen content, the phosphate concentration, the TOC, the sulphate concentration, the ammonium and ammonia concentration, the protein, the carbohydrate and the VFA concentration (Table 3.4.2) of the feed mixture are measured.

Table 3.4.2 Typical values for the feed mixtures used

Name	$C_{Substrate}$ [g.l^{-1}]	pH []	COD [mg.l^{-1}]	SS [g.l^{-1}]	VSS [g.l^{-1}]	Total-N [mg.l^{-1}]	Total-P [mg.l^{-1}]	TOC [mg.l^{-1}]	SO_4^{2-} [mg.l^{-1}]
Maissilage "FM2-280705"	32.1	8.12	18508.47	18.11	17.29	222.86	85.75	-	34.36
Maissilage "FM1-280705"	64.2	7.61	40592.20	45.95	44.93	511.47	169.55	2117.50	39.30
Maissilage "FM3-280705"	128.4	7.69	86864.00	97.28	94.80	1870.85	320.41	6143.40	96.30
Maisesilage "721.5"	144.2	7.61	99867.25	99.45	95.52	994.65	378.78	-	129.55
Maissilage "1442"	288.4	7.65	223313.41	226.74	220.47	2478.85	697.93	15527.93	188.14
Maissilage "256.8"	256.8	8.10	139962.72	216.15	225.27	2908.73	321.55	11132.00	133.51
Sunflower	144.2	7.49	147978.92	120.72	113.16	3861.18	1422.59	16829.43	188.41
GP-FM2	64.2	7.55	34289.13	56.03	52.67	664.90	126.99	4390.14	42.44

3.5 Experimental procedure

The main focus of the project was to gain data for model calibration and validation and to estimate kinetic parameters. Further experiments were made to run fuzzy-logic based control algorithms. The aim of this control is to reach maximum methane production rates and organic loading rates by keeping the system stable.

3.5.1 Inoculum

The reactors working in the mesophilic temperature range were inoculated with sludge from the Abwasserverband Klosterneuburg, a waste water treatment plant nearby Vienna. The reactors working in the thermophilic temperature range were inoculated with thermophilic sludge from the waste water treatment plant Altenburg (Germany), later on with a mixture of sludge from the waste water treatment plant Altenburg and Klosterneuburg.
The sludge is stored at 4 °C and to prevent clogging it id sieved to 1mm before used in the lab-scale CSTRs.

3.5.2 Sampling

Sampling was done every work day (partly also on the weekends) at a certain time, by taking about 100 ml sludge sample. The samples were either processed immediately or stored in the fridge at 4 °C for later processing.

3.5.3 Degradation experiments

To gain kinetic data, batch experiments in 100 ml plastic syringes (VWR, BD Plastipak) were performed. This test arrangement has the advantage of being simple, fast and space-saving.
The test is carried in triplicate plus a reference (RO-water) in duplicate for each time step. Time steps of 6 h, 12 h, 24 h, 2 d and 3 d were used.
The opening of the syringe is sealed with an airtight plastic tube (VWR, ISO-Versinic (5 x 8)) and a small wedge.
About 30 g of sludge from one of the reactor systems (FM1 to FM4) is weighted into the syringe and 30 g of the test solution (here 10 gl^{-1} BSA solution) is as well added into the syringe. Then the air is removed from the syringe and the test vessel is closed with the wedge. The syringes are hung in a breeding room at 37 °C.
After each time step the gas production of the syringe is estimated by using the scale on the syringe and afterwards the syringe is weighted. Subsequently the syringe is opened and the sludge put in a beaker for further processing. Like all other sludge samples the pH, SS, VSS, TOC, VFA and COD are determined.

3.6 Data processing

3.6.1 Statistical methods

Different supporting statistical parameters are used for data processing: the average (x_{Mean}) (Equation (60)), the standard deviation (σ) (Equation (61)) and the variance (σ^2) (Equation (62)).

$$x_{mean} = \frac{\sum_{i=1}^{n} x_i}{n} \quad \textbf{(60)}$$

$$\sigma = \sqrt{\frac{n\Sigma x^2 - (\Sigma x)^2}{n(n-1)}} \quad \textbf{(61)}$$

$$\sigma^2 = \frac{n\Sigma x^2 - (\Sigma x)^2}{n(n-1)} \quad \textbf{(62)}$$

where:
x measurement value
n number of measurements

Regression analysis and co-variance analysis are used for the comparison of the different reactor systems (FM1 – FM2).

Regression analysis

A regression analysis exam if a linear correlation between two variables (dependent and independent) exists (Equation (63)). The intercept (a) and slope (b) are calculated according the principle of the least square (Equation (64) and (65)) (von d. Lippe 1993).

$$y = a + bx \quad \textbf{(63)}$$

$$a = \frac{\Sigma y - b\Sigma x}{n} \quad \textbf{(64)}$$

$$b = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}} \quad \textbf{(65)}$$

where:
x dependent variable
y independent variable
n number of measurements

The regression analysis is performed with SigmaStat 3.1 (Sysstat Software Inc.). The program not only gives the result of the regression analysis, but also a value for the correlation coefficient and the Root Square Error. The correlation coefficient is a measure of the strength and direction of a linear correlation and has a value between –1 and 1.

Covariance analysis

Part of a covariance analysis is the adjustment of the variable observed for the effect of a covariate variable (Dubocowski *et al*. 2008). Thus covariance is a method that is adjustable for the effect of an uncontrollable nuisance variable (Dubocowski *et al*. 2008). This method is a combination of a regression analysis and a variance analysis (Dubocowski *et al*. 2008).

The variance is the measure of the difference (deviation) between the actual measurement and the expected value (Spafford 2003) and the variance analysis is the process to determine the variance.

The covariance analysis is determined with SAS Enterprise Guide 4.1 (SAS Institute Inc.).

3.6.2 Sensitivity analysis

The sensitivity analysis gives information on the sensitivity of model outputs to changes in a parameter's initial conditions (De Pauw 2005). One can distinguish between local and global sensitivity, whereby local sensitivity refers to only small changes in the parameter values (De Pauw 2005).

There are various different methods for determining the local sensitivity analysis. In this work the centralised sensitivity function (Equation (66) and (67)) was used (De Pauw 2005):

$$\frac{\partial x}{\partial k_{i,+}} = \frac{x(t, k + \xi k_i) - x(t, k_i)}{\xi k_i} \quad \textbf{(66)}$$

$$\frac{\partial x}{\partial k_{i,-}} = \frac{x(t, k - \xi k_i) - x(t, k_i)}{\xi k_i} \quad \textbf{(67)}$$

where

ξ perturbation factor

$x(t, k_i)$ value of state variable with parameter k_i

$x(t, k_i \pm \xi k_i)$ value of state variable with the parameter k_i changed for ξ_{k_i}

The perturbation factor defines the quality of the sensitivity function (De Pauw 2005). The centralised sensitivity function is calculated as the average of both the negative and the positive sensitivity function (Equation (66) and (67)) (De Pauw 2005).

The quality of the sensitivity calculation can be estimated by several criteria, such as the sum of squared errors (SSE) (Equation (68)), the sum of absolute errors (SAE) (Equation (69)) and the maximum relative error (MRE) (Equation (70)) (De Pauw 2005).

$$SSE = \frac{\sum \left(\frac{\partial x}{\partial k_+} - \frac{\partial x}{\partial k_-} \right)}{n} \quad \textbf{(68)}$$

$$SAE = \frac{\sum \left| \frac{\partial x}{\partial k_+} - \frac{\partial x}{\partial k_-} \right|}{n} \quad \textbf{(69)}$$

$$MRE = \max \left| \frac{\frac{\partial x}{\partial k_+} - \frac{\partial x}{\partial k_-}}{\frac{\partial x}{\partial k_+}} \right| \quad \textbf{(70)}$$

where:

$\frac{\partial x}{\partial k_+}$ positive sensitivity function (state variable x to parameter k_i)

$\frac{\partial x}{\partial k_-}$ negative sensitivity function (state variable x to parameter k_i)

n number of measurement points

Whereby the SRE was found to be the most useful parameter (De Pauw 2005).

The sensitivity coefficient of parameter j for state variable i ($S_{k_j}^{x_i}$)can be calculated according (Equation (71)) (De Pauw 2005):

$$S_{k_j}^{x_i} = \frac{\frac{1}{n}\sum_{l=1}^{n} \left| \frac{\partial x_i(k)}{\partial k_j} \right|}{\frac{1}{n}\sum_{l=1}^{n} x_i(l, k_0)} * k_{j,0} \quad \textbf{(71)}$$

where:

n number of measurement points

$x_i(l,k_0)$ model output using the nominal parameter value

$\frac{\partial x_i(k)}{\partial k_j}$ Sensitivity function for the output x to the parameter k

$k_{j,0}$ nominal parameter value

The average sensitivity coefficient (S_{k_j}) is then defined as (Equation (72)) by (De Pauw 2005):

$$S_{k_j} = \frac{\sum_{i=1}^{n} S_{k_j}^{x_i}}{n} \quad \textbf{(72)}$$

where:

$S_{k_j}^{x_i}$ Sensitivity coefficient of parameter j for state variable i

n number of state variables

The sensitivity coefficients can now be ranked in order to determine the most sensitive parameters.

3.6.3 Model Evaluation

The model performance is evaluated using different statistical indicators: First of all the "*most widely used statistical indicators of the goodness of fit*…" (Elias *et al*. 2006) is used: the square of the correlation coefficient (r^2) (Equation (73)):

$$r^2 = \left(\frac{\sum_{i=1}^{n} \left(x_{pre,i} - x_{mean\_pre}\right) * \left(x_{mes,i} - x_{mean\_mes}\right)}{n * \sigma_{pre} * \sigma_{mes}} \right)^2 \quad \textbf{(73)}$$

where (for Equation (73) to (76)):

$x_{pre,i}$	predicted values
$x_{mes,i}$	measured values
x_{mean_pre}	average of predicted values
x_{mean_mes}	average of measured values
σ_{pre}	standard deviation of the predicted values
σ_{mes}	standard deviation of measured values
n	number of values

Moreover several other statistical indicators suggested by Elias *et al*. (2006) are in use, like the:

Ratio of means (R_{mean}) (Papanastasiou *et al*. 2007) (Equation (74)):

$$R_{mean} = \frac{x_{mean\_pre} - x_{mean\_mes}}{x_{mean\_mes}} \quad \textbf{(74)}$$

The total roots mean squared error (RMSE) (Equation (75)):

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} \left(x_{pre,i} - x_{mes,i}\right)^2}{n}} \quad \textbf{(75)}$$

And the index of agreement (d) (Papanastasiou *et al*. 2007) (Equation (76)):

$$d = 1 - \frac{\sum_{i=1}^{n} \left(x_{mes,i} - x_{pre,i}\right)^2}{\sum_{i=1}^{n} \left(\left| x_{pre,i} - x_{mean\_mes} \right| + \left| x_{mes,i} - x_{mean\_mes} \right| \right)^2} \quad \textbf{(76)}$$

A correlation coefficient of 1 would describe an ideal model and a absolute value of < 0.3 for the ratio of means (R_{mean}) indicates that the model predicts the observation with acceptable accuracy (Elias *et al*. 2006). A negative sign of R_{mean} signifies that the values measured are underestimated in the model and a positive one that the values measured are overestimated (Elias *et al*. 2006). The index of agreement normally lies between 0 and 1, for good models the value for *d* is higher than 0.6 (Elias *et al*. 2006).

4 Results and Discussion

4.1 Anaerobic digestion of energy crops

To obtain data for model validation and calibration and also to obtain kinetic data, four completely stirred tank reactors (CSTR), as described before, later noted as FM1 to FM4 were operated at 35°C (mesophilic) (FM1, FM3) and at 60°C (thermophilic) (FM2, FM4). Whereat three different substrates were used in single crops experiments: Maize silage (only corn, "*Maize*"), whole crop corn silage ("*GPS*") and sunflower press residues ("*Sunflower*").
The main focus was to reach an optimal methane production in a stable reactor. Thus it was necessary to push the envelope of stable conditions. Therefore the organic loading rate (OLR) was increased 20 % per day 5 days a week, then left equal for 2 days and then the procedures was repeated. In the case of process disturbances the OLR was reduced or no substrate at all was fed into the reactor. The pH was not regulated, except in the case of a drop in pH to 5. In this instance the pH was stabilized with 10 M NaOH. For short term experiments e.g. kinetic experiments, pulse experiments, automatic control with a Fuzzy Logic control software, this manual control was interrupted.
The influence of the HRT, the substrates and temperature of the anaerobic digestion of energy crops in CSTRs were examined in order to better understand the reactor behaviour and to find optimal conditions.

4.1.1 Influence of the hydraulic retention time (HRT) and OLR

Firstly the influence of the HRT was tested (Table 4.1.1, Table 4.1.2 and Table 4.1.3), as the hydraulic retention time is one of the most important factors for the control of the process (De la Rubia *et al.* 2006). It has to be mentioned that the HRT in this study is only a calculated statistical value, depending on the OLR and the reactor volume.
For lower HRTs (< 20 days) the data had a high fluctuation range, which could be a result of the partial wash-out of the biomass at HRTs lower < 15 days and/or inhibitory effects.
Mackie and Bryant (1995) found that the methane production (per reactor volume) rises with increasing OLR (= decreasing HRT). Furthermore, they detected a decrease in the biological conversion efficiency in the methane production with decreasing HRT, working with HRT of 3 to 12 days. The same effect was also found by Varel and co-workers (1980), working a HRT range of 3 to 18 days using cattle waste. Other authors (Kiyohara et al. 2000; De la Rubia *et al.* 2006) demonstrated the same negative correlation between the methane production and the HRT as well as the positive correlation with the OLR. Bolzonella and co-workers (2005) examined data from an Italian WWTP. They registered the highest gas production at the lowest SRT, for SRTs of between 10 to 45 days, with a degree of the specific gas production ($m^3.kg_{vs\_Feed}$) of 25 % (Bolzonella *et al.* 2005). De la Rubia *et al.* (2001) observed an increase in the biogas production with an

increase in the OLR in the thermophilic temperature range when using sewage sludge as substrate in a pilot-scale CSTR. Bouallagui *et al.* (2004) found the best daily gas production at the lowest HRT, testing HRTs of 10, 15 and 20 days for thermophilic conditions.

For all temperature ranges observed (mesophilic and thermophilic) and all substrates (*Maize*, *GPS* and *Sunflower*) in this study the same effect was observed. For the gas and methane production per volume of reactor in the thermophilic reactor digesting *GPS* the gas production declines by -0.06 $m^3_{Biogas}.m^{-3}_{Reactor}.d^{-1}$ per 10 d HRT and by -0.04 $m^3_{Methane}.m^{-3}_{Reactor}.d^{-1}$ per 10 d HRT. The determination coefficient is generally low (R = 0.288 in average), due to the large amount of the data examined. Using *Maize* as substrate in the same temperature range the gas production sinks by -0.03 $m^3_{Methane}.m^{-3}_{Reactor}.d^{-1}$ per 10 d HRT (Figure 4.1.1) and the methane production per volume of reactor by -0.02 $m^3_{Methane}.m^{-3}_{Reactor}.d^{-1}$ per 10 d HRT. In the mesophilic temperature range using the same substrate the gas production drops by -0.02 $m^3_{Biogas}.m^{-3}_{Reactor}.d^{-1}$ per 10 d HRT and the methane production falls by –0.01 $m^3_{Methane}.m^{-3}_{Reactor}.d^{-1}$ per 10 d HRT. In the mesophilic reactor system using *Sunflower* as substrate the methane production decreases by -0.13 $m^3_{Methane}.m^{-3}_{Reactor}.d^{-1}$ per 10 d HRT.

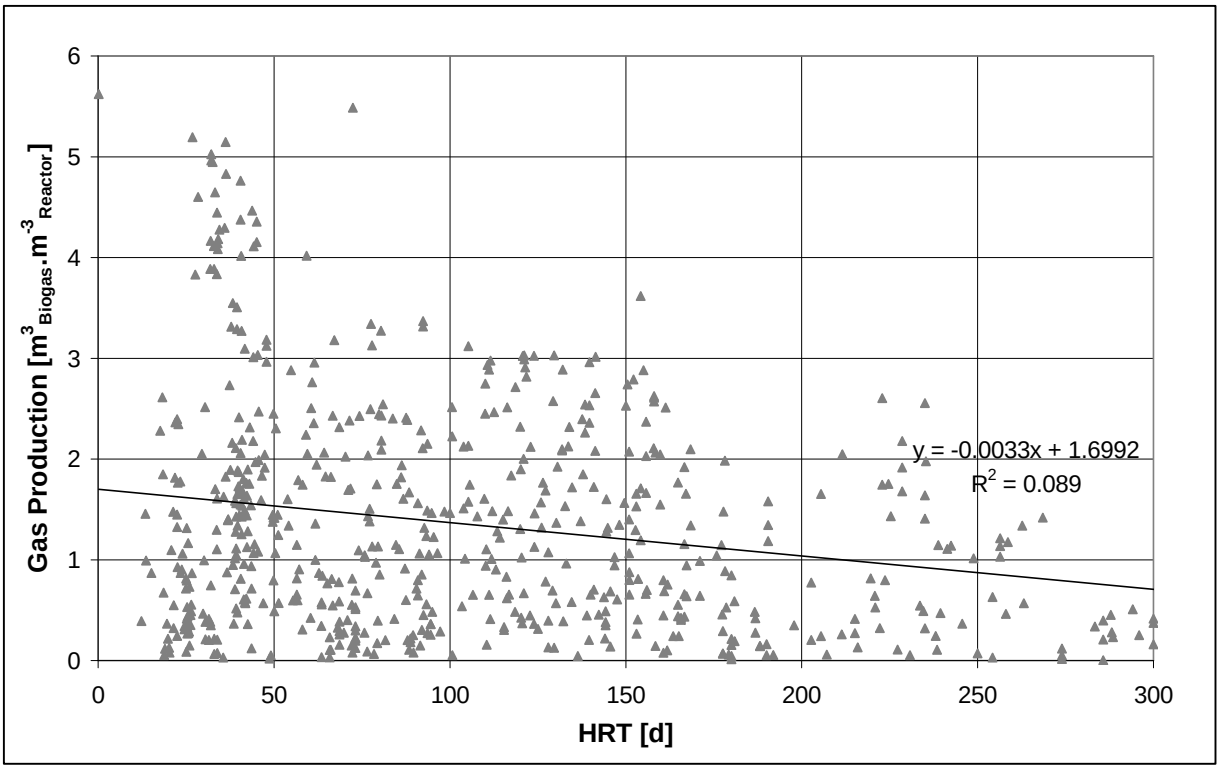

Figure 4.1.1: Gas production in the thermophilic reactors systems (FM2 and FM4) – Showing the influence of the HRT

However, for the gas production and the methane production per COD in most of the cases studied no significant correlation to the HRT was observed. Only for the methane production per COD in the thermophilic system using *GPS* as substrate and the mesophilic system digesting *Maize* was a significant influence of the HRT observed. Yet in these cases an increased correlation to the HRT was found (0.05 $m^3_{Methane}.kg_{COD}$

(P = 0.003, n = 41) in the first case and 0.004 $m^3_{Methane}.kg_{COD}$ (P = 0.002, n = 647) in the second case). This behaviour seems to be a result of the inhibitory effects.

No significant correlation between the HRT and the methane content was found, either. Yet, Farhan (1997) discovered that low HRTs affect the composition of the biogas, due to the high level of solubility of the carbon dioxide compared to methane, as the effluent stream acts as removal mechanism for the CO_2.

The pH significantly increases with higher HRTs. So the pH increases by 0.045 per 10 d HRT using *Maize* as substrate in the thermophilic temperature range. In the mesophilic reactor using *Maize* (Figure 4.1.2) the pH only rises by 0.036 per 10 d. The steepest gradient was found in the reactor using *GPS* as substrate (thermophilic range), here the pH rises by 0.055 per 10 d HRT. If *Sunflower* is used as substrate at 35 ± 1 °C no significant change in the pH is found, which seems to be a result of the high buffer capacity in this system due to the high TAN concentration.

It is interesting that with higher HRTs the acetic acid, the propionic acid and the VFA increases significantly, as well. In the thermophilic system the acetic acid rises by 0.036 $g.l^{-1}$ per 10 d HRT (*Maize* as substrate), the acetic acid increases a bit slower (0.033 $g.l^{-1}$/10 d) in the mesophilic reactor system using *Maize* as substrate. Digesting *GPS* the acetic acid increases by 0.042 $g.l^{-1}$ per 10 d HRT. Using *Sunflower* as substrate no significant influence of the HRT on the acetic acid, propionic acid and VFA concentration is found. The propionic acid increases faster in the mesophilic system (0.079 $g.l^{-1}$/10 d HRT) than in the thermophilic (0.063 $g.l^{-1}$/10 d HRT) reactor using *Maize*. However the average and maximum propionic acid concentration is higher in the thermophilic reactor system (see 4.1.3). For the systems using *GPS* and *Sunflower* no significant influence of the HRT on the propionic acid is detected. The VFA concentration rises by 0.084 $g.l^{-1}$ (thermophilic) and 0.056 $g.l^{-1}$ (mesophilic) using *Maize* as substrate and by 0.184 $g.l^{-1}$ using *GPS* as substrate.

In their study De la Rubia and co-workers (2006) found an increase of the acetic acid concentration with increasing HRT, however, the propionic acid and VFA concentrations decreased with increasing HRT. In anaerobic upflow filters Ahn and Forster (2002) have shown that the VFA increases as the HRT increased in the mesophilic temperature range. On the other hand Varel and co-workers (1980) detected a decrease in the acetate and propionate with increasing HRTs.

The COD degradation is high (> 70 %) if the reactor has a long HRT (Elmitwalli *et al.* 2006). De la Rubia (2006) found that the COD removal efficiency in % is positively correlated to the HRT, as well. Thus the COD removal efficiency increases in the mesophilic reactors using *Maize* by 1.28 % per 10 d HRT and by 1.87 % per 10 d HRT in the thermophilic reactor. The COD removal efficiency is augments by 1.04 % per 10 d HRT using *GPS* as substrate in the thermophilic reactor.

Table 4.1.1: Influence of the HRT on the gas production, methane production, acetate, propionate and VFA concentration, COD Reduction, VSS content, TOC, TAN, Sulphate and H_2S concentration in the mesophilic reactor using *Maize* and *Sunflower* as substrate. Showing the change per 10 d HRT and the significance of the change. P < 0.05 and a Power > 0.8 means that the correlation is significant. "n" gives the number of measurements used in this analysis.

Substrate		Maize				Sunflower			
		Δx per 10 d HRT	P	Power	n	Δx per 10 d HRT	P	Power	n
pH	[]	0.036	0.001	1.000	443	-	0.331	0.161	121
Gas Production/Volume	$m^3_{Biogas}.m^{-3}_{Reactor}.d^{-1}$	-0.020	0.001	1.000	683	-	0.006	0.792	99
Methane Production/Volume	$m^3_{Methane}.m^{-3}_{Reactor}.d^{-1}$	-0.009	0.001	1.000	678	-0.134	0.002	0.865	93
Acetate	$[g.l^{-1}]$	0.033	0.001	0.999	408	-	0.821	0.041	120
Propionate	$[g.l^{-1}]$	0.079	0.001	1.000	449	-	0.997	0.025	117
VFA	$[g.l^{-1}]$	0.056	0.001	1.000	399	-	0.891	0.034	118
COD Reduction	[%]	1.280	0.001	1.000	433	-	0.065	0.455	124
VSS	$[kg.kg^{-1}]$	0.720	0.001	1.000	449	-	0.027	0.600	123
TOC	$[mg.l^{-1}]$	161.620	0.001	0.999	443	-	0.953	0.029	125
TAN	$[mg.l^{-1}]$	-	0.532	0.091	74	-	0.470	0.107	58
Sulphate	$[mg.l^{-1}]$	-	0.898	0.033	69	-	0.786	0.045	55
H_2S	$[mg.l^{-1}]$	-	0.111	0.357	74	-	0.411	0.127	61

Table 4.1.2: Influence of the HRT on the gas production, methane production, acetate, propionate and VFA concentration, COD Reduction, VSS content, TOC, TAN, Sulphate and H_2S concentration in the thermophilic reactor using *Maize* and *GPS* as substrate. Showing the change per 10 d HRT and the significance of the change. P < 0.05 and a Power > 0.8 means that the correlation is significant. "n" gives the number of measurements used in this analysis.

Substrate		Maize				GPS			
		Δx per 10 d HRT	P	Power	n	Δx per 10 d HRT	P	Power	n
pH	[]	0.046	0.001	1.000	366	0.055	0.001	1.000	173
Gas Production/Volume	$[m^3_{Biogas}.m^{-3}_{Reactor}.d^{-1}]$	-0.033	0.001	1.000	656	-0.058	0.002	0.871	38
Methane Production/Volume	$[m^3_{Biogas}.m^{-3}_{Reactor}.d^{-1}]$	-0.017	0.001	1.000	656	-0.043	0.005	0.806	41
Acetate	$[g.l^{-1}]$	0.036	0.001	1.000	341	0.042	0.001	0.999	153
Propionate	$[g.l^{-1}]$	0.063	0.001	0.990	370	-	0.031	0.581	102
VFA	$[g.l^{-1}]$	0.084	0.001	1.000	346	0.184	0.001	1.000	173
COD Reduction	[%]	1.870	0.001	1.000	370	1.040	0.001	1.000	150
VSS	$[kg.kg^{-1}]$	-	0.310	0.172	320	0.246	0.002	0.863	143
TOC	$[mg.l^{-1}]$	-	0.021	0.635	350	60.270	0.001	0.998	138
TAN	$[mg.l^{-1}]$	-	0.154	0.297	82	-	0.114	0.352	158
Sulphate	$[mg.l^{-1}]$	33.410	0.001	0.985	284	-	0.300	0.174	15
H_2S	$[mg.l^{-1}]$	-	0.079	0.420	86	-	0.774	0.047	24

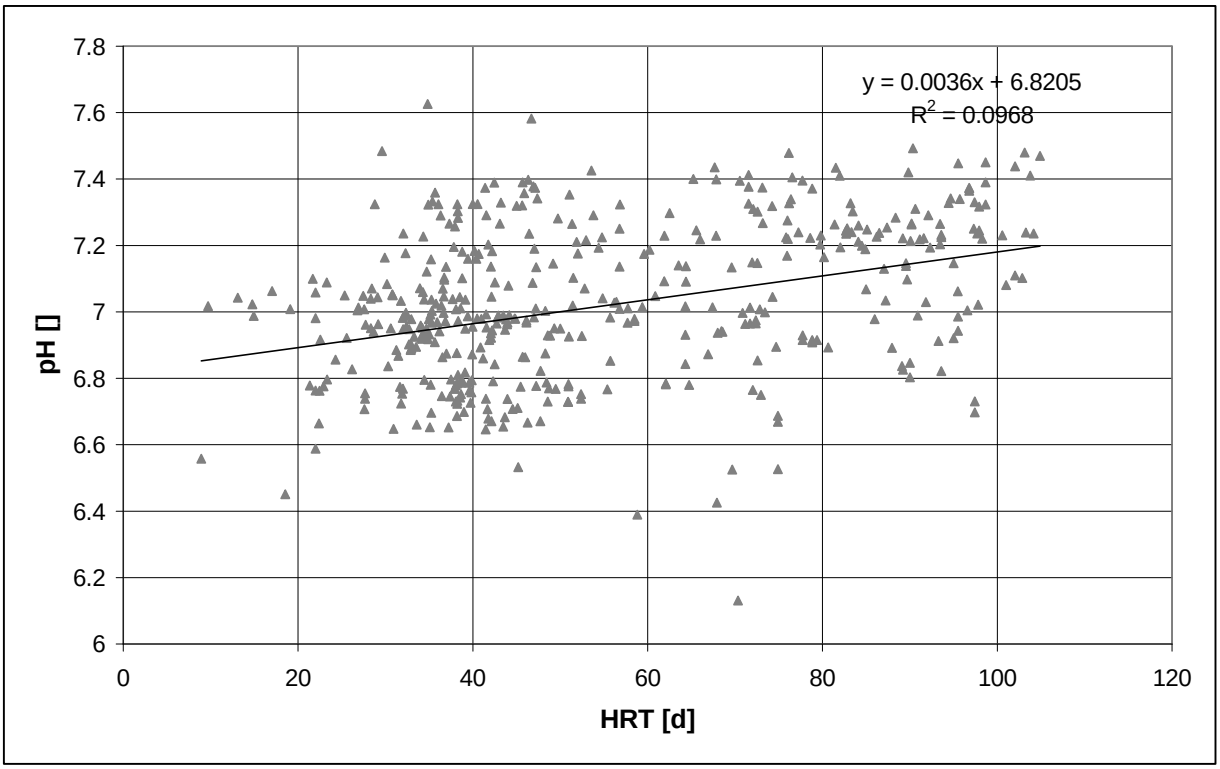

Figure 4.1.2: pH in the mesophilic reactors systems (FM1 and FM3) – Showing the influence of the HRT

For the mesophilic reactor using *Sunflower* the COD removal rate also increases with the HRT, yet this correlation is not significant.

An interesting effect can be observed, as the influence of the HRT on the COD removal efficiency can be separated in two parts – for a HRT < 40 d the efficiency is lower and fluctuates a lot and for a HRT of 40 to 100 d the COD is higher and fluctuates less. This effect is found especially in the thermophilic system using *Maize* as substrate (FM2), here a COD removal efficiency of 52 % to 90 % is found for a HRT < 40 d and a COD removal of between 77 % and 92 % is estimated for a HRT of 40 to 100 d (Figure 4.1.3).

Kiyohara and co-worker (2000) detected an increase of ammonium with a increasing HRT. In this study only a significantly positive correlation between the HRT and the TAN (Total ammonium nitrogen) was observed for the thermophilic reactor digesting *Maize*, here the TAN increases by 33.41 $mg.l^{-1}$ per 10 d HRT. For all other experiments no significant correlation between the HRT and the TAN concentration is found, yet in these cases the TAN concentration is also increasing with higher HRTs.

No significant influence of the HRT on the sulphate and the H_2S in the biogas has been detected.

In the mesophilic system digesting *Maize* the TOC increases by 161.82 $mg.l^{-1}$ per 10 d HRT and in the thermophilic reactor using *GPS* the TOC rises by 60.27 $mg.l^{-1}$ per 10 d HRT. In the thermophilic system using *Maize* and in the mesophilic reactor using *Sunflower* the TOC is positively correlated with the HRT, as well, but not significantly.

Table 4.1.3: Influence of the HRT on the gas production, methane production, acetate, propionate and VFA concentration and COD Reduction; Showing the change of the variables by increasing HRT for different reactor types, temperature ranges and substrates - comparison between literature data and this study

Gas Production	Methane Production	Acetate	Propionate	VFA	COD Reduction	HRT	Reactor typ	Reactor Working Volume	Temperature Range	Substrate	Reference
-	decrease	increase	increase	increase	increase	3 - 12 d	bench-top fermentor	3 l	mesophile	cattle waste	Mackie and Bryant, 1995[1)]
-	decrease	increase	increase	increase	increase	3 - 12 d	bench-top fermentor	3 l	thermophile	cattle waste	
-	decrease	decrease	decrease	decrease	-	3 - 18 d	CSTR	3 l	mesophile	beef cattle waste	Varel et al., 1980[2)]
-	decrease	decrease	decrease	decrease	-	3 - 18 d	CSTR	3 l	thermophile	beef cattle waste	
-	decrease	-	-	-	increase	2.5 - 40 d	CSTR	-	mesophile	sewage sludge	Kiyohara et al., 2000[3)]
-	decrease	-	-	-	constant	2.5 - 40 d	CSTR	-	thermophile	sewage sludge	
-	decrease	increase	decrease	decrease	increase	15 - 75 d	CSTR	150 l	thermophile	primary and secondary waste sludge	De la Rubia et al., 2006[4)]
decrease	-	-	-	-	-	10 - 45 d	WWTP	divers	mesophile	waste activated sludge	Bolzonella et al., 2005[5)]
decrease	decrease	-	-	-	-	15 - 75 d	CSTR	150 l	mesophile	raw sludge	De la Rubia et al., 2001[6)]
decrease	-	-	-	-	-	10 - 20 d	tubular reactor	18 l	thermophile	fruit and vegetable waste	Bouallagui et al., 2004[7)]
-	decrease	-	-	increase	constant	10 - 30 h	upflow filter	1.52 l	mesophile	paper-pulp-liquors	Ahn and Forster, 2002[8)]
-	constant	-	-	constant	increase	10 - 30 h	upflow filter	1.55 l	thermophile	paper-pulp-liquors	
decrease	decrease	increase	increase	increase	increase	10 - 100 (300) d	CSTR	18 l	mesophile	maize silage (Maize)	This Study[9)]
-	decrease	-	-	-	-	10 - 100 (300) d	CSTR	18 l	mesophile	sunflower press residues (Sunflower)	
decrease	decrease	increase	increase	increase	increase	10 - 100 (300) d	CSTR	18 l	thermophile	maize silage (Maize)	
decrease	decrease	increase	increase	-	increase	10 - 100 (300) d	CSTR	18 l	thermophile	whole plant maize silage (GPS)	

[1)] Methane production per volume reactor; the methane production per VS is positivley correlated with the HRT
[2)] Methane production per volume reactor; methane production per VS fed is positively correleated with the HRT
[3)] Methane production per volume reactor
[4)] Methane production per volume reactor
[5)] Gas productionper VS fed
[6)] Gas and methane production per volume reactor; methane production is per VS is negativley correlated with the HRT
[7)] Gas production in $l.d^{-1}$
[8)] Methane production per COD removed
[9)] Gas and methane production per volume reactor; production per COD removed is positivley correlated with HRT (mesophile, Maize and thermophile, GPS)

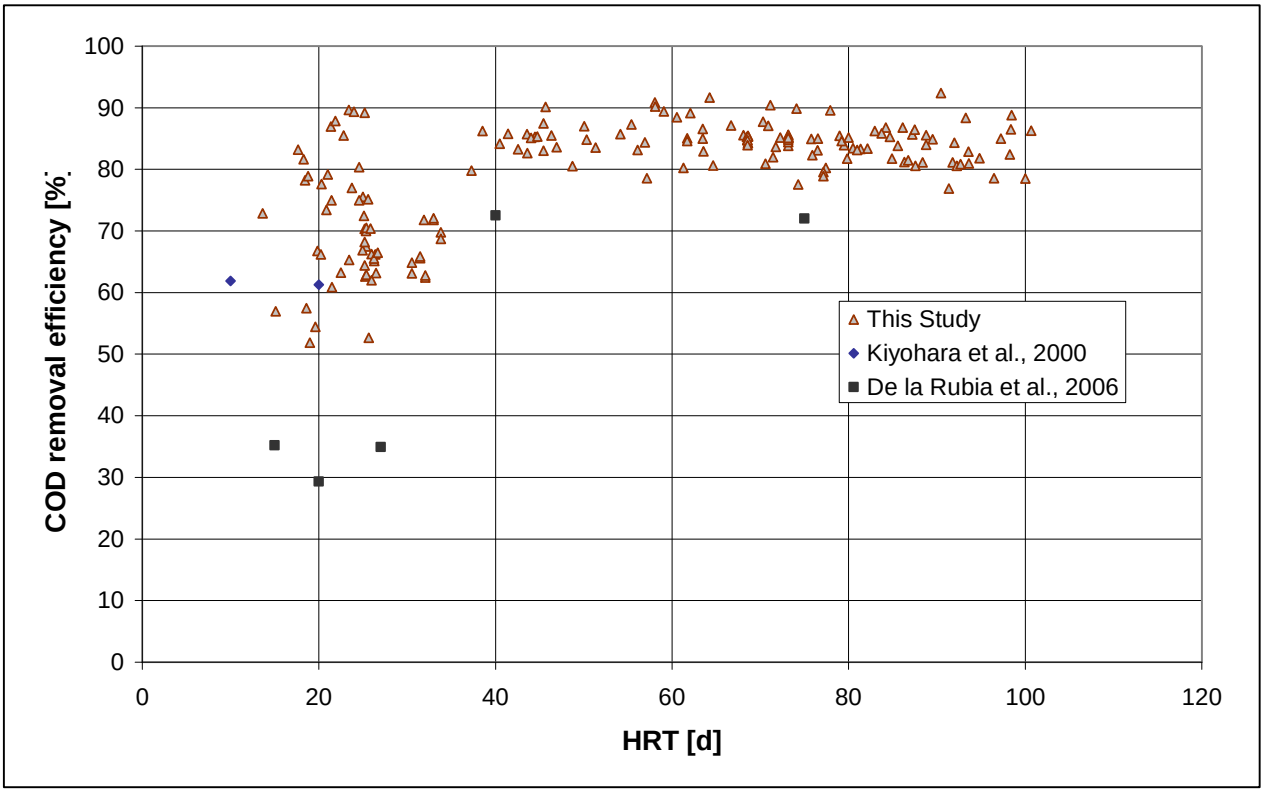

Figure 4.1.3: Influence of the HRT on the COD removal efficiency in % in the thermophilic reactor FM2 using *Maize* as substrate – compared with data from literature: Kiyohara *et al.* 2000: thermophile CSTR using sewage sludge as substrate and De la Rubia *et al.*, 2006: CSTR working in the thermophilic temperature range digesting primary and secondary waste sludge

The same applies for the VSS – here the VSS rises in the mesophilic reactor system by 0.72 kg.kg^{-1} per 10 d HRT and in the thermophilic reactor system using *GPS* as substrate the VSS increases by 0.25 kg.kg^{-1} per 10 d HRT.

Summarizing the above knowledge discussed about influence of the HRT in anaerobic systems it can be concluded that the optimal HRT lies around 30 d. The gas and methane production is highest at lower HRTs, however at a HRT < 15 d a wash out of the biomass is observed, moreover the COD removal is lower and fluctuates more at lower HRTs.

4.1.2 Comparison of different substrates

Analysing the use of substrates in Austria, it can be found that ¾ of the energy plants used are maize (either as silage or as grains). Grass silage, whole plant silage, sun flower silage and many more are also used (Amon et al. 2007; Laaber 2007). Other substrates used in agricultural biogas plants are, for example, left-overs, debris, Lecithin and sugar beets refuses, and manures and dung (Laaber 2007).

In these experiments now, as mentioned before, three different substrates were compared in single crops experiments: Maize silage (only corn, "*Maize*"), whole crop corn silage ("*GPS*") and sunflower press residues ("*Sunflower*") (Table 4.1.4).

Table 4.1.4: Composition of different substrates from literature (arranged by Brändle, J.)[14]

Substrate	Yield [t.ha^{-1}]	DM [g.kg^{-1}$_{FM}$]	ash [g.kg^{-1}$_{TS}$]	XP [g.kg^{-1}$_{TS}$]	XF [g.kg^{-1}$_{TS}$]	XL [g.kg^{-1}$_{TS}$]	NDF [g.kg^{-1}$_{TS}$]	ADF [g.kg^{-1}$_{TS}$]	ADL [g.kg^{-1}$_{TS}$]	Nfe [g.kg^{-1}$_{TS}$]	starch [g.kg^{-1}$_{TS}$]	Note	Reference
		373	38	97			463	224			262		McAllister et al., 2000
		321	42	76	34	210							Richter, 2003
Maize	12.6	232	75	82		262	526	295	45			milk	Abreu et al., In press
		20	53	89	23	226				609	158	milk	Jeroch et al., 1993
		320	47	83	31	193				646	300	dough	Jeroch et al., 1993
	11.3	277		91		292	470	388				mature	Abreu et al., In press
Sunflower	10.7	240		103		287	461	385				outer sseds soft	Abreu et al., In press
		173	91	92	70			313				seeds milky ripe	McDonald et al., 1991
		171	112	133	89			333				dough seed stage	McDonald et al., 1991
			58	86	24	305				416		dough	DLG, 1997
Rye		415		58			536	348				soft dough	Bohle et al., 2003
		412		66			544	316				soft dough	Juskiw et al., 2000
Triticale		511	39	103	24	158				676			Richter, 2003
	11.8	458	75		59		340	605	390	44		dough	Abbreu wt al., In press
		110	147	194	37	133				489			DLG, 1997
Rape		120	152	194	38	184				432		blossom	Muck et al., 2003
		110	153	216	40	132				459		before blossom	Jeroch et al., 1993

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Abreu *et al.,* In press. Intake and Nutritive value of Mediterranean Forages and Diets. 20 years of experimental data

Bohle *et al.*, 2003. Winter Cereal Forage Varieties for Central Oregon, 2002 Central Oregon Agricultural Research Center Annual Report

DLG, 1997. DLG Futterwerttabellen. Hrsg. Universität Hohenheim-Dokumentation

Jeroch *et al.*, 1993. Futtermittelkunde, Urban & Fischer

Juskiw *et al.*, 2000. Forage Yield and Quality for Monocrops and Mixtures of Small Grain Cereals. Crop Sci. 40:138–147

McAllister *et al.*, 2000. The Fundamentals of Making Good Quality Silage. Proceedings of the Western Canadian Dairy Seminar.

McDonald et al., 1991. The biochemistry of Silage. Second Edition. Chalcombe Publications, Aberystwyth

Muck *et al*., 2003. Effects of Breeding for Quality on Alfalfa Ensilability. Transactions of the ASAE. Vol 46 (5):pp 1305-1309

Richter, 2003. Triticale-Ganzpflanzensilage (GPST), Gärsäureentwicklung, aerobe Stabilität und Mykotoxinreduktion. Bayerische Landesanstalt für Landwirtschaft (LfL)/Institut für Tierernährung und Futterwirtschaft (ITE).

The substrates all came from the biogas plant Pfiehl, Sitzenberg-Reidling, Austria in different charges.

Sunflower oil cakes (= sunflower press residues, "*Sunflower*") have about 27 % crude protein, whereby dehulled Sunflower consists of up to 40 % protein and 10 % fibre (Ramachandran *et al.* 2007). Sunflower can be divided into three main fractions a lignocellulosic (23.2 - 25.3 %), a proteinaceous (55.4 – 57.6 %) and a soluble (17.1 – 21.4 %) one (Ramachandran *et al.* 2007) (Table 4.1.5).

Table 4.1.5: Composition of sunflower oil cake from literature, cited by (Ramachandran *et al.* 2007)[15]

Composition		
Dry matter	[%]	91.00
Crude protein	[%]	34.10
Crude fibre	[%]	13.20
Ash	[%]	6.60
Calcium	[%]	0.30
Phosphorus	[%]	1.30

Looking at the feed mixtures used in this study, so following characteristics can be found (Table 4.1.6): The pH_{mean} lies between 7.49 to 8.12 for all substrate mixtures, though the pH was slightly higher for the *Maize* mixtures. The COD_{mean} for *Sunflower* (1.03e+06 $mg.kg^{-1}$) is definitely higher than for *Maize* (6.50e+05 $mg.kg^{-1}$) and *GPS* (5.34e+05 $mg.kg^{-1}$).

The total nitrogen concentration is higher for the *Sunflower* feed mixture than for *Maize* using the same substrate concentration (994.65 $mg.l^{-1}$ for *Maize* and 3861.18 $mg.l^{-1}$ for *Sunflower* at a concentration of 144.2 $g.l^{-1}$ substrate (marked red in Table 4.1.6). Moreover, it was found that the proteins concentration of *Sunflower* is about 14 times higher than for *Maize*. Comparing the Total-N content of *Maize* and *GPS* it can be found that for the *GPS* feed mixture the N-content is slightly higher (664.90 $mg.l^{-1}$) than for *Maize* (511.47 $mg.l^{-1}$) (compared at a substrate concentration of 64.2 g.l-1 (marked blue in Table 4.1.6)).

The SS and VSS content is slightly higher for the *Sunflower* substrate (SS = 120.72 $kg.kg^{-1}$ and VSS = 113.16 $kg.kg^{-1}$ (at 144.2 $g_{Substrate}.l^{-1}$)) compared to *Maize* (SS = 99.45 $kg.kg^{-1}$ and VSS = 95.52 $kg.kg^{-1}$ (at 144.2 $g_{Substrate}.l^{-1}$)), also for the *GPS* substrate (SS = 56.03 $kg.kg^{-1}$ and VSS = 52.67 $kg.kg^{-1}$ (at 64.2 $g_{Substrate}.l^{-1}$)) the SS and VSS is higher than for *Maize* (SS = 45.95 $kg.kg^{-1}$ and VSS = 44.93 $kg.kg^{-1}$ (at 64.2 $g_{Substrate}.l^{-1}$)). The total organic carbon (TOC) content is much higher for *GPS* (TOC = 4319.14 $mg.l^{-1}$ (at 64.2 $g_{Substrate}.l^{-1}$)) than for *Maize* (TC = 2117.5 $mg.l^{-1}$ (at 64.2 $g_{Substrate}.l^{-1}$)). The TOC for *Sunflower* could not be compared, as there is no value available for *Maize* at the same substrate concentration.

[15] Brendon, R.M., 1957, Uganda Protectorate. Department of Veterinary Services and Animal Industry. Occasional Bulletin No.1

Table 4.1.6: Composition of the feed mixtures used– Comparison of the total nitrogen concentration - the Total-N for *Sunflower* feed mixture is higher as for *Maize* using the same substrate concentration (994.65 mg.l^{-1} for *Maize* and 3861.18 mg.l^{-1} for *sunflower* at a concentration of 144.2 g.l^{-1} substrate (marked red)) - for the *GPS* feed mixture the N-content is slightly higher (664.90 mg.l^{-1}) as for *Maize* (511.47 mg.l^{-1}) (compared at a substrate concentration of 64.2 g.l-1 (marked blue)

Substrate	$C_{Substrate}$ [g.l^{-1}]	pH []	COD [mgl^{-1}]	SS [kg.kg^{-1}]	VSS [kg.kg^{-1}]	Total-N [mgl^{-1}]	Total-P [mgl^{-1}]	TOC [mgl^{-1}]	SO_4^{2-} [mgl^{-1}]
Maize	32.1	8.12	18508.47	18.11	17.29	222.86	85.75		34.36
	64.2	7.61	40592.20	45.95	44.93	511.47	169.55	2117.50	39.30
	128.4	7.69	86864.00	97.28	94.80	1870.85	320.41	6143.40	96.30
	144.2	7.61	99867.25	99.45	95.52	994.65	378.78		129.55
	288.4	7.65	223313.41	226.74	220.47	2478.85	697.93	15527.93	188.14
	256.8	8.10	139962.72	216.15	225.27	2908.73	321.55	11132.00	133.51
Sunflower	144.2	7.49	147978.92	120.72	113.16	3861.18	1422.59	16829.43	188.41
GPS	64.2	7.55	34289.13	56.03	52.67	664.90	126.99	4390.14	42.44

Comparison between *Maize* and *GPS* in the thermophilic reactor system

In the thermophilic reactor FM2 *Maize* and *GPS* were compared. The pH, the biogas production, the methane production, the acetate concentration, the total volatile fatty acid concentration (VFA), the degraded COD, the volatile suspended solids, the total nitrogen concentration (TAN), sulphate concentration and the H_2S concentration.

To be comparable all variables were based on the degraded COD and statistical outliers were removed, to get typical reactor values. “Wrong” data from reactor failure, due to broken tubes, leaking reactor, … was removed manually. The first 50 days were also removed, as this data was designated as the “start-up” of the reactor.

The results found, were further compared to literature data. Yet a comparison with literature data is always difficult, due to inconsistencies in study conditions (Farhan *et al.* 1997).

Table 4.1.7 shows the average values (Mean), the standard deviation (STD), the variance (VAR), the minimum and maximum (MIN and MAX) and the number of samples (n) for *Maize* and *GPS*. In order to include the influence of the HRT in the comparison of the different substrates a covariance analysis was carried out. To simplify the calculations a covariance analysis (Table 4.1.8) was done for all variables, ignoring the fact that it would not have been necessary, as the HRT has no influence on all variables.

The pH is significantly higher using *GPS* as substrate (Figure 4.1.4). This influences the enzymatic activity and as each enzyme has its maximum activity at a specific pH (El-Mashad *et al.* 2004) the optimum pH for the AD process lies within a narrow range. The optimum pH for the methanogenesis lies within the range of 6.5 to 7.5, acidogenic bacteria are less sensitive to bothhigher and lower pH values (Leitao *et al.* 2006).

Table 4.1.7: Comparison based on the different substrates (*Maize* and *GPS*; under consideration of the HRT) of the variables pH, Gas Production, Methane Production, Acetic and Propionic Acid and VFA Concentration, VSS, TOC, TAN concentration, Sulphate and H_2S concentrations based on the degraded COD in the thermophilic reactor FM2. Showing the average values (Mean), the standard deviation (STD), the variance (VAR), the minimum and maximum (MIN and MAX) and the number of samples (n) for both substrates

		MAIZE					
		Mean	STD	VAR	MIN	MAX	n
pH	[]	7.08E+00	3.40E-01	1.16E-01	6.20E+00	7.67E+00	176
Gas Production/COD	$[m^3 \cdot kg^{-1}_{CODdeg.} d^{-1}]$	3.90E-01	2.68E-01	7.18E-02	1.81E-02	1.27E+00	172
Methane Production/COD	$[m^3 \cdot kg^{-1}_{CODdeg.} d^{-1}]$	1.98E-01	1.38E-01	1.92E-02	8.83E-03	6.63E-01	172
Acetate/COD	$[g.kg^{-1}_{Coddeg}]$	3.48E+00	2.73E+00	7.46E+00	1.45E-02	1.09E+01	169
Propionate/COD	$[g.kg^{-1}_{Coddeg}]$	4.13E+00	6.41E+00	4.11E+01	0.00E+00	3.45E+01	178
VFA/COD	$[g.kg^{-1}_{Coddeg}]$	1.07E+01	8.35E+00	6.96E+01	1.33E-03	3.96E+01	165
VSS/COD	$[kg.kg^{-1}_{Coddeg}]$	1.19E+02	3.70E+01	1.37E+03	0.00E+00	2.36E+02	139
TOC/COD	$[g.kg^{-1}_{Coddeg}]$	2.19E+01	1.03E+01	1.06E+02	5.14E+00	4.90E+01	171
TAN/COD	$[g.kg^{-1}_{Coddeg}]$	2.68E+00	1.90E+00	3.61E+00	1.97E-01	1.17E+01	118
Sulphate/COD	$[g.kg^{-1}_{Coddeg}]$	6.58E-01	1.61E-01	2.58E-02	3.95E-01	8.43E-01	24
H_2S/COD	$[g.kg^{-1}_{Coddeg}]$	1.24E+00	1.03E+00	1.05E+00	1.91E-02	4.32E+00	25

		GPS					
		Mean	STD	VAR	MIN	MAX	n
pH	[]	7.34E+00	1.25E-01	1.56E-02	7.01E+00	7.60E+00	35
Gas Production/COD	$[m^3 \cdot kg^{-1}_{CODdeg.} d^{-1}]$	4.72E-01	2.47E-01	6.10E-02	5.09E-02	9.55E-01	40
Methane Production/COD	$[m^3 \cdot kg^{-1}_{CODdeg.} d^{-1}]$	2.24E-01	1.20E-01	1.43E-02	1.45E-02	4.74E-01	40
Acetate/COD	$[g.kg^{-1}_{Coddeg}]$	1.25E+01	1.03E+01	1.06E+02	6.82E-02	3.69E+01	37
Propionate/COD	$[g.kg^{-1}_{Coddeg}]$	8.04E+00	1.45E+01	2.11E+02	0.00E+00	7.00E+01	40
VFA/COD	$[g.kg^{-1}_{Coddeg}]$	1.62E+01	1.39E+01	1.92E+02	4.07E-02	5.74E+01	37
VSS/COD	$[kg.kg^{-1}_{Coddeg}]$	2.09E+02	6.31E+01	3.99E+03	1.03E+02	3.56E+02	37
TOC/COD	$[g.kg^{-1}_{Coddeg}]$	2.25E+01	3.54E+00	1.25E+01	1.23E+01	2.98E+01	28
TAN/COD	$[g.kg^{-1}_{Coddeg}]$	1.60E+01	8.53E+00	7.27E+01	2.46E+00	3.21E+01	40
Sulphate/COD	$[g.kg^{-1}_{Coddeg}]$	7.31E-01	2.91E-01	8.46E-02	2.38E-01	1.50E+00	30
H_2S/COD	$[g.kg^{-1}_{Coddeg}]$	6.56E+00	4.77E+00	2.28E+01	3.24E-01	1.74E+01	39

Table 4.1.8: Comparison based on the different substrates (*Maize* and *GPS*; under consideration of the HRT) of the variables pH, Gas Production, Methane Production, Acetic and Propionic Acid and VFA Concentration, VSS, TOC, TAN concentration, Sulphate and H_2S concentrations based on the degraded COD in the thermophilic reactor FM2. Showing the p-value (P) of the Covariance-analysis and the number of samples used (n). A p-value < 0.05 means that the analysed difference is significant

	P	n
pH	0.003	211
Gas Production/COD	0.000	212
Methane Production/COD	0.001	212
Acetate/COD	0.031	206
Propionate/COD	0.000	218
VFA/COD	0.149	202
VSS/COD	0.821	176
TOC/COD	0.041	199
TAN/COD	0.002	158
Sulphate/COD	0.544	54
H_2S/COD	0.330	64

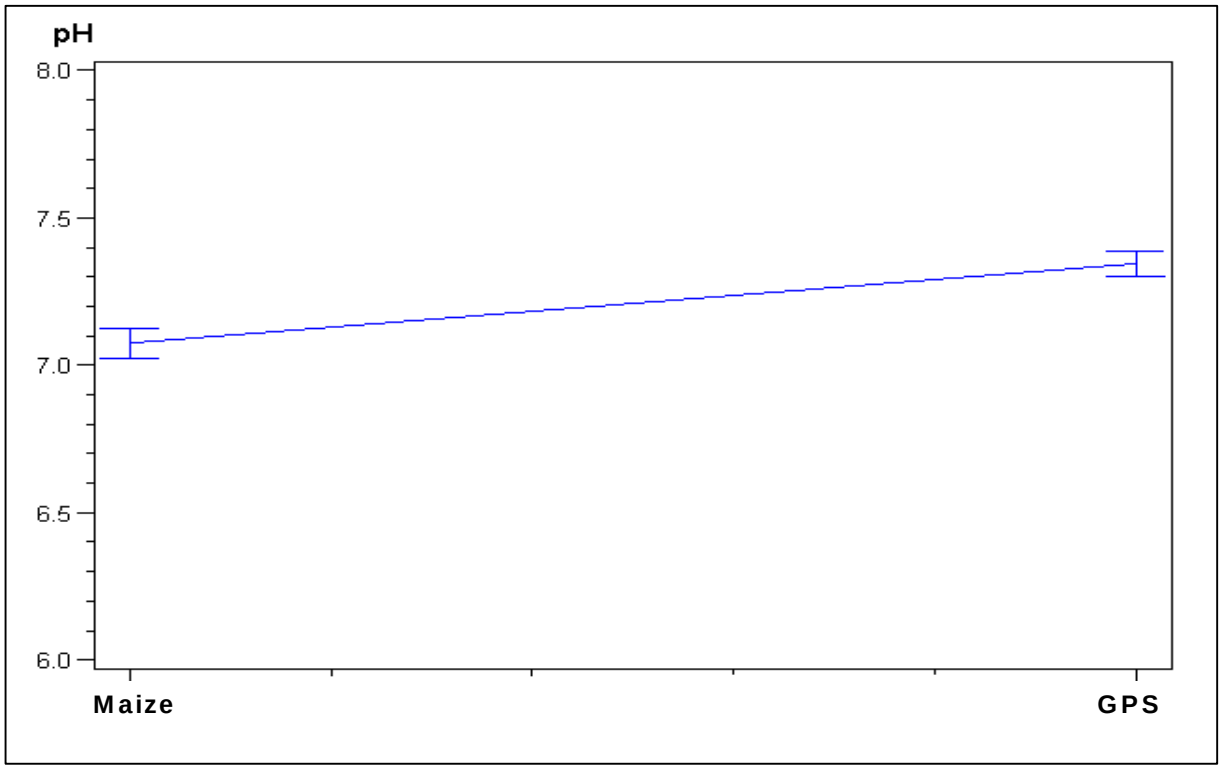

Figure 4.1.4: Results of the co-variance analysis - comparison of pH using *Maize* respectively *GPS* as substrate in the thermophilic reactor

The gas production and methane production (per COD) is on average lower for *Maize* compared to using *GPS* as feed. Amon and co-workers (2007) observed a specific methane yield of 0.398 $m^3_N.kg^{-1}_{VSS}$ (± 0.023 $m^3_N.kg^{-1}_{VSS}$) for the digestion of maize in BMP tests, testing different maize varieties.

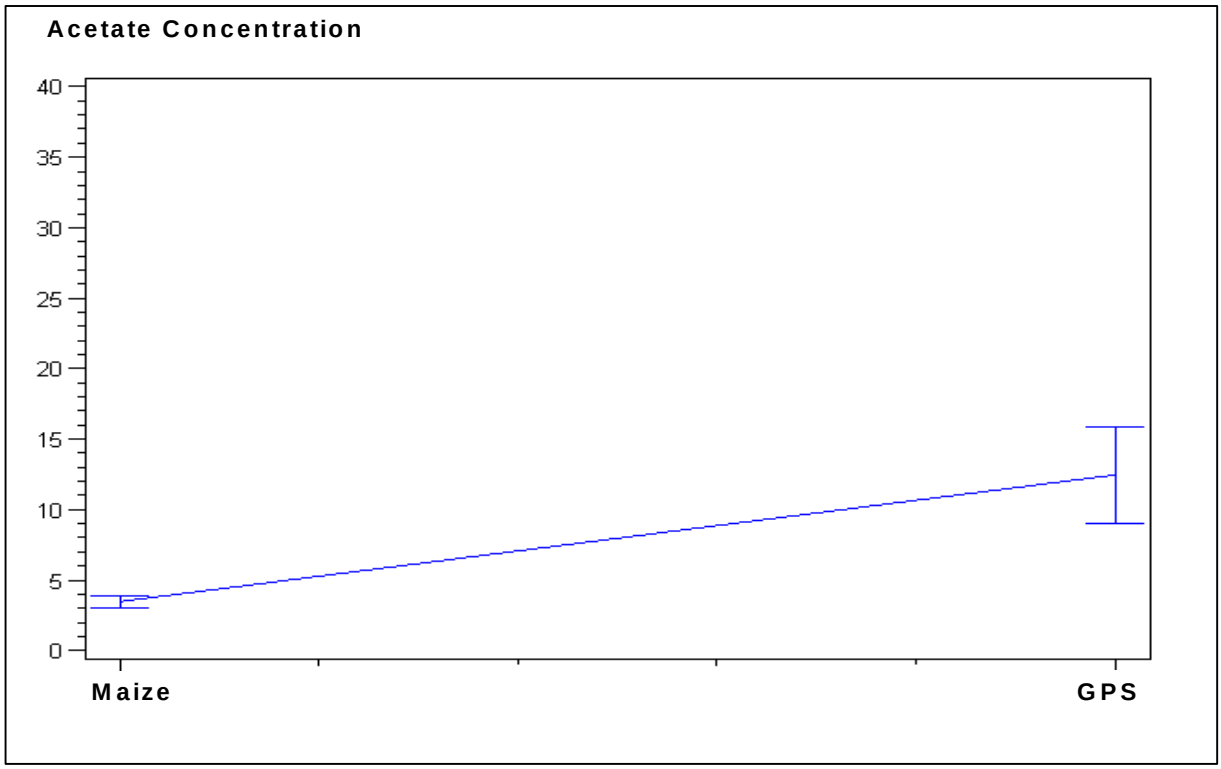

Figure 4.1.5: Results of the co-variance analysis - comparison of acetate concentration using *Maize* and GPS as substrates in the thermophilic reactor

Yet, for the methane yield per hectare differences for the different maize varieties were found, whereby the time of harvesting seems to be the key influencing factor (Amon *et al.* 2007). For wheat a specific methane yield of 0.140 – 0.343 $m^3_N.kg^{-1}_{VSS}$ using BMP test was detected (Amon *et al.* 2007).

The gas production observed is in the same range as the results for other energy crops and substrates. Chynoweth *et al.* (2001)[16] found for Kelp a specific methane production of 0.39 – 0.41 $m^3.kg^{-1}_{VSS}$, for Sorghum 0.26 – 0.39 $m^3.kg^{-1}_{VSS}$, 0.19 – 0.34 $m^3.kg^{-1}_{VSS}$ for Napiergrass, 0.23 – 0.30 $m^3.kg^{-1}_{VSS}$ for Sugarcane, 0.13 – 0.30 $m^3.kg^{-1}_{VSS}$ for Willow and for Avicel Cellulose 0.37 $m^3.kg^{-1}_{VSS}$. Chanakya and co-workers (1997) examined different feed stocks in solid-phase stratified bed fermenter at an ambient temperature of 26 °C. They found for Paddy Straw a gas production of 0.39 $m^3.kg^{-1}_{vss}$, for Bagasse a gas production of 1.24 $m^3.kg^{-1}_{VSS}$ and for cane trash a gas production of 0.46 $m^3.kg^{-1}_{VSS}$ (Chanakya *et al.* 1997) (Table 4.1.9).

Table 4.1.9: Gas and methane production of different energy crops – comparison with data found in literature

Gas Production [$m^3.kg^{-1}_{VSS}$]	Methane Production [$m^3.kg^{-1}_{VSS}$]	Substrate	Temperature Range	Reference
	0.375 - 0.421	maize	mesophile	Amon et al., 2007
	0.140 - 0.343	wheat		
	0.39 - 0.41	Kelp		
	0.26 - 0.39	Sorghum		
	0.19 - 0.34	Napiergrass		Chynoweth et al., 2001
	0.23 - 0.30	Sugarcane		
	0.13 - 0.30	Willow		
	0.37	Avicel Cellulose		
0.39		Paddy Straw		
1.24		Bagasse	mesophile	Chanakya et al., 1997
0.46		cane trash		
0.81 (0.63)	0.41 (0.32)	maize silage	thermophile	
0.55 (0.28)	0.26 (0.14)	whole plant maize silage	thermophile	This Study
0.65 (0.40)	0.32 (0.21)	maize silage	mesophile	
0.70 (0.49)	0.45 (0.34)	sunflower press residues	mesophile	

The acetic acid, the propionic acid and the VFA are lower for *Maize*, hereby the difference is significant for acetic and propionic acid (Figure 4.1.5). The propionic acid concentration shows a very high standard deviation, which is a result of the fact that in most cases the propionic acid concentration is not traceable, especially for lower HRTs. The VSS and TOC concentrations are significantly lower in respect to for the TOC

[16] original paper: Chynoweth, D.P., Turick, C.E., Owens, J.M., Jerger, D.E., Peck, M.W., „Biochemical methane potential of biomass and waste feedstocks", Biomass and Bioenergy, 1993;5:95-111

concentration using *Maize* as substrate, as well. This can be a result of on the one side undegraded material from former experiments remaining in the reactor or higher biomass formed, as no new inoculum was used if a new substrate was tested.
The TAN concentration is significantly higher if *Maize* is used as feed, whereby the sulphate concentration in the reactor and the H_2S concentration in the biogas are higher for the second case, where GPS is used as feed.

These results indicate that it is more efficient to use GPS as substrate and not Maize. First of all the gas production and methane production are higher and considering additionally the harvest per hectare it is much more economical to use the whole plant. However some disadvantages have to be considered, as well. Higher VFA concentrations are found for the GPS, which can easily lead to reactor failure if the process is not controlled properly. Efficient digestion with low volatile fatty acids leads to a higher methane content in the biogas (Farhan et al. 1997).
Yet, the TAN concentration is higher for the Maize, due to the higher protein content, which can lead on the one hand to dangerous NH_3 concentrations in the reactor and on the other hand stabilize the reactor pH.
The Sulphate and H_2S concentration is higher using GPS as feed. This can lead to toxic concentrations in the reactor and therefore to an inhibition of the process (toxic effects appear from 30 mg.l^{-1} (Bischofsberger et al. 2005)). Increasing the process temperature can decrease inhibitory effects (Bischofsberger et al. 2005), because higher temperature means higher solubility of the H_2S in the sludge. Yet, a high H_2S content in the biogas is a problem for CHP or gas turbines. A removal of the H_2S from the gas is thus absolutely necessary.

Comparison between *Maize* and *Sunflower* in the mesophilic reactor system

In the mesophilic reactor FM3 *Maize* and *Sunflower* were compared. The procedure was the same as for the comparison between *Maize* and GPS.
First the mesophilic reactors FM1 and FM3 were compared, to demonstrate that there was no difference between the two mesophilic reactors using *Maize* as substrate, since in the mesophilic reactor FM3 TAN, NH_3 and H_2S concentrations were not measured and thus the data from the mesophilic reactor FM1 was used for the comparison.
Comparing the two mesophilic reactors the pH in FM1 is on average slightly higher compared to the pH in FM3. It is interesting that the acetic acid, propionic acid and the VFA concentrations were also higher in FM1. The gas and methane production is also higher in FM1. The VSS and TOC concentrations were baser in FM1 compared to FM3 (Table 4.1.10).
However no significant difference could be found (Table 4.1.11), except for the VSS concentration, which could be the result of residual biomass in the reactor which was not entirely degraded. Looking at these results the behaviour of the two mesophilic reactors can be seen as equal, thus also the behaviour regarding TAN and H_2S can be taken as similar.

Table 4.1.10: Comparison based on different mesophilic reactors (FM1 and FM3) using *Maize* as substrate (under consideration of the HRT) of the variables pH, Gas Production, Methane Production, Acetic and Propionic Acid and VFA Concentration, VSS and TOC concentration based on the degraded COD. Showing the average values (Mean), the standard deviation (STD), the variance (VAR), the minimum and maximum (MIN and MAX) and the number of samples (n) for both temperature ranges

		MAIZE - FM1					
		Mean	STD	VAR	MIN	MAX	n
pH	[]	7.05E+00	1.93E-01	3.74E-02	6.43E+00	7.58E+00	309
Gas Production/COD	$[m^3 \cdot kg^{-1}_{CODdeg.} d^{-1}]$	9.20E-01	6.54E-01	4.28E-01	1.93E-02	3.08E+00	300
Methane Production/COD	$[m^3 \cdot kg^{-1}_{CODdeg.} d^{-1}]$	4.60E-01	3.31E-01	1.09E-01	0.00E+00	1.56E+00	300
Acetate/COD	$[g.kg^{-1}_{Coddeg}]$	5.90E+00	3.81E+00	1.45E+01	0.00E+00	1.69E+01	284
Propionate/COD	$[g.kg^{-1}_{Coddeg}]$	3.09E+00	5.38E+00	2.90E+01	0.00E+00	3.42E+01	315
VFA/COD	$[g.kg^{-1}_{Coddeg}]$	8.31E+00	6.02E+00	3.63E+01	2.11E-02	2.43E+01	276
VSS/COD	$[kg.kg^{-1}_{Coddeg}]$	1.42E+02	5.74E+01	3.30E+03	4.51E+01	3.47E+02	277
TOC/COD	$[g.kg^{-1}_{Coddeg}]$	2.17E+01	9.86E+00	9.72E+01	5.83E+00	5.24E+01	255

		MAIZE - FM3					
		Mean	STD	VAR	MIN	MAX	n
pH	[]	6.99E+00	2.70E-01	7.32E-02	6.39E+00	7.48E+00	124
Gas Production/COD	$[m^3 \cdot kg^{-1}_{CODdeg.} d^{-1}]$	8.78E-01	5.39E-01	2.90E-01	4.94E-03	2.78E+00	137
Methane Production/COD	$[m^3 \cdot kg^{-1}_{CODdeg.} d^{-1}]$	4.39E-01	2.91E-01	8.48E-02	9.89E-04	1.58E+00	137
Acetate/COD	$[g.kg^{-1}_{Coddeg}]$	3.63E+00	1.91E+00	3.67E+00	2.16E-01	8.75E+00	113
Propionate/COD	$[g.kg^{-1}_{Coddeg}]$	2.31E+00	2.55E+01	6.51E+02	0.00E+00	2.85E+02	125
VFA/COD	$[g.kg^{-1}_{Coddeg}]$	6.73E+00	5.94E+00	3.52E+01	8.27E-03	2.55E+01	118
VSS/COD	$[kg.kg^{-1}_{Coddeg}]$	2.02E+02	6.24E+01	3.89E+03	3.16E+01	3.33E+02	124
TOC/COD	$[g.kg^{-1}_{Coddeg}]$	2.70E+01	1.57E+01	2.47E+02	3.15E+00	6.66E+01	117

Table 4.1.11: Comparison based on different mesophilic reactors (FM1 and FM3) using Maize as substrate (under consideration of the HRT) of the variables pH, Gas Production, Methane Production, Acetic and Propionic Acid and VFA Concentration, VSS and TOC concentration based on the degraded COD. Showing the p-value (P) of the Covariance-analysis and the number of samples used (n). A p-value < 0.05 means that the analysed difference is significant

	P	n
pH	0.897	433
Gas Production/COD	0.325	437
Methane Production/COD	0.659	437
Acetate/COD	0.163	397
Propionate/COD	0.782	440
VFA/COD	0.914	394
VSS/COD	0.000	401
TOC/COD	0.054	372

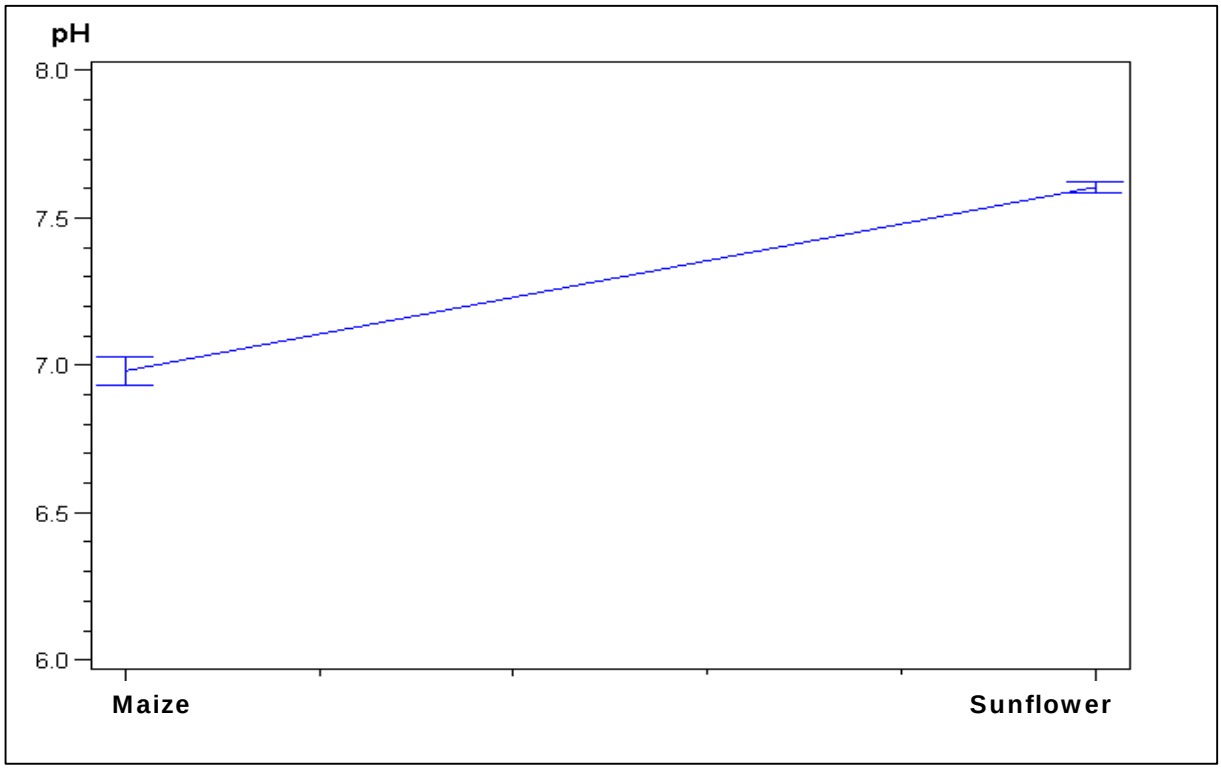

Figure 4.1.6: Results of the co-variance analysis - comparison of pH using *Maize* and *Sunflower* as substrates in the mesophilic reactor

Comparing now the different substrates, it can be observed that the gas production (Table 4.1.12) digesting *Maize* and *Sunflower* shows no significant difference, however the methane production is significantly higher when digesting *Maize* (Table 4.1.13).

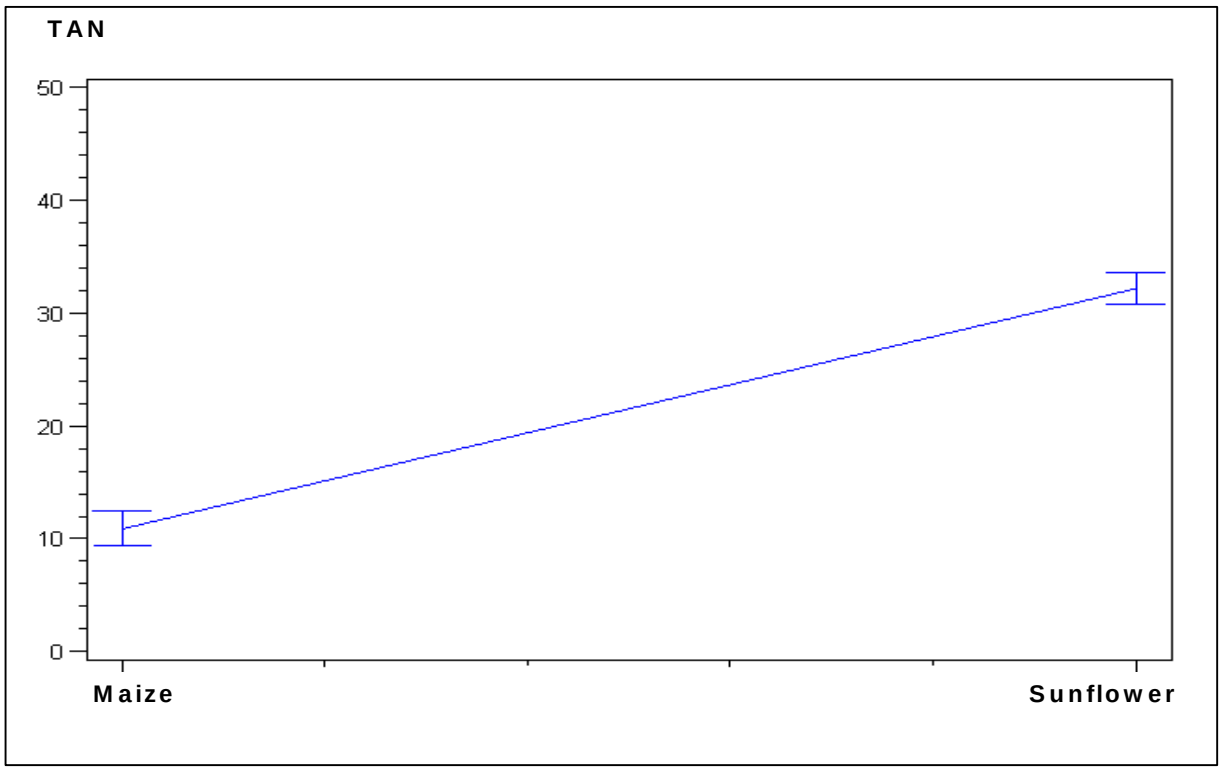

Figure 4.1.7: Results of the co-variance analysis - comparison of TAN concentration using *Maize* and *Sunflower* as substrates in the mesophilic reactor

Table 4.1.12: Comparison based on the different substrates (*Maize* and *Sunflower*; under consideration of the HRT) of the variables pH, Gas Production, Methane Production, Acetic and Propionic Acid and VFA Concentration, VSS, TOC, TAN concentrations based on the degraded COD in the mesophilic reactor FM3. Showing the average values (Mean), the standard deviation (STD), the variance (VAR), the minimum and maximum (MIN and MAX) and the number of samples (n) for both temperature ranges

MAIZE							
		Mean	STD	VAR	MIN	MAX	n
pH	[]	6.98E+00	2.74E-01	7.49E-02	6.39E+00	7.48E+00	125
Gas Production/COD	$[m^3 \cdot kg^{-1}_{CODdeg.} d^{-1}]$	8.88E-01	5.45E-01	2.97E-01	4.94E-03	2.78E+00	126
Methane Production/COD	$[m^3 \cdot kg^{-1}_{CODdeg.} d^{-1}]$	4.25E-01	2.60E-01	6.78E-02	9.89E-04	1.21E+00	124
Acetate/COD	$[g.kg^{-1}_{Coddeg}]$	3.63E+00	1.91E+00	3.67E+00	2.16E-01	8.75E+00	113
Propionate/COD	$[g.kg^{-1}_{Coddeg}]$	2.29E+00	2.54E+01	6.45E+02	0.00E+00	2.85E+02	126
VFA/COD	$[g.kg^{-1}_{Coddeg}]$	6.73E+00	5.94E+00	3.52E+01	8.27E-03	2.55E+01	118
VSS/COD	$[kg.kg^{-1}_{Coddeg}]$	2.02E+02	6.24E+01	3.89E+03	3.16E+01	3.33E+02	124
TOC/COD	$[g.kg^{-1}_{Coddeg}]$	2.70E+01	1.57E+01	2.47E+02	3.15E+00	6.66E+01	117
TAN/COD*	$[g.kg^{-1}_{Coddeg}]$	1.09E+01	6.41E+00	4.11E+01	1.36E+00	2.12E+01	68
NH_3/COD*	$[g.kg^{-1}_{Coddeg}]$	1.26E+00	1.25E+00	1.57E+00	2.69E-02	4.24E+00	65
H_2S/COD*	$[g.kg^{-1}_{Coddeg}]$	4.39E-03	1.85E-03	3.42E-06	1.04E-03	9.04E-03	61

SUNFLOWER							
		Mean	STD	VAR	MIN	MAX	n
pH	[]	7.60E+00	1.08E-01	1.17E-02	7.38E+00	7.97E+00	124
Gas Production/COD	$[m^3 \cdot kg^{-1}_{CODdeg.} d^{-1}]$	9.77E-01	1.26E+00	1.60E+00	6.85E-05	5.20E+00	107
Methane Production/COD	$[m^3 \cdot kg^{-1}_{CODdeg.} d^{-1}]$	3.65E-01	3.05E-01	9.30E-02	4.58E-05	1.36E+00	95
Acetate/COD	$[g.kg^{-1}_{Coddeg}]$	9.89E+00	7.95E+00	6.32E+01	1.41E-01	3.28E+01	118
Propionate/COD	$[g.kg^{-1}_{Coddeg}]$	4.29E+00	5.25E+00	2.76E+01	0.00E+00	2.33E+01	125
VFA/COD	$[g.kg^{-1}_{Coddeg}]$	1.34E+01	1.21E+01	1.47E+02	1.62E-02	5.23E+01	115
VSS/COD	$[kg.kg^{-1}_{Coddeg}]$	3.00E+02	8.06E+01	6.50E+03	1.22E+02	4.41E+02	123
TOC/COD	$[g.kg^{-1}_{Coddeg}]$	1.84E+01	6.36E+00	4.04E+01	8.70E+00	3.78E+01	113
TAN/COD	$[g.kg^{-1}_{Coddeg}]$	3.26E+01	3.88E+00	1.51E+01	2.46E+01	4.19E+01	57
NH_3/COD	$[g.kg^{-1}_{Coddeg}]$	1.52E+01	9.26E+00	8.57E+01	6.95E-01	3.23E+01	60
H_2S/COD	$[g.kg^{-1}_{Coddeg}]$	2.54E-01	4.46E-02	1.99E-03	1.80E-01	3.76E-01	58

Table 4.1.13: Comparison based on the different substrates (*Maize* and *Sunflower*; under consideration of the HRT) of the variables pH, Gas Production, Methane Production, Acetic and Propionic Acid and VFA concentration, VSS, TOC, TAN concentration, NH3 and H2S concentration based on the degraded COD in the mesophilic reactor FM3. Showing the p-value (P) of the Covariance-analysis and the number of samples used (n). A p-value < 0.05 means that the analysed difference is significant

	P	n
pH	0.000	249
Gas Production/COD	0.297	233
Methane Production/COD	0.010	219
Acetate/COD	0.063	231
Propionate/COD	0.261	251
VFA/COD	0.151	233
VSS/COD	0.003	247
TOC/COD	0.041	230
TAN/COD	0.000	125
NH_3/COD	0.000	125
H_2S/COD	0.000	119

No significant difference could be found for the fatty acid concentration. The main difference was the higher pH (Figure 4.1.6), TAN (Figure 4.1.7), NH3 and H_2S concentrations if *Sunflower* was used as feed, which is a result of the high protein content in the sunflower press residues.

Large increases in the ammonia nitrogen can cause inhibition of hydrogen and gas production (Sterling Jr. *et al.* 2001). The very high ammonia values for *Sunflower* (up to 4900 $mg_{NH4}l^{-1}$ and 260 $mg_{NH3}l^{-1}$) (NH_3 is inhibiting from 100 – 200 $g_N.l^{-1}$ (Henze and Harremoes 1983), however, is only in part a problem as the reactor got used to it. As adaptation can increase the ammonia tolerance of the micro-organism (Sung and Liu 2003). On the other hand increases in already high TAN concentration can stabilise the pH in the reactor. Nevertheless attention has to be paid to this high ammonia and ammonium concentrations during reactor operation. A further problem is the high H_2S concentration when digesting *Sunflower*.

This comparison shows that Maize as a substrate has an advantage over Sunflower – for the gas production no difference was found, however the methane production is higher using Maize (no yields per hectare are considered in this comparison). Moreover high ammonia nitrogen values and the high H_2S concentrations can lead to fatal reactor failure if the reactor is not adapted to the Sunflower substrate. Yet if sunflower press residues are digested it is advisable to mix them with substrates of low protein content, for example potatoes or Sudan grass.

4.1.3 Mesophilic vs. thermophilic

Temperature is an important factor among the many factors affecting the anaerobic digestion process (El-Mashad *et al.* 2004). Traditionally one distinguishes between psychrophile (< 20 °C), mesophile (20 – 40 °C) and thermophile (> 40 °C) temperature ranges (Bischofsberger *et al.* 2005).

As with all chemical reactions, bio-chemicals reactions are also strongly temperature dependant (Bischofsberger *et al.* 2005). Increasing the temperature from 15 °C to 35°C already results in an increase in the gas production of about 20 % to 60 % (Braun 1982). This can be observed in the hydrolysis efficiency, as well, which is much greater in the mesophilic and thermophilic temperature ranges than in the psychrophilic temperature range (Bolzonella *et al.* 2005). Nevertheless the same processes take place in the mesophilic as in the thermophilic plant operation (Ahring 1994).

Two temperature optima are given in mesophilic range at 35 °C and in thermophilic temperature range at 48 to 55 °C (Bischofsberger *et al.* 2005), whereby most technical plants work in the mesophile range (35 – 37 °C) (Ahn and Forster 2000; Gavala *et al.* 2003). This seemed to be the best compromise, as the kinetic of the biogas process in the psychrophile range is too slow to be economical and the thermophilic process has a lower stability compared to the mesophilic process (van Lier *et al.* 1996; Gavala *et al.* 2003). Equally the thermophilic anaerobic digestion process is more sensitive to toxicity and environmental changes (Ahn and Forster 2000; Gerardi 2003; Sung and Liu 2003), has a lack of diversity of anaerobes (Gerardi 2003), has relatively high residuals values

for VFAs (Gerardi 2003) and additionally needs longer start-up times (Henze and Harremoes 1983; Ahring 1994; El-Mashad *et al.* 2004). This is a result of the low net yield (Henze and Harremoes 1983; Speece 1996; Gerardi 2003). But this instability is often due too few safety factors and very poor process control (Henze and Harremoes 1983).

But this start-up time can be minimized by using thermophilic sludge as an inoculum (Ahring 1994). Yet, reactors operating at lower temperatures are more sensitive to organic variations (Leitao et al. 2006)

However, in the last years a new phenomena in biogas plants using protein rich substrates (normally energy crops, mostly maize silage) was detected, the self heating of the plants (Lindorfer 2007). Here reactors operating in the mesophilic temperature range heated themselves up to about 40 °C (Lindorfer 2007), which lies already in the lower boundary of the thermophilic temperature range. This raises the question of whether it is more economical and efficient to cool down the reactor or to operate the plant in the thermophile temperature range.

To find the optimum temperature range, two mesophilic reactors (FM1 and FM3), operating at 35 °C (± 1 °C), were compared with the two thermophilic reactors (FM2 and FM4), operating at 60 °C (± 1 °C) (Table 4.1.4). The procedure was the same as before for the comparison of the different substrates.

The gas production and the methane production are significantly higher in the thermophilic reactors (Figure 4.1.10). This is in accordance with the results of Mackie and Bryant (1995). They also found a higher gas methane production in the thermophilic reactor using cattle waste as substrate. Bouallagui *et al.* (2004) came to similar results for the digestion of fruit and vegetable wastes. Ahn and Forster (2000) showed in their comparative study in anaerobic upflow filters that at low loading rates no difference in the methane yield could be found, however, at higher OLR the mesophilic filter showed a significantly lower efficiency. The same effect was found for the SCOD removal rate (Ahn and Forster 2000). Gavala and co-workers (2003) observed no significant difference in their work between the mesophilic and the thermophilic when digesting primary and secondary sludge. This, however, seemed to be the result of operation at relatively high retention times in this study and they further concluded that a significant difference would be evident at lower retention times (Gavala *et al.* 2003). This is consistent with Varel *et al.* (1980), they concluded from their results that the effect of temperature is more apparent at short than at long retention times, for HRTs of longer than 6 days no difference between the methane production rate at 40 °C and 60 °C was registered. Gallert and Winter (2002) observed a specific biogas production of 0.6 – 1.1 $m^3.kg^{-1}_{COD}$ for different substrates (sewage sludge, biowaste, manure, food production residues,…), whereby they discovered no significance difference between the digestion in the mesophilic and thermophilic temperature ranges. De la Rubia and co-workers (2001) determined a higher methane production in the mesophilic reactor for HRTs > 20 d, but better values for the thermophilic reactor at low HRTs (< 20 d) using sewage sludge as substrate in a pilot-scale CSTR. Depending on the substrate the methane production rate

is about 25 to 50 % higher in the thermophilic process compared to the mesophilic process (Henze and Harremoes 1983).

The pH showed no significant difference. Nevertheless, on average the pH in the mesophilic reactors was lower than in the thermophilic reactors.

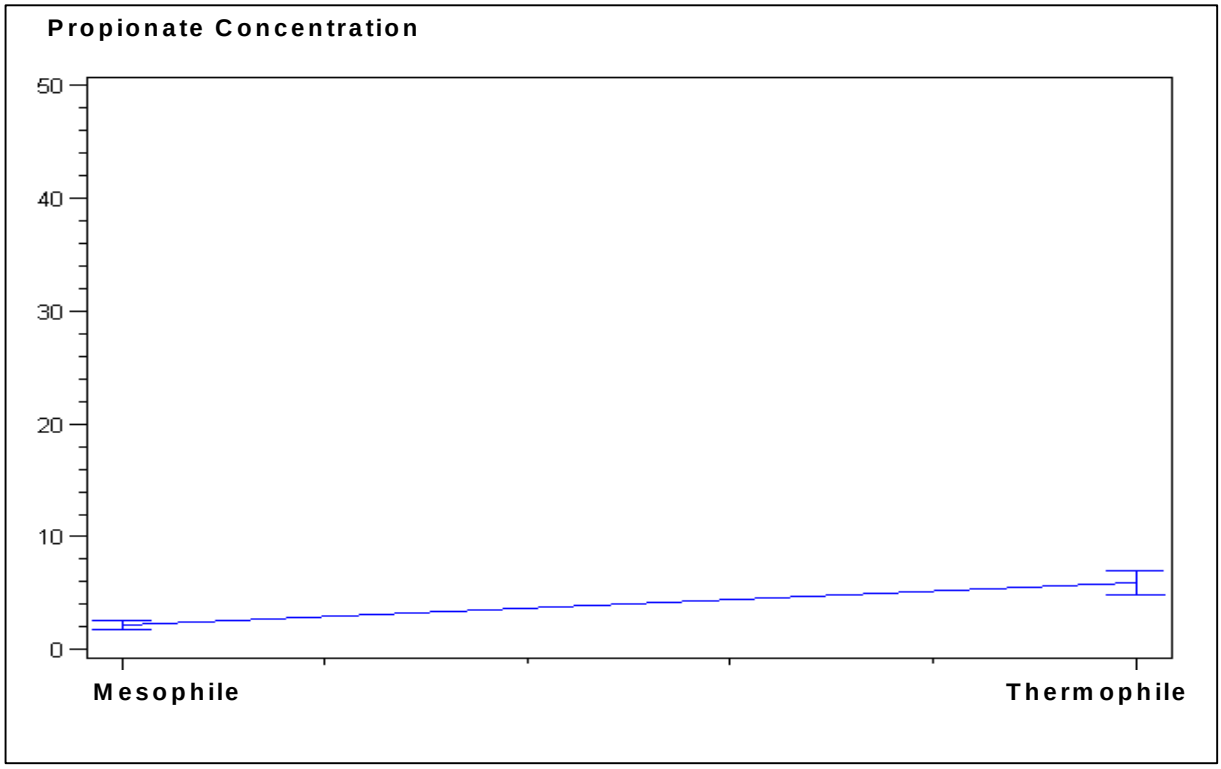

Figure 4.1.8: Results of the co-variance analysis - comparison of propionate concentration using *Maize* as substrate in the mesophilic and thermophilic reactors

The higher pH in the thermophilic reactor seems to be a result of the higher TAN concentration in the thermophilic reactor. The higher ammonia concentration is a result of the more complete breaking down of protein (Speece 1996). The VFA concentration is, on average, in both cases nearly the same, but shows a small, but nevertheless a significant difference. While, the acetic concentration is higher in the mesophilic reactor than in the thermophilic reactor on the other hand the propionic acid concentration in the thermophilic reactor is significantly higher than in the mesophilic reactor. Mackie and Bryant (1995) also registered in their study a generally higher VFA content in the mesophilic reactor. However, in the thermophilic AD process a vastly higher VFA content often manifests itself (Speece 1996), in particular propionate, when compared to the mesophilic process, which has its origin amongst others in the non-ideal conditions for all members of the bacteria consortium (Kim *et al.* 2002). Kiyohara *et al.* (2000) found that, even though the activity of thermophilic bacteria was higher, the thermophilic bacteria tended to remove propionic acid more slowly than mesophilic bacteria. A decrease in the temperature normally correspond to a decrease in the VFA production (Bolzonella *et al.* 2005). De la Rubia (2001) registered that the VFA concentration in the effluent, under the same conditions, was higher in the thermophilic reactor than in the mesophilic reactor, where most of these VFA was propionate in the thermophilic process.

The VSS and TOC concentration were higher in the mesophilic reactor compared to the reactors with higher temperatures. The H_2S concentration in the gas phase is significantly higher in the thermophilic reactor than in the mesophilic, which can be explained by the lower solubility of H_2S in sludge at higher temperatures.

The COD removal is higher in the thermophilic reactor compared to the mesophilic reactor (Kiyohara *et al.* 2000).

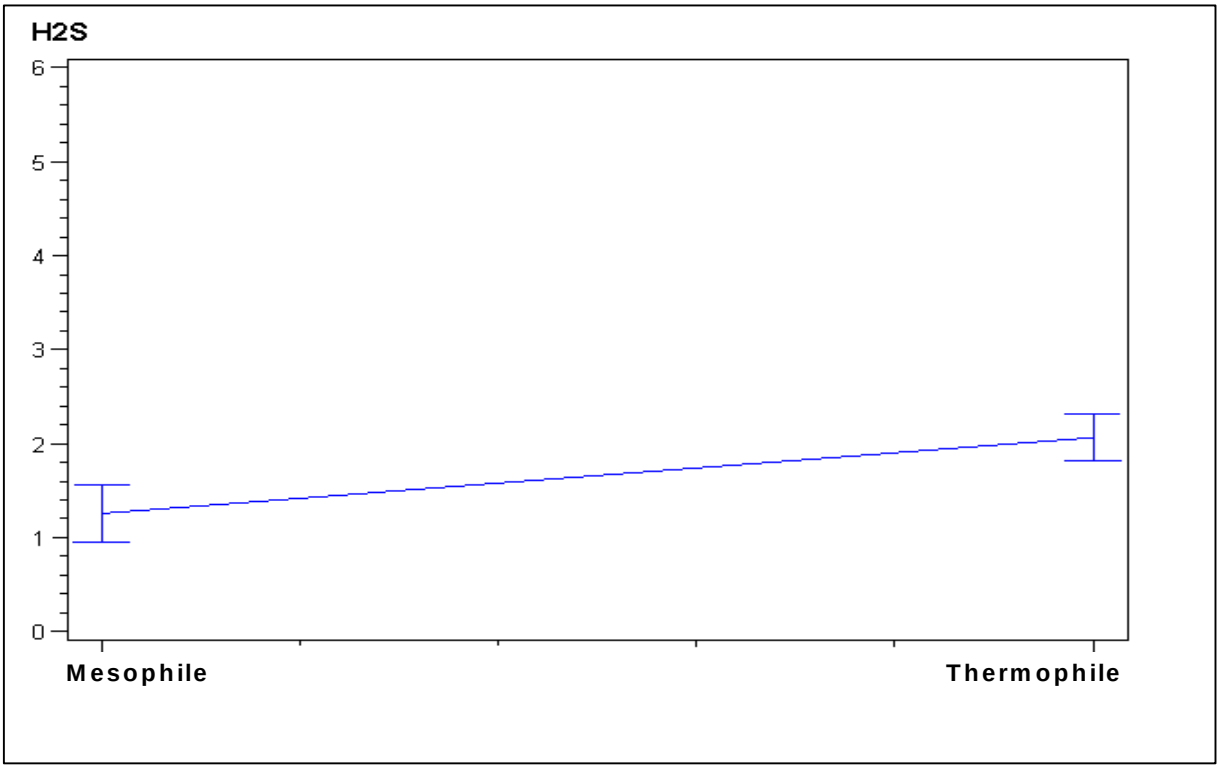

Figure 4.1.9: Results of the co-variance analysis - comparison of H_2S concentration using *Maize* as substrate in the mesophilic and thermophilic reactors

These results again confirm the higher gas yield when operating the plant in the thermophilic temperature range. Kim and co-workers (2006), for example, found the optimum temperature to be at 50 °C, whereas Ahring (1994) detected 60 °C as the optimum temperature to digest manure, but recommended that the reactor be operated at under 60 °C to ensure that fluctuations in temperature have no disastrous effect on the microbial activity. Varel *et al.* (1980) suggested that there is no advantage in increasing the fermenting temperature of waste from 50 to 60 °C.

Generally it can be said, AD in the thermophilic temperature range is more efficient in terms of COD removal and methane production than operation in the mesophilic temperature range (Gavala *et al.* 2003). Moreover the thermophilic process has the benefit, to a great extent, it destroys pathogens (El-Mashad *et al.* 2004). A further advantage is the less viscous sludge in the thermophilic reactor compared to the mesophilic reactor, therefore less energy is needed for stirring.

Table 4.1.14: Comparison based on the different temperature ranges (mesophile and thermophile; under consideration of the HRT) of the variables pH, Gas Production, Methane Production, Acetic and Propionic Acid and VFA Concentration, VSS, TOC, TAN concentrations based on the degraded COD using *Maize* as substrate. Showing the average values (Mean), the standard deviation (STD), the variance (VAR), the minimum and maximum (MIN and MAX) and the number of samples (n) for both temperature ranges

		MESOPHILE					
		Mean	STD	VAR	MIN	MAX	n
pH	[]	7.03E+00	2.28E-01	5.18E-02	6.13E+00	7.63E+00	445
Gas Production/COD	$[m^3.kg^{-1}_{CODdeg}.d^{-1}]$	8.63E-01	4.37E-01	1.91E-01	4.94E-03	2.15E+00	439
Methane Production/COD	$[m^3.kg^{-1}_{CODdeg}.d^{-1}]$	4.27E-01	2.19E-01	4.81E-02	0.00E+00	1.08E+00	437
Acetate/COD	$[g.kg^{-1}_{Coddeg}]$	5.25E+00	3.48E+00	1.21E+01	0.00E+00	1.58E+01	409
Propionate/COD	$[g.kg^{-1}_{Coddeg}]$	2.18E+00	4.72E+00	2.23E+01	0.00E+00	3.42E+01	449
VFA/COD	$[g.kg^{-1}_{Coddeg}]$	7.86E+00	6.11E+00	3.74E+01	8.27E-03	2.69E+01	405
VSS/COD	$[kg.kg^{-1}_{Coddeg}]$	1.66E+02	7.51E+01	5.64E+03	3.16E+01	4.39E+02	420
TOC/COD	$[g.kg^{-1}_{Coddeg}]$	2.00E+01	1.32E+01	1.75E+02	0.00E+00	6.25E+01	430
TAN/COD	$[g.kg^{-1}_{Coddeg}]$	1.09E+01	6.41E+00	4.11E+01	1.36E+00	2.12E+01	68
H_2S/COD	$[g.kg^{-1}_{Coddeg}]$	1.26E+00	1.25E+00	1.57E+00	2.69E-02	4.24E+00	65
		THERMOPHILE					
		Mean	STD	VAR	MIN	MAX	n
pH	[]	7.13E+00	2.91E-01	8.49E-02	6.20E+00	7.72E+00	369
Gas Production/COD	$[m^3.kg^{-1}_{CODdeg}.d^{-1}]$	1.12E+00	8.86E-01	7.84E-01	0.00E+00	4.09E+00	367
Methane Production/COD	$[m^3.kg^{-1}_{CODdeg}.d^{-1}]$	5.70E-01	4.48E-01	2.01E-01	0.00E+00	1.97E+00	366
Acetate/COD	$[g.kg^{-1}_{Coddeg}]$	3.69E+00	3.05E+00	9.30E+00	0.00E+00	1.52E+01	332
Propionate/COD	$[g.kg^{-1}_{Coddeg}]$	5.93E+00	1.02E+01	1.05E+02	0.00E+00	4.51E+01	373
VFA/COD	$[g.kg^{-1}_{Coddeg}]$	7.73E+00	7.75E+00	6.01E+01	0.00E+00	3.41E+01	329
VSS/COD	$[kg.kg^{-1}_{Coddeg}]$	1.99E+02	9.90E+01	9.80E+03	0.00E+00	5.44E+02	371
TOC/COD	$[g.kg^{-1}_{Coddeg}]$	2.60E+01	1.53E+01	2.33E+02	0.00E+00	7.59E+01	360
TAN/COD	$[g.kg^{-1}_{Coddeg}]$	7.15E+00	7.14E+00	5.10E+01	0.00E+00	2.68E+01	322
H_2S/COD	$[g.kg^{-1}_{Coddeg}]$	2.06E+00	1.17E+00	1.37E+00	1.91E-02	5.47E+00	86

Table 4.1.15: Comparison based on the different substrates (*Maize* and *Sunflower*; under consideration of the HRT) of the variables pH, Gas Production, Methane Production, Acetic and Propionic Acid and VFA concentration, VSS, TOC, TAN concentration, NH_3 and H_2S concentrations based on the degraded COD in the mesophilic reactor FM3. Showing the p-value (P) of the Covariance-analysis and the number of samples used (n). A p-value < 0.05 means that the analysed difference is significant

	P	n
pH	0.316	814
Gas Production/COD	0.000	806
Methane Production/COD	0.000	803
Acetate/COD	0.000	741
Propionate/COD	0.000	822
VFA/COD	0.007	734
VSS/COD	0.000	791
TOC/COD	0.000	790
TAN/COD	0.351	390
H_2S/COD	0.000	151

However, more problematic is the higher propionic acid concentration, which supports the argument of the instable process in the thermophilic temperature range. Nevertheless with adequate reactor control this is not a real problem. El-Mashard and co-workers (2004) also concluded, from their results that a stable digester operation under thermophilic conditions is very possible.

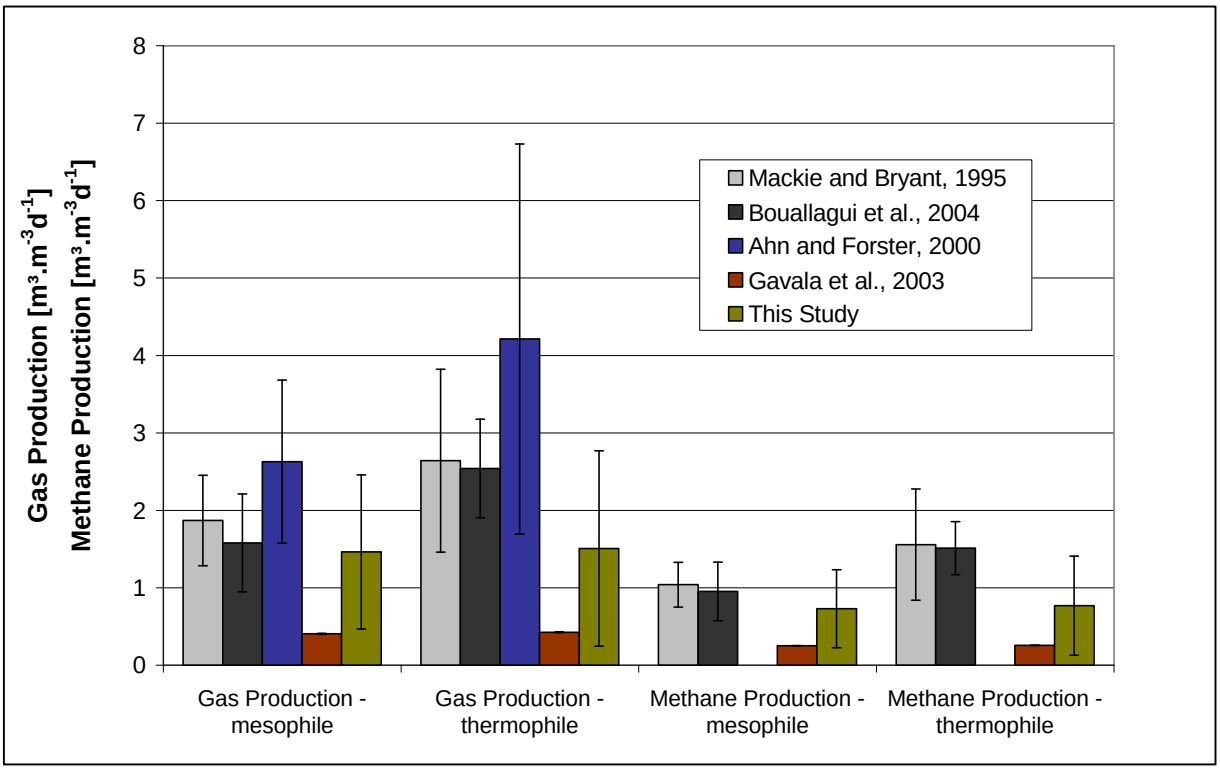

Figure 4.1.10: Comparison of the gas production and methane production in the mesophilic and thermophilic temperature range. Comparison of literature data (mean values): Bryant and Mackie (1995) digested cattle waste in 5 l bench-top fermenters. Bouallagui (2004) examined the performance of an anaerobic tubular reactor treating fruit and vegetable waste. Ahn and Forster (2000) used an anaerobic upflow filter digesting a starch based feed. Gavala *et al.* (2003) digested primary and secondary sludge in 1 l digesters; with data measured in this study (mean values, *Maize*, thermophile and mesophile). This data is compared to data measured in this study using *Maize* as substrate – as only the mean values over all HRTs is shown, the difference between the mesophilic and the thermophilic temperature ranges is rather small and the standard deviation high.

The TAN and H_2S problematic was already mentioned previously. Free ammonia is the active component causing an inhibiting effect (Angelidaki and Ahring 1993).

The ammonia inhibition is normally a type of self regulative process, as the inhibition of the process increases the fatty acid concentration and therefore decreases the pH in the reactor. This effect, in turn, shifts the equilibrium to the ammonium and thus reduces the inhibiting effect. Not only the pH influences the ammonia concentration, but also the temperature (Angelidaki and Ahring 1993)– which leads to higher ammonia levels in thermophilic reactors. But Angelidaki and Ahring (1993) detected that a stable reactor performance can be attained if the reactor is adapted to high ammonia concentrations.

In the thermophilic reactor, the solubility of the H_2S is worse; this reduces the concentration of the toxic H_2S in the sludge. Higher H_2S concentrations in the biogas, however, can lead to problems of corrosion in the CHP or gas turbine. So, the H_2S has to be removed from the gas. This is normally done by blowing air in the gas phase of the reactor.

The main disadvantage of reactor operation in the thermophilic range is the high energy consumption required to heat up the reactor. But if self heating occurs there is no need to heat the reactor. On the other hand the reactor has to be cooled down to keep the process within the mesophilic temperature range. But taking into account the results

observed as well as the literature it can be concluded that it make no sense to waste energy in order to keep the process within the mesophilic temperature range. Yet, one should ensure that there are no abrupt changes in the temperature, as variations in the temperature can dramatically affect the performance of the AD process (Leitao *et al.* 2006).

No general temperature optimum can be found. In the thermophilic temperature range the gas and methane production is significantly higher then in the mesophilic temperature range, however the VFA concentration is also significantly higher in the thermophilic range. Yet the choice of temperature depends on several criteria: one important criteria is the bio-climatic conditions (Bouallagui et al. 2004) – which have an important influence on the energy balance of the reactor. The substrate used also has a great impact on the optimum temperature, especially its sulphate and ammonia content.

4.1.4 Pulse experiments

In order to obtain, on the one hand, a data set with a wide range for the purpose of modelling and on the other hand to glean further information on the process kinetics, pulse experiments were performed.

Pulse experiment in the mesophilic reactor system FM1

One experiment was accomplished in the mesophilic reactor system FM1 (Figure 4.1.11), where the reactor was fed at intervals of 2 to 4 days with a feed peak. Hereby an OLR of 4.6 to 9.4 $kg_{COD}m^{-3}_{Reactor}d^{-1}$ with *Maize* and an OLR of 3.4 to 7 $kg_{COD}m^{-3}_{Reactor}d^{-1}$ with *Sunflower* was attained.

During the pulse experiment the overall methane content in the biogas decreased from 72.53 % to 31.42%, whereby the methane content always fell one day after a feed peak by about 5 to 25 percentage points and returned to a “normal” level afterwards. The overall biogas production also declined in the period observed, as an inverse effect to the methane content was noticed, one day after a feed peak the biogas production increased by about 0.2 $m^3_{Biogas}.m^{-3}_{Reactor}.d^{-1}$ to 2.3 $m^3_{Biogas}.m^{-3}_{Reactor}.d\text{-}^{1}$, and subsequently returned to the overall average.

The VFA concentration (Figure 4.1.12) increased during the whole experiment from 5578 $mg.l^{-1}$ to 12649 $mg.l^{-1}$, while the acetic acid concentration went from 2341 $mg.l^{-1}$ to 4396 $mg.l^{-1}$ and the propionic acid rose from 2451 $mg.l^{-1}$ to 5578 mg.l-1, at the same time the pH decreased from 7.06 to 6.53. In the same manner, the COD degradation efficiency fell from 84 % to 62 %:

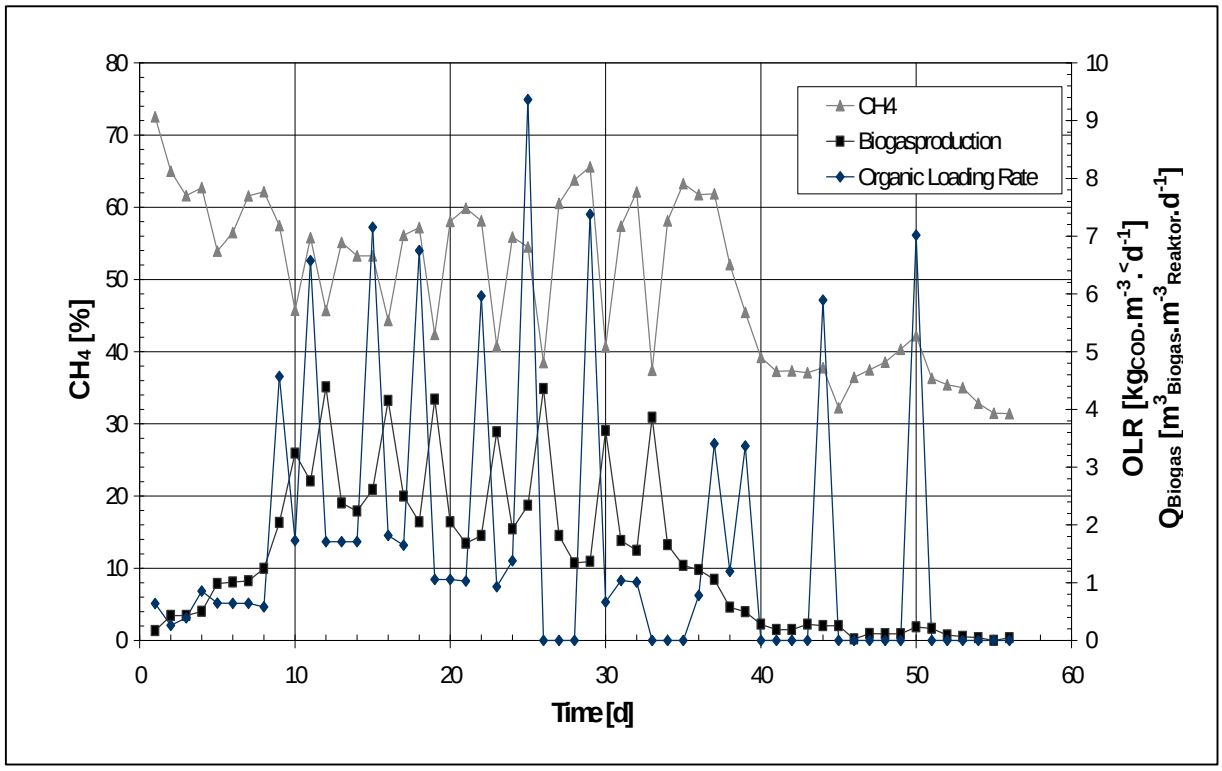

Figure 4.1.11: Results of the pulse experiments with maize silage and sunflower residues in the mesophilic reactor system FM1

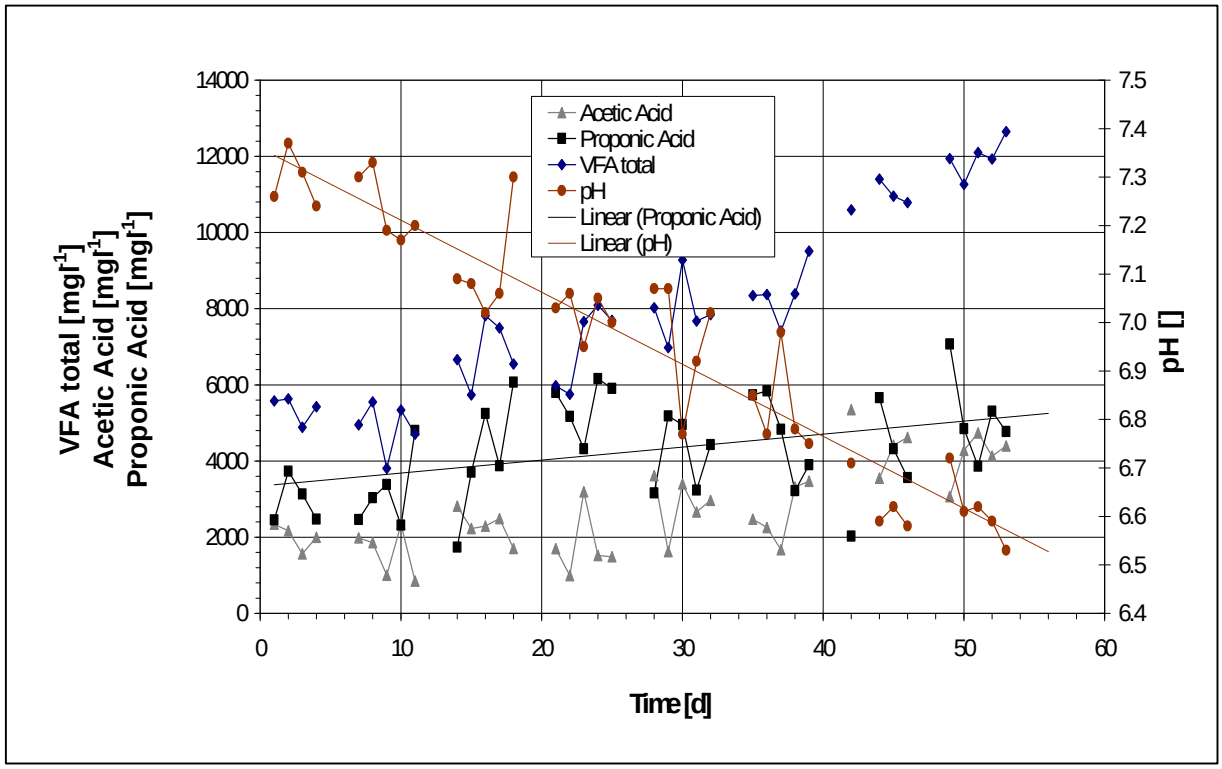

Figure 4.1.12: Results of the pulse experiments with maize silage and sunflower residues in the mesophilic reactor system FM1

The correlation between the decrease of the methane content, increase of the biogas production and the feed peak can be clearly seen, whereby the system reacts rather slowly. As a change in the biogas production and the methane content was only observed after one day. Borja and Banks (1995) tested shock loads in a fluidised bed reactor treating ice cream waste water.

They observed a decrease in the pH, an increase in the effluent VFA and COD and an increase in the gas production, but a decrease in the methane production, as well (Borja and Banks 1995). This change in the methane-carbon dioxide ratio indicates a process overload and can be explained by an inhibition of the methanogenesis (Borja and Banks 1995) and the decreased solubility of CO_2 at low pH values.

Observing the VFA and the pH no direct response to the feed peaks was noticed, except the overall accumulation of the fatty acids and the decrease of the pH over time, which explains also the fall in the methane production.

It was further found that after the change of the substrate from *Maize* to *Sunflower* at day 35 the H_2S content in the gas increased up to 2,000 ppm and ammonium concentration rose from 825.66 ± 124.93 $mg.l^{-1}$ on average to 2287.48 ± 1191.63 $mg.l^{-1}$ on average, whereas the ammonia concentration remained nearly the same due to the change in pH. The increase of the H_2S and the ammonium concentration at the end of the experiment is a result of the high protein content of *Sunflower*.

The amount of SS (33 ± 4.06 on average) and VSS (24.38 ± 4.32 on average) did not vary very much during the experiment time.

Pulse experiment in the thermophilic reactor system FM2

A second experiment was carried out in the thermophilic reactor system FM2 (Figure 4.1.13), whereas the reactor was charged as before at intervals from 2 to 4 days with a feed peak, here an OLR of 4.6 to 6.2 $kg_{COD}m^{-3}_{Reactor}d^{-1}$ with *Maize* was reached.

As in the mesophilic reactor the overall methane content in the biogas decreased during the pulse experiment - from 61.08 to 40.05. Here, contrary to the mesophilic reactor system no distinctive change in the methane content could be observed. This could have its origin in the lower solubility of gases at higher temperatures.

All anaerobic reactors react in a similar way, if exposed to short changes in process conditions. These change of conditions results in an increase in the VFA concentration, a drop in the pH and a rush of the CO_2 and H_2 content in the biogas, due to incomplete methanogenesis (Leitao *et al.* 2006).

The overall biogas production increased over the period observed. Furthermore one day after a feed peak the biogas production increased like in the mesophilic reactor, here however for about 0.6 $m^3_{Biogas}.m^{-3}_{Reactor}.d^{-1}$ to 3.0 $m^3_{Biogas}.m^{-3}_{Reactor}.d\text{-}^{1}$, and subsequently returned to the overall average.

Yet, the pulse experiments also lead in this case to a break-down of the process. The VFA concentration (Figure 4.1.12) increased during the whole experiment from 567 $mg.l^{-1}$ to 7172 $mg.l^{-1}$, while the acetic acid concentration went from 456 $mg.l^{-1}$ to 3560 $mg.l^{-1}$ and the propionic acid rose from 0 $mg.l^{-1}$ to 4348 $mg.l^{-}1$. The accumulation of VFAs is a typical effect during reactor overload in the organic loading rates (Leitao *et al.* 2006). The pH decreased from 7.56 to 5.64, which resulted in an inhibition of the process and a drop in the COD degradation efficiency, which fell from 90 % to 76 %.

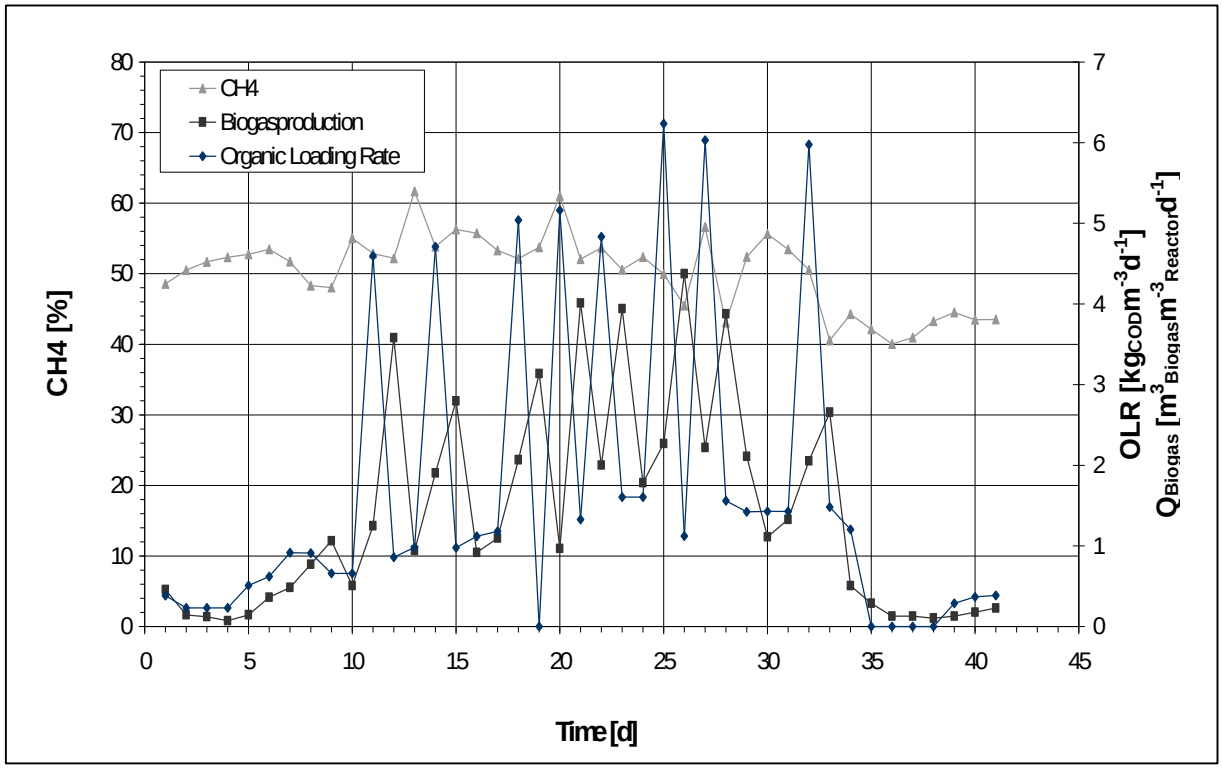

Figure 4.1.13: Results of the pulse experiments with maize silage in the thermophilic reactor system FM2

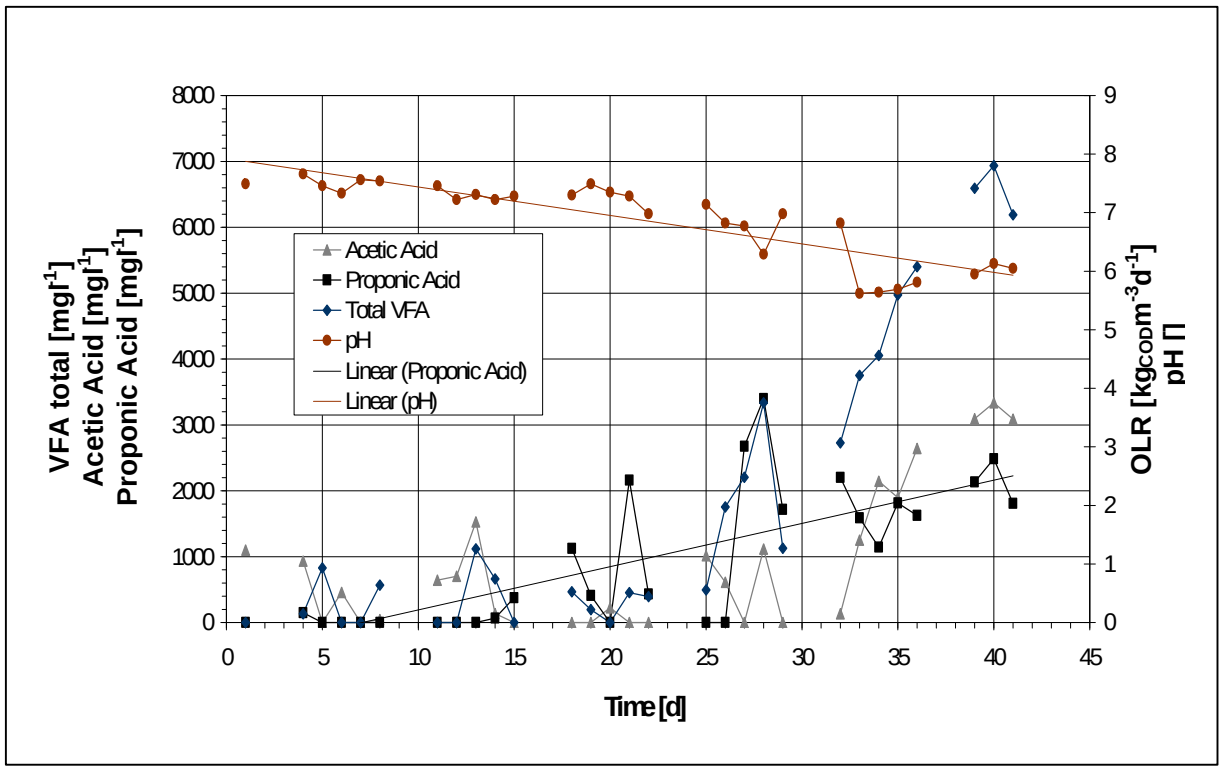

Figure 4.1.14: Results of the pulse experiments with maize silage in the thermophilic reactor system FM2

Normally the gas produced from a days feed emerges within one day (Gerardi 2003). This can also be seen in both experiments since most of the highest gas production levels are found within 24h of the prior feed peak.

4.2 Calibration of an AD model

Before a model can be used for any application it has to be calibrated, in the sense that the parameter values are chosen in such a way that the best possible conformity between the model predictions and the data measured can be obtained (Madsen 2000).
The easiest case for calibration is, if all parameters are known and no optimisation is necessary (De Pauw 2005) - however this is not normally the case. Model calibration can be done in different ways either manually (trial and error), following a calibration protocol or using automatic optimisation and calibration tools (Madsen 2000). No matter how the calibration is done, calibrating a model is always time consuming.
Most of the models which describe such complicated dynamic processes show a high degree of uncertainty, as the knowledge of the process is incomplete and the kinetic parameters are not normally known (Kremling and Saez-Rodriguez 2007). The prediction capability of a model like the ADM1 can be further lowered in the case that some parameters are unknown or inaccurate, as well as if the model structure itself is not accurate (Aceves-Lara *et al.* 2005).
This study endeavoured to address comprehensively all issues of the calibration problem; however the calibration of such a model entails a lot of different questions and problems, which makes it impossible to deal with all issues in detail within the framework of this study.
One of the first problems is: which variables have to be measured, which variables can be measured directly or which variables can be determined within the framework of the project. Moreover what possibilities exist for estimating variables and parameters that cannot be measured directly? The data measured has to be further processed for use in the model due to measurement errors, missing data, experimental problems, and so on as there is no possibility for modelling unexpected disturbances (Olsson and Newell 1999).
The original ADM1 entails around 100 different parameters that make it necessary to pre-select the parameters which are de facto calibrated in the model. This pre-selection can either be done manually or with the assistance of a sensitivity analysis.
The parameter estimation itself can be done by trial and error, using data from literature or by determining the parameter required through experimentation. Whereby one has to pay attention to the high range for some parameters in literature and on the other hand to the fact that standard experiments for estimating different parameters are in the most cases rather voluminous.
A further question is the best way to calibrate the model. Automatic calibration algorithms are a very “elegant” solution, however these algorithms have to be used with care. Due to the fact that such complex biological models as the ADM1 normally have too many state variables to be observable and too many parameters to be identifiable – as there are too many parameters as feasible measurements (Olsson and Newell 1999). Whereby one can distinguish between structural identifiability – if there is only one set of

parameter valid for the model and practical identifiability – if there are no local minimums (Bernard *et al.* 2001) and thus the parameters can be estimated from a given set of experimental data ((Gadkar, 2005) cited in (Kremling and Saez-Rodriguez 2007))[17].
The problem of model identifiability could not be covered in more depth within the framework of this project. Yet differently to literature dealing with the similar complex models ((Petersen 2000), (Olsson and Newell 1999)) it can be concluded that the ADM1 is non-identifiable. This fact greatly restricts the application of automatic calibration algorithms for the ADM1. In addition calibration algorithms often have convergence problems if a large number of parameters occurs (Olsson and Newell 1999).
Yet the problem of non-identifiability equally applies if calibration protocols are applied or parameters are calibrated by trial and error. Whereby it has to be mentioned that, to our knowledge, no complete calibration protocols exist until now for the ADM1. One approach to avoiding the problem of non-identifiability is to divide the problem in smaller fitting problems (Olsson and Newell 1999).

4.2.1 Suggested Measurements for the calibration of the ADM1

Generally it can be said that models of biotechnological processes are normally in need of a substrate characterization in a specific form (Kleerebezem and Van Loosdrecht 2004). The ADM1 requires the identification of the concentrations of particulate carbohydrates, proteins, lipids, soluble, soluble sugars, amino acids and fats and VFAs (Kleerebezem and Van Loosdrecht 2004).
Erashin and co-workers (2005) found, moreover, that the influent characterization values had a great impact upon the model outputs and therefore more accurate results could be obtained by experimental studies of these variables. Kleerebezem and Van Loodsrecht (2004) came to the same conclusion, as well. Yet, due to the complexity of the model only a restricted number of variables can be determined via measurement (Wett *et al.* 2007).
For manure and agricultural wastes knowledge about substrate composition in terms of ADM1 components is missing (in literature), therefore characterisation of the input becomes very important (Wett *et al.* 2007). For energy crops however data is found in the table of the nutrient requirements (for example, for dairy cattle) see for example Harris (2003) or Wiedner *et al.* (2001).
A study of the influence of modification in the influent variables and the initial condition state variables (by sensitivity analysis, see chapter 4.2.4) was done to gain a better insight into the coherences in the model and to make it easier to estimate the impacts on the ADM1 of changing influent variables or the initial conditions. This is important, as it was not possible to measure all influent composites and therefore a part of the influent and initial condition variables had to be calculated or in the worst case guessed at.

[17] Gadkar, K. G., J. varner, et al. (2005). "Model idenfication of signal transduction networks from data using a state regulator problem." Syst. Biol. **2**(1): 17-30.

It was found that the concentration of inorganic nitrogen ($S_{IN,in}$) has the greatest impact on the model output, followed by the anion concentration ($S_{an,in}$) and cation concentration ($S_{cat,in}$) as well as the hydrogen concentration ($S_{H2,in}$). Hereby $S_{IN,in}$ has the greatest effect on the hydrogen concentration in both the liquid and the gaseous phase and the propionic acid concentration. The same applies to $S_{an,in}$ and $S_{cat,in.}$ The hydrogen concentration in the gaseous phase is the state variable that is most influenced by a change in most of the influent variables ($X_{su,in}$, $X_{aa,in}$, $X_{fa,in}$, $X_{c4,in}$, $X_{ac,in}$ and $X_{h2,in}$).
Furthermore, the effect on the model output of a change of the initial conditions was investigated. Here the propionic acid and propionate concentration (S_{pro} and S_{pro-}) have the most influence, followed by S_{IN} and S_{H+}. The propionic acid and propionate concentration and the hydrogen anion have a strong ascendancy on S_{H2}, S_{CH4} and $S_{gas,CH4}$. $S_{IN,}$ on the other hand, has the most sway over the acetate concentration, the hydrogen concentration in both liquid and gas phase and on the propionate concentration, as well. The methane concentration in the liquid phase is most of all effected by most of the state variables, as by S_{bu}, S_{pro}, S_{ac}, S_{H2}, S_{CH4}, X_{li}, S_{va-}, S_{bu-}, S_{pro-}, S_{HCO3-}, S_{H+}, $S_{gas,H2}$ and $S_{gas,CO2.}$ A change in the particulate inert concentration only has an effect on the particulate inert and the soluble inert concentrations in the output, as expected.

In order to determine experimentally all state variables required for the model (input, initial) a lot of measurements are necessary. But to determine the waste (or crop) composition from standard measurements as needed for the ADM1 is not an easy task (Kleerebezem and Van Loosdrecht 2004). The same is true for the reactor conditions. In particular the intermediate products and the bacteria concentrations are difficult to estimate.
However the widespread utilization of the ADM1 stands or falls with an easy application of the model (Kleerebezem and Van Loosdrecht 2004).
Kleerebezem and Van Loodsrecht (2004) proposed a method to determine the full influent substrate composition for direct implementation in ADM1 from a minimal set of measurements – the COD, TOC, organic nitrogen and the alkalinity. Yet this guidance is not convenient for this study as it is only suitable for wastewater without any particulates.
Table 4.2.1 shows the measurement methods used in this study to estimate the input state variables and initial conditions and gives suggestions for alternative methods and further analytical methods.
The COD and $COD_{soluble}$ can be measured by standard methods (DEV S9 (DEV 1997)) or a test kit (for example LCK 114 (Dr. Lange)). These are very important parameters when using the ADM1, as the ADM1 is COD based.
The gas production is normally a standard measurement in every biogas plant and easily defined on a lab scale with any kind of gas flow meter or by displacement measurement.

Table 4.2.1: Parameters measured for the estimation of the "Input"/"Initial Condition" in the model

Measured variable	ADM1 state variable	Measurement method
Acetic Acid, Propionic Acid, VFA	S_{pro}, S_{ac}, S_{va}, S_{bu}, S_{va-}, S_{bu-}, S_{pro-} and S_{ac-}	HPLC, FTIR, GC, test kit
COD, $COD_{soluble}$	ADM1 variables are based on COD	standard method (DEV S9), test kit
gas production	$S_{CH4,gas}$, $S_{CO2,gas}$, $S_{H2,gas}$, S_{CH4}, S_{CO2}, S_{H2},	flow meter, displacement method
gas components (CH_4, CO_2, H2, (H_2S))	($S_{H2S,gas}$ and S_{H2S})	infrared sensors, dräger tubes, GC
VSS, SS	X_{xc}, X_I, X_{su}, X_{aa}, X_{fa}, X_{c4}, X_{pro}, X_{ac} and X_{H2}	standard method (DEV H1, DIN 38414-10)
Glucose	S_{su}	enzymatic tests, HPLC, titration, polarimetry
Amino Acids	S_{aa}	Ninhydrin method, HPLC
Lipids	X_{li}	solvent extraction
Carbohydrates	X_{CH}	HPLC, Anthrone method
Protein	X_{pr}	Kjeldahl, Lowry, Bradford
NH_4^+, NH_3	S_{IN}, S_{NH3}	single measurement rod
Total Nitrogen		Kjeldahl, test kit
Total Alkalinity, Partial Alkalinity	S_{IC}	standard method (DIN EN ISO 9963-1)
Total Carbon, Total Organic Carbon		TC Analyser
pH	S_{H+}	pH electrode, standard method (DIN 19265, DIN 19268)
Sulphate	S_{S2-}, S_{SO42-}, S_{HS-}	Standard method (ISO/DIS 10304), test kit
Sulphide		Standard method (DIN 38405-26)

The CH_4, CO_2, H_2S and H_2 content in the gas phase can be determined by online measurement (for example infrared sensors), “dräger” tubes and or by GC. Using the gas components and the total gas production the amount of CH_4, CO_2 and H_2 (esp. H_2S) in the gas ($S_{gas,CH4}$, $S_{gas,CO2}$ and $S_{gas,H2}$) and the liquid phase (S_{CH4}, S_{CO2} and S_{H2}) can be estimated using Henry’s law.

The VSS content was used to estimate the biomass in the reactor DEV H1 (DEV 1997). Yet, the measurement of biomass as VSS is a significant limitation in studies of the kinetic of the process, since substrate which is not degraded is also measured. Direct count procedures (microscopy methods), however, yield the highest estimate of micro-organisms (De la Rubia *et al.* 2006), this method however is very extensive and definitely only suitable for laboratory use. A further problem is that substrate which is not degraded is also included in the VSS content – thus X_{xc}, X_{ch}, X_{pr} and $X_{li.}$ Moreover the VSS content only gives an overall content for the biomass that has to be separated into the single bacteria groups X_{su}, X_{aa}, X_{fa}, X_{c4}, X_{pro}, X_{ac} and X_{H2}, which means that the VSS content is rather a rough estimation of the different bacteria groups used in the model. An alternative is therefore the determination of single bacteria groups: Methanogens, for example, can be counted by auto-fluorescence microscopy, due to their UV-induced blue-green auto-fluorescence (Doddema and Vogels 1978). This method, however, only shows methanogens with a high F420 content (De la Rubia *et al.* 2006).

X_{xc} (complex particulates) and X_I (inert particulates) can be estimated using the SS content.

Different methods for the estimation of organic acids can be used, such as titration, GC, HPLC, test kits (for example LCK 365 (Dr. Lange)) or FTIR. Yet using a test kit has the disadvantage that normally only VFA can be measured. In this study propionic (S_{pro}) and acetic acid concentrations (S_{ac}) are measured directly, whereas valeric (S_{va}) and butyric acid concentrations (S_{bu}) are estimated from the VFA concentration. Having measured the pH (using a pH-electrode), S_{H+} is estimated directly and thus S_{va-}, S_{bu-}, S_{pro-} and S_{ac-} can be estimated, as well.

Inorganic carbon (S_{IC}) can be determined with a TC/TOC-Analyser. Alternatively S_{IC} can be estimated using the total alkalinity and the VFA concentration.

The inorganic nitrogen is estimated by measuring the total nitrogen (Kjeldahl-nitrogen, test kits (e.g. LCK 338 (Dr. Lange)), and the measurement of NH_4 and NH_3 by a sensitive single-rod measuring cell.

Protein (X_{pr}), carbohydrates (X_{CH}), lipids (X_{li}), monosaccharides (S_{su}) and amino acids (S_{aa}) can be ascertained directly using an adequate measuring method (Table 4.2.1). An alternative would be to perform a standard nutrient analysis procedure, such as the “Weender” analysis – where crude ash (CA), crude fibre (CF), crude protein (CP), crude lipids (CL) and nitrogen free extract (NfE) are measured or the “Van Soest” analysis in the area of the carbohydrate analysis.

The estimation of the cations (S_{cat}) and anions (S_{an}) is very sensitive and also difficulty (Feng *et al.* 2006). Cations and anions were estimated using the data used by Rosen and Jeppsson (2002).

4.2.2 Processing of raw data measured

Due to measurement errors and other disturbances in reactor operation the data gets noisy, whereby extreme disturbances in the reactor operation (for example: broken tubes, leaky reactor,…) were removed manually from the data set.
Thus, a filter was used to smooth the noisy data. In order to obtain, but still relevant data three different low pass filters (Olsson and Newell 2001) were tested:

- a simple low pass filter (1)
- a modified low pass filter (2) and
- an exponential filter (3) (Figure 4.2.1)

The (1) simple low pass filter (Equation (77)) corresponds to a moving average, not based on future measurements and can be written in the following form (Olsson and Newell 2001):

$$\hat{y}_i = \frac{1}{n}\left(y_i + y_{i-1} + y_{i-2} + ... + y_{i-n+1}\right) \quad \textbf{(77)}$$

The (2) modified low pass filter also uses the moving average, but data before and after the current time (Equation (78)) (Olsson and Newell 2001):

$$\hat{y}_i = \frac{1}{n}\left(y_{i+n/2-1} + ... + y_{i+2} + y_{i+1} + y_i + y_{i-1} + y_{i-2} + ... + y_{i-n/2+1}\right) \quad \textbf{(78)}$$

Another possibility is the (3) exponential filter, which is very commonly used (Olsson and Newell 2001). The exponential filter has the advantage that older data is less important for defining the current data output. This filter can be described as (Equation (79)) (Olsson and Newell 2001):

$$\hat{y}_i = (1-\alpha) * y_i + \alpha * (1-\alpha) * y_{i-1} + \alpha^2 * (1-\alpha) * y_{i-2} + ... \quad \textbf{(79)}$$

The simple low pass filter and the modified low pass filter were tested for different n-values (n = 1…9), here a high n-value means a very smooth, but also more delayed value (Olsson and Newell 2001). The exponential filter was tested for α – values from 0.1 to 0.9 (Figure 4.2.2). Where a low α - values means that a short data history is considered.

It was finally decided to use the exponential filter, due to the fact that here historic data is less considered in the calculation depending on the "age", which conforms more to the constantly changing time series.

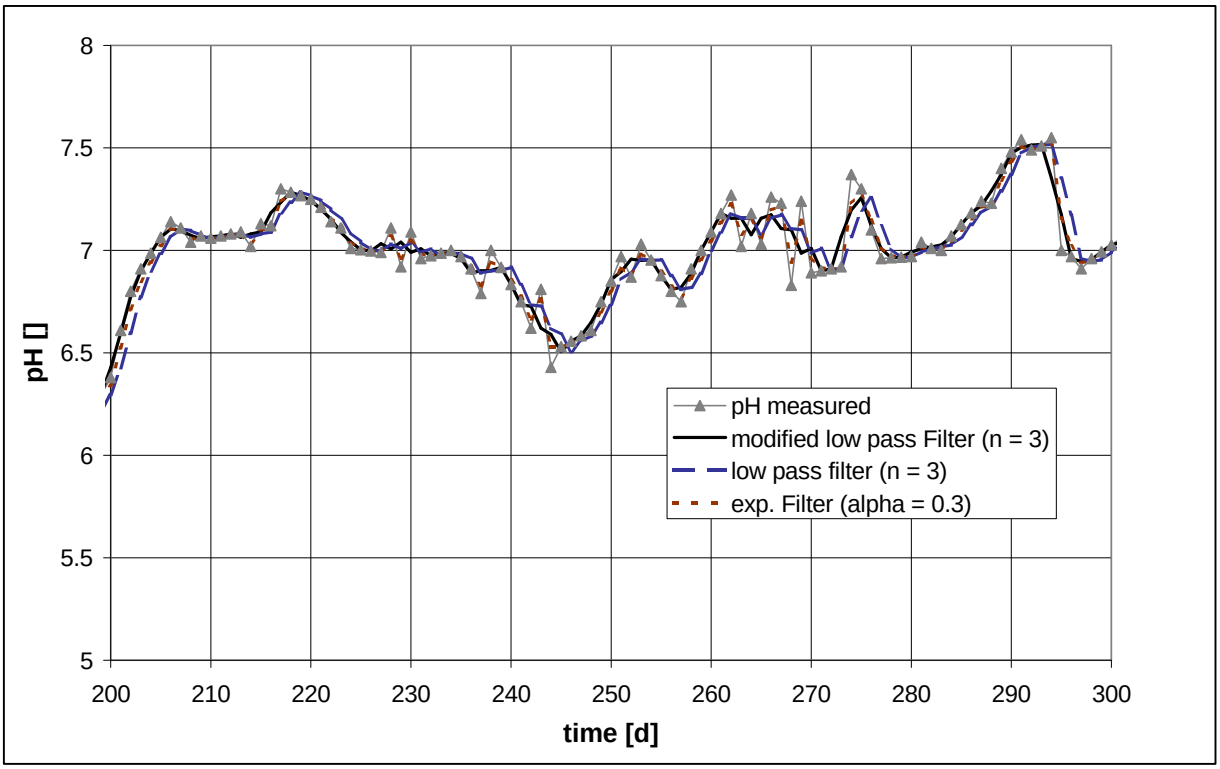

Figure 4.2.1: Comparison of different filters: low pass filter, modified low pass filter and exponential filter – on the example of the pH in the mesophilic reactor system FM1 from day 200 to 300

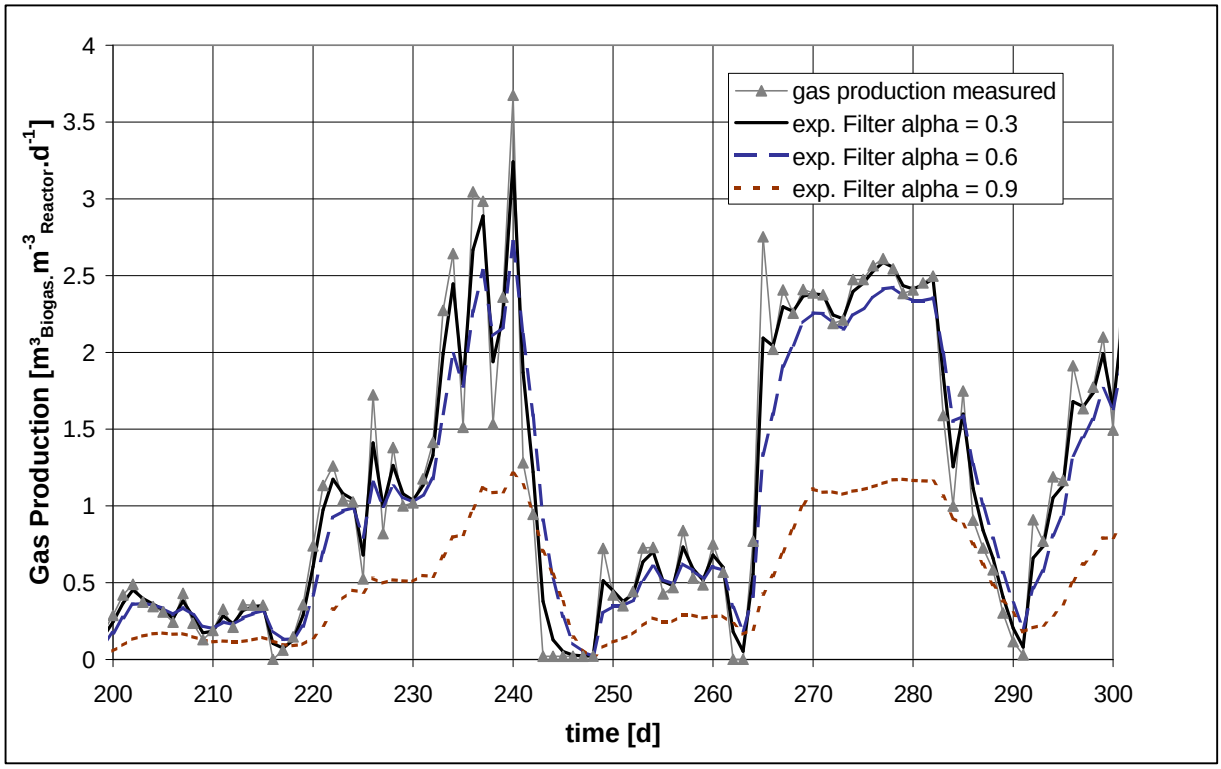

Figure 4.2.2: Comparison of different □-values (0.3, 0.6, 0.9) for the exponential filter – on the example of the gas production of the mesophilic reactor system FM1 from day 200 to 300

For the exponential filter the data with an α – value of 0.3 was found be the best compromise between smooth data and considering all relevant changes in the data (Figure 4.2.1). It has further to be mentioned that to obtain a continued time series

missing data was displaced through linear interpolation of surrounding data, which is not completely consistent with the biochemical behaviour in the reactor, that is non-linear, but as the interpolated time is small (two days on average), the resulting error is negligible.

4.2.3 Mass balance of the AD process

A mass balance was used to estimate the initial conditions for the ADM1. This estimation is especially applied in the Virtual Laboratory 1.2.
The basis of this mass balance is the ADM1 model itself, where the calculation of the initial conditions happens iteratively. For these calculations a MATLB®-Script was written ("mass_balance") (Figure 4.2.3).
The estimation of the methane production was done according to Baserga (1998)[18] using the protein, fat and carbohydrate content in the feed and the reactor sludge.
The carbohydrate, protein and lipid content in step n is then (Equation (80) to (82)):

$$X_{ch\_n} = \left(X_{ch\_in} + X_{ch\_n-1}\right) * 0.938 \quad \textbf{(80)}$$

$$X_{pr\_n} = \left(X_{pr\_in} + X_{pr\_n-1}\right) * 0.695 \quad \textbf{(81)}$$

$$X_{li\_n} = \left(X_{li\_in} + X_{li\_n-1}\right) * 0.350 \quad \textbf{(82)}$$

where:
X_{ch_in} carbohydrate content in the feed [kg_{COD}]
X_{ch_n-1} carbohydrate content in the sludge after last iteration step [kg_{COD}]
X_{pr_in} protein content in the feed [kg_{COD}]
X_{pr_n-1} protein content in the sludge after the last iteration step [kg_{COD}]
X_{li_in} lipid content in the feed [kg_{COD}]
X_{li_n-1} lipid content in the sludge after the last iteration step [kg_{COD}]

The gas production is now calculated as (Baserga, 1998)[18] (Equation (83) to (85)):

$$GY_{ch} = 0.7 \left[m^3.kg^{-1}\right] \quad \textbf{(83)}$$

$$GY_{pr} = 0.79 \left[m^3.kg^{-1}\right] \quad \textbf{(84)}$$

$$GY_{li} = 1.25 \left[m^3.kg^{-1}\right] \quad \textbf{(85)}$$

Baserga (1998)[18] gives for the methane content digesting carbohydrates 50 %, for proteins 71 % and for lipids 68 %, thus the methane production arises as (Equation (86) to (88)):

$$MY_{ch} = 0.395 \left[m^3.kg^{-1}\right] \quad \textbf{(86)}$$

$$MY_{pr} = 0.497 \left[m^3.kg^{-1}\right] \quad \textbf{(87)}$$

$$MY_{li} = 0.85 \left[m^3.kg^{-1}\right] \quad \textbf{(88)}$$

[18] Baserga, U., 1998, Landwirtschaftliche Co-Vergärungsanlagen, FAT-Berichte Nr. 12

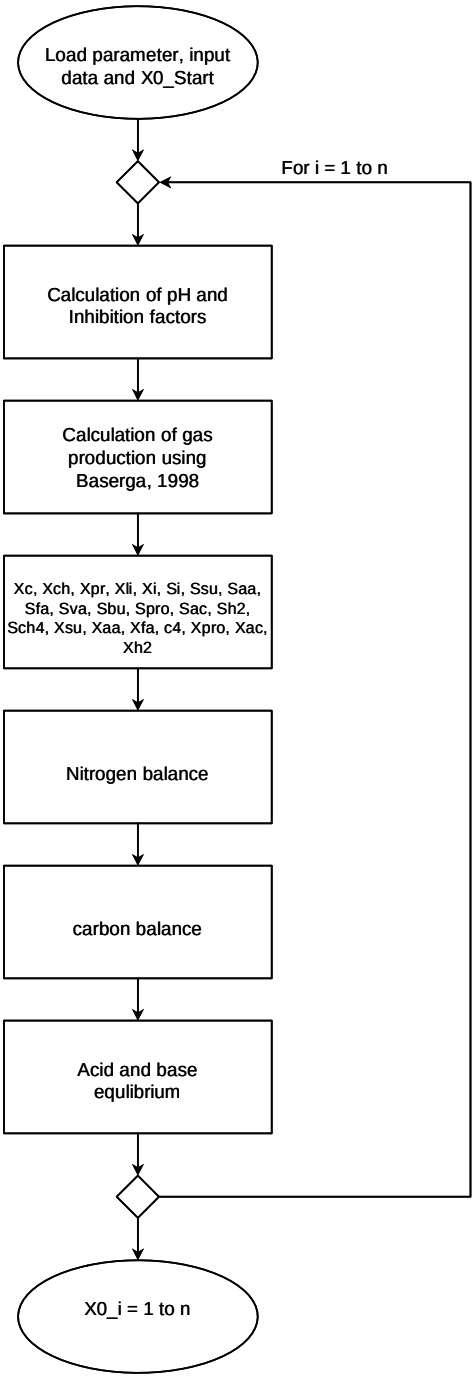

Figure 4.2.3: Structure of the "massbalance" program

Using the overall gas and methane production, the methane content (Equation (89) and (90)) is calculated (Equation (91)):

$$GP = X_{ch\_n} * GY_{ch} + X_{pr\_n} * GY_{pr} + X_{li\_n} * GY_{li} \quad \textbf{(89)}$$

$$MP = X_{ch\_n} * MY_{ch} + X_{pr\_n} * MY_{pr} + X_{li\_n} * MY_{li} \quad \textbf{(90)}$$

$$MC = (100 / GP) * MP \quad \textbf{(91)}$$

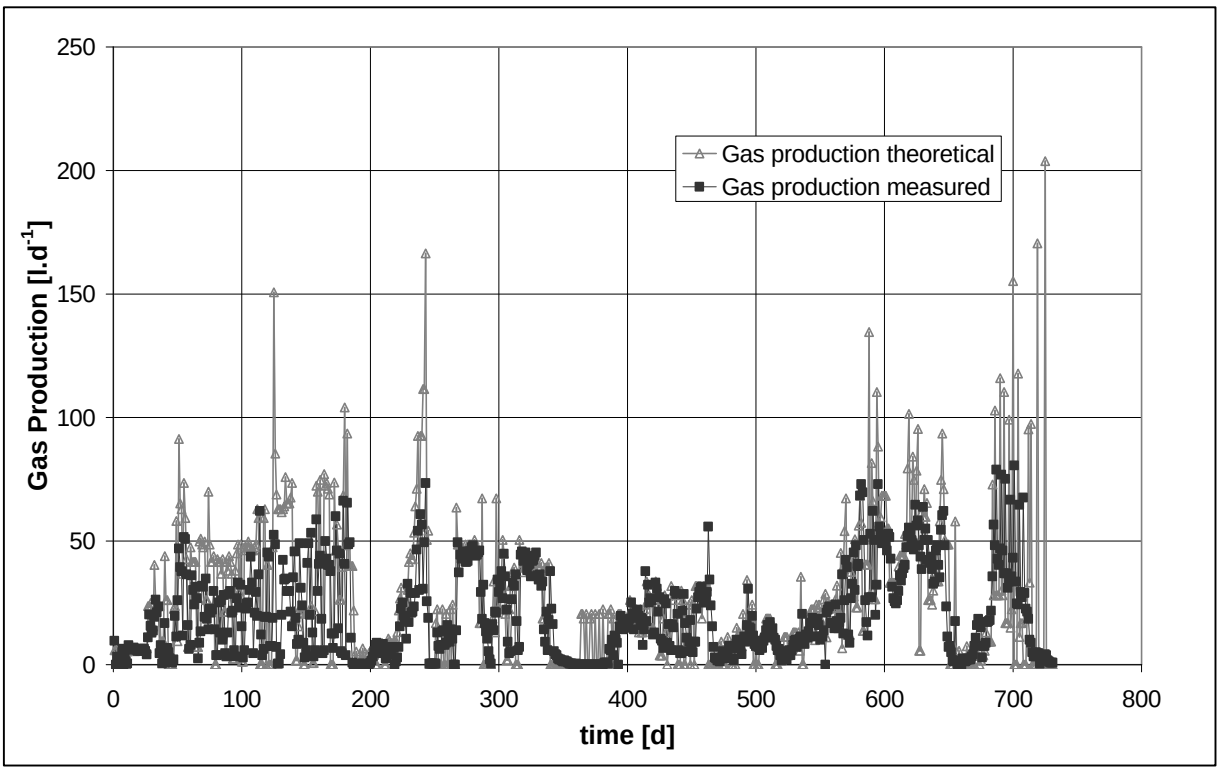

Figure 4.2.4: Gas production measured in the mesophilic reactor FM1 compared to the theoretical gas production according to Baserga, 1998

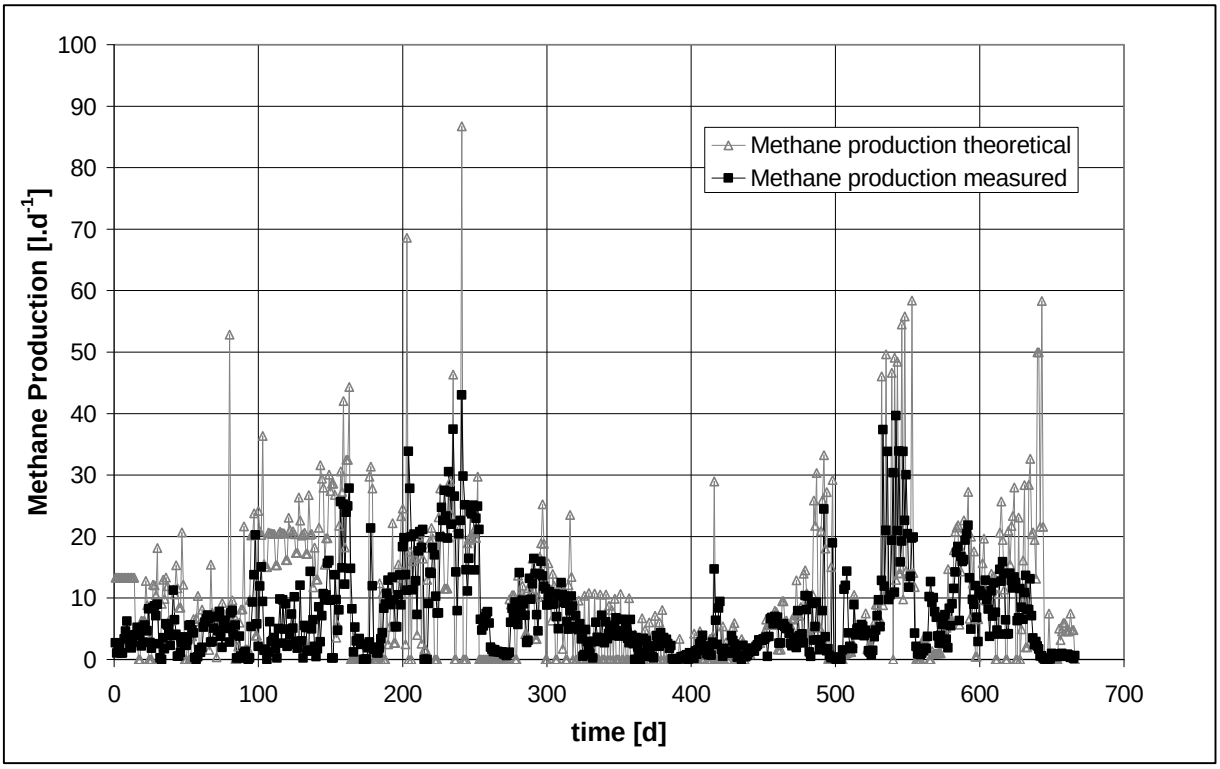

Figure 4.2.5: Methane production measured in the thermophilic reactor FM2 compared to the theoretical methane production according to Baserga, 1998

The gas and methane production calculated was now compared with values measured in the mesophilic reactor FM1 and the thermophilic reactor FM2 (Figure 4.2.4 and Figure 4.2.5) in order to prove that the estimation using the data from Baserga (1998) is accurate (Table 4.2.2). For the comparison the same statistical indicators as for the

investigation of the model performance are used: the square of the correlation coefficient (r^2), the ratio of means (R_{mean}), the root mean square error (RSME) and the index of agreement (d). R_{mean} shows, as was expected, an overestimation of the gas and methane production using the conversion factor from Baserga (1998)[18]. As the index of agreement in both cases (mesophile and thermophile) for both the gas production and the methane production is > 0.6 and the r^2 is acceptable as well.

Table 4.2.2: A) Correlation coefficient, Ratio of means, RMSE and the index of agreement (d) of the mesophilic reactor and B) Correlation coefficient, Ratio of means, RMSE and the index of agreement (d) of the thermophilic reactor

A	Gas Production	Methane Production
r^2	0.557	0.573
R_{mean}	0.295	0.301
RSME	23.968	11.912
d	0.690	0.693

B	Gas Production	Methane Production
r^2	0.472	0.456
R_{mean}	0.343	0.395
RSME	20.428	10.570
d	0.650	0.628

The Hydrogen content was given as a constant value by HC = 0.05 %, as hydrogen was now measured in this study. The Carbon Dioxide content is so given by (Equation (92)):

$$CC = 100 - MC - HC \quad \textbf{(92)}$$

The initial conditions are then calculated based on the ADM1 (Batstone *et al.* 2002) (Equation (93) to (100)):

$$S_{gas,CH_4} = ((((MC * 10)/\mathrm{VM}) * 64)/1000) * 0.25 \quad \textbf{(93)}$$

$$S_{gas,CO_2} = ((\mathrm{CC} * 10)/VM)/1000 \quad \textbf{(94)}$$

$$S_{gas,H_2} = ((((\mathrm{HC} * 10)/\mathrm{VM})) * 16/1000) * 0.125 \quad \textbf{(95)}$$

$$S_{CH_4} = S_{gas,CH_4} * \mathrm{R} * \mathrm{Temp} * K_{H,CH_4} \quad \textbf{(96)}$$

$$S_{CO_2} = S_{gas,CO_2} * \mathrm{R} * \mathrm{Temp} * K_{H,CO_2} \quad \textbf{(97)}$$

$$S_{H_2} = S_{gas,H_2} * R * Temp * K_{H,H_2} \quad \textbf{(98)}$$

$$S_{HCO_3} = (S_{CO_2} * K_{a,CO_2})/S_{H^+} \quad \textbf{(99)}$$

$$S_{IC} = S_{CO_2} + S_{HCO_3} \quad \textbf{(100)}$$

where:

$S_{gas,CH4}$ CH_4 in the gas phase [$kg_{COD}.m^{-3}$]
$S_{gas,CO2}$ CO_2 in the gas phase [$kmol_C.m^{-3}$]
$S_{gas,H2}$ H_2 in the gas phase [$kg_{COD}.m^{-3}$]
S_{CH4} CH_4 in the liquid phase [$kg_{COD}.m^{-3}$]
S_{CO2} CO_2 in the liquid phase [$kmol_C.m^{-3}$]
S_{H2} H_2 in the liquid phase [$kg_{COD}.m^{-3}$]
S_{HCO3} Hydrogencarbonate [$kg_{COD}.m^{-3}$]
S_{IC} inorganic carbon [$kg_{COD}.m^{-3}$]
VM molar Volume [$m^3.mol^{-1}$]
R gas constant [$bar.M^{-1}.K^{-1}$]
Temp temperature in the reactor [K]

$K_{H,CH4}$ Henry constant for CH_4 [M.bar^{-1}]
$K_{H,CO2}$ Henry constant for CO_2 [M.bar^{-1}]
$K_{H,H2}$ Henry constant for H_2 [M.bar^{-1}]

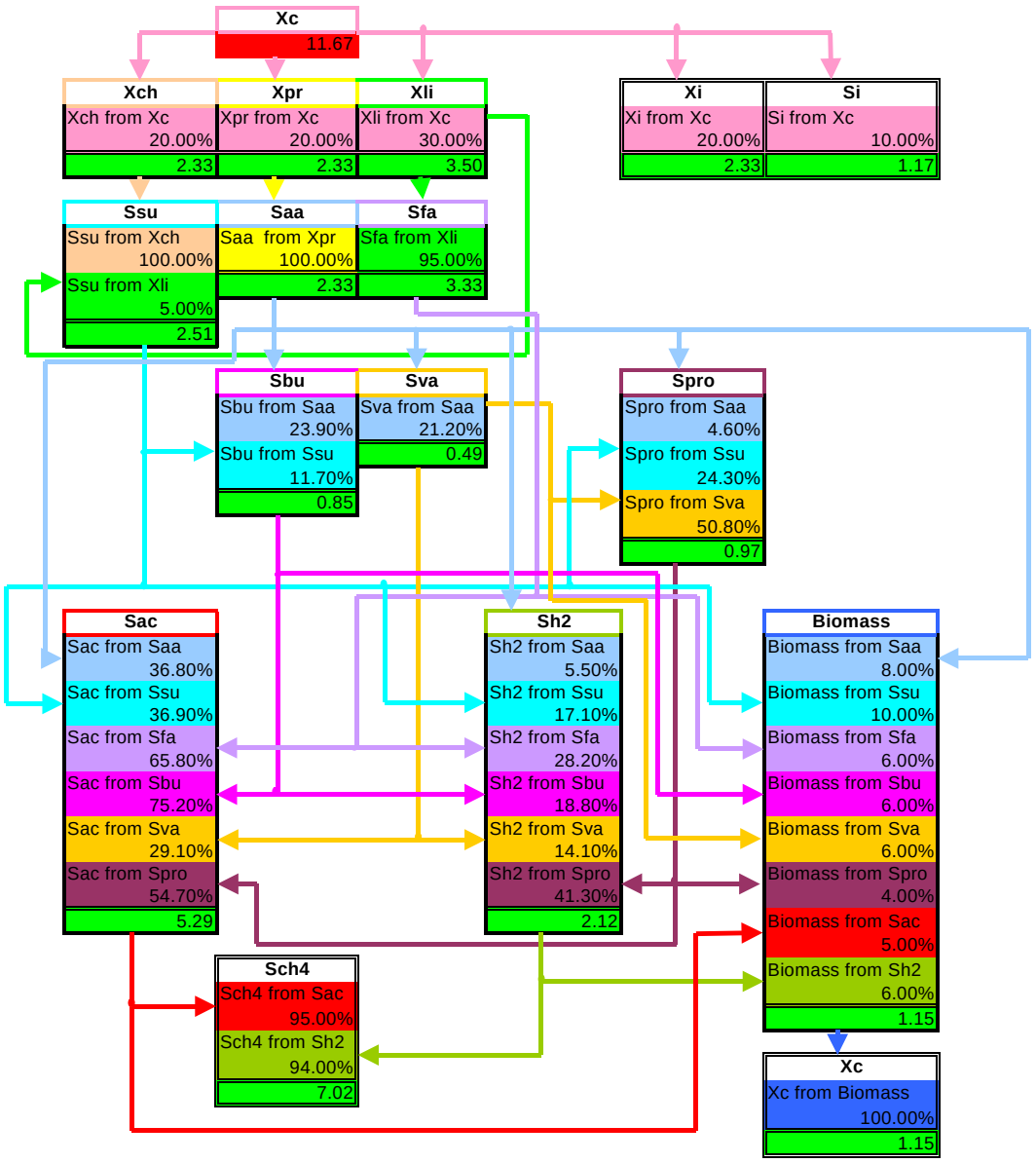

Figure 4.2.6: Stoechiometric mass balance of the ADM1

Complex particulates (X_c), carbohydrates (X_{ch}), proteins (X_{pr}), lipids (X_{li}), particulate inerts (X_I), soluble inerts (S_I), soluble sugar (S_{su}), soluble amino acids (S_{aa}), soluble LCFA (S_{fa}), valeric acid (S_{va}), butyric acid (S_{bu}), propionic acid (S_{pro}), acetic acid (S_{ac}), hydrogen (S_{H2}), methane (S_{CH4}), sugar degraders (X_{su}), amino acid degraders (X_{aa}), LCFA using bacteria (X_{fa}), valeric acid and butyric acid using bacteria (X_{c4}), propionic acid degrading bacteria (X_{pro}), acetate degraders (X_{ac}) and hydrogen using bacteria (X_{H2}) are estimated using a stoechiometric mass balance (Figure 4.2.6) and parts of the ADM1.

Box 4.2.1 shows a part of the “mass_balance” MATLAB®-Script and illustrates the assumptions made in this estimation of the initial conditions using the example of complex particulate matter (X_c). X_C at the current iteration step (X_{c_all}) arises from the input fraction (X_{C_in}) and the mass fraction of X_c calculated in the last iteration step. It is

now assumed that at the same time as X_c is formed it is also degraded. X_{c_out} is the actual mass fraction of this iteration step. X_{c_abbau} is the degraded part of X_{c_all}. This degraded part is divided into particulate carbohydrates ($X_{ch\_xc\_all}$), particulate proteins ($X_{pr\_xc\_all}$) and particulate lipids ($X_{li\_xc\_all}$) according to Figure 4.2.6. Here again it is assumed that a part of the carbohydrates, proteins and lipids formed is degraded at the same moment as they are arising, and so on. Moreover from a part of all degraded products biomass is formed, which is equally partly degraded at the same moment as it is formed.

```
…
Xc_all = Xc_in + Xc_tminus1;
Xc_out = (Xc_all)*exp(-k_dis);
Xc_abbau = Xc_all - (Xc_out);
%--------------------------------------------------------------------------
%--------------------------------------------------------------------------
Xch_xc_all = f_ch_xc*Xc_abbau;
Xch_xc_out = Xch_xc_all*exp(-k_hyd_ch);
Xch_xc_abbau = Xch_xc_all - Xch_xc_out;
C_xch_xc_out = C_ch*Xch_xc_out;
%--------------------------------------------------------------------------
Xpr_xc_all = f_pr_xc*Xc_abbau;
Xpr_xc_out = Xpr_xc_all*exp(-k_hyd_pr);
Xpr_xc_abbau = Xpr_xc_all - Xpr_xc_out;
N_xpr_xc_out = N_aa*Xpr_xc_out;
C_xpr_xc_out = C_pr*Xpr_xc_out;
%--------------------------------------------------------------------------
Xli_xc_all = f_li_xc*Xc_abbau;
Xli_xc_out = Xli_xc_all*exp(-k_hyd_li);
Xli_xc_abbau = Xli_xc_all - Xli_xc_out;
C_xli_xc_out = C_li*Xli_xc_out;
%--------------------------------------------------------------------------
Xi_xc_out = f_xi_xc*Xc_abbau;
Si_xc_out = f_si_xc*Xc_abbau;
N_xI_xc_out = N_I*Xi_xc_out;
N_sI_xc_out = N_I*Si_xc_out;
C_xi_xc_out = C_xI*Xi_xc_out;
C_si_xc_out = C_sI*Si_xc_out;
%--------------------------------------------------------------------------
%--------------------------------------------------------------------------
Ssu_xc_all = Xch_xc_abbau + (1 - f_fa_li)*Xli_xc_abbau;
Ssu_xc_out     =     Ssu_xc_all*exp(-km_su*(Ssu_xc_all/((Ks_su*Volume)     +
Ssu_xc_all))*Xsu_all*I5);
Ssu_xc_abbau = Ssu_xc_all - Ssu_xc_out;
C_ssu_xc_out = C_su*Ssu_xc_out;
%--------------------------------------------------------------------------
Saa_xc_all = Xpr_xc_abbau;
Saa_xc_out     =     Saa_xc_all*exp(-km_aa*(Saa_xc_all/((Ks_aa*Volume)     +
```

```
Saa_xc_all))*Xaa_all*I6);
Saa_xc_abbau = Saa_xc_all - Saa_xc_out;
N_saa_xc_out = N_aa*Saa_xc_out;
C_saa_xc_out = C_aa*Saa_xc_out;
%-------------------------------------------------------------------------
Sfa_xc_all = f_fa_li*Xli_xc_abbau;
Sfa_xc_out     =     Sfa_xc_all*exp(-km_fa*(Sfa_xc_all/((Ks_fa*Volume)     +
Sfa_xc_all))*Xfa_all*I7);
Sfa_xc_abbau = Sfa_xc_all - Sfa_xc_out;
C_sfa_xc_out = C_fa*Sfa_xc_out;
%-------------------------------------------------------------------------
Sbu_xc_all = (1-Yaa)*f_bu_aa*Saa_xc_abbau + (1-Ysu)*f_bu_su*Ssu_xc_abbau;
Sva_xc_all = (1-Yaa)*f_va_aa*Saa_xc_abbau;
Sbu_xc_out     =     Sbu_xc_all*exp(-km_c4*(Sbu_xc_all/((Ks_c4*Volume)     +
Sbu_xc_all))*Xc4_all*I8*(Sbu_xc_all/(Sbu_xc_all+Sva_xc_all+1*10-6)));
Sva_xc_out     =     Sva_xc_all*exp(-km_c4*(Sva_xc_all/((Ks_c4*Volume)     +
Sva_xc_all))*Xc4_all*I9*(Sva_xc_all/(Sbu_xc_all+Sva_xc_all+1*10-6)));
Sbu_xc_abbau = Sbu_xc_all - Sbu_xc_out;
Sva_xc_abbau = Sva_xc_all - Sva_xc_out;
C_sbu_xc_out = C_bu*Sbu_xc_out;
C_sva_xc_out = C_va*Sva_xc_out;
%-------------------------------------------------------------------------
Spro_xc_all = (1-Yaa)*f_pro_aa*Saa_xc_abbau + (1-Ysu)*f_pro_su*Ssu_xc_abbau
+ (1-Yc4)*0.54*Sva_xc_abbau;
Spro_xc_out    =   Spro_xc_all*exp(-km_pro*(Spro_xc_all/((Ks_pro*Volume)   +
Spro_xc_all))*Xpro_all*I10);
Spro_xc_abbau = Spro_xc_all - Spro_xc_out;
C_spro_xc_out = C_pro*Spro_xc_out;
%-------------------------------------------------------------------------
Sac_xc_all = (1-Yaa)*f_ac_aa*Saa_xc_abbau + (1-Ysu)*f_ac_su*Ssu_xc_abbau +
...
             (1-Yfa)*0.7*Sfa_xc_abbau + (1-Yc4)*0.8*Sbu_xc_abbau + ...
             (1-Yc4)*0.31*Sva_xc_abbau + (1-Ypro)*0.57*Spro_xc_abbau;
Sac_xc_out     =     Sac_xc_all*exp(-km_ac*(Sac_xc_all/((Ks_ac*Volume)     +
Sac_xc_all))*Xac_all*I11);
Sac_xc_abbau = Sac_xc_all - Sac_xc_out;
C_ac_xc_out = C_ac*Sac_xc_out;
%-------------------------------------------------------------------------
Sh2_xc_all = (1-Yaa)*f_h2_aa*Saa_xc_abbau + (1-Ysu)*f_h2_su*Ssu_xc_abbau +
(1-Yfa)*0.3*Sfa_xc_abbau + ...
             (1-Yc4)*0.2*Sbu_xc_abbau  +  (1-Yc4)*0.15*Sva_xc_abbau  +  (1-
Ypro)*0.43*Spro_xc_abbau;
Sh2_xc_out     =     Sh2_xc_all*exp(-km_h2*(Sh2_xc_all/((Ks_h2*Volume)     +
Sh2_xc_all))*Xh2_all*I12)      +      Sh2_xc_all*exp(-kLa*(Sh2_xc_all      -
16*K_H_h2*pgas_h2));
Sh2_xc_abbau = Sh2_xc_all - Sh2_xc_out;
%-------------------------------------------------------------------------
Sch4_xc_all = (1-Yac)*Sac_xc_abbau + (1-Yh2)*Sh2_xc_abbau;
```

```
Sch4_xc_out = Sch4_xc_all*exp(-kLa*(Sch4_xc_all - 64*K_H_ch4*pgas_ch4));
C_sch4_xc_out = C_ch4*Sch4_xc_out;
%-------------------------------------------------------------------------
%-------------------------------------------------------------------------
Xaa_xc_all = Yaa*Saa_xc_abbau;
Xaa_xc_out = Xaa_xc_all*exp(-kdec_aa);
Xaa_xc_abbau = Xaa_xc_all - Xaa_xc_out;
N_xaa_xc_out = N_bac*Xaa_xc_out;
C_xaa_xc_out = C_bac*Xaa_xc_out;
%-------------------------------------------------------------------------
Xsu_xc_all = Ysu*Ssu_xc_abbau;
Xsu_xc_out = Xsu_xc_all*exp(-kdec_su);
Xsu_xc_abbau = Xsu_xc_all - Xsu_xc_out;
N_xsu_xc_out = N_bac*Xsu_xc_out;
C_xsu_xc_out = C_bac*Xsu_xc_out;
%-------------------------------------------------------------------------
Xfa_xc_all = Yfa*Sfa_xc_abbau;
Xfa_xc_out = Xfa_xc_all*exp(-kdec_fa);
Xfa_xc_abbau = Xfa_xc_all - Xfa_xc_out;
N_xfa_xc_out = N_bac*Xfa_xc_out;
C_xfa_xc_out = C_bac*Xfa_xc_out;
%-------------------------------------------------------------------------
Xc4_xc_all = Yc4*Sbu_xc_abbau + Yc4*Sva_xc_abbau;
Xc4_xc_out = Xc4_xc_all*exp(-kdec_c4);
Xc4_xc_abbau = Xc4_xc_all - Xc4_xc_out;
N_xc4_xc_out = N_bac*Xc4_xc_out;
C_xc4_xc_out = C_bac*Xc4_xc_out;
%-------------------------------------------------------------------------
Xpro_xc_all = Ypro*Spro_xc_abbau;
Xpro_xc_out = Xpro_xc_all*exp(-kdec_pro);
Xpro_xc_abbau = Xpro_xc_all - Xpro_xc_out;
N_xpro_xc_out = N_bac*Xpro_xc_out;
C_xpro_xc_out = C_bac*Xpro_xc_out;
%-------------------------------------------------------------------------
Xac_xc_all = Yac*Sac_xc_abbau;
Xac_xc_out = Xac_xc_all*exp(-kdec_ac);
Xac_xc_abbau = Xac_xc_all - Xac_xc_out;
N_xac_xc_out = N_bac*Xac_xc_out;
C_xac_xc_out = C_bac*Xac_xc_out;
%-------------------------------------------------------------------------
Xh2_xc_all = Yh2*Sh2_xc_abbau;
Xh2_xc_out = Xh2_xc_all*exp(-kdec_h2);
Xh2_xc_abbau = Xh2_xc_all - Xh2_xc_out;
N_xh2_xc_out = N_bac*Xh2_xc_out;
C_xh2_xc_out = C_bac*Xh2_xc_out;
%-------------------------------------------------------------------------

…
```

Box 4.2.1: Part of the "mass_balance" program – Calculation of the state variable X_c

Comparing now the results of the iteration process using 500 iterations with the measured values, starting with a X0_Start of 0 for all state variables except S_{cat}, S_{an} and S_{H+}.

Table 4.2.3: Comparison of the state variables measured with the state variables calculated using the "mass_balance"

state variable		measured value	value calculated in the mass balance	Difference [%]
S_{va}	[$kg_{COD}m^{-3}$]	3.18E-01	4.17E-01	30.92
S_{bu}	[$kg_{COD}m^{-3}$]	2.83E-01	8.40E-01	197.04
S_{pro}	[$kg_{COD}m^{-3}$]	1.22E-02	3.58E+00	29341.24
S_{ac}	[$kg_{COD}m^{-3}$]	5.96E-01	3.07E+00	414.17
S_{CH4}	[$kg_{COD}m^{-3}$]	1.12E-02	9.96E-03	11.46
S_{IC}	[$kmol_{C}m^{-3}$]	1.02E-02	2.08E-02	103.41
Xxc	[$kg_{COD}m^{-3}$]	4.00E+00	2.85E+00	28.85
S_{NH3}	[$kmolNm^{-3}$]	5.41E-05	1.20E-06	97.79
$S_{gas,CH4}$	[$kg_{COD}m^{-3}$]	3.79E-01	3.35E-01	11.46
$S_{gas,CO2}$	[$kmol_{C}m^{-3}$]	4.30E-03	5.04E-03	17.18

Some differences can be found; especially the propionic acid and acetic acid concentrations are overestimated. This makes it clear that some further developments which where not possible within the frame of this study are still necessary. Especially as some simplifications are made in the mass balance: thus the pH (pH = 7) is taken as constant, because the pH calculation in the program was too unstable. The same is true for the calculation of some bacteria concentrations (X_{aa}, X_{pro} and X_{H2}). A further difficulty is the nitrogen balance.

Yet, the results of the mass balance for nearly all state variables lay in the same range as the state variables for the initial conditions measured or otherwise determined and therefore within an acceptable range. These results indicate that the mass balance is definitely suitable for a first estimation of the initial conditions.

4.2.4 Sensitivity analysis

In order to obtain more information for the model calibration a sensitivity analysis was done to find the most sensitive parameter.

Similarly to DePauw (2005), a pre-selection of parameters which have to be calibrated was carried out in order to reduce the calculation effort: it will be not necessary to calibrate stoechiometric parameters (as they are well known), influent composition parameters, and temperature correction factors (De Pauw 2005).

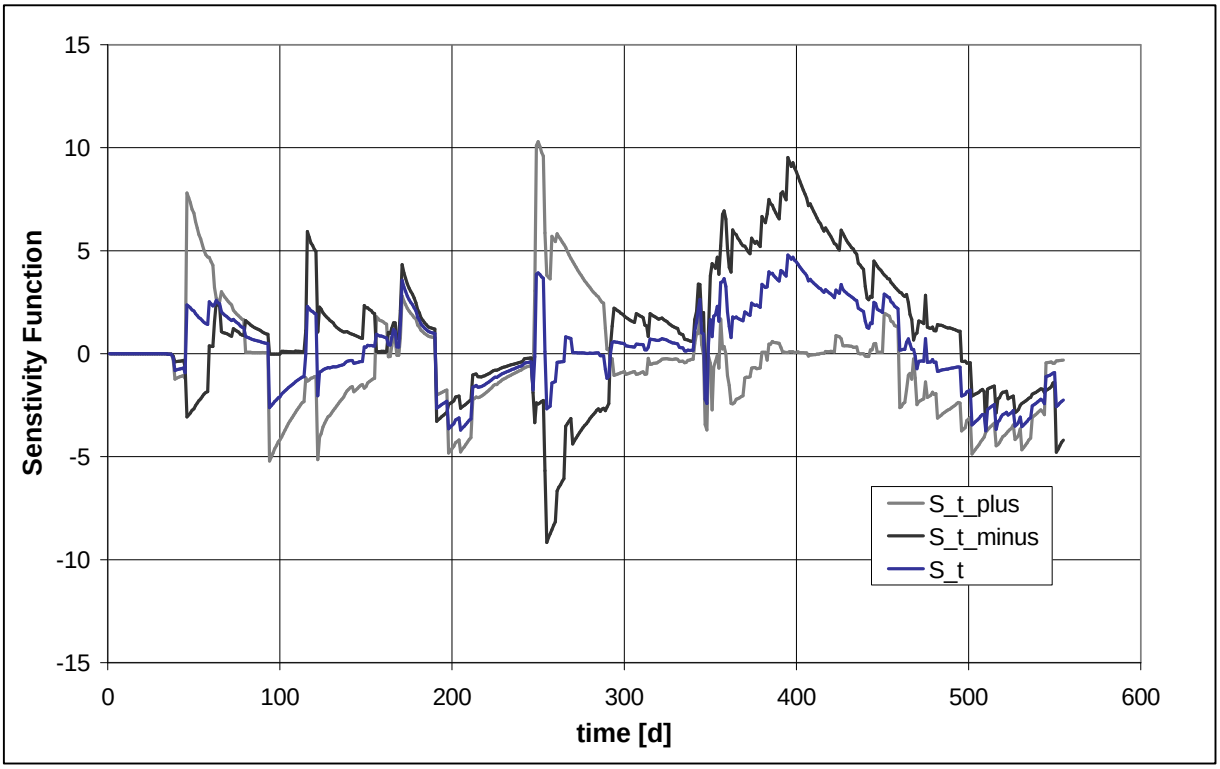

Figure 4.2.7: Sensitivity functions "plus" and "minus" for a perturbation factor of 1e-8 for kdis as parameter and XH2 as variable – showing the effect of non-linearity effects of the ADM1 using a perturbation factor of 1e-8. Sensitivity function "plus" and "minus" differ from each other to the nonlinearties.

So, for example, k_La was also excluded from the sensitivity analysis: for the k_La Pauss *et al.* (1990) reported for H_2 and CH_4 values of 3.84 and 2.16 d^{-1} for anaerobic digestion in a CSTR. Whereas (Siegrist *et al.* 2002) gained a k_La value for CO2 of over 100 d^{-1}. But in the range between 1 to 1000 d^{-1} the k_La value has no influence on the model output. It is only if the k_La value is less than 1 d^{-1} that the model output is affected (Feng *et al.* 2006).

Sensitivity functions (mean value of sensitivity function "plus" and "minus") were calculated for different perturbation values (Figure 4.2.7), from $5.00*10^{-1}$ to $1.00*10^{-8}$. Subsequent error values (Sum of squared errors (SSE), Sum of absolute errors (SAE), Maximum relative errors (MRE) (Zaher 2005)) were determined, to find the best perturbation value (Figure 4.2.8 to Figure 4.2.11).

Optimal perturbation values are parameter dependant, the values can be found when the criteria value (here named "error") reaches the minimum (De Pauw 2005). SSE was used in this study as the actual criteria value. Thus the optimal perturbation value was found to be 5.00e-1.

Looking at Figure 4.2.8 respectively Figure 4.2.9 and Figure 4.2.10 respectively Figure 4.2.11 it comes clear that the perturbation factor is not only dependant on the parameters but also on the variables. DePauw (2005) came to the same conclusion in his study.

The parameter with the most influence on the original ADM1 (Table 4.2.4) according to the sensitivity analysis carried out is the hydrolysis rate of the proteins ($k_{hyd,pr}$), further the hydrolysis rate of the carbohydrates ($k_{hyd,ch}$) and the hydrolysis rate of the lipids ($k_{hyd,li}$).

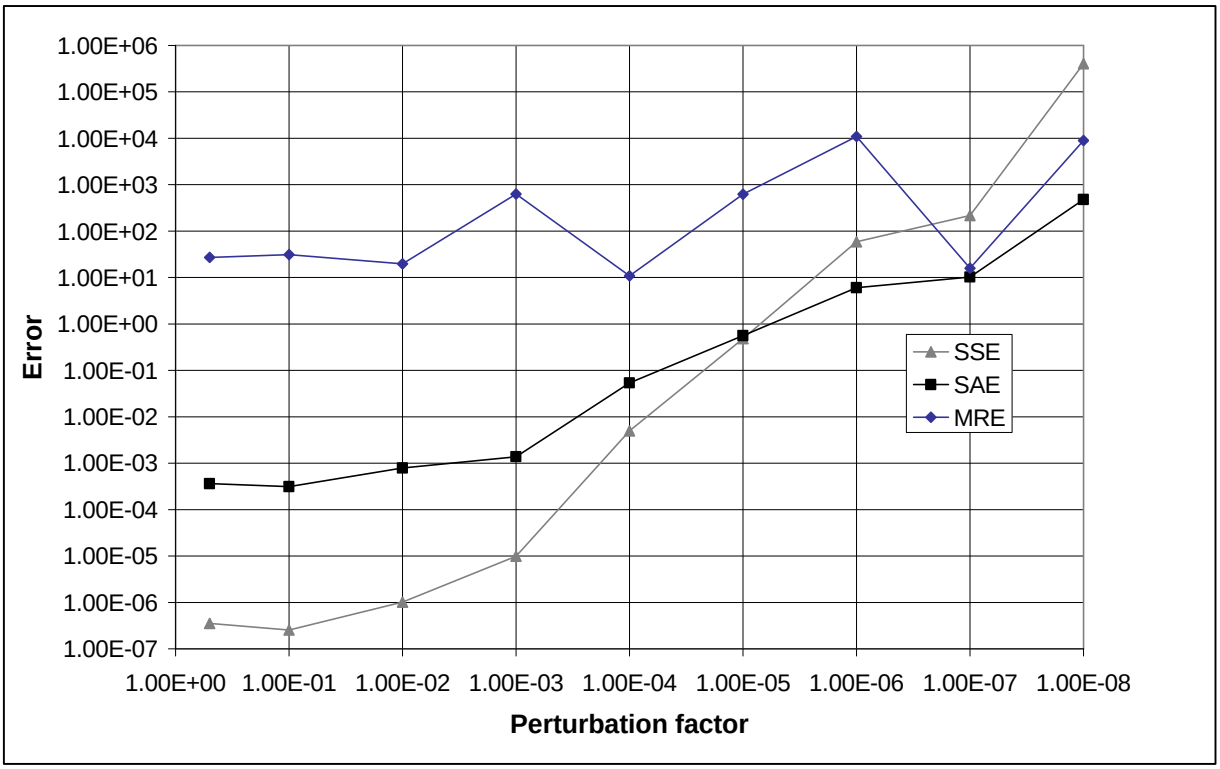

Figure 4.2.8: Errors (SSE, SAE and MRE) for the parameter k_{dis} and variable S_{ac}

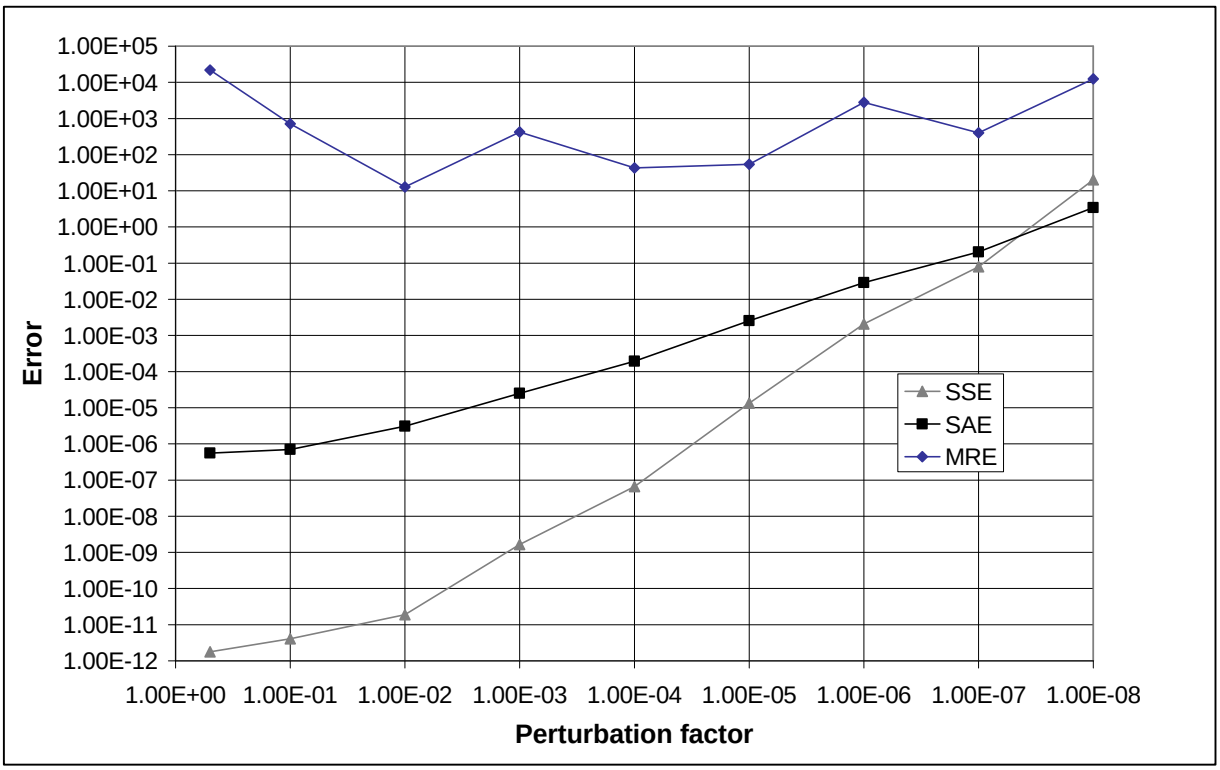

Figure 4.2.9: Errors (SSE, SAE and MRE) for the parameter k_{dis} and variable X_{H2}

Further the disintegration rate (k_{dis}) and the substrate uptake rate of amino acids ($k_{m,aa}$). The protein hydrolysis rate mostly affects the particulate protein concentration (X_{pr}) and the soluble amino acid concentration (S_{aa}). This parameter also has a relatively important influence on the hydrogen concentration (S_{H2}) and the hydrogen concentration in the gas phase ($S_{gas,H2}$).

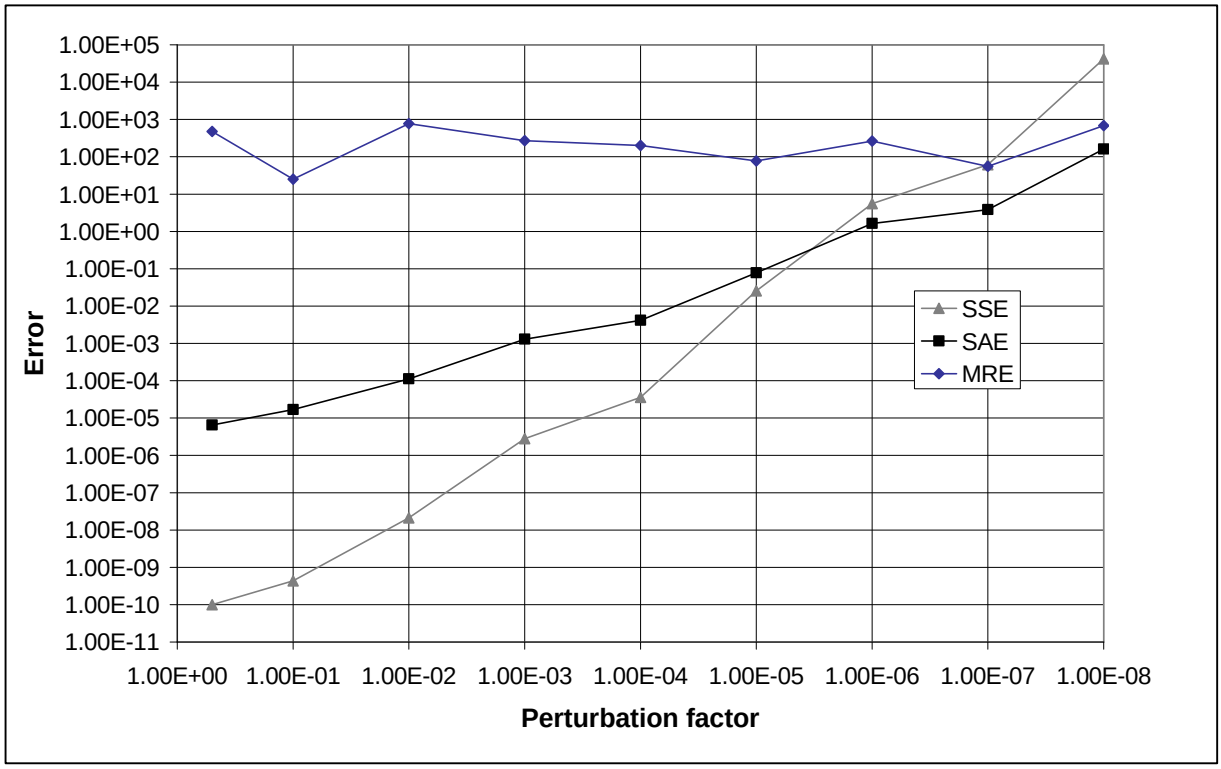

Figure 4.2.10: Errors (SSE, SAE and MRE) for parameter $k_{hyd,li}$ and variable S_{ac}

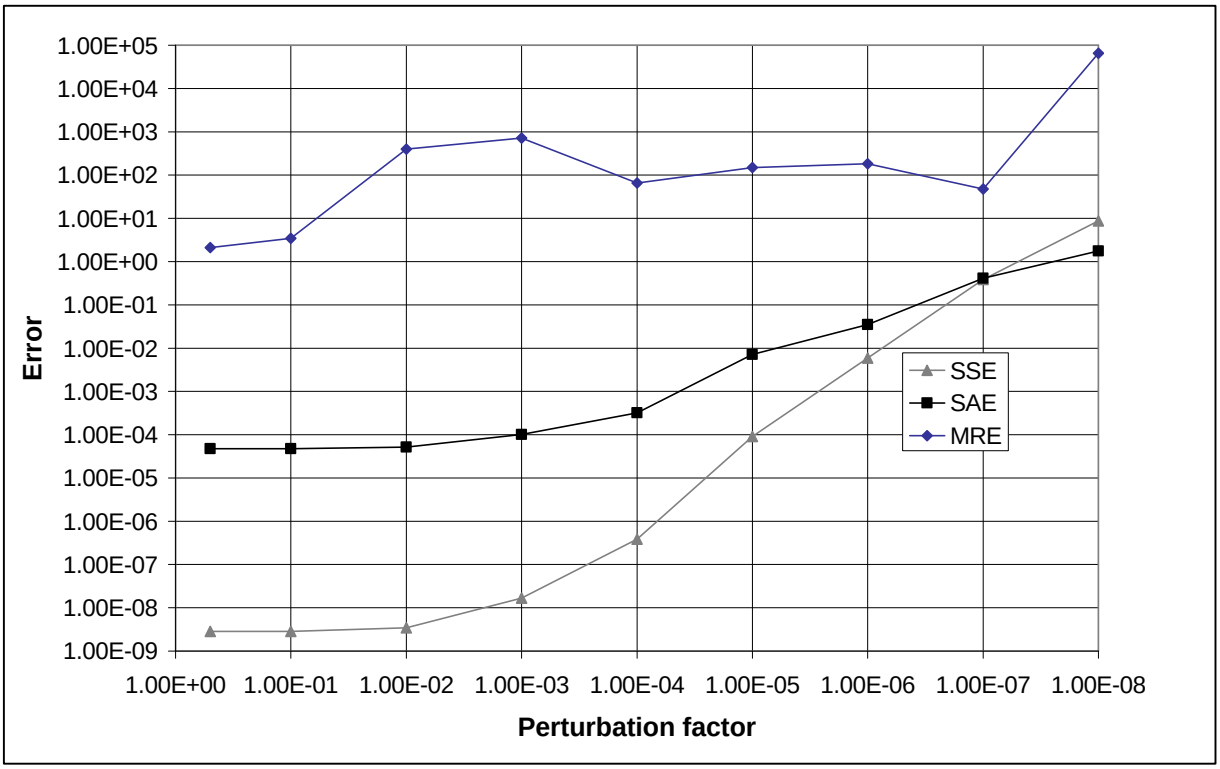

Figure 4.2.11: Errors (SSE, SAE and MRE) for parameter $k_{m,c4}$ and variable S_{ac}

The carbohydrate hydrolysis rate has the greatest impact on the particulate carbohydrate (X_{ch}) and on the sugar concentration (S_{Su}), but also on the hydrogen concentration in the gas phase and the hydrogen concentration.

Whereby the lipid hydrolysis rate mostly influences the particulate lipid concentration (X_{li}) and long chain fatty acid concentration (S_{fa}), as well as the methane concentration in the gas ($S_{gas,ch4}$) and the methane concentration (S_{ch4}).
The disintegration rate is affected most of all by the complex particulate concentration (X_{xc}) as well as the methane concentration in the gas ($S_{gas,ch4}$) and the methane concentration (S_{ch4}) in the liquid phase, and finally the complex particulate concentration.
The hydrogen concentration in the gaseous phase is the variable that is affected most by most of all parameters. For instance by all half saturation constants ($K_{S,IN}$, $K_{S,su}$, $K_{S,aa}$, $K_{S,fa}$, $K_{S,c4}$, $K_{S,pro}$, $K_{S,ac}$ and $K_{S,H2}$) and other parameters, too, such as Y_{fa}, $K_{I,H2,fa}$, Y_{c4}, $K_{I,H2,c4}$, $k_{m,pro}$, Y_{pro}, $K_{I,H2,pro}$, $k_{m,ac}$, Y_{ac}, and Y_{H2}. Followed by the hydrogen concentration in the liquid phase, which is governed by the sugar and amino acid substrate uptake rate and the biomass yield of monosaccharides and amino acids.
All other variables are most influenced by one parameter only, thus the bacteria are mostly effected by their decay rates, for example X_{su} is mostly influenced by $k_{dec,su}$, X_{aa} by $k_{dec,aa}$, X_{fa} and so on. In the same way the particulate components are most of all influenced by their hydrolysis and disintegration rate (i.e. X_{xc} by k_{dis}, X_{ch} by $k_{hyd,ch}$, X_{pr} by $k_{hyd,pr}$ and X_{li} by $k_{hyd,li}$). Interesting here is that X_{xc} is also most of all influenced by $k_{m,H2}$, X_{ch} also by $k_{m,fa}$ and X_{li} by $k_{m,c4}$.
The IWA Task group came to a similar conclusion in the ADM1 report (Batstone *et al.* 2002) investigating different data from literature, they found that k_{dis}, $k_{hyd,ch}$, $k_{hyd,pr}$, $k_{m,ac}$, $K_{S,ac}$ and $pH_{UL,ac}$ have a significant sensitivity.
Wett *et al.* (2007) found that the most sensitive parameters are the disintegration rate, the saturation coefficient for monosaccharides, the decay rate of sugar degraders and also the carbon and nitrogen contents of the particulate fraction X_c.
Insensitive parameters should be omitted from the parameter estimation for reasons of simplification (Olsson and Newell 1999). Following the results of the sensitivity analysis the disintegration and hydrolysis rates are determined and calibrated up front in the model.

Table 4.2.4: Results of the sensitivity analysis of the ADM1 (Parameter ranking according to the calculated Sensitivity coefficients). Furthermore: comparison with the literature data: Most sensitive parameter of the ADM1 found by Wett *et al.*, 2007 and Batstone *et al.*, 2002 (shown as circles (without ranking)).

#	Parameter	Senstivity Coefficient	Wett et al. 2007	Batstone et al., 2002	#	Parameter	Senstivity Coefficient	Wett et al. 2007	Batstone et al., 2002
1	$k_{hyd,pr}$	1.12E-02		o	20	$K_{S,aa}$	1.27E-10		
2	$k_{hyd,ch}$	1.12E-02		o	21	$K_{S,pro}$	6.30E-11		
3	$k_{hyd,li}$	1.11E-02			22	$K_{S,H2}$	4.92E-11		
4	k_{dis}	9.82E-03	o	o	23	$k_{m,fa}$	4.75E-11		
5	$k_{m,aa}$	1.54E-03			24	$k_{m,H2}$	3.61E-11		
6	$k_{dec,pro}$	1.29E-03			25	$K_{S,su}$	3.12E-11	o	
7	$k_{dec,su}$	1.11E-03	o		26	$K_{S,fa}$	3.03E-11		
8	$k_{dec,ac}$	1.08E-03			27	$k_{m,c4}$	2.45E-11		
9	$k_{dec,c4}$	1.07E-03			28	Y_{fa}	2.18E-11		
10	$k_{dec,fa}$	9.45E-04			29	$K_{S,IN}$	1.66E-11		
11	$k_{dec,h2}$	9.23E-04			30	$K_{S,ac}$	1.36E-11		o
12	$k_{dec,aa}$	9.09E-04			31	Y_{c4}	1.32E-11		
13	$k_{m,su}$	1.97E-07			32	Y_{pro}	1.10E-11		
14	$K_{I,H2,pro}$	1.80E-08			33	$K_{S,c4}$	8.22E-12		
15	Y_{su}	9.52E-09			34	Y_{H2}	8.18E-12		
16	Y_{aa}	8.09E-09			35	Y_{ac}	6.66E-12		
17	$K_{I,H2,c4}$	7.70E-09			36	$K_{I,NH3,ac}$	6.21E-12		
18	$K_{I,H2,fa}$	1.70E-09			37	$k_{m,ac}$	3.21E-12		o
19	$k_{m,pro}$	9.49E-10							

4.2.5 Parameter estimation

Parameters have to be chosen in such a way that the likelihood that measured data can be predicted by the model is the highest (Olsson and Newell 1999). Yet, most of the parameters suggested in the ADM1 are not fully suitable for energy crops. Therefore a literature search for appropriate parameters was done for the most important and sensitive parameters (Table 4.2.5 and Table 4.2.6). This data includes experimental data and data used in modeling (Henze and Harremoes 1983).

Pavlostathis and Gossett (1985) found that the limiting steps in anaerobic digestion are those related to the conversion of substrate into a soluble form and the formation of methane from acetate and propionate. The IWA task-group came to a similar conclusion in their description of the Anaerobic Digestion Model No.1 (Batstone *et al.* 2002).

As there is generally a great lack of specific data on energy crop digestion in literature (Lindorfer 2007) and the values found in the literature have a great range of margin, the parameters were also experimentally estimated (from other project partners, too) for different crops, particularly maize silage.

Looking at the hydrolysis (disintegration) rates (first order) from literature, a mean value of 1.42 d^{-1} ± 8.10 is found, with a minimum value of $4.10*10^{-3}$ d^{-1} and maximum of $1.06*10^{2}$ d^{-1} independent from substrate and temperature range. Normally a hydrolysis rate is valid for a certain substrate under certain conditions and other factors such as e.g. the particle shape (Bolzonella *et al.* 2005). This seems the reason why even for one and the same substrate at the same temperature great differences are noticed, for example for the hydrolysis rate of carbohydrates (n = 9) at a temperature of 35 °C a minimum of $1.30*10^{-1}$ d^{-1} and maximum of $1.06*10^{2}$ d^{-1} is found. For the proteins hydrolysis rate (n = 10) and lipids hydrolysis rate (n = 12) a minimum of $9.60*10^{-3}$ d^{-1} and $5.00*10^{-3}$ d^{-1} and a maximum of 2.70 d^{-1} and $1.00*10^{1}$ d^{-1} respectively were determined. Thus estimated rates should be seen as indicative and not as universal (Bolzonella *et al.* 2005).

The same applies for the substrate uptake rate – here a mean value of $7.90*10^{1}$ $kg_{COD}kg_{COD}d^{-1}$ ± $4.68*10^{2}$ with a minimum of $1.40*10^{-1}$ $kg_{COD}kg_{COD}d^{-1}$ and a maximum of $5.07*10^{3}$ $kg_{COD}kg_{COD}d^{-1}$ independent from substrate and temperature was detected. As before even for the same substrate at the same temperature great differences can be observed, for example for substrate uptake rate of acetate (n = 7) at 35 °C a minimum of 3.10 $kg_{COD}kg_{COD}d^{-1}$ and maximum of $1.30*10^{1}$ $kg_{COD}kg_{COD}d^{-1}$ were spotted. For butyrate at 35 °C, as well, a minimum of 3.50 $kg_{COD}kg_{COD}d^{-1}$ and a maximum of $4.10*10^{1}$ $kg_{COD}kg_{COD}d^{-1}$ were found.

The yield coefficients of anaerobic processes are generally lower than of aerobic processes, which is a result of the small ATP yield (Henze and Harremoes 1983).

During the project hydrolysis rates for energy crops and other substrates were determined in batch experiments over a temperature range from 35 °C to 55 °C by

project partners, such as the hydrolysis rates for Bracken, Buckwheat, Carrots, Hay, Jerusalem artichoke, Knotweed, Lawn, Lupine, Maize silage, Oil seed rape, Sweet clover and more (Table 4.2.7).

Table 4.2.5: Hydrolysis rates (disintegration rates) of different substrates from literature

Substrate	$k_{hyd(first\ order)}$ [d^{-1}]	Temperature [°C]	Reference
Bark	7.60E-02	30	Veeken and Hamelers, 1999
Blackwater + Kitchen refuse	4.50E+00	38	Feng et al., 2006
Cattle manure	1.30E-01	37	Vavilin et al., 1997
Cellulose	9.00E-02	37	Myint et al, 2007
Diapers	2.50E-02	35	Jokela et al., 2005
Excrement wih gelatine	1.00E+00	55	Angelidaki et al., 1999
food waste	4.10E-01	55	Vavilin et al., 1998
forest soil	5.40E-01	30	Lokshina and Vavilin, 1999
Grass	3.50E-02	20	Veeken and Hamelers, 1999
Grey waste	3.10E-02	35	Jokela et al., 2005
Hemicellulose	1.40E+00	37	Myint et al, 2007
Leaves	6.80E-02	20	Veeken and Hamelers, 1999
Lipids	1.00E+01	38	Feng et al., 2006
Manure/Oil	2.40E-01	55	Angelidaki et al., 1992
Meat peptone	2.30E+00	37	Gonzalez et al., 2005
Newsprint	5.60E-02	35	Jokela et al., 2005
Newsprints	5.68E-02	35	Vavilin et al., 2004
Office paper	3.60E-02	35	Jokela et al., 2005
Orange peelings	1.45E-01	20	Veeken and Hamelers, 1999
Packaging	5.80E-02	35	Jokela et al., 2005
Packagings	5.60E-02	35	Vavilin et al., 2004
Pig manure	9.60E-02	6	Vavilin et al., 1997
Pond silt	1.30E-02	28	Lokshina and Vavilin, 1999
Primary Sludge	2.50E-01	55	Siegrist et al., 2002
Protein	1.00E+01	38	Feng et al., 2006
Putrescibles	1.07E-01	35	Jokela et al., 2005
Slaughterhouse	7.00E-02	35	Salminen et al., 2000
Straw	2.40E-02	20	Veeken and Hamelers, 1999
Textiles	2.10E-02	35	Jokela et al., 2005
Two-phase olive pomace	5.40E-02	35	Borja et al., 2005
Wholewheat bread	1.95E-01	30	Veeken and Hamelers, 1999

Table 4.2.6: Substrate uptake rate, half saturation constant, growth rate, yield and decay rate for different substrate from literature

Substrate	Input Substrate	k_m [$kg_{COD} kg_{COD} d^{-1}$]	K_S [$kg_{COD} m^{-3}$]	Y [$kg_{CODX} kg_{CODS}^{-1}$]	k_{dec} [d^{-1}]	Temperature [°C]	Reference
Acetate	Pig slaughterhouse wastewater	1.28E+01	3.00E+00	2.60E-01	1.99E-02		Batstone et al., 2000
Butyrate	Blackwater + Kitchen refuse	1.80E+01	1.10E+02			38	Feng et al., 2006
Formate	Cassava starch wastewater	6.00E+00				35	Zaher et al., 2006
Glucose		1.77E+02	2.46E-02		2.00E-02	20	Costello et al., 1991
Hydrogen	Pig slaughterhouse wastewater	1.77E+01	5.50E-02	1.93E+01	9.00E-03		Batstone et al., 2000
Lactate	Pig slaughterhouse wastewater	1.39E+02	1.14E+00		2.06E-02		Batstone et al., 2000
LCFA	Pig slaughterhouse wastewater	1.11E+00	3.10E+00	3.14E-02	3.00E-02		Batstone et al., 2000
Propionate	Blackwater + Kitchen refuse	1.40E+01	1.20E+02			38	Feng et al., 2006
Sugar	Manure/Oil	4.93E+01	5.33E-01	1.00E-01	1.00E-02	55	Angelidaki et al., 1992
Valerate	Manure/Oil	1.37E+01	3.57E-01	5.00E-02	1.00E-02	55	Vavilin et al., 1996

Table 4.2.7: Hydrolysis rates of different substrate determined during CROPGEN by project partners

Substrate	$k_{hyd(first\ order)}$ [d^{-1}]	Temperature [°C]	Reference
Braken	1.81E-01	35(+-2)	Publication in preparation. Results Environmental Technology dpt. Wageningen University. Pabon, van Lier et al. 2007
Carrot	1.00E+00	35(+-2)	Publication in preparation. Results Environmental Technology dpt. Wageningen University. Pabon, van Lier et al. 2007
Grass hay 2 since day 0	1.90E-02		Lehtomäki et al., 2005
Jerusalem artichoke 1 since day 0[b]	2.10E-02		Lehtomäki et al., 2005
Knotweed 1	5.60E-02		Lehtomäki et al., 2005
Lawn since day 49[a]	9.40E-02		Lehtomäki et al., 2005
Lupine 1 since day 25[a]	6.70E-02		Lehtomäki et al., 2005
Maize silage	2.70E-01	35(+-2)	Publication in preparation. Results Environmental Technology dpt. Wageningen University. Pabon, van Lier et al. 2007
Marrow kale 1since day 0	9.00E-03		Lehtomäki et al., 2005
Mix Market waste (fruit+vegetable)	2.10E-01	37	Bolzonella
Mixed Agro-wastes	2.80E-01		Bolzonella
Mixed market waste and sewage sludge	3.05E+00	55	UNIVE-DSA, Cavinato
Nettle 2 since day 0	3.00E-02		Lehtomäki et al., 2005
Oil seed rape	1.61E-01	35(+-2)	Publication in preparation. Results Environmental Technology dpt. Wageningen University. Pabon, van Lier et al. 2007
Red clover 1 since day 10	3.90E-02		Lehtomäki et al., 2005
Reed canary grass 1	3.90E-02		Lehtomäki et al., 2005
Rhubarb 1 since day 0	3.40E-02		Lehtomäki et al., 2005
Sewage Sludge + Market waste (1305.7 $g_{COD}d^{-1}$)	4.28E+00		Pavan
Spartina-Cordgrass	1.67E-01	35(+-2)	Publication in preparation. Results Environmental Technology dpt. Wageningen University. Pabon, van Lier et al. 2007
Straw of oats since day 17	4.30E-02		Lehtomäki et al., 2005
Tops of sugar beet since day 0	1.90E-02		Lehtomäki et al., 2005
Triticale	1.21E-01	35(+-2)	Publication in preparation. Results Environmental Technology dpt. Wageningen University. Pabon, van Lier et al. 2007
Vetch 2 since day 12	4.70E-02		Lehtomäki et al., 2005
Yellow lupin	3.18E-01	35(+-2)	Publication in preparation. Results Environmental Technology dpt. Wageningen University. Pabon, van Lier et al. 2007

As it is not possible to get suitable kinetic parameters in the CSTR. Syringe experiments were used to estimate hydrolysis rates. Simultaneous batch experiments were used as a basic method for the estimation of parameters (Flotats *et al.* 2006). In this approach the evolution of different substances in a set of batch experiments were distinguished by different initial conditions for known substances and constant initial concentrations for others (Flotats *et al.* 2006).
In this study a hydrolysis rate for protein was determined in batch experiments. The batch experiments were performed in 100 ml plastic syringes (VWR, BD Plastipak). This test arrangement has the advantage of being simple, fast and space-saving.
Here a hydrolysis rate for proteins of 0.702 d^{-1} could be found (Figure 4.2.12).

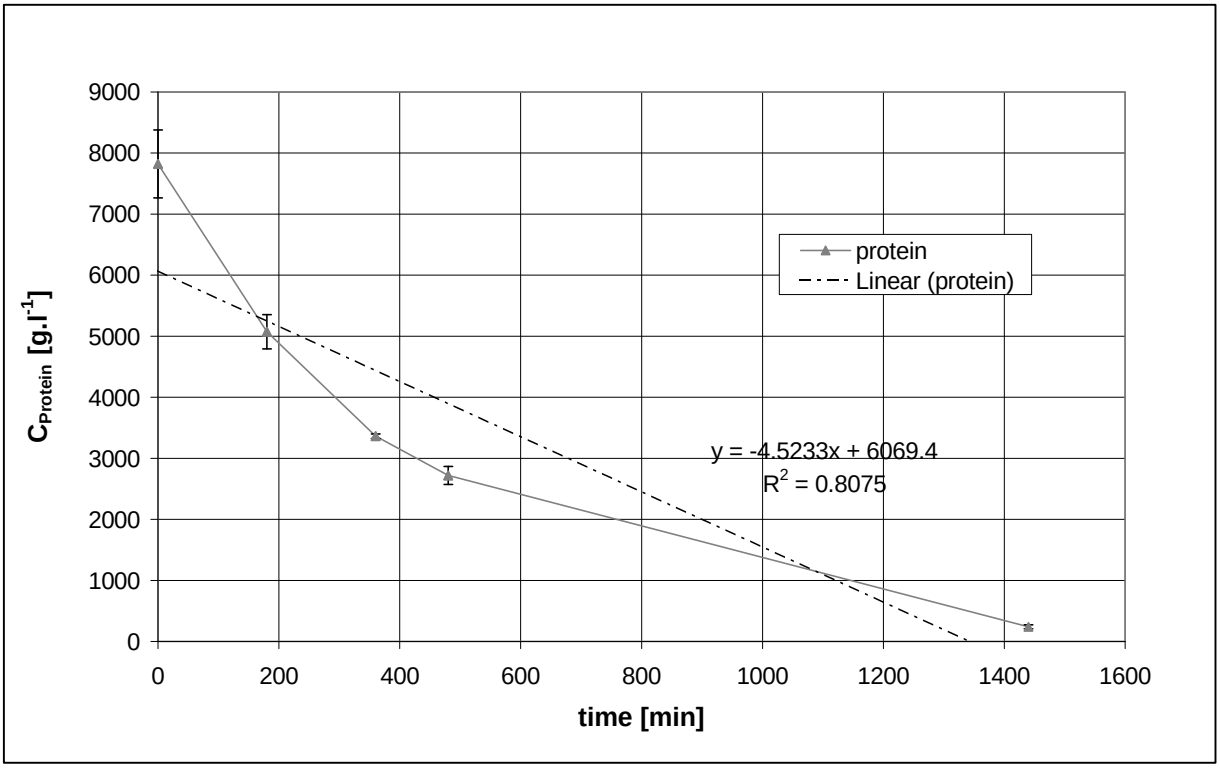

Figure 4.2.12: Results of the degradation test to obtain kinetic data: protein concentration

In the ADM1 now a value of 10 d^{-1} is suggested - It is assumed that under certain circumstances the same rate can be used for carbohydrates, proteins and lipids (Feng *et al.* 2006). The high value makes the model easier to manipulate, as the influence of the hydrolysis is excluded from the model (Feng *et al.* 2006). It also makes the model less sensitive to distribution ratios between the three, if the hydrolysis rates are the same (Feng *et al.* 2006).

4.2.6 Calibration algorithms

The application of an automatic calibration algorithm is especially interesting for simplified AD models that are identifiable. Automatic control algorithms are applicable for the ADM1if the model is divided in smaller sub-models, which again are identifiable.
In this study now three different automatic control algorithms were adapted for the use with AD models:

- a Genetic algorithm,
- Simulated Annealing and
- a Luus-Jaakola algorithm.

All three algorithms were implemented in script-form in MATLAB®.

Parameter estimation can primarily seen as an optimisation problem, where the parameters sought can be determined by minimizing a so called “objective function” (Linga *et al.* 2006). This function is a measure of the difference between the values observed and the values predicted (Laquerbe *et al.* 2001; Linga *et al.* 2006; Kremling and Saez-Rodriguez 2007). It has to be recognized that model predicted values will never fit perfectly to measurement data, due to measurement errors or noise (De Pauw 2005). In the literature a lot of different object functions are found. A frequently used objective function is the sum of squares (De Pauw 2005) (Equation (101)):

$$OF(k) = \sum_{i=1}^{n} \left(x_{i,mes} - x_{i,pre}(k) \right)^2 \quad \textbf{(101)}$$

A feasible multiple objective function (OF(k)) is according to Linga *et al.* (2006) (Equation (102)):

$$OF(k) = \left[\sum_{i=1}^{n} \left(x_{i,mes} - x_{i,pre(ti,k)} \right)^T * w_i * \left(x_{i,mes} - x_{i,pre(ti,k)} \right) \right] \quad \textbf{(102)}$$

where

k	vector of unknown parameter
$x_{,, mes}$	measured values
x_i, pre	predicted values
w_i	weighting matrix
n	number of measured values

Possible weighting matrices are (Kremling and Saez-Rodriguez 2007) (Equation (103)):

$$w_i = \frac{1}{\left(\frac{\left(x_{i,1} + x_{i,2} \right)}{2} \right)^2} \quad (103)$$

Here the OF is weighted by the average of two models. Another approach is (Kremling and Saez-Rodriguez 2007) (Equation (104)):

$$w_i = \left(C + VC_1 + VC_2 \right)^{-1} \text{ with } VC = WF^{-1}W^T \quad (104)$$

Where C is the variance of measured values and VC is the covariance-variance of the values predicted, determined from the sensitivity matrix W and the Fisher Information matrix (FIM). Only the diagonal elements of C and VC are used here (Kremling and Saez-Rodriguez 2007).

Another possibility for an objective function is (Kremling and Saez-Rodriguez 2007) (Equation (105)):

$$OF = \sum_{n} \sum_{i} \sum_{j} \frac{\left(x_{i,j,mes} - x_{i,j,pre}(k) \right)^2}{\sigma_{i,j}^2} \quad (105)$$

where

$x_{i,j,mes}$ measured values
$x_{i,j,pre}$ predicted values
k parameter vector
$\sigma_{i,j}$ standard deviation of the measured values

In this OF the measurement error is considered by adding the variance of the measured values, where $1/\sigma_{i,j}^2$ acts as a weight to compensate for the measurement errors (De Pauw 2005).

Laquerbe and co-workers (2001) define the objective function similar to the De Pauw (2005) as (Equation (106)):

$$OF = \frac{1}{n}\sum_{i=1}^{n}\left[x_{i,mes} - x_{i,pre}\right]^2 \quad \textbf{(106)}$$

where
n Number of experimental values
$x_{i,j,mes}$ measured values
$x_{i,j,pre}$ predicted values

they further weighted the objective function with a outer penalty term ($\rho \cdot \dim(p)$) (Laquerbe *et al.* 2001) (Equation (107)):

$$OF' = OF + \rho \dim(p) \quad \textbf{(107)}$$

where
p parameters that are optimized
ρ penalty factor

In the case that the parameters chosen are close to the "true" parameter values the difference between the predicted and the observed values approaches zero and thus the OF gets small. This minimization of the OF can be done by trial and error by an automatic optimization method. In order to be effective an optimisation method must solve the optimisation problem in passable time and the global minimum/maximum must be found after the smallest possible number of evaluations. (Kaczmarski and Antos 2006).

Optimisation methods can be distinguished in two classes: gradient methods and direct search methods (Linga *et al.* 2006). These methods are a set of functions with adjustable parameters and approximate the optima by varying these variables (Tsoulos and Lagaris 2006). It has to be indicated the gradient search methods require derivatives of the objective function (Linga *et al.* 2006). It has further to be mentioned that these methods often fail to find the global optimum for "hard-to-optimise" objective functions (Kaczmarski and Antos 2006).

An example of a gradient search method is the Gauss-Newton method (Linga *et al.* 2006). This method should always be associated with the Marquardt-Levenberg modification to enhance robustness (Linga *et al.* 2006).

Frequently used direct search algorithms are neural networks, genetic algorithms, iterative dynamic programming (Bojkov and Luus 1994) and Luus_Jaakola (LJ) optimization (Linga *et al.* 2006). Further alternatives are "simulated annealing" or "taboo search" (Kaczmarski and Antos 2006). These search algorithms are especially suited to

solve optimisation problems, where the optima is surrounded by a lot of local minima (Kaczmarski and Antos 2006). Other alternatives are, for example, the Simplex algorithm and the Praxis optimisation algorithm (De Pauw 2005).

Genetic Algorithm (GA)

Genetic Algorithms (GA) are loosely based on the mechanics of natural selection and genetics (Laquerbe *et al.* 2001; Alcock and Burrage 2004; Mwembeshi *et al.* 2004; De Pauw 2005; Coleman and Block 2006). Convergence to the exact minima cannot be guaranteed, but it is a robust method for a lot of objective functions to find at least a "near-optimum" (Alcock and Burrage 2004). In the GA a potential solution is called individual or chromosome – each is represented by a sequence of "genes" and ranked corresponding to their objective function value (= fitness) (Laquerbe *et al.* 2001; Alcock and Burrage 2004; Mwembeshi *et al.* 2004). A population is then a set of chromosomes with their fitness values (Alcock and Burrage 2004). The GA is iteratively improving the fitness either by reproduction, cross-over or mutation (Laquerbe *et al.* 2001; Alcock and Burrage 2004) – that means that the GA emulates the evolutionary theory (Laquerbe *et al.* 2001) and simulates a digital survival of the fittest (Mwembeshi *et al.* 2004). Reproduction means the copying of the genes of a chromosome from one generation to another, cross-over stands for the mixing of genes of individuals to form new individuals in the next generation and mutation is a random switching of genes in an individual (Alcock and Burrage 2004).

The algorithm consists of four phases: Initialisation, Fitness evaluation, genetic operations and the Termination control (Tsoulos and Lagaris 2006). In the first phase the mutation (control of changes inside a chromosome) and replication rate (rep_rate, number of chromosomes that will not be changed) are defined (Tsoulos and Lagaris 2006). The cross-over rate is therefore determined as 1 – rep_rate (Tsoulos and Lagaris 2006). Tsoulos and Lagaris (2006) chose 10 % for the replication rate and 5% for the mutation rate. To define the GA parameter (n_{pop}, $n_{gen,}$ r_{mut} and r_{rep}) can be quite difficult and can also lead to unusual values "*to insure good convergence*" (Laquerbe *et al.* 2001).

Initialisation is performed once in the 1st generation (Tsoulos and Lagaris 2006). The initial population is normally generated using a random procedure (Laquerbe *et al.* 2001). In the next step the fitness value is calculated (= objective function).

The genetic operations are then performed in all generations (Tsoulos and Lagaris 2006). First the individuals are sorted according to there fitness values (best to worse) (Tsoulos and Lagaris 2006). Then (1 – rep_rate)*num_in[19] new chromosomes are reproduced by cross-over (Laquerbe *et al.* 2001; Tsoulos and Lagaris 2006). For cross-over a group of N ≥ 2 randomly selected individuals is chosen and from this group the chromosome with the best fitness is taken (Tsoulos and Lagaris 2006). There are

[19] Num_in = number of individuals

different possibilities for cross-over and mutation operators: simple crossover (variables of two solutions are exchanged), arithmetic cross-over (two complimentary linear combinations of two solutions), heuristic cross-over (linear exploration of two solutions), uniform mutation (one variable is exchanged with a uniformly random number), non-uniform mutation (one variable is swapped with a non-uniformly random number) and boundary mutation (a randomly selected variable is set to its lower or upper limit (Alcock and Burrage 2004).

Mutation is applied for all chromosomes except those that are replicated (Tsoulos and Lagaris 2006).

The probability P_i that an individual survives can be formulated as (Laquerbe *et al.* 2001) (Equation (108)):

$$P_i = \frac{f_i}{\sum_{k=1}^{n_{pop}} f_k} \quad \textbf{(108)}$$

where

f_i fitness of the individium

f_k overall fitness

The 2nd and the 3rd steps are repeated until the termination criteria is met or the maximum number of generations is reached (Laquerbe *et al.* 2001; Mwembeshi *et al.* 2004; Tsoulos and Lagaris 2006). The fittest individual of each generation stays either the same or is gets better, the same applies for the average fitness function (Mwembeshi *et al.* 2004).

The size of the populations is critical factor – if too small the method is ineffective and if too big it becomes slow (Tsoulos and Lagaris 2006). Tsoulos and Lagaris (2006) found that a population size of between 200 and 1000 is proper (they used 1000, with a chromosome length of 50).

Mwembeshi and co-workers (2004) formulated the fitness function in the maximisation process of the GA optimization procedure as the following (Equation (109)):

$$f_i = \frac{l}{(1+OF_i)^m} \quad \textbf{(109)}$$

where:

OF Objective Function, which has to be minimized

m sensitivity integer

i positive constant that scales the fitness values

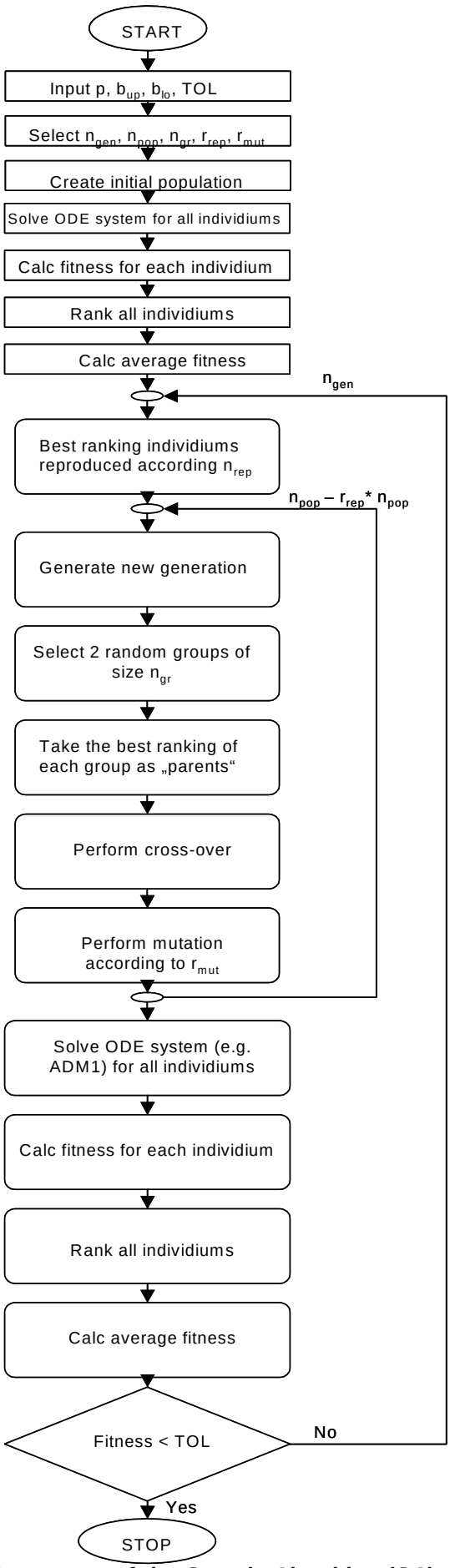

Figure 4.2.13: Adapted structure of the Genetic Algorithm (GA) optimisation algorithm for use with AD models

Mwembeshi *et al.* (2004) was using the integral absolute error (IAE) as objective function. Laquerbe and co-worker (2001) set the fitness function to (Equation (110)):

$$f_i = \left(\frac{OF_i}{\sum_{k=1}^{n_{pop}} \frac{OF_k}{n_{pop}}} \right)^{-1} \quad \textbf{(110)}$$

where

n_{pop} Size of population

Here the fitness of each individium is scaled by the average fitness of the whole population (Laquerbe *et al.* 2001).

The size of the "search space" (SESP) of the GA can be estimated according to (Laquerbe *et al.* 2001) (Equation (111)):

$$SESP = n_{pop} * \left[1 + n_{gen} * \left(r_{mut} + r_{cross-over}\right)\right] \quad \textbf{(111)}$$

The adapted GA algorithm is partly based on a MATLAB®-Sript by Houck and co-workers (1995) (see also (Houck *et al.* 1995)).

Simulated Annealing (SA)

A further optimisation technique is the simulated annealing (SA) procedure. SA is a heuristic method (Schramm *et al.* 2004) and was first introduced by Kirkpatrick and co-workers in 1983[20] (Lee *et al.* 2008) and copies the annealing of solid, where the reordering of the crystals follows the laws of probability rules (Laquerbe *et al.* 2001; Kaczmarski and Antos 2006). The aim is to reach the atomic configuration, which minimizes the internal energy (Laquerbe *et al.* 2001; Kaczmarski and Antos 2006). In a SA algorithm not only downhill moves, but also uphill moves are permitted (Lee *et al.* 2008). The SA procedure distinguishes between different local optima (Roytman and Safro 1998) – as it finds the global optima, as slow annealing leads to the lowest internal state, and avoids stopping at local minima, as fast cooling generates a metastable state (Laquerbe *et al.* 2001; Kaczmarski and Antos 2006).

If during the gradual temperature decrease the energy (= object function) of the material is lower than the energy in the current state, then the new state replaces the current state (Lee *et al.* 2008). Yet, if the energy of the new state is higher than the energy in the current state a probability factor decides whether the current state is replaced by the new state or not (Lee *et al.* 2008).

The method makes only few assumptions regarding the function, which is optimised and can be thus used as an optimiser for difficult functions (Roytman and Safro 1998).

[20] Kirkpatrick, S, Gelatt, C.D. and Vecchi, M.P., 1983, Optimization by Simulated Annealing, Science, 220, 4598, 671-680

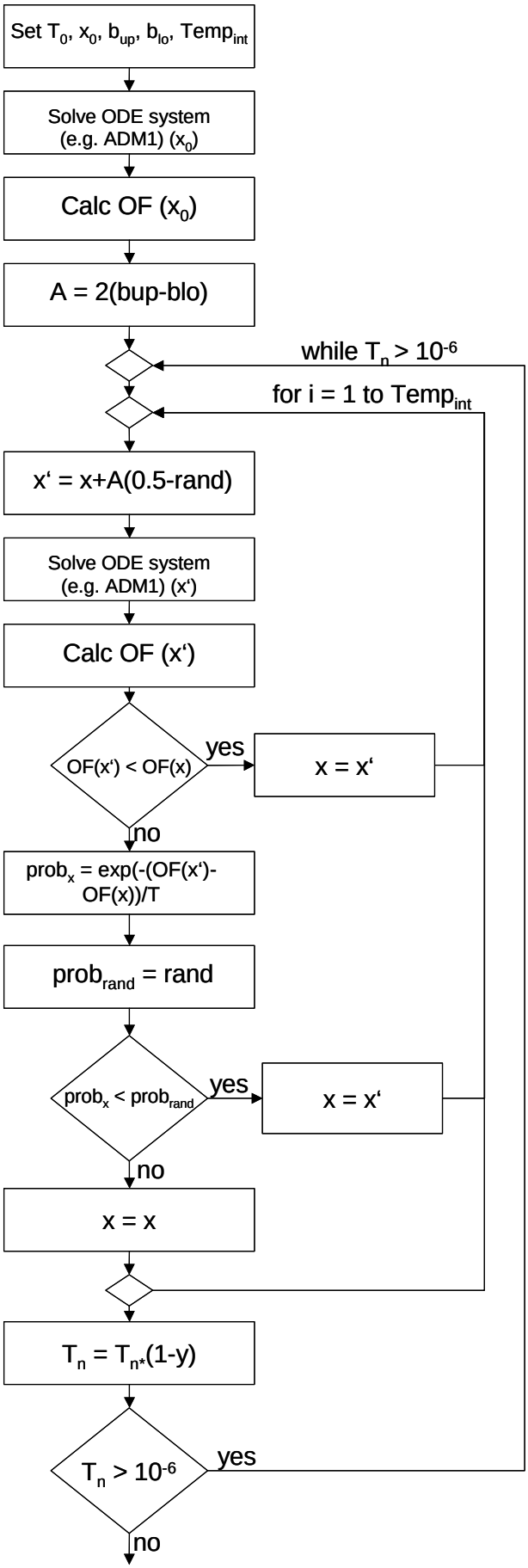

Figure 4.2.14: Adapted structure of the Simulated Annealing (SA) optimisation algorithm for use with AD models

Luus-Jaakola Algorithm (LJ)

The LJ method (Figure 4.2.15) is an optimisation method using random search points and region reduction (Peng Lee *et al.* 1999) and was introduced by Luus and Jaakola (1973). With the LJ method even optimisation problems with multiple local minima could be solved (Linga *et al.* 2006).

Linga and co-workers (2006) found an optimum for the number of randomly chosen points (n_{points}) of 30, by a chosen value of 100 iterations (n_{iter}). The concentration factor (ε) is 0,95, the Tolerance (TOL) is set to 10^{-6} and ϕ, the p-dimensional vector of random numbers between -0,5 and 0,5 (Linga *et al.* 2006). Whereas Luus and Hennessy (1999) where using random numbers between -1 and 1.
As there is always the possibility of a local optimum (minimum), it useful to use a second optimisation algorithm (Luus and Hennessy 1999). It has to be mentioned that Luus (2001) changes the region size for the first passes (n_{passes} = 10) following the formula $r^{q+1} = \eta * r^{q}$, with $\eta = 0{,}7$, after 10 passes the region value is restored to r^{q} = 0,01. Further parameters are TOL = 10^{-8}, n_{passes} = 200 and n_{iter} = 15 (Luus 2001). Luus (1999) found that using a multipass procedure with a smaller number of iterations was more effective, than using a single pass system with a large number of iterations.
It was shown that the efficiency of the method can be increased, when at the beginning of the passes the region size is determined from the size of the variation of the corresponding variables during the previous pass (Peng Lee *et al.* 1999).
The chance to approach the global minimum is improved by using an increased initial search region (Peng Lee *et al.* 1999). Especially for the first pass the initial search region should be sufficiently large to cover the entire feasible region (Peng Lee *et al.* 1999).

Unfortunately it was only possible to test these control algorithms cursorily within the framework of the project. For practical application further tests and possibly some improvements are still necessary (e.g. adaptation of optimisation parameters).
The development of more than one optimisation algorithm was necessary as in a lot of practical applications many local minima exist, this applies also for the ADM1. The global optimum, is therefore difficult to determine, a cross-checking with different optimisation procedures is important (Bojkov and Luus 1992).

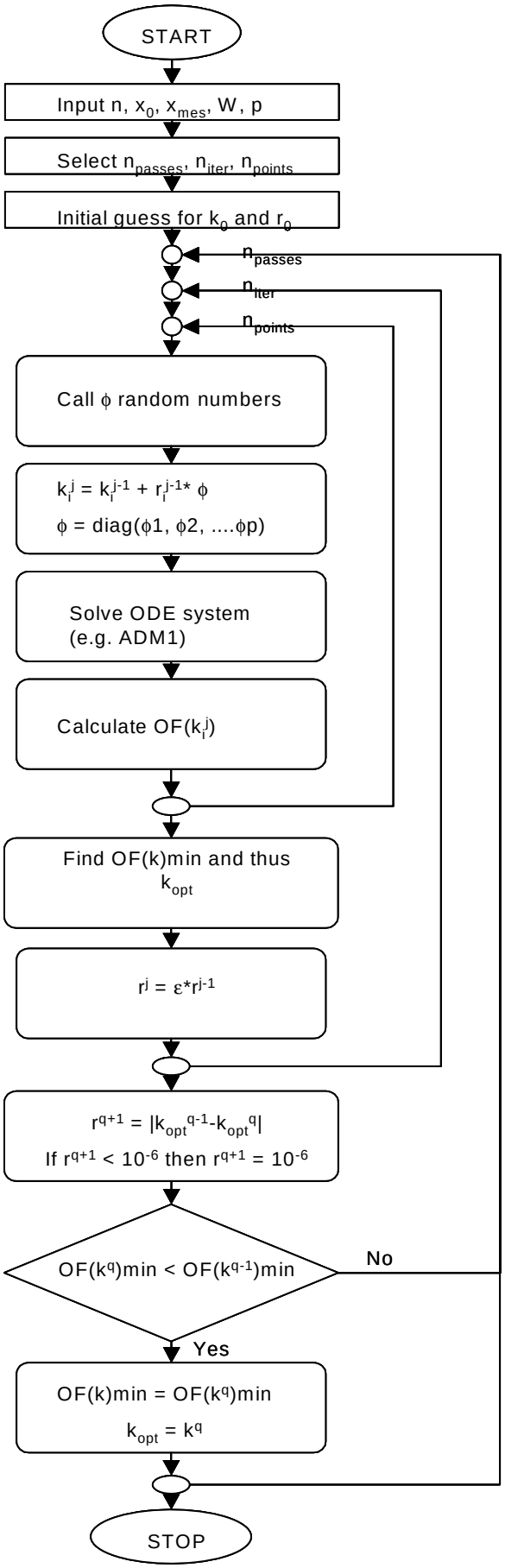

Figure 4.2.15: Adapted structure of the LJ optimization procedure according to Linga (Linga *et al.* 2006) for use with AD models

4.3 AD models for energy crop digestion

By far the majority of the existing anaerobic digestion models are developed or implemented for use with wastewater or sludge as substrate. AD models especially for the use of energy crops as substrate were not found in literature. Therefore the ADM1 was adapted to model the biogas process using crops as feed input. For use in the Virtual Laboratory the sulphate reduction process was added, as well. Furthermore a simpler model, based on the ADM1 (Batstone *et al.* 2002) and the AD model by Marsili-Libelli and Beni (1996), was developed for implementation in a model based decision support system.

4.3.1 Adaptation of the ADM1

The original ADM1 does not specify all the mechanisms of anaerobic digestion, for instance solid precipitation, homoacteogenesis, glucose alternative products, sulphate reduction and sulphide inhibition, nitrate, weak acid and base inhibition, LCFA inhibition and acetate oxidation (Batstone *et al.* 2002), but encourages the extension and development of it (Strik 2004).
The main limitations of the ADM1 are the incomplete regulation of the products from glucose, the uncertainty in parameter values and the lack of satisfactory understanding of related processes (Batstone *et al.* 2005).

Table 4.3.1: Cellulose and Hemicellulose content of different substrates (presented as crude fibre content), partly assembled by Julia Brändle21

Substrate	CF-content [g.kg-1]	Type	Reference
Maize	208	fresh	DLG, 1997
	260	silage	Preston, 2002
Rye	328	fresh	DLG, 1997
	328	silage	National Academy Press, 2001
Triticale	227	fresh	DLG, 1997
	300	silage	Preston, 2002
Sunflower	210	fresh	Jeroch et al., 1993
	310	silage	Putnam et al., 1990
Lucerne	286	fresh	DLG, 1997
	276	silage	LFL, 2005

[21] DLG, 1997, DLG Futterwerttabellen Wiederkäuer. Hrsg: Universität Hohenheim-Dokumentationsstelle. DLG-Verlags-GesmbH. Frankfurt am Main
Preston, 2002, 2002 feed composition guide
National Academy Press, 2001, Requirements of Diary Cattle, Seventh Revised Edition
Jeroch *et al.*, 1993, Futtermittelkunde
Putnam *et al.*, 1990, Sunflower; in: Alternative Field Crops Manual. University of Wisconsin-Extension, Cooperative Extension, University of Minnesota: Center for Alternative Plant and Animal Products and the Minnesota Extension Service
LFL, 2005, Gruber Tabelle zur Fütterung der Milchkühe, Zuchtrinder, Mastrinder, Schafe, Ziege, Bayrische Landesanstalt für Landwirtschaft

Moreover some of the model hypotheses restricts the ability of the model to simulate every application, for example the definition of the particulate component X_{xc}, which consists of both the influent particulate and the biomass decay products (Huete *et al.* 2005). Huete *et al.* (2005) solved this restriction by uncoupling the biomass decay from the influent particulate component.

Addition of a second hydrolysis rate

Energy crops, such as rye, triticale, sunflower…, have a high content of cellulose and hemicellulose (Table 4.3.1). A second hydrolysis rate for slow degradable carbohydrates was added in order to compensate for the slower degradation of this material (Figure 4.3.1 and equation (112), (113) and (114)).

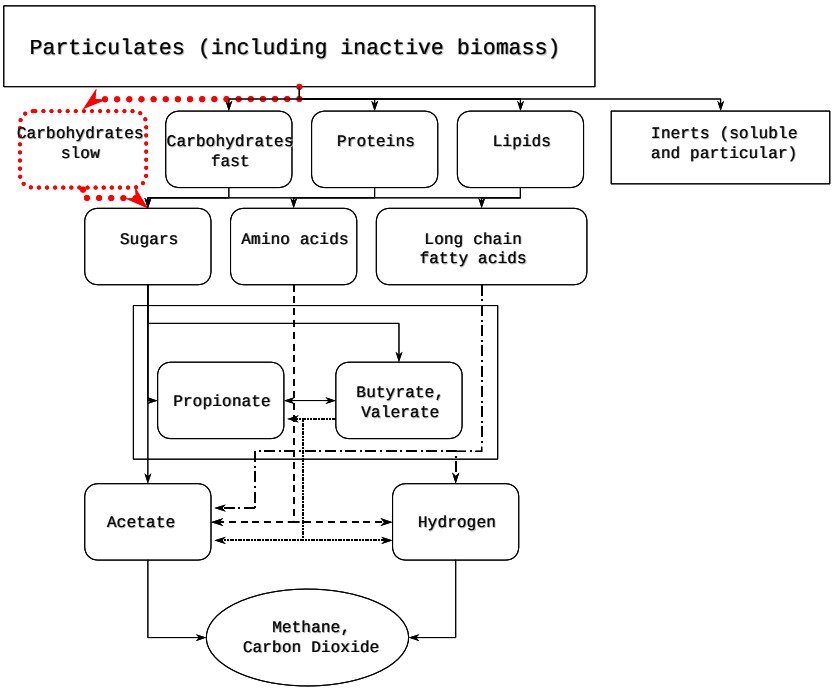

Figure 4.3.1: Schema of the biochemical processes of the adapted model. The red dots (…) shows the changes compared to the original model

Therefore a new process rate was added (Equation (112)) and the existing differential equation for sugar (S_{su} respectively X_i) was changed (Equation (113)) and a new differential equation was also added (equation (114)).

$$\rho_{20} = k_{hyd\_ch\_s} * X(37) \quad \textbf{(112)}$$

$$\frac{dX_1}{dt} = ... + \rho_{20} + ... \quad \textbf{(113)}$$

$$\frac{dX_{37}}{dt} = \frac{q_{in}}{V_{liq}} * \left(X_{in,28} - X_{37}\right) + f_{chs,xc} * \rho_1 - \rho_{20} \quad \textbf{(114)}$$

This minor adaptation was encouraged by a comparison of the adapted model and the original model (Figure 4.3.2, Table 4.3.2 and Table 4.3.3) using the original parameters, suggested by the IWA Task group for the mathematical modelling of anaerobic digestion

(Batstone *et al.* 2002). It was observed that using the adapted model better results can be achieved compared to the original model using different statistical methods, such as the determination coefficient (= square of the correlation coefficient) (r^2), the index of agreement (d) and the Ratio of means (R_{Mean}). Looking at the methane production and COD reduction – here for the original model a value for $r^2_{CH4,original}$ = 0.19 and $d_{CH4,original}$ = 0.60 can be found, though for the adapted model the determination coefficient $r^2_{CH4,adapted}$ = 0.40 and $d_{CH4,adapted}$ = 0.62. For the COD reduction $r^2_{COD,original}$ = 0.06 and $d_{COD,original}$ = 0.06 and $r^2_{COD,adapted}$ = 0.62 and $d_{COD,adapted}$ = 0.88. Whereby a correlation coefficient of 1 would describe an ideal model (Elias *et al.* 2006).

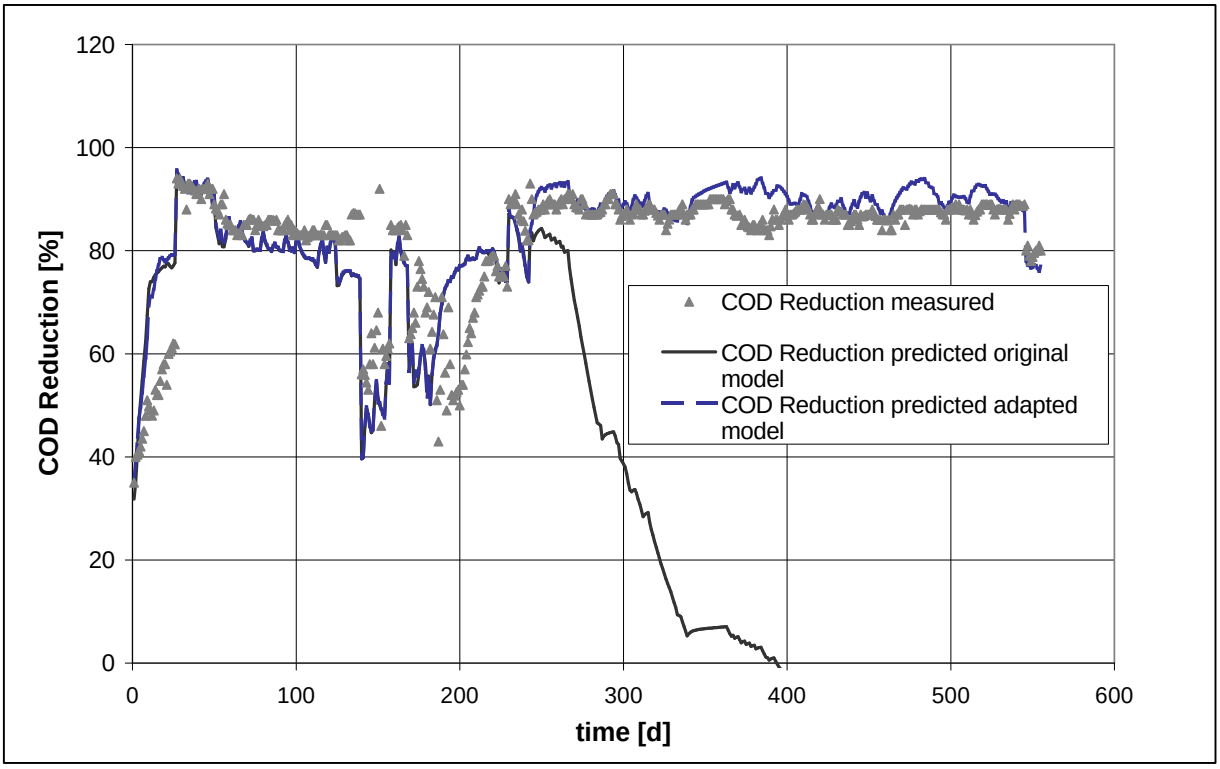

Figure 4.3.2: Comparison of the original and the adapted model using the parameter suggested by the IWA Task group for mathematical modelling of Anaerobic Digestion (Batstone *et al.* 2002)

In accordance with Elias *et al.* (2006) in this study it was established that a value > 0.6 indicates a accurate model. Even though the determination coefficient is rather low in both cases, the index of agreement indicates a good model, as the values of d > 0.6. Values of d between 0.5 and 0.6 would be within an acceptable range. A value of < 0.3 (absolute) for the Ratio of means (R_{Mean}) indicates that the model predicts the observation with acceptable accuracy (Elias *et al.* 2006). The determination coefficient for the pH and the gas production is higher for the original model (Table 4.3.2 and Table 4.3.3), however the original cannot follow the development of the data. The model predicts the correct magnitude of the acetate, propionate and VFA concentration, but the model cannot follow the developing of the data, as well, which results in the low determination coefficient and index of agreement (Table 4.3.2 and Table 4.3.3). This applies for both cases.

Table 4.3.2: Values of the statistical indicators r^2, d and R_{Mean} for the original model using the parameter suggested by the IWA Task group for mathematical modelling of Anaerobic Digestion (Batstone *et al.* 2002) (green = good, yellow = okay)

	r^2	d	R_{Mean}	
Gas production	0.26	**0.54**	0.87	okay
Methane production	**0.40**	**0.62**	0.90	okay
Acetate Concentration	0.00	0.23	1.41	bad
Propionate Concentration	0.03	0.43	-0.99	bad
VFA	0.00	0.36	**0.00**	bad
pH	0.18	**0.53**	**0.05**	okay
COD Reduction	**0.62**	**0.88**	**0.02**	good

Table 4.3.3: Values of the statistical indicators r^2, d and R_{Mean} for the adapted model using the parameter suggested by the IWA Task group for mathematical modelling of Anaerobic Digestion (Batstone *et al.* 2002) (green = good, yellow = okay)

	r^2	d	R_{Mean}	
Gas production	0.35	**0.68**	**0.39**	okay
Methane production	0.19	**0.60**	**0.06**	okay
Acetate Concentration	0.05	0.00	41143.52	bad
Propionate Concentration	0.07	0.00	26121.59	bad
VFA	0.02	0.00	32915.33	bad
pH	**0.42**	0.05	**-0.22**	okay
COD Reduction	0.06	0.06	-0.59	bad

The parameters recommended in the technical report (Batstone *et al.* 2002) are a reasonable compromise and case-specific adaptations may be necessary (Batstone *et al.* 2005) and, as in both cases, the model cannot predict the values accurately enough, a calibration of the model is absolutely necessary. For the calibration the data set was divided into two groups – one group for the calibration (about 2/3 of the data) and the other group for the validation of the model (1/3 of the data set).

In the same way as for the original ADM1, a sensitivity analysis was done for the adapted anaerobic digestion model (Table 4.3.4), to find the most sensitive parameters. The most sensitive parameters will be calibrated up front. The result of this analysis was rather similar to the one made for the original model, as the disintegration rate (k_{dis}) and hydrolysis rates ($k_{hyd,ch}$, $k_{hyd,pr}$, $k_{hyd,li}$ and $k_{hyd,chs}$) have the most impact on the model output.

Whereby a change of k_{dis} has the greatest impact on X_{xc}, further on $S_{gas,H2}$ and S_{H2}. $K_{hyd,ch}$ has the most influence on X_{ch}, moreover the carbohydrate hydrolysis rate has a great influence on the sugar concentration and the methane concentration in the liquid phase. In the same way $k_{hyd,pr,}$ $k_{hyd,li}$ and $k_{hyd,chs}$ have the most influence on X_{pr}, X_{li} and $X_{ch,s}$.

The hydrogen concentration in the gas is the variable that is above all influenced by most of the parameters, such as $K_{S,su}$, $K_{S,aa}$, $k_{m,aa}$, Y_{aa}, $k_{m,fa}$ and $K_{S,H2}$.

Table 4.3.4: Results of the sensitivity analysis of the adapted ADM1

#	Parameter	Sensitivity Coefficient	#	Parameter	Sensitivity Coefficient
1	k_{dis}	1.52E-02	23	$k_{m,c4}$	2.24E-08
2	$k_{hyd,li}$	1.11E-02	24	Y_{su}	2.20E-08
3	$k_{hyd,ch}$	1.10E-02	25	$K_{S,fa}$	2.18E-08
4	$k_{hyd,pr}$	1.10E-02	26	$K_{S,c4}$	1.89E-08
5	$k_{hyd,ch,s}$	1.07E-02	27	Y_{aa}	1.79E-08
6	$k_{dec,su}$	1.60E-03	28	$K_{I,H2,fa}$	1.31E-08
7	$k_{dec,pro}$	1.25E-03	29	$K_{I,H2,c4}$	9.33E-09
8	$k_{dec,ac}$	1.05E-03	30	$k_{m,H2}$	9.07E-09
9	$k_{dec,c4}$	1.02E-03	31	$k_{A,B,va}$	3.85E-09
10	$k_{dec,fa}$	9.16E-04	32	$k_{A,B,bu}$	2.08E-09
11	$k_{dec,h2}$	8.97E-04	33	$K_{S,su}$	3.72E-10
12	$k_{dec,aa}$	6.65E-04	34	Y_{H2}	2.52E-10
13	$k_{m,aa}$	2.34E-05	35	$K_{S,ac}$	5.76E-11
14	$K_{S,aa}$	1.58E-05	36	$k_{m,pro}$	5.73E-11
15	$k_{A,B,co2}$	9.51E-06	37	Y_{fa}	3.77E-11
16	$k_{m,su}$	6.02E-06	38	$K_{I,NH3,ac}$	3.06E-11
17	$K_{S,H2}$	6.64E-08	39	$K_{S,pro}$	2.72E-11
18	$k_{A,B,pro}$	5.74E-08	40	$K_{I,H2,pro}$	2.25E-11
19	$K_{S,IN}$	4.41E-08	41	$k_{m,ac}$	1.86E-11
20	$k_{A,B,IN}$	3.64E-08	42	Y_{pro}	1.67E-11
21	$k_{A,B,ac}$	3.09E-08	43	Y_{c4}	1.10E-11
22	$k_{m,fa}$	2.46E-08	44	Y_{ac}	4.88E-12

For the parameter calibration, literature data, data from our own experiments and from experiments performed by project partners were used. Yet, the problem is that the data found in literature and also those determined in the laboratory experiments have a large margin of deviation, makes the identification of adequate parameters rather difficult.

Moreover some of the influent components in the ADM1 with a different defining characters are difficult to estimate, such as the soluble and particulates substrate, biomass and the inerts (Huete *et al.* 2005). Whereby the particulate component X_{xc} and the inerts (S_I and X_I) are the variables with greatest degree of uncertainty (Huete *et al.* 2005). Huete (2005) suggests therefore that X_{xc}, X_I and S_I should be chosen so that the elemental mass fractions of X_{xc} are consistent with the stoichiometric parameters and the model predicts nitrogen as well as possible.

Furthermore after acclimatisation to high ammonium and ammonia levels the process became less sensitive to changes in NH_3 and NH_4^+ concentrations and also changes in the pH, but the ADM1 cannot simulate this acclimatisation (Feng *et al.* 2006).

Fifteen parameters were changed during the calibration of the model (Table 4.3.5): For the N_I and the N_{XC} content the values suggested by Rosen and Jeppsson (2006) and their implementation of the ADM1 into the BSM2 framework were used. The value of 6 $g_N.g_{COD}$-1 for N_I was chosen to be consistent with the ASM1 model (Henze *et al.* 1986),

thus the N_{XC} value had to be adapted to close the nitrogen balance (Rosen and Jeppsson 2006). As the stoechiometric relationships were updated to keep track of excess nitrogen and carbon, as suggested by Rosen and Jeppson (2006) – the term N_{bac} - N_{xc} and C_{bac}-C_{xc} was added to the Peterson matrix – the C_{XC} value has to be changed to 0.02786 $kmol_C.kg_{COD}^{-1}$ to avoid excess amounts of inorganic carbon and thus low values of methane in the biogas (Rosen and Jeppsson 2006). Batstone and co-workers suggest that $k_{A,B}$ should "*be at least one order of magnitude larger than the fastest time constant"* and proposed a value of 1.10^8 $M^{-1}d^{-1}$ (Rosen and Jeppsson 2006). Yet, Rosen and Jeppsson (2006) found that a value of 1.10^8 is not sufficient and recommended a value of 1.10^{10} $M^{-1}d^{-1}$, this value was used for all $k_{A,B}$ in this study. All other parameters were calibrated by trial and error.

Table 4.3.5: Original and calibrated parameters in the adapted ADM1

Parameter		original values	adapted values
$f_{XI,Xc}$	[]	2.50E-01	3.00E-01
$f_{li,xc}$	[]	2.50E-01	2.00E-01
N_{xc}	[]	2.00E-03	2.69E-03
N_i	$[kmol_N kg_{COD}^{-1}]$	2.00E-03	4.29E-03
k_{dis}	$[d^{-1}]$	5.00E-01	4.10E-01
$k_{m,aa}$	$[d^{-1}]$	5.00E+01	3.00E+01
$k_{m,c4}$	$[d^{-1}]$	2.00E+01	2.20E+01
$k_{A,B,va}$	$[M^{-1}d^{-1}]$	1.00E+08	1.00E+10
$k_{A,B,bu}$	$[M^{-1}d^{-1}]$	1.00E+08	1.00E+10
$k_{A,B,pro}$	$[M^{-1}d^{-1}]$	1.00E+08	1.00E+10
$k_{A,B,ac}$	$[M^{-1}d^{-1}]$	1.00E+08	1.00E+10
$k_{A,B,co2}$	$[M^{-1}d^{-1}]$	1.00E+08	1.00E+10
$k_{A,B,IN}$	$[M^{-1}d^{-1}]$	1.00E+08	1.00E+10
C_{xc}	$[kmol_C kg_{COD}^{-1}]$	3.00E-02	2.79E-02
$k_{hyd,ch,s}$	$[d^{-1}]$	1.20E+00	8.50E-01

The adapted and calibrated model predicts most of the values accurately enough (Table 4.3.6 and Figure 4.3.3). The COD reduction is predicted very well by the model (r^2 = 0.57, d = 0.85 and R_{Mean} = 0.03). The low determination coefficients are a result of the large amount of data, in similar studies a data range of 66 days on average (Angelidaki *et al.* 1993; Christ *et al.* 2000; Kalyuzhnyi *et al.* 2000; Seok and Komisar 2002; Siegrist *et al.* 2002; Aceves-Lara *et al.* 2005; Bernard *et al.* 2005; Flotats *et al.* 2006) is shown. If only part of the data is examined higher determination coefficients can be found, for example for the final period (day 501 – 555) an r^2 = 0.92 can be found for the COD reduction. Yet, as biogas plants are planned and constructed as long term processes, it seemed more reasonable to make also a long term simulation. The prediction of the gas and methane production (Figure 4.3.3) as well as the pH was done accurately. The negative value of R_{Mean} of the acetate, propionate and VFA concentration, and the COD reduction signifies that the values measured are underestimated in the model (Elias *et al.* 2006). It seems that the model cannot handle

the high VFA concentration which appeared in this study, which leads to an underprediction of these values – the addition of a new inhibition function could improve the performance of the model. Yet, the implementation of a new inhibition function was not possible within the frame of this study.

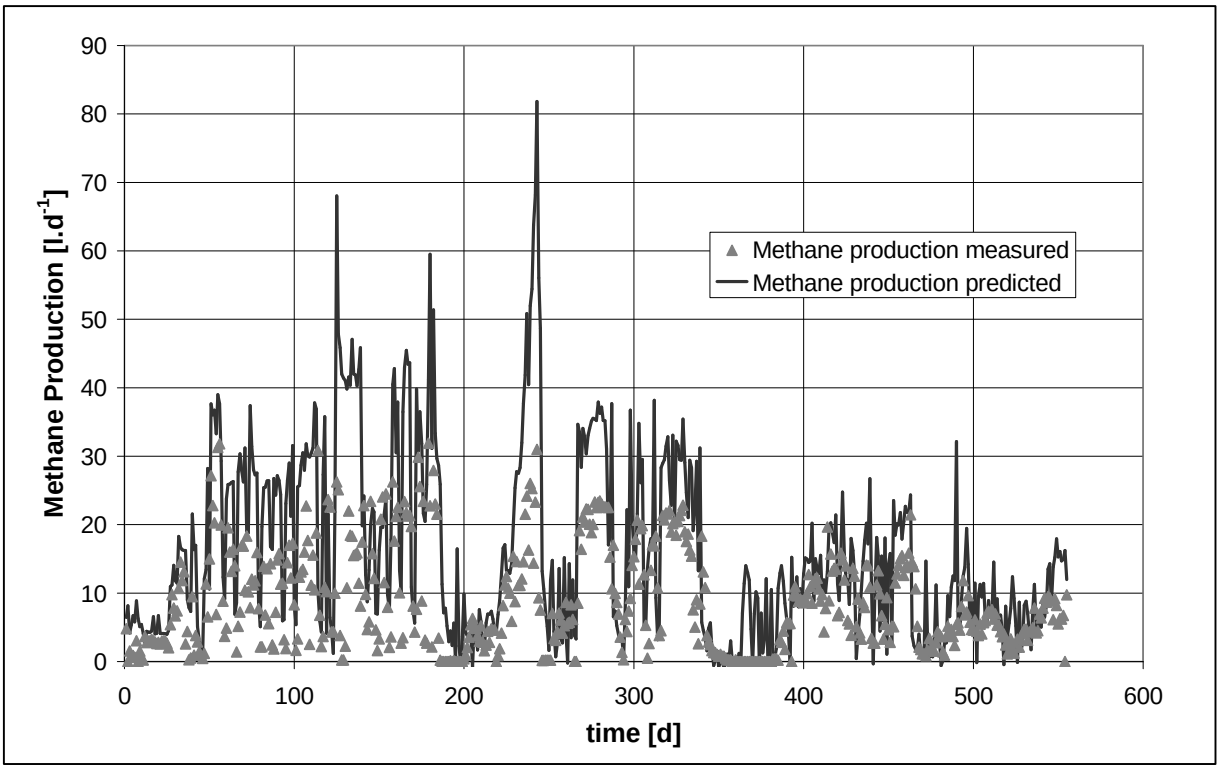

Figure 4.3.3: Methane production predicted using the calibrated adapted model

Table 4.3.6: Values of the statistical indicators r^2, d and R_{Mean} for the adapted model using the calibrated parameter (green = good, yellow = okay)

	r^2	d	R_{Mean}	
Gas production	**0.48**	**0.70**	0.73	okay
Methane production	**0.48**	**0.68**	0.84	okay
Acetate Concentration	0.00	0.36	-0.46	bad
Propionate Concentration	0.03	0.43	-0.99	bad
VFA	0.01	0.40	-0.77	bad
pH	**0.43**	**0.72**	**0.03**	good
COD Reduction	**0.57**	**0.85**	**0.03**	good

The results of the calibrated adapted model were compared with results from the calibrated original model (Table 4.3.7). In the original model sixteen parameters were adapted for this study. Whereby $N_{xc,}$ N_I, C_{xc}, and $k_{A,B}$ were adapted according to Rosen and Jeppson (2006), in the same way as for the adapted model. All other parameters were calibrated by trial and error. Whereby the hydrolysis rates had to be chosen as 1 d^{-1} instead of 10 d^{-1} (suggested by Batsone and co-workers (2002)), to obtain reasonable results.

Comparing the adapted and the original calibration it was found that rather similar results are achieved using the calibrated original model.

The gas and methane production was predicted less adequately using the adapted model ($r^2_{gas,adapted}$ = 0.48 and $d_{gas,adapted}$ = 0.70 and $r^2_{methane,adapted}$ = 0.51 and $d_{methane,adapted}$ = 0.68

respectively) compared to the original model ($r^2_{gas,original}$ = 0.48 and $d_{gas,original}$ = 0.74 and $r^2_{methane,original}$ = 0.51 and $d_{methane,orignal}$ = 0.70 respectively). On the other hand the pH and the COD reduction were predicted slightly better using the adapted model compared to the original model. The VFA could not be predicted better in the original model than in the adapted model.

Table 4.3.7: Calibrated and original parameters in the original ADM1

Parameter		original values	adapted values
$f_{XI,Xc}$	[]	2.50E-01	3.00E-01
$f_{li,xc}$	[]	2.50E-01	2.00E-01
Nxc	[]	2.00E-03	2.69E-03
Ni	[$kmol_N kg_{COD}^{-1}$]	2.00E-03	4.29E-03
k_{dis}	[d^{-1}]	5.00E-01	3.00E-01
$k_{hyd,ch}$	[d^{-1}]	1.00E+01	1.00E+00
$k_{hyd,pr}$	[d^{-1}]	1.00E+01	1.00E+00
$k_{hyd,li}$	[d^{-1}]	1.00E+01	1.00E+00
$k_{m,ac}$	[d^{-1}]	6.00E+00	8.00E+00
$k_{A,B,va}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
$k_{A,B,bu}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
$k_{A,B,pro}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
$k_{A,B,ac}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
$k_{A,B,co2}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
$k_{A,B,IN}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
C_{xc}	[$kmol_C kg_{COD}^{-1}$]	3.00E-02	2.79E-02

Table 4.3.8: Values of the statistical indicators: square of the correlation coefficient (r^2), index of agreement (d) and Ratio of means (R_{Mean}) for the original model using measured values to estimate the initial conditions (case 1) (green = good, yellow = okay)

	r^2	d	R_{Mean}	
Gas production	**0.51**	**0.74**	0.67	okay
Methane production	**0.51**	**0.70**	0.81	okay
Acetate Concentration	0.00	0.40	-0.76	bad
Propionate Concentration	0.03	0.43	-0.99	bad
VFA	0.01	0.42	-0.90	bad
pH	**0.42**	**0.70**	**0.03**	good
COD Reduction	**0.52**	**0.84**	**-0.01**	good

In order to prove that the “mass_balance” was completely suitable for a first estimation of the initial conditions especially if no or only few measurements are available, a comparison was made predicting the gas (Figure 4.3.4) and methane production, the VFA, pH and COD reduction using the measured values as estimations for the initial conditions (case 1) and using the values determined by the mass balance to estimate the initial conditions (case 2) (Figure 4.3.4, Table 4.3.8 and Table 4.3.9). Whereby the measured values were used for the acetic and propionic acid concentrations as well as the inorganic nitrogen.

Using the values estimated by the “mass_balance” to determine the initial conditions, the pH and the COD reduction were predicted as acceptably as using the measured values. Only the prediction of the gas and methane production displayed a lower determination

coefficient (Table 4.3.9) compared to case 1 (Table 4.3.8). Also in “case 2”, the VFA concentration was predicted far too low and very low values for the determination coefficient were found. A value of < 0.3 for R_{Mean}, which is acceptable, was only obtained for the pH and the COD reduction in both cases. Yet, the index of agreement for the gas and methane production and the pH and COD reduction was higher in case 1 than 0.6, which indicates a good model (Elias *et al.* 2006). In case 2 for the prediction of pH and COD reduction values of > 0.6 were found. For the prediction of the gas and methane production acceptable values of between 0.5 and 0.6 were found.

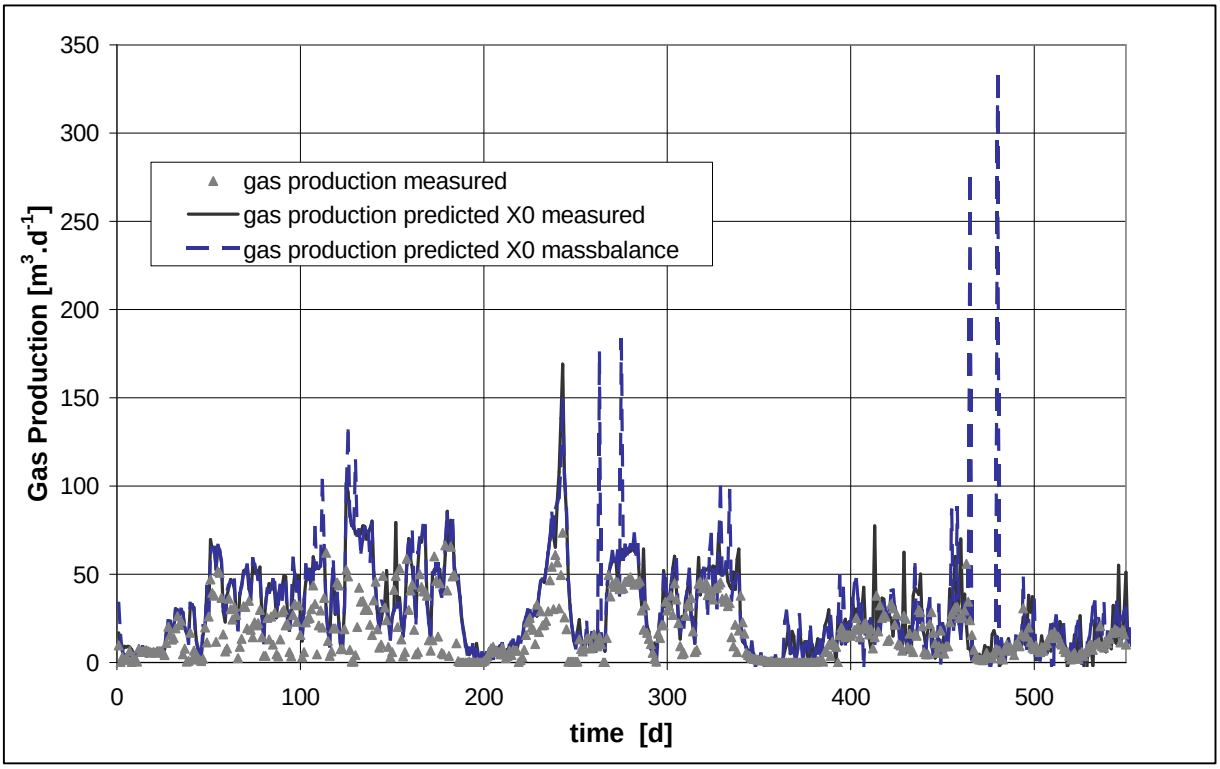

Figure 4.3.4: Comparison of the original ADM1 using measured values as initial conditions and using values from the mass balance as initial conditions.

Table 4.3.9: Values of the statistical indicators: square of the correlation coefficient (r^2), index of agreement (d) and Ratio of means (R_{Mean}) for the original model using measured values to estimate the initial conditions (case 2) (green = good, yellow = okay)

	r^2	d	R_{Mean}	
Gas production	0.31	**0.59**	0.76	okay
Methane production	0.28	**0.54**	0.90	okay
Acetate Concentration	0.00	0.41	-0.79	bad
Propionate Concentration	0.01	0.43	-0.98	bad
VFA	0.00	0.42	-0.90	bad
pH	**0.40**	**0.68**	**0.03**	good
COD Reduction	**0.55**	**0.85**	**-0.01**	good

These results indicate that the mass balance is definitely suitable for a first estimation of the initial conditions.

After calibration of the model a validation and examination of the estimated parameters is necessary. Since parameter estimation means the fitting of the model to data from a specific process, whereas validation is the testing of the fitted model (Olsson and Newell 1999).

Model validation was done with one third of the data, which was not used in model calibration.

Figure 4.3.5 shows the results of the validation of the adapted model. R_{Mean} indicates in nearly all cases an accurate model – for the gas and methane production, the acetate concentration, the pH and the COD reduction $R_{Mean} < 0.3$. The index of agreement indicates for the gas and methane production and surprisingly for the acetate concentration a good model. For the VFA are the values acceptable for the index of agreement. Yet the determination coefficient shows very low values, except for the COD reduction (Table 4.3.10).

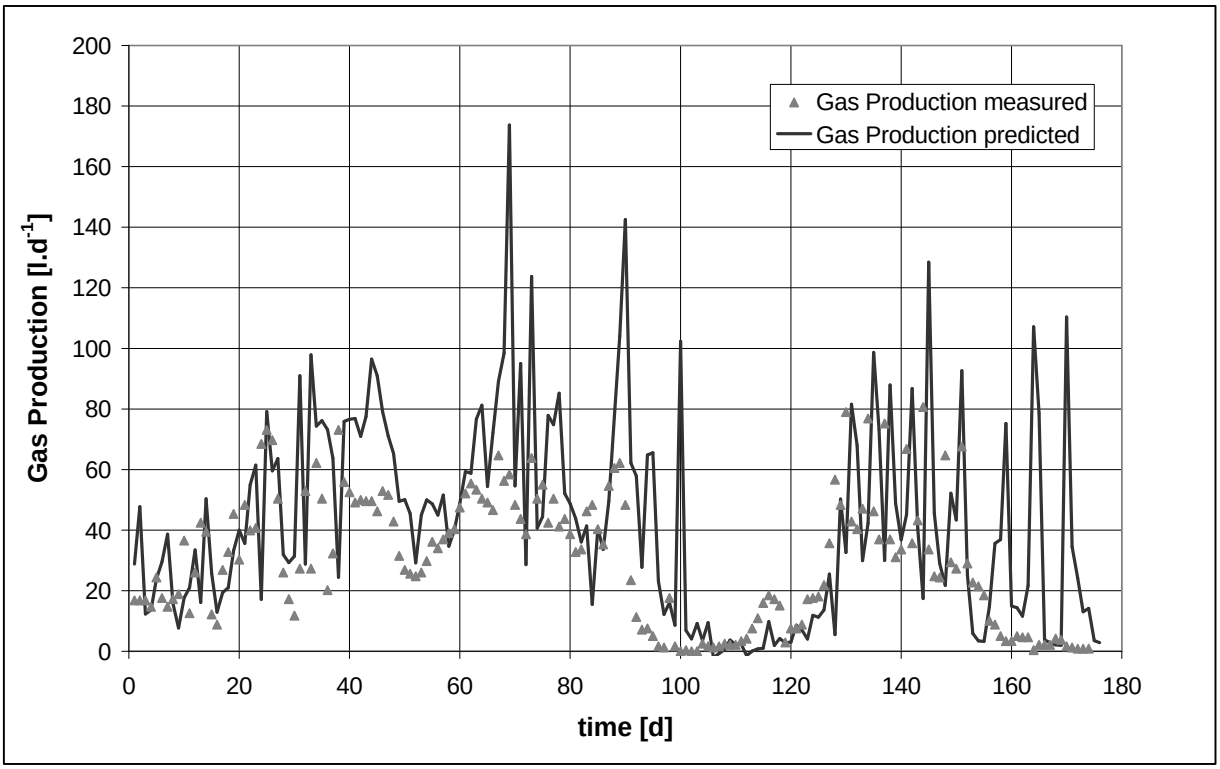

Figure 4.3.5: Prediction of the gas production during model validation of the adapted ADM1

Table 4.3.10: Values of the statistical indicators r^2, d and R_{Mean} during validation of the adapted model (green = good, yellow = okay)

	r^2	d	R_{Mean}	
Gas production	0.21	**0.69**	**-0.01**	okay
Methane production	0.22	**0.70**	**-0.08**	okay
Acetate Concentration	0.15	**0.61**	**-0.02**	okay
Propionate Concentration	0.03	0.45	-0.99	bad
VFA	0.27	**0.58**	-0.73	bad
pH	0.11	0.27	**0.07**	okay
COD Reduction	**0.42**	0.49	**0.16**	okay

Summarizing it can be said that no real difference could be found between the original and the adapted model. This indicates that the original model was not structurally wrong for this application – except for the problem of predicting VFA values, which is true for both models. Yet, the adapted model is a better illustration of the reality.

The model predicts the measured data accurately enough to predict the rough development of the data and the correct range of the data, however not precisely enough

to predict the "correct" value and thus the model is not suitable for a Decision Support System (DSS).
Moreover for practical application in technical plants the large amount of data needed in the ADM1 is a core issue – as only a small number of the values are normally measured in technical plants. Sure it is useful to calibrate the model using a laboratory reactor and it is possible to estimate most of the values, for example using the "mass_balance". Yet, the more the values are estimated the more imprecise the prediction is, as changes in the input and the initial conditions have a great impact on the model output.
Surely better results could be achieved if more state variables could have been measured during the study. A further core issue is also that the adaptation to high ammonium and ammonia values can not be modelled and that apparently the high, in part, VFA concentrations cannot be modelled.

Addition of the Sulphate reduction process according to Federovich *et al.* (2003) and according to Batstone (2006)

In particular when substrates with high protein content are used, e.g. sunflower press residues, high amounts of sulphate are found in the liquid and gas phases. These high levels of sulphate and sulphide result in different problems. First of all, sulphate reducing bacteria (SRB) compete with the methanogenic bacteria for the same substrate. In addition the H_2S in the sludge causes an inhibition of the metabolic activity of the bacteria (Gerardi 2003). The first inhibition effects have to be taken into account beginning at an H_2S concentration of 30 $mg.l^{-1}$. An H_2S content of more than 10 % in the biogas disrupted the acetate production (Bischofsberger *et al.* 2005). An elimination of the H_2S is not only important for the successful operation of the AD process, but also to prevent problems of corrosion in the plant or minimize SO_2 emissions during the combustion of the biogas (Bischofsberger *et al.* 2005).
Thus the ADM1 augmented by the sulphate reduction process was added into the Virtual Laboratory 1.2 in two versions: the sulphate reduction process according to Federovich (2003), already described in (Strik 2004), and the extension with the SR according to Batstone (2006).

The addition of the sulphate reduction (SR) process is very complex as the sulphate acts as electron acceptor for the oxidation of VFAs and reacts with hydrogen (Batstone *et al.* 2005). Moreover sulphide is inhibitory and affects the pH (Batstone *et al.* 2005). Thus for a comprehensive model of sulphate reduction every part of the ADM1 has to be modified (Batstone *et al.* 2005). Such an implementation of the SR was done by Federovich and co-workers (2003), also described in (Strik 2004) (Figure 4.3.6). Batstone (2006) suggests a simpler model, with only one group of sulphate reducers, however this model works only with an influent S:COD ratio of up to 0.1 $kg_S.kg_{COD}^{-1}$.
SRB are capable of using many intermediates formed during the AD process and therefore competes for with the acidogenic bacteria for sugars and amino acids,

acetogenic bacteria for VFAs and methanogenic bacteria for acetate and hydrogen (Fedorovich *et al.* 2003).

For the sugars and amino-acids the competition is won by the acidogenic bacteria (Fedorovich *et al.* 2003). The ADM1 should be therefore augmented by VFA, acetate and hydrogen removal by sulphate reduction (Fedorovich *et al.* 2003).

Four bacteria groups are considered in the extension for the SR process: the butyrate-degrading sulphate-reducing bacteria (X_5), propionate-degrading sulphate-reducing process (X_6), acetotrophic sulphate-reducing process (X_7) and hydrogenotrophic bacteria (X_8) (Fedorovich *et al.* 2003).

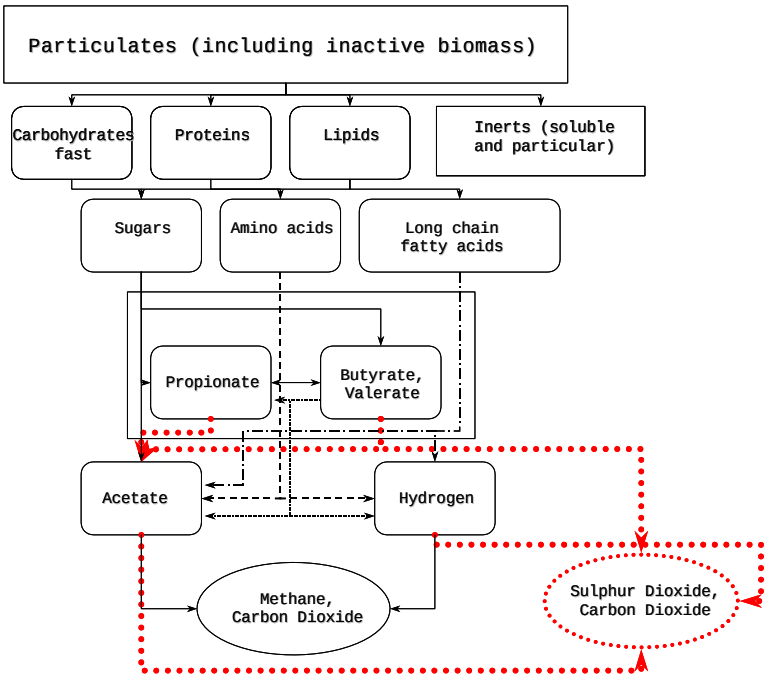

Figure 4.3.6: Schema of the biochemical processes of the adapted model (implementation of the SR process according to (Fedorovich *et al.* 2003) - ····· Shows changes compared to the original model

The extension of the ADM1 to include sulphate reducing processes is described in more detail by Federovich and co-workers (2003) and by Strik (2004), as well.

To find the most sensitive parameter a sensitivity analysis was done for the model augmented with the SR process according to Federovich and co-workers (2003) (Table 4.3.11).

The most sensitive parameters should be calibrated up front. The acetate uptake rate ($k_{m,ac}$), the half saturation rate ($K_{S,ac}$) and the 50% inhibitory concentration ($K_{I,H2S,c4}$) have the most impact on the model output.

Table 4.3.11: Sensitivity coefficients of the ADM1 augmented with the Sulphate reduction process according to Federovich *et al.*, 2003

#	Input variable	Sensitivtiy coefficient	#	Input variable	Sensitivtiy coefficient
1	$k_{m,ac}$	1.17E+15	42	$K_{I,h2s,h2}$	4.74E+03
2	$K_{S,ac}$	1.30E+14	43	$K_{S,c4}$	4.73E+03
3	$K_{I,h2s,c4}$	1.89E+12	44	$K_{I,h2s,x5}$	3.72E+03
4	$K_{S,H2}$	1.55E+12	45	$k_{dec,ac}$	3.49E+03
5	$K_{I,h2s,fa}$	4.92E+11	46	$K_{a,hs}$	3.03E+02
6	$pH_{LL,srb}$	1.27E+10	47	$K_{I,h2s,su}$	3.44E+01
7	k_La_{h2s}	8.95E+09	48	$k_{dec,aa}$	2.24E+01
8	$K_{H,CH4}$	4.37E+09	49	k_{dis}	1.03E+01
9	$k_{A,Bhs}$	3.39E+09	50	$k_{A,B,co2}$	9.36E+00
10	$K_{H,h2s}$	2.37E+09	51	$k_{m,so4,bu}$	6.78E+00
11	Y_{su}	2.17E+09	52	$k_{m,so4,h2}$	5.98E+00
12	$k_{m,so4pr}$	9.24E+08	53	$K_{I,h2s,x8}$	4.87E+00
13	$K_{I,h2s,ac}$	5.26E+08	54	$K_{S,IN}$	4.66E+00
14	Y_{c4}	3.58E+08	55	$K_{I,H2,fa}$	4.24E+00
15	Y_{x7}	2.64E+08	56	Y_{ac}	2.57E+00
16	$K_{I,h2s,x6}$	1.61E+08	57	$k_{dec,X7}$	2.09E+00
17	$K_{I,H2,c4}$	7.66E+07	58	$K_{S,aa}$	1.97E+00
18	Y_{x6}	5.41E+07	59	$K_{I,h2s,aa}$	1.64E+00
19	$k_{m,fa}$	3.08E+07	60	$k_{dec,X5}$	9.12E-01
20	Y_{aa}	2.83E+07	61	Y_{x5}	8.36E-01
21	$k_{m,so4,ac}$	2.39E+07	62	$k_{dec,h2}$	4.14E-01
22	$K_{a,h2s}$	2.38E+07	63	$k_{hyd,li}$	1.68E-01
23	$K_{S,fa}$	9.77E+06	64	$k_{dec,su}$	1.27E-01
24	$K_{I,h2s,x7}$	5.67E+06	65	$K_{S,so4,h2,h2}$	1.24E-01
25	$K_{I,NH3,ac}$	3.59E+06	66	$pH_{UL,srb}$	1.01E-01
26	$k_{dec,fa}$	2.61E+06	67	$k_{dec,X8}$	9.05E-02
27	$k_{m,H2}$	8.20E+05	68	Y_{H2}	3.74E-02
28	$K_{S,so4,ac,ac}$	6.21E+05	69	$k_{hyd,pr}$	8.96E-03
29	$k_{dec,pro}$	4.57E+05	70	$k_{hyd,ch}$	8.92E-03
30	Y_{fa}	1.41E+05	71	$K_{S,so4,pr,so4}$	1.75E-44
31	$k_{dec,X6}$	9.65E+04	72	$K_{S,so4,bu,so4}$	2.75E-45
32	$k_{m,aa}$	9.45E+04	73	$k_{m,pro}$	0.00E+00
33	$k_{dec,c4}$	9.01E+04	74	$K_{S,pro}$	0.00E+00
34	$K_{S,so4,bu,bu}$	8.66E+04	75	Y_{pro}	0.00E+00
35	$k_{m,su}$	6.23E+04	76	$K_{I,H2,pro}$	0.00E+00
36	k_La	4.21E+04	77	$K_{S,so4,h2,so4}$	0.00E+00
37	$k_{m,c4}$	3.00E+04	78	$K_{I,h2s,pro}$	0.00E+00
38	$K_{S,so4,ac,so4}$	1.57E+04	79	$\Delta H0_{Ka,hs}$	0.00E+00
39	$K_{S,su}$	9.04E+03	80	$\Delta H0_{Ka,h2s}$	0.00E+00
40	$K_{S,so4,pr,pr}$	6.66E+03	81	$\Delta H0_{KH,h2s}$	0.00E+00
41	Y_{x8}	5.65E+03			

These parameter have the greatest influence on the sulphate concentration ($S_{SO4,2-}$), hydrogen sulphide anion concentration (S_{HS-}) and the hydrogen carbonate concentration (S_{HCO3-}). The sulphate concentration state ($S_{SO4,2-}$) is moreover the variable that is the most influenced by the majority of the parameters, followed by the hydrogen sulphide anion concentration (S_{HS-}) and the sugar concentration (S_{SU}).

As described above the implementation of the SR process according to Batstone (2006) is much simpler (Figure 4.3.7).

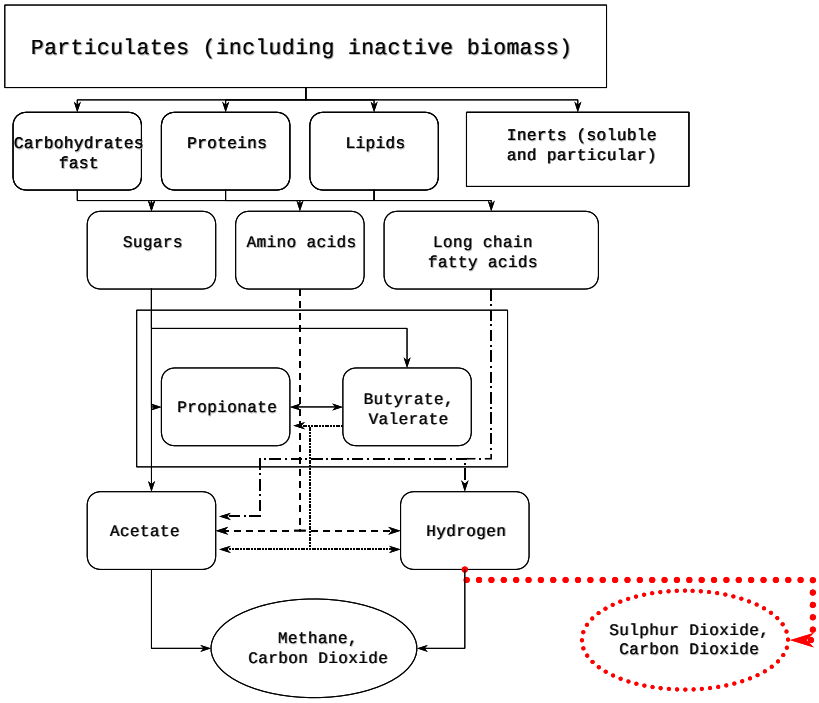

Figure 4.3.7: Schema of the biochemical processes of the adapted model (implementation of the SR process according to (Batstone 2006) - ····· Shows changes compared to the original model

The following equations show the extension of the ADM1 with sulphate reducing processes (according to (Batstone 2006)). The algebraic equation for the pH calculation was extended to (Equation (115)):

$$\Theta = S_{cat^+} + S_{nh4^+} - S_{hco3^-} - \frac{S_{ac^-}}{64} - \frac{S_{pr^-}}{112} - \frac{S_{bu^-}}{160} - \frac{S_{va^-}}{208} - \frac{S_{s^{2-}}}{32} - \left(\frac{S_{so4^{2-}}}{96*2}\right) - S_{an^-} \quad \textbf{(115)}$$

The inhibition function describes the influence of excessive amounts of sulphides (Fedorovich *et al.* 2003) (Equation (116)):

$$I_{13} = \frac{1+2\times10^{0.5(\mathrm{pH_{LL}}-\mathrm{pH_{UL}})}}{1+10^{(pH-pH_{UL})}+10^{(pH_{LL}-pH)}} \quad \textbf{(116)}$$

Subsequent process rates were added to the ADM1 (Equation (117) to (119)):

$$\rho_{20} = \frac{k_{m,SO_4^{2-}} * S_{S=_4^{2-}}}{K_{S,SO_4^{2-}} + S_{SO_4^{2-}}} * \frac{S_{H_2}}{K_{S,H_2} + S_{H_2}} * X_{SO_4^{2-}} * I_{13} \quad \textbf{(117)}$$

$$\rho_{21} = k_{dec,SO_4^{2-}} * X_{SO_4^{2-}} \quad \textbf{(118)}$$

$$\rho_{T,11} = k_{L\,a_{H_2S}} * (S_{2^-} - 80 * K_{H,H_2S} * p_{gas,H_2S}) \quad \textbf{(119)}$$

New gas phase equations for H_2S were added as well (Equation (120) and (121)):

$$p_{gas,\,h2s} = S_{gas,\,h2s} \frac{R \cdot T_{op}}{80} \quad \textbf{(120)}$$

$$q_{gas} = ... \cdot \left(..... + \frac{P_{T,11}}{80} \right) \quad \textbf{(121)}$$

The carbon balance was extended with subsequent equations, too (Equation (117) to (118)):

$$\sum s_k \rho_k = ... + s_{20} * \rho_{20} + s_{13,19,21} \left(\sum_{i=13}^{19} \rho_i + \rho_{21} \right) \quad \textbf{(122)}$$

$$s_{20} = Y_{SO_4^{2-}} * C_{bac} \quad \textbf{(123)}$$

Following new or adapted differential equations were implemented in the model (Equation (124) to (129)):

$$\frac{dS_{h2}}{dt} = \frac{q_{in}}{V_{liq}} \left(S_{h2,\,in\,-} S_{h2} \right) + \rho ..... - \rho_{20} \quad \textbf{(124)}$$

$$\frac{dS_{IN}}{dt} = ... + \left(N_{bac} - N_{xc} \right) \sum_{i=13}^{19} \rho_i + \rho_{21} - Y_{SO_4^{2-}} * N_{bac} \quad \textbf{(125)}$$

$$\frac{dS_{SO_4^{2-}}}{dt} = \frac{q_{in}}{V_{liq}} \left(S_{SO_4^{2-},in} - S_{SO_4^{2-}} \right) - \left(\left(1 - Y_{SO_4^{2-}} \right) \Big/ 64 \right) * \rho_{20} \quad \textbf{(126)}$$

$$\frac{dS_{S^{2-}}}{dt} = \frac{q_{in}}{V_{liq}} \left(S_{S^{2-},in} - S_{S^{2-}} \right) + \left(1 - Y_{S^{2-}} \right) * \rho_{20} \quad \textbf{(127)}$$

$$\frac{dX_{SO_4^{2-}}}{dt} = \frac{q_{in}}{V_{liq}} \left(X_{SO_4^{2-},in} - X_{SO_4^{2-}} \right) + Y_{SO_4^{2-}} * \rho_{20} - \rho_{21} \quad \textbf{(128)}$$

$$\frac{dS_{gas,h2s}}{dt} = -\frac{S_{gas,h2s} * q_{gas}}{V_{gas}} + \rho_{T,11} * \frac{V_{liq}}{V_{gas}} \quad \textbf{(129)}$$

A sensitivity analysis was done, in order to find the most sensitive parameters for the extension of the ADM1 with the SR process according to Batstone (2006) (Table 4.3.12). In this case the upper and lower limit of pH inhibition ($pH_{UL,srb}$ and $pH_{LL,srb}$) and the sulphate uptake rate ($k_{m,SO4}$) are the most sensitive parameters, followed by the disintegration rate (k_{dis}) and the hydrolysis rates ($k_{hyd,ch}$, $k_{hyd,pr}$, $k_{hyd,li}$ and $k_{hyd,chs}$).
Whereby a change in the limits of pH inhibition or $k_{m,SO4}$ has the highest impact on the hydrogen in the liquid and gas phases. The disintegration rate has the highest impact on the complex particulates and the methane concentration in the liquid and in the gas phase. The hydrogen concentration in the gas is the variable that is most influenced by the majority of the parameters, such as $K_{S,IN}$, $k_{m,su}$, Y_{su}, $k_{m,aa}$, $K_{S,fa}$, $k_{m,c4}$ and $k_{m,H2}$.

The calibration and validation of the implementation of the SR process in the ADM1 according to Federovich and co-workers (2003) and according to Batstone (2006) was

not in the frame of this study. Moreover, validation of the SR process according to Federovich *et al.* (2003) has been done in the thesis by Strik (2004). However, both ADM1 adaptations were implemented in the Virtual Laboratory 1.2.

Table 4.3.12: Sensitivity coefficients of the ADM1 extended with the Sulphate reduction process according to Batstone, 2005

#	Parameter	Sensitivity Coefficient	#	Parameter	Sensitivity Coefficient
1	$pH_{LL,srb}$	1.49E+10	27	$k_{dec,aa}$	5.05E-04
2	$k_{m,so4}$	3.49E+00	28	$k_{m,H2}$	5.01E-04
3	$pH_{UL,srb}$	2.65E-02	29	Y_{aa}	4.55E-04
4	k_{dis}	1.37E-02	30	$k_{dec,h2}$	4.36E-04
5	$k_{hyd,ch}$	1.05E-02	31	Y_{so4}	4.35E-04
6	$k_{hyd,pr}$	9.70E-03	32	$k_{dec,fa}$	4.29E-04
7	$k_{hyd,ch,s}$	9.67E-03	33	$k_{A,B,va}$	2.60E-04
8	$k_{hyd,li}$	9.38E-03	34	$k_{m,ac}$	2.19E-04
9	$k_{m,su}$	6.11E-03	35	$K_{I,NH3,ac}$	2.19E-04
10	$k_{m,aa}$	2.40E-03	36	Y_{ac}	2.19E-04
11	$k_{m,c4}$	1.95E-03	37	$K_{S,ac}$	2.19E-04
12	$K_{S,c4}$	1.88E-03	38	Y_{H2}	2.19E-04
13	$k_{A,B,co2}$	1.76E-03	39	$k_{dec,pro}$	1.88E-04
14	$k_{A,B,IN}$	1.76E-03	40	$K_{S,fa}$	1.22E-04
15	$K_{S,IN}$	1.66E-03	41	Y_{fa}	7.61E-05
16	$k_{A,B,pro}$	1.49E-03	42	Y_{c4}	1.65E-05
17	$K_{S,H2}$	1.28E-03	43	$K_{I,H2,c4}$	1.12E-05
18	$k_{m,fa}$	1.28E-03	44	$K_{S,so4}$	1.02E-05
19	$K_{S,aa}$	1.27E-03	45	k_La_{h2s}	6.14E-06
20	Y_{su}	7.59E-04	46	$K_{I,H2,fa}$	9.07E-07
21	$k_{A,B,ac}$	7.19E-04	47	$K_{S,su}$	6.66E-07
22	$k_{dec,XSO4}$	6.85E-04	48	$K_{S,pro}$	1.53E-12
23	$k_{dec,su}$	5.68E-04	49	Y_{pro}	9.27E-15
24	$k_{dec,c4}$	5.48E-04	50	$K_{I,H2,pro}$	3.02E-15
25	$k_{A,B,bu}$	5.39E-04	51	$k_{m,pro}$	4.77E-18
26	$k_{dec,ac}$	5.35E-04			

4.3.2 AD model based on the ADM1 (Batstone *et al.* 2002) and the model by Marsili-Libelli and Beni (1996) (ADMML)

Since the ADM1 is too complex and the results gained by the ADM1 not precisely enough to be suitable for a DSS, a simpler model was developed. The model developed (Figure 4.3.8) was based on the anaerobic digestion model by Marsili-Libelli and Beni (1996) and the ADM1 (Batstone *et al.* 2002).

The model by Marsili-Libelli and Beni (1996) is a simplified mathematical AD model, especially developed to describe the behaviour of anaerobic digesters under shock loading conditions with a special emphasis on bicarbonate alkalinity. This model was

designed for the implementation of control strategies to minimize the effects of organic load shocks (Marsili-Libelli and Beni 1996).

In contrast to the model by Marsili-Libelli and Beni (1996) in the ADMML the substrate is not only soluble, but also in a particulate form. All complex particulates are hydrolysed to one soluble component, idealist in the form of glucose. The glucose is then degraded to the fatty acids, idealist in the form of acetate and CO_2. The simplification is done as in a digester under normal conditions. The fatty acids are present for the most part as acetic acid (Marsili-Libelli and Beni 1996). Finally methane and carbon dioxide is produced. The decayed biomass becomes a part of the complex and the inert particulates. As methane is not very soluble and not used in the biochemical processes only a gaseous methane component is included in the model (Sötemann *et al.* 2006). That means that the acetoclastic methanogenesis process produces methane in the gas phase directly (Sötemann *et al.* 2006).

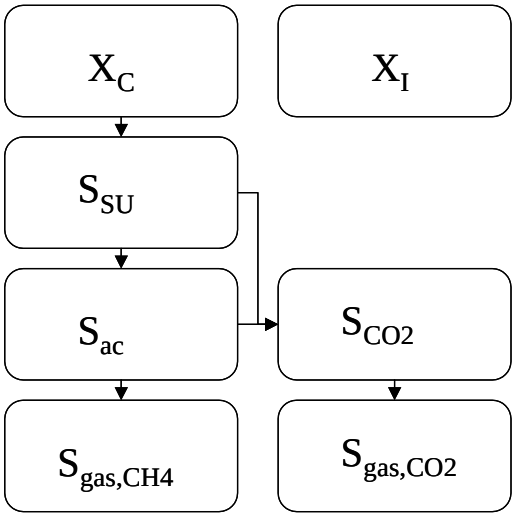

Figure 4.3.8: ADMML based on ADM1 (Batstone *et al.* 2002) and the AD model by Marsili-Libelli and Beni (1996)

Only one inhibition function was included in the model (Equation (130)):

$$I_{ac3} = \frac{K_{IAC}}{K_{IAC} + S_{ac}} \quad \textbf{(130)}$$

The following process rates were implemented in the model (Equation (131) to (136)):

$$\rho_1 = k_{hyd} * X_{xc} \quad \textbf{(131)}$$

$$\rho_2 = \frac{k_{m,su} \cdot S_{su}}{K_{s,su} + S_{su}} * \frac{S_{su,in} + K_{s,su}}{S_{su,in}} * X_{su} \quad \textbf{(132)}$$

$$\rho_3 = \frac{k_{m,ac} * S_{ac}}{K_{s,ac} + S_{ac}} \cdot \frac{S_{ac,in} + K_{s,ac}}{S_{ac,in}} * X_{ac} * I_{ac3} \quad \textbf{(133)}$$

$$\rho_4 = k_{dec,su} * X_{su} \quad \textbf{(134)}$$

$$\rho_5 = k_{dec,ac} * X_{ac} \quad \textbf{(135)}$$

$$\rho_{l,co2} = k_L a * \left(S_{CO_2} - S_{gas,CO_2}\right) \quad \textbf{(136)}$$

The model comprises the subsequent differential equations (Equation (137) to (145)):

$$\frac{dX_{xc}}{dt} = \frac{q_{in}}{V_{liq}} * \left(X_{xc,in} - X_{xc}\right) + f_{xc,dec} * \sum_{i=4}^{5} \rho_i - \rho_1 \quad \textbf{(137)}$$

$$\frac{dS_{su}}{dt} = \frac{q_{in}}{V_{liq}} * \left(S_{su,in} - S_{su}\right) + \rho_1 - \rho_2 \quad \textbf{(138)}$$

$$\frac{dX_{su}}{dt} = \frac{q_{in}}{V_{liq}} * \left(X_{su,in} - X_{su}\right) + Y_{su} * \rho_2 - \rho_4 \quad \textbf{(139)}$$

$$\frac{dS_{ac}}{dt} = \frac{q_{in}}{V_{liq}} * \left(S_{ac,in} - S_{ac}\right) + (1 - Y_{su}) * f_{ac,su} * \rho_2 - \rho_3 \quad \textbf{(140)}$$

$$\frac{dX_{ac}}{dt} = \frac{q_{in}}{V_{liq}} * \left(X_{ac,in} - X_{ac}\right) + Y_{ac} * f_{ch4,ac} * \rho_3 - \rho_5 \quad \textbf{(141)}$$

$$\frac{dS_{co2}}{dt} = \frac{q_{in}}{V_{liq}} \left(S_{co2,in} - S_{co2}\right) + (1 - Y_{su}) * f_{co2,su} * \rho_2 + (1 - Y_{ac}) * f_{co2,ac} * \rho_3 - \rho_{l,co2} \quad \textbf{(142)}$$

$$\frac{dS_{gas,co2}}{dt} = -\frac{q_{in}}{V_{liq}} * S_{gas,co2} + \rho_{l,co2} * \frac{V_{liq}}{V_{gas}} \quad \textbf{(143)}$$

$$\frac{dS_{gas,ch4}}{dt} = -\frac{q_{in}}{V_{liq}} * S_{gas,ch4} + (1 - Y_{ac}) * f_{ch4,ac} * \rho_3 \quad \textbf{(144)}$$

$$\frac{dX_{XI}}{dt} = \frac{q_{in}}{V_{liq}} \left(X_{XI,in} - X_{XI}\right) + f_{XI,dec} * \sum_{i=4}^{5} \rho_i \quad \textbf{(145)}$$

It was not possible to calibrate or validate the ADMML within the framework of this study, however a sensitivity analysis was carried out (Table 4.3.13).

Table 4.3.13: Sensitivity coefficients of the simplified Model ADMML

#	Parameter	Sensitivtiy Coefficient
1	$K_{S,ac}$	1.57E-06
2	$k_{dec,su}$	1.53E-06
3	$k_{dec,ac}$	1.52E-06
4	Y_{su}	1.25E-06
5	$k_{m,su}$	1.23E-06
6	Y_{ac}	1.07E-06
7	$k_{m,ac}$	1.03E-06
8	k_{hyd}	2.16E-07
9	$K_{S,su}$	1.57E-09
10	K_{IAC}	1.82E-12

The half saturation rate of acetate $K_{S,ac}$ and the decay rate of sugar $k_{dec,su}$ and acetate $k_{dec,ac}$ are the parameters that have the most impact on the model output. $K_{S,ac}$, $k_{dec,su}$ and $k_{dec,ac}$ have the greatest impact on the acetate concentration S_{ac}, the sugar concentration S_{su} and the complex particulates X_c. The complex particulates is the state

variable that is most influenced by most of the parameters, followed by the acetate concentration and the sugar concentration.

This simplified model was implemented in a control tool. The control tool was developed, but could only be cursorily tested as it was not within the framework of the study to calibrate the model.
The input data of the current day is first increased by 20% and then the reactor data of the next day is predicted using the data measured of the current day as initial conditions. The output is then compared with “ideal” values. The increase of the feed input is decreased as long as the “ideal” values are not reached. And so the organic loading rate of the next day can be predicted.

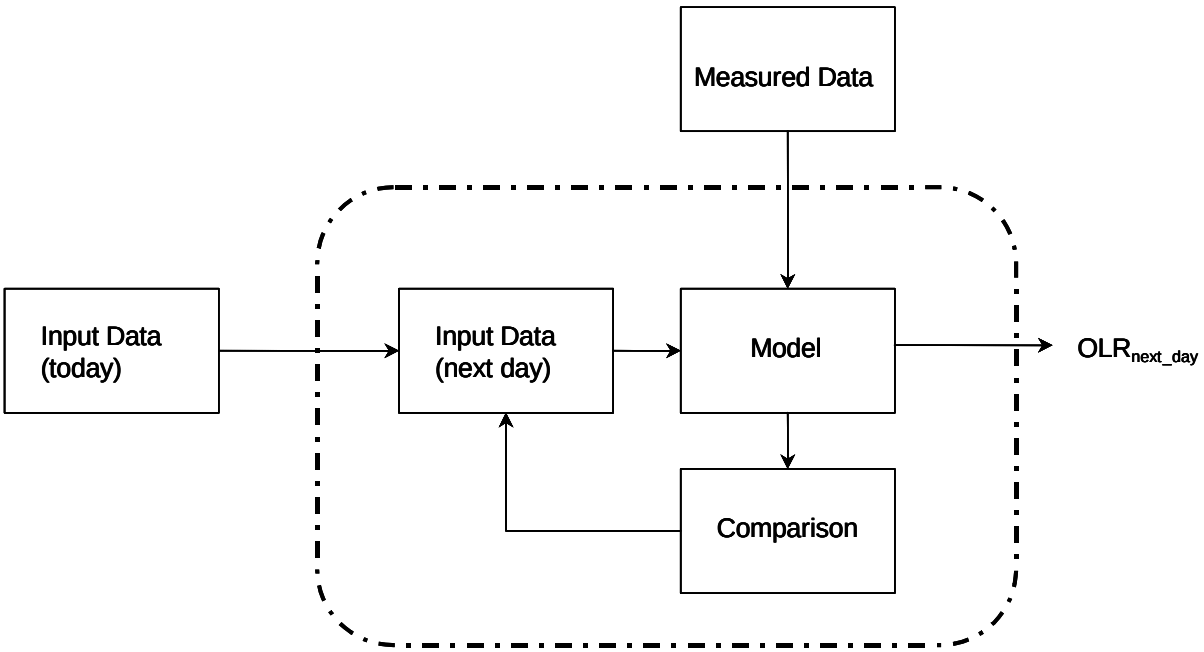

Figure 4.3.9: Control tool based on the ADMML

4.4 Virtual Laboratory (VL)

One output of this study was the development of a so-called “Virtual Laboratory” (VL), which was one of the goals of the CROPGEN project, for data processing and interpretation as well as the mathematical model formulation of the biogas process. Two different VLs were developed. The basis of the VLs is the ADM1 (Batstone *et al.* 2002). The VL is conceived of as a software training tool to provide users with a more detailed insight into the AD process.

4.4.1 Virtual Laboratory VL 1.1

The first version of the VL (VL 1.1) is a software training tool for the simulation of the AD process, using different energy crops (maize, lupine (blue, white), soy, sunflower, rape, rye and triticale) as input substrate and the original ADM1 (Batstone *et al.* 2002). Additionally the “mass balance” was implemented in the tool to estimate the initial conditions.
The VLs are written in the graphical programming system LABVIEW®, with an implementation of the ADM1 as MATLAB® script, compiled in MATLAB® executable. The MATLAB® code for the VL was written, based on an existing MATLAB®-SIMULINK® file of the original ADM1 (Rosen and Jeppsson 2002). The LABVIEW® program serves as a user interface.

Structure of the VL 1.1

The VL is organized into several levels (Figure 4.4.1). The possibility to choose different reactor types has not been implemented in the frame of this work, but is foreseen in the program structure. First of all there is the possibility to choose a substrate or a mixture of substrates. Next there is a characterization of the reactor used. Then the selected case is modelled using the ADM1. The output of the model is then presented as diagrams and/or tables and can be printed out.

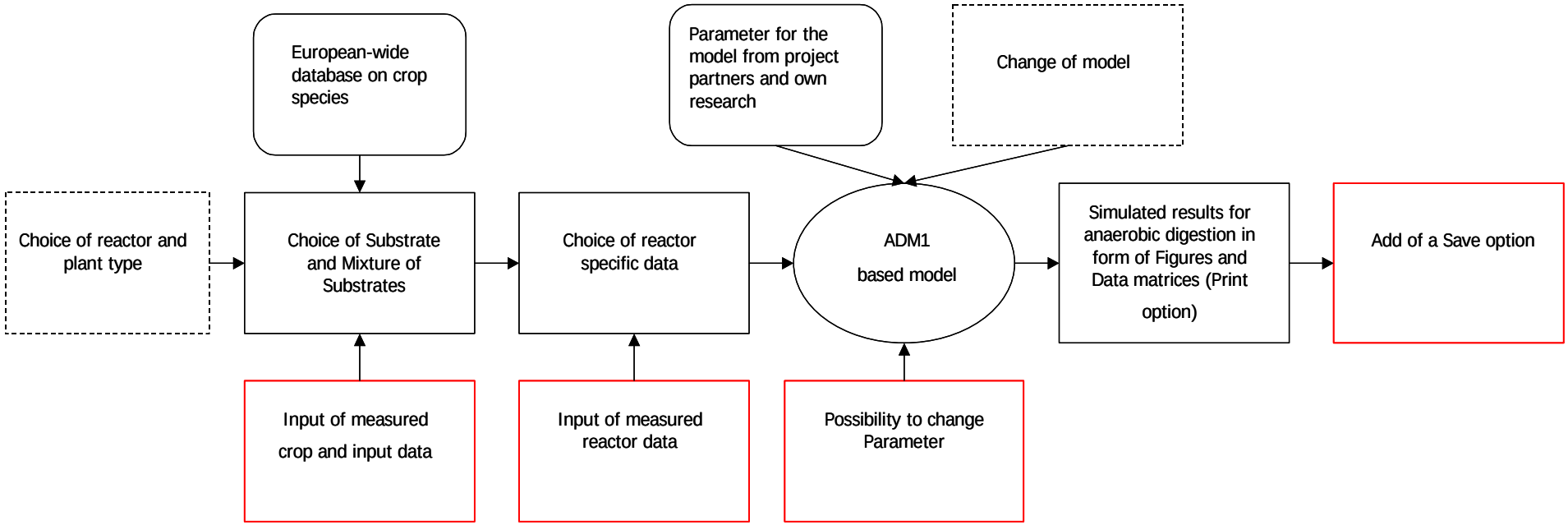

Figure 4.4.1: Structure of the Virtual Laboratory. Options marked red in the flow sheet are not available in the Version 1.1, but will be available in later versions. Boxes with a dashed line will not be realized in the frame of CROPGEN project.

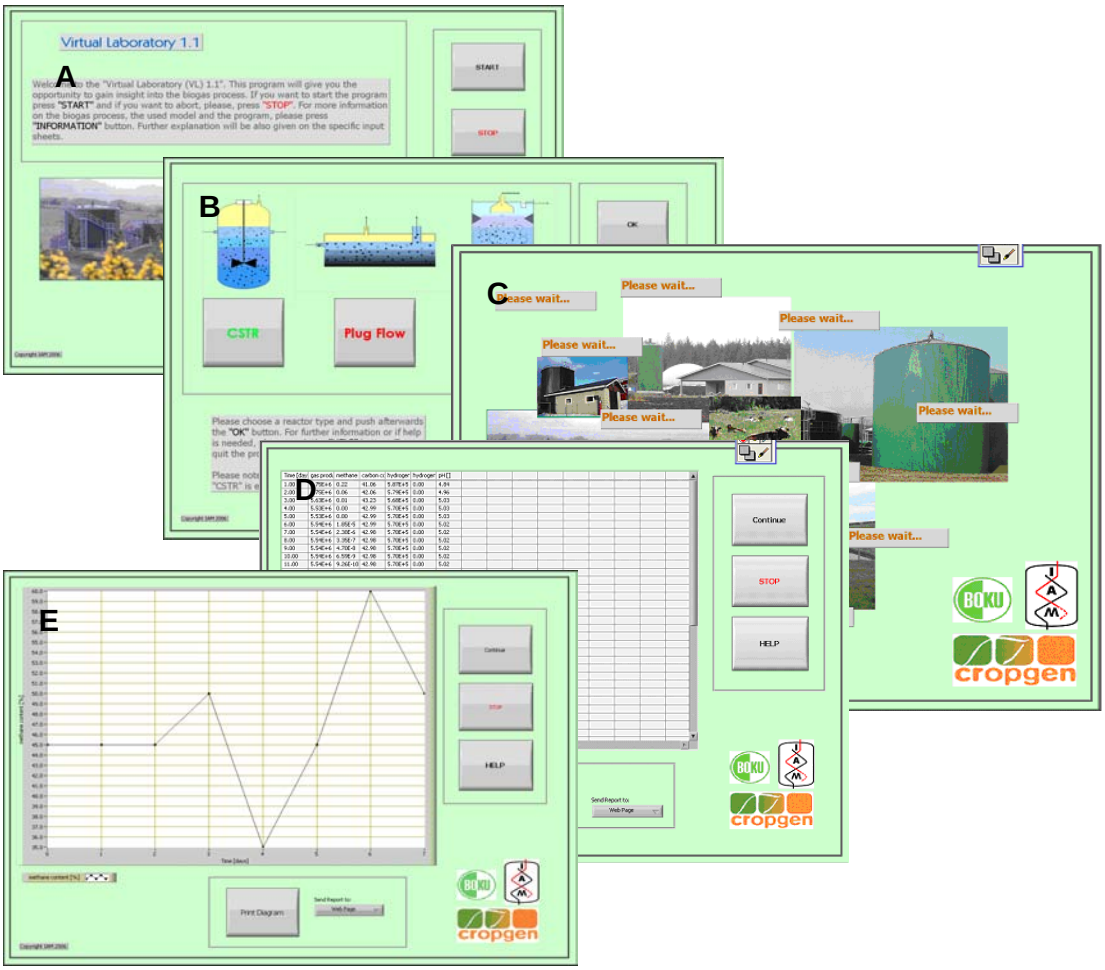

Figure 4.4.2: Screenshots of the redesigned Virtual Laboratory 1.1: A) Start of program, B) Choose of Reactor type, C) Simulation of the AD process, D) and E) Output of the simulation results in form of tables and graphs

Furthermore the user can choose different values for the disintegration rate and the hydrolysis rates. Since, according to literature, the limiting steps in anaerobic digestion are the hydrolysis of macromolecules and the methanogenesis from acetate (Pavlostathis, 1986).

4.4.2 Virtual Laboratory VL 1.2

The Virtual Laboratory (VL) version 1.2 is software tool for the simulation of the AD process with mathematical models based on the ADM1.
The VL 1.2 is written, like the VL 1.1, in the graphical programming system LABVIEW®, with an implementation of the ADM1 as MATLAB® script, compiled in MATLAB® executable.

Structure of the VL 1.2

The VL 1.2 is organized into several levels (Figure 4.4.3), similar to the VL 1.1. First of all there is the possibility to choose the model version and model alternative (batch or continuous). Next there is a characterization of the reactor used and subsequently the parameters. Then the selected case is modelled using the ADM1. The output of the model is then presented as diagrams and/or tables and can be saved and printed out.

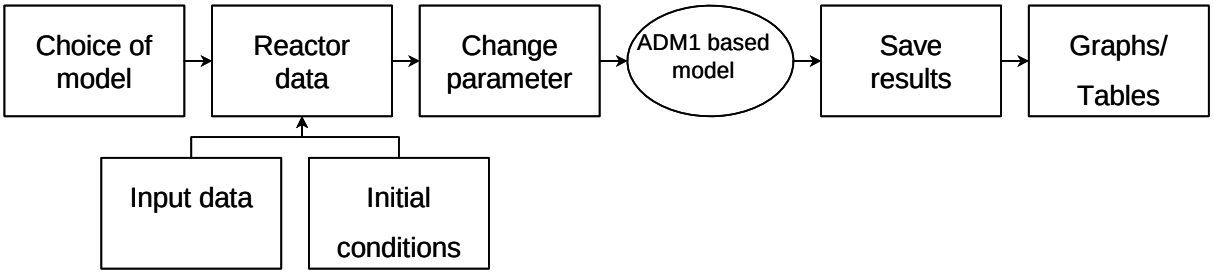

Figure 4.4.3: Structure of the VL 1.2

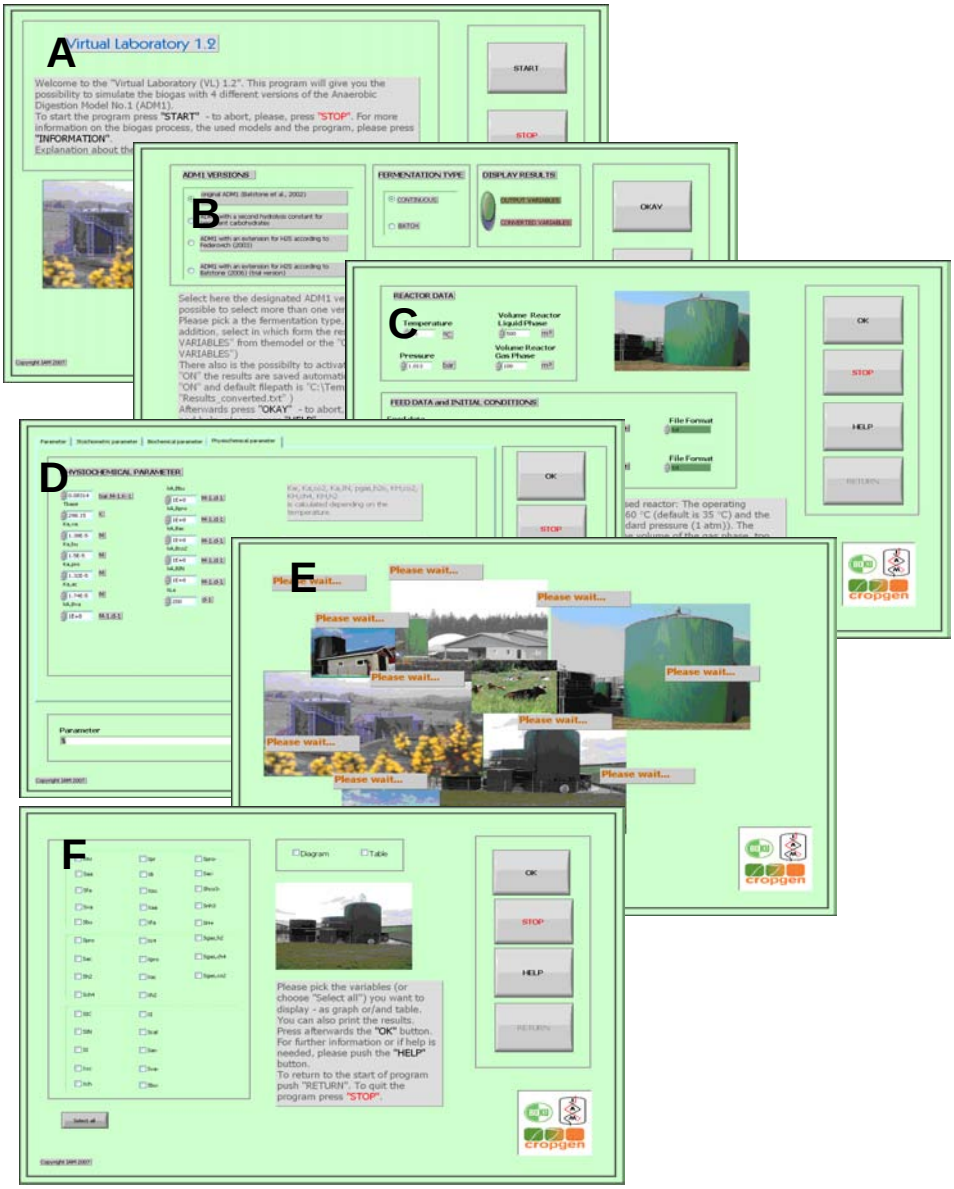

Figure 4.4.4: Screenshots of the User Interface of the VL 1.2: A) Start of program, B) Choice of model, C) Reactor data, D) Choice of parameter, E) Simulation of the AD process and F) Output of the simulation results – choice of table and / or graphs

4.4.3 Virtual Laboratory VL 2.1

The Virtual Laboratory VL 2.1 (Figure 4.4.5) is also, similarly to the VL 1.1, conceived as a training instrument for students to gain an insight into the biogas process.

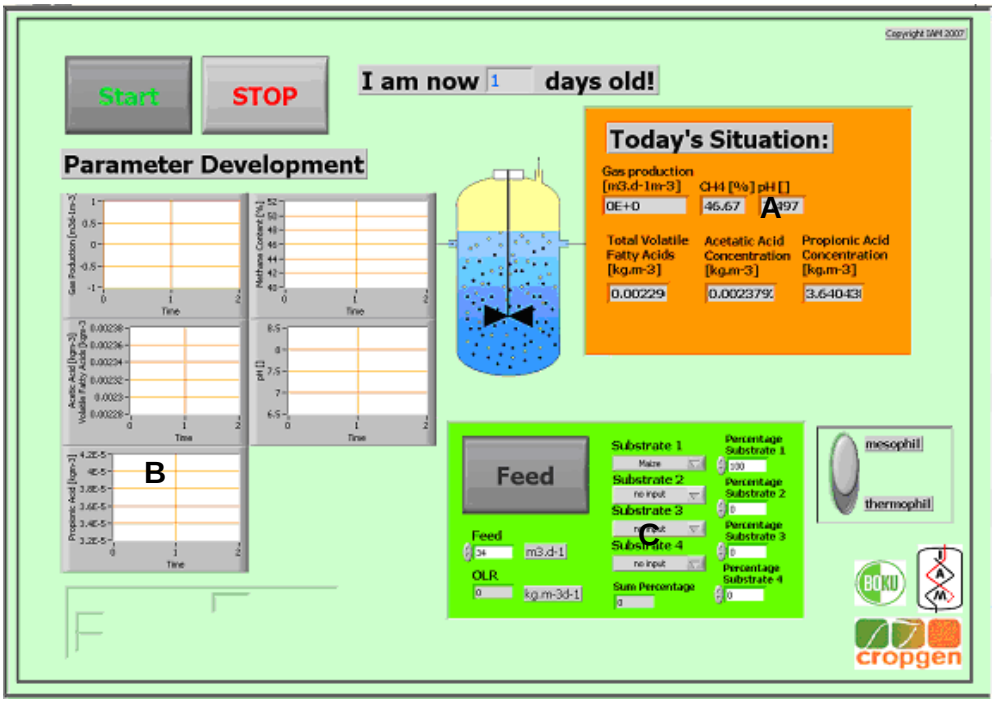

Figure 4.4.5: Screenshot of the VL 2.1

Structure of the VL 2.1

The basis of the VL 2.1, is like in the VL 1.1, the Anaerobic Digestion Model No.1 (ADM1) (Batstone *et al.* 2002). However in comparison to the VL 1.1 the structure is much simpler (Figure 4.4.6): At the beginning the temperature range can be chosen (either mesophile or thermophile), after pressing start the program begins to run. It first waits 20s – during this time it is possible to chose the substrate type and also the feed amount, afterwards the feed button has to be pressed – then the biogas process is simulated for one day and the program gives the data for the current (Figure 4.4.5 (A)) and also the development of the parameters since the program was started (Figure 4.4.5 (B)) – then the program starts again and waits again. If the feed button is not pressed within the "wait" period, the program calculates the input array with a feed amount of zero.

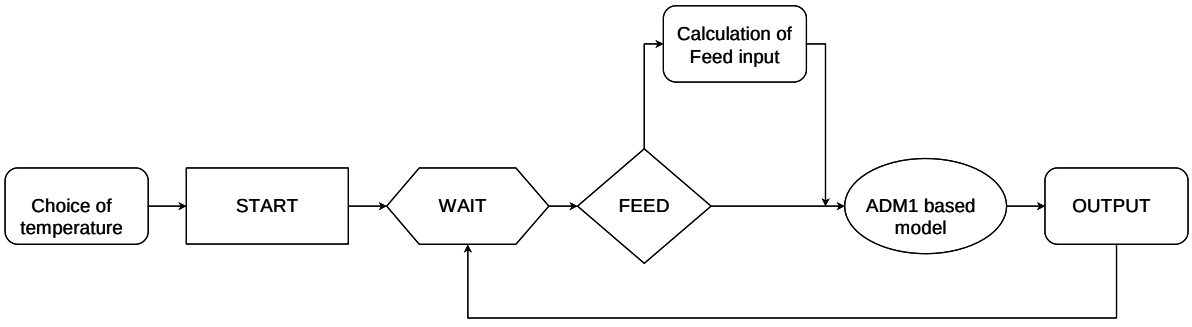

Figure 4.4.6: Structure of the VL 2.1

The reactor condition is defined by the gas production (gp), the methane content (mc), the pH (ph), the volatile fatty acid concentration (vfa), the propionic acid concentration

(pro), the acetic acid concentration (ac) and the COD Reduction (abbau), are indicated by different colours (green = “good”, orange = “okay” and red = “bad”).
As in the VL 1.1, in the VL 2.1 eight different substrates (maize, lupine (blue, white), soy, sunflower, rape, rye and triticale)) can be chosen, where up to four different substrates can be mixed.

4.5 Decision Support System based on Fuzzy Control

To make the biogas process economically attractive, the process has to be optimised. There are different ways to improve biogas production, such as substrate pre-treatment (thermal methods, chemical methods, biochemical methods and mechanical methods), removal of toxic components, co-digestion different substrates, innovative reactor designs and an advanced process control. Advanced process control can be realized using a decision support system (DSS).

"*DSS are computer-based systems used to assist and aid decision makers in their decision making processes*" (Bogardi 2004) Normally such a system consists of several dimensions, where the actual decision-making is important, but nevertheless only a part of the whole DSS (Bogardi 2004).

For DSS based on complex mathematical models (e.g ADM1), a significant problem is the amount of data required, which is often a problem for technical biogas plants. This is due to the fact that there is a lack of online sensors available and existing sensors need extensive maintenance (Olsson and Newell 1999). Moreover operators are in most cases not sufficiently educated to deal with the instrumentation and control adequately (Olsson and Newell 1999). One possibility is to use a simpler model as the basis for the DSS, which was shown before. Another alternative is a fuzzy logic based control tool.

4.5.1 Comparison of different Fuzzy Tools using the ADM1 and a composite programming based ranking method

A fuzzy logic tool, which was designed during another EU project (AMONCO project), was now enhanced to improve the control performance. This DSS is designed to identify process control strategies to yield a high methane content final product.

The original tool used either the (i) total fatty acid concentration (from the current day and the day before) or the (ii) pH (from the current day and the day before) and also the methane content, the gas production rate and the organic loading rate of the current day as input and gave the organic loading rate of the following day as output. The fuzzy methodology used was the fuzzy interference method by Mamdani (for all used Fuzzy Tools). (Mandani and Assilian, 1975).

Seven different tools were developed. These tools and the original tool developed (i) were tested with the ADM1 (Batstone *et al.* 2002) and compared with a composite programming based ranking method, not only to find the best structure, but also to find a possibility for supporting experts in the development of fuzzy tools.

Composite Programming is an extension of Compromise Programming, which is itself an approach to multiple criterion decisions making (Bogardi 2004). The principle of multiple decisions making is to maximize the satisfaction of the different conflicting objectives in order to find the best option (Bogardi 2004). Compromise Programming is measuring the distance between the actual solution and the ideal solution and then selecting the

minimum distance. Composite Programming works with a hierarchical and normalized distance type. Here, the process is broken down into its elementary components, and then grouped in broader and higher leveled indicators (Bogardi 2004).
Firstly the tools themselves were tested to find the ideal membership functions. This examination is done by calculating one day with the ADM1. The output values of the ADM1 are then used as the input for the Fuzzy Tool. Herewith the amount of feed for the next day is calculated. This is considered in the input for the ADM1 and the next day is calculated. The output is again used as the input for the Fuzzy Tool and so on. The calculation is done for 50 days. Hereby 3 to 47 different scenarios were tested, changing either one or more “Fuzzy Blocks” (either changing only the input membership functions of the block or also changing the output membership functions, with 60 different values for each membership function (also varying between trapezoid and triangle forms).
The gas production, the methane content, the concentration of the acetic acid, the concentration of the propionic acid, the total concentration of volatile fatty acids, the COD reduction and the pH were chosen as appraisal factors.
Minima and maxima and a weight were defined for these criteria, depending on the range of the membership functions used (the range remained equal for all membership functions in all scenarios). For the evaluation here, the mean value of each criterion in each scenario was used, under consideration of the standard deviation. The mean values were normalized, multiplied by the weight and summarized. Thus each scenario gets one value, thus the scenarios can be ranked depending on this value.

Fuzzy Tool 1: F_DSS_051006

The input of this tool (Figure 4.5.1) is the VFA concentration of the current day and the difference between the VFA concentration of the current day and the day before. The difference in the VFA concentration of the current day and the day before is added to the fuzzy tool as a second input factor, as not only the current VFA concentration is important, but the change in the VFA concentration. This is due to the fact that, if the reactor is adapted to high levels of organic acids, they have less effects on the reactor performance (Braun 1982).

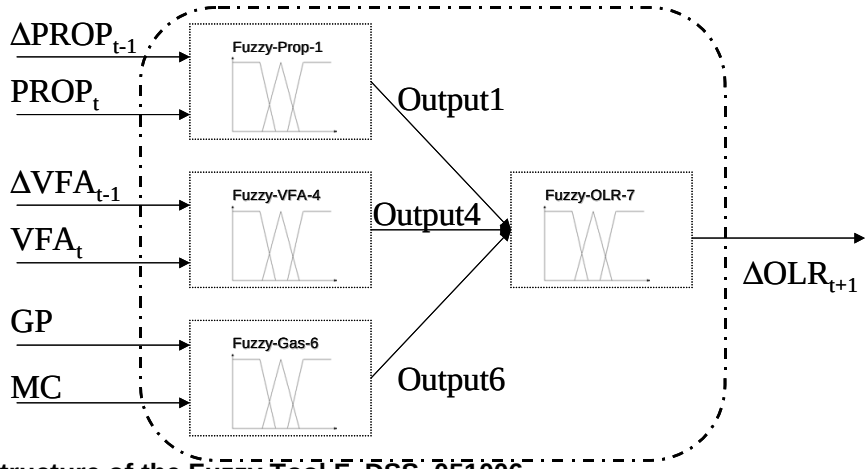

Figure 4.5.1: Structure of the Fuzzy Tool F_DSS_051006

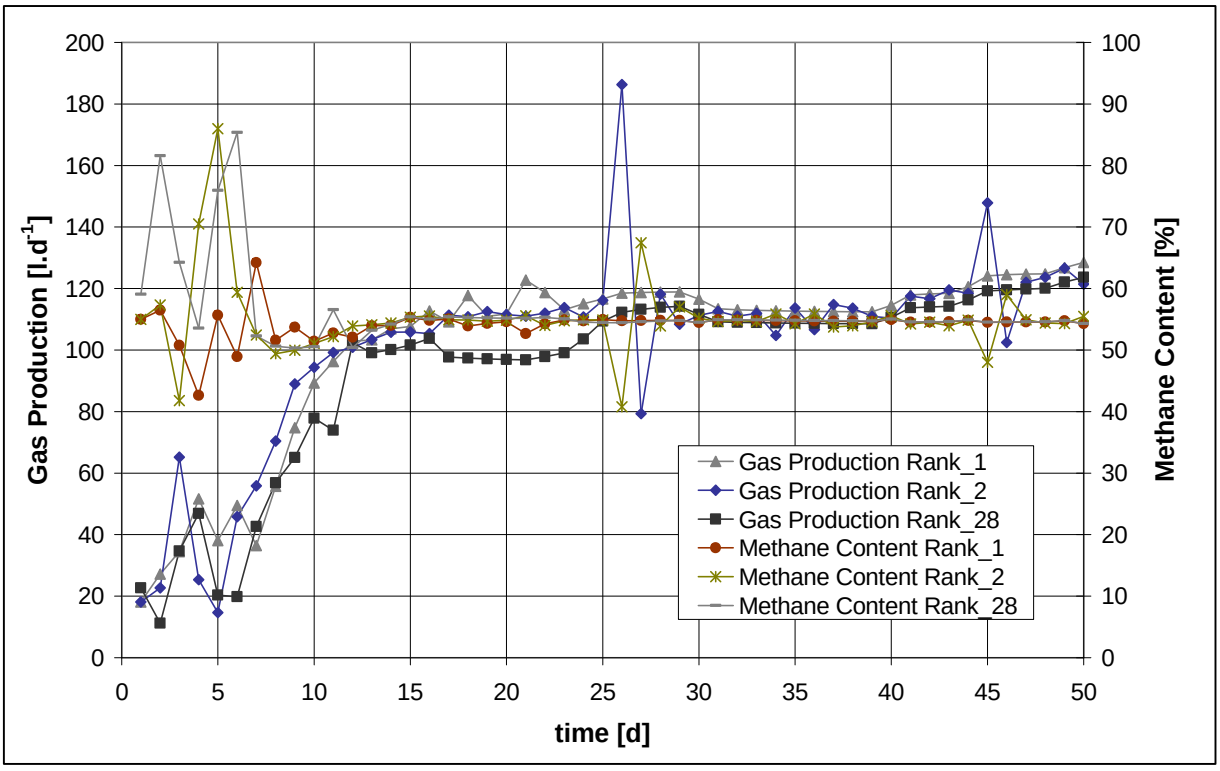

Figure 4.5.2: Result of the comparison of different combinations of membership functions F_DSS_051006

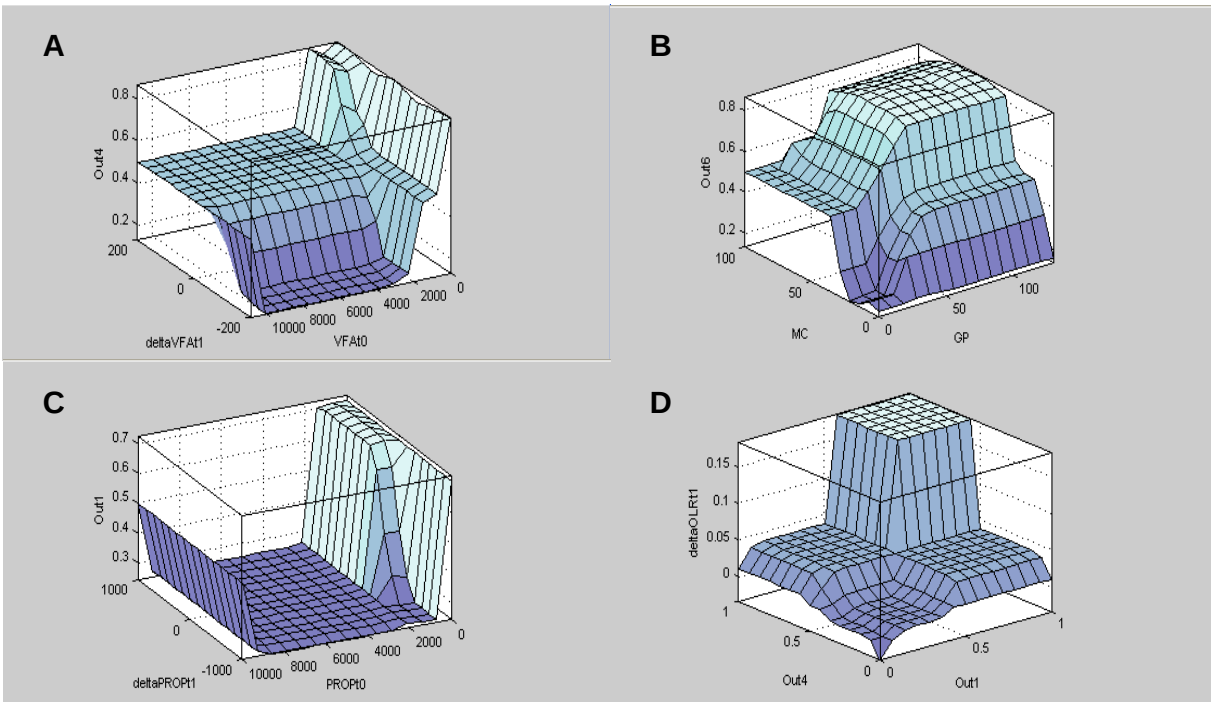

Figure 4.5.3: Structure of the best combination of the membership functions of F_DSS_051006; A) Membership function for the VFA concentration, B) membership function for the propionic acid concentration, C) membership functions for gas production and methane content and D) membership function for OLR.

The VFA concentration alone has his weaknesses as indicator, not the amount alone, but the composition is of importance, since the acetic acid concentration is less toxic than propionic acid (Henze and Harremoes 1983). During an inhibition of aceticlastic

methanogens an increase of the propionic acid can always be observed (Angelidaki *et al.* 1993; Gerardi 2003; Bischofsberger *et al.* 2005). Thus a further input block using propionic acid was added to the fuzzy tool, due to the fact that exalted values of propionic acid may indicate difficulties in any of the metabolic steps of anaerobic treatment (Speece 1996). Here the propionic acid of the current day and difference in the propionic acid of the current day and the day before are used as input.
Additionally the gas production and methane content of the current day were also chosen as inputs. These factors are used as input factors as one of the aims of the object tool is to reach a stable reactor, with as high a methane production as possible. The methane content is, like the VFA and propionic acid concentration, an indicator for the performance of the reactor. Due to the fact that Methanogenic bacteria are very sensitive to the toxicity of fatty acids and also to changes in pH, temperature and alkalinity (Gerardi 2003). The output of the tool is the organic loading rate of the next day and the feed volume.
1320 different combinations of membership functions were compared to each other (Figure 4.5.2). Figure 4.5.3 shows the structure of the best combination of the membership functions for F_DSS_051006.

Fuzzy Tool 2: F_DSS_051006b

The structure of this tool (Figure 4.5.4), is simpler than F_DSS_051006 and consists of only two input blocks – one using the concentration of the propionic acid of the current day and the difference between the propionic acid concentration of the current day and the day before and the other the gas production of the current day and the methane content of the current day. The output is the organic loading rate of the next day and the feed volume.

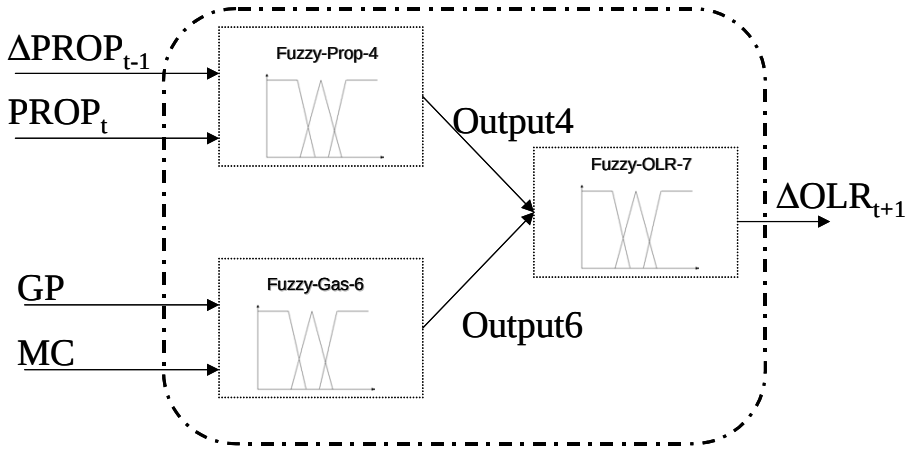

Figure 4.5.4: Structure of the Fuzzy Tool F_DSS_051006b

In this case 1020 different combinations of membership functions were compared to each other (Figure 4.5.5) in order to find the best combination (Figure 4.5.6).

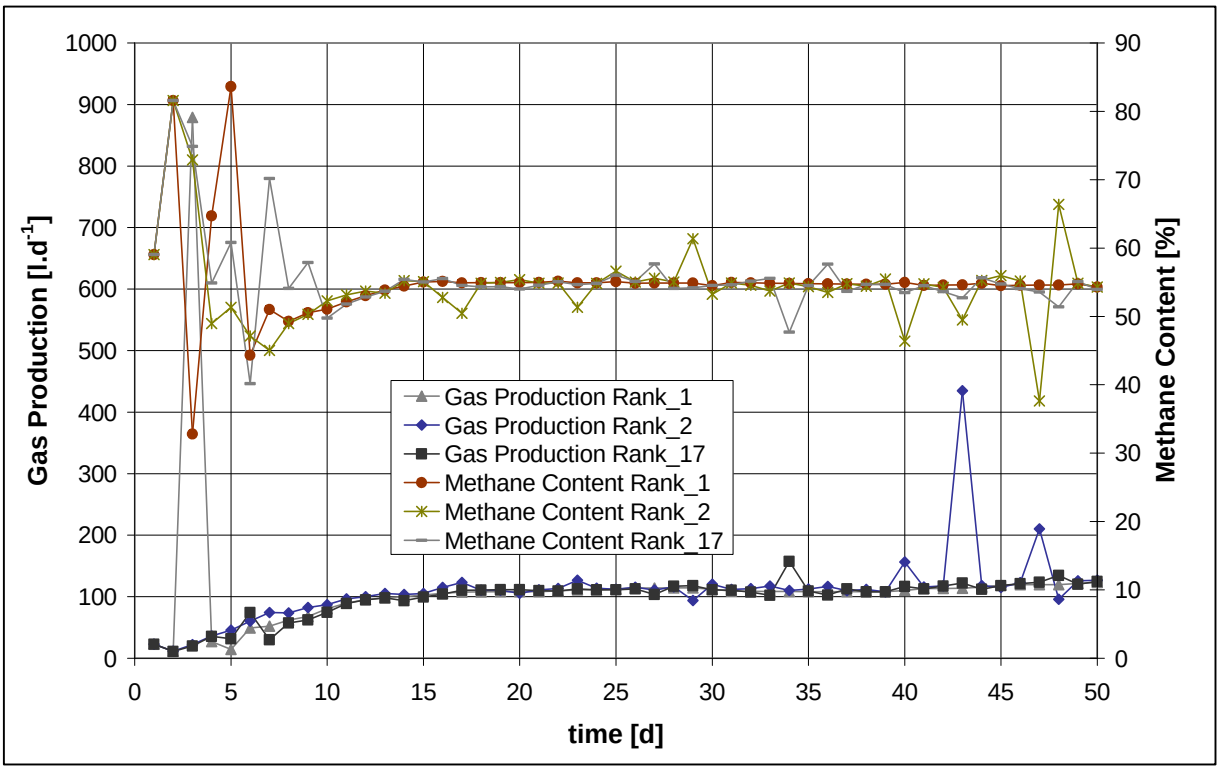

Figure 4.5.5: Result of the comparison of different combinations of membership functions F_DSS_051006b

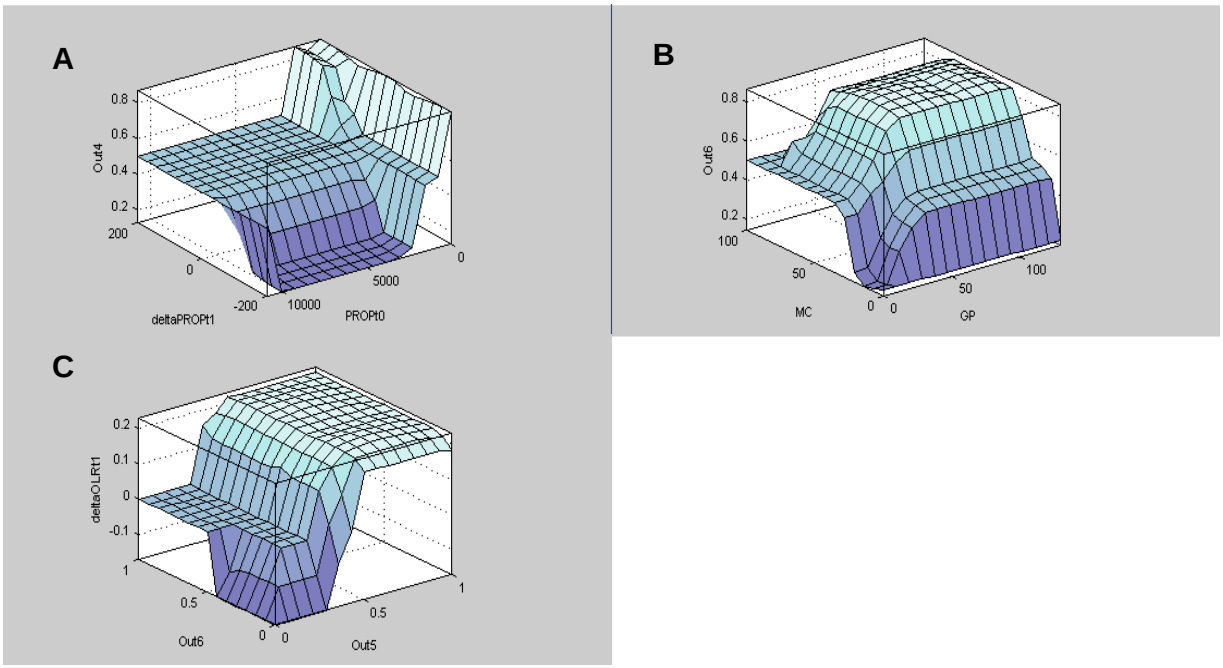

Figure 4.5.6: Structure of the best combination of the membership functions of F_DSS_051006b; A) Membership function for the propionic acid concentration, B) membership functions for gas production and methane content and C) membership function for OLR.

Fuzzy Tool 3: F_DSS_090806

This tool (Figure 4.5.7) is, in principal, equal to the one developed in the AMONCO project. The input of this tool is the concentration of the VFA concentration of the current day and the difference between the VFA concentration of the current day and day before as well as the gas production and methane content of the current day. The output is the

organic loading rate of the next day and the feed volume. This tool is interesting, if only the total concentration of fatty acids is measured.

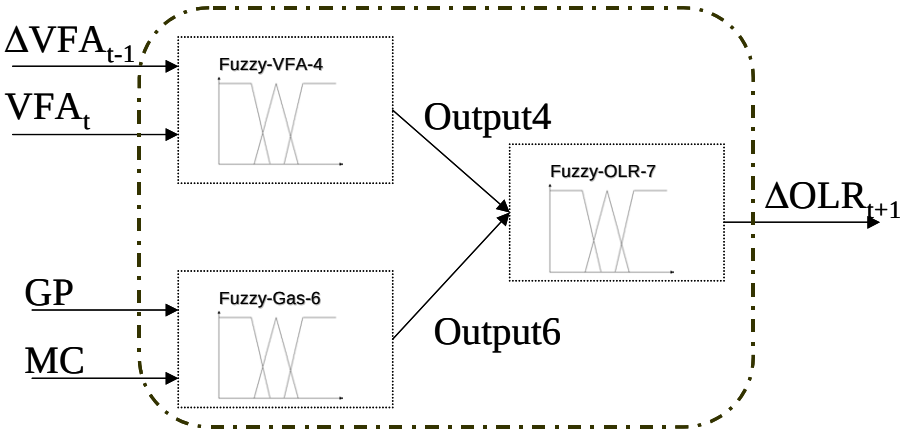

Figure 4.5.7: Structure of the Fuzzy Tool F_DSS_090806

Figure 4.5.8 shows the results of the comparison of the different membership function for the tool F_DSS_090806. In the figure the gas production and the methane content of the combination of membership functions of rank 1, rank 2 and rank 17 are shown. The final membership functions chosen are shown in Figure 4.5.9.

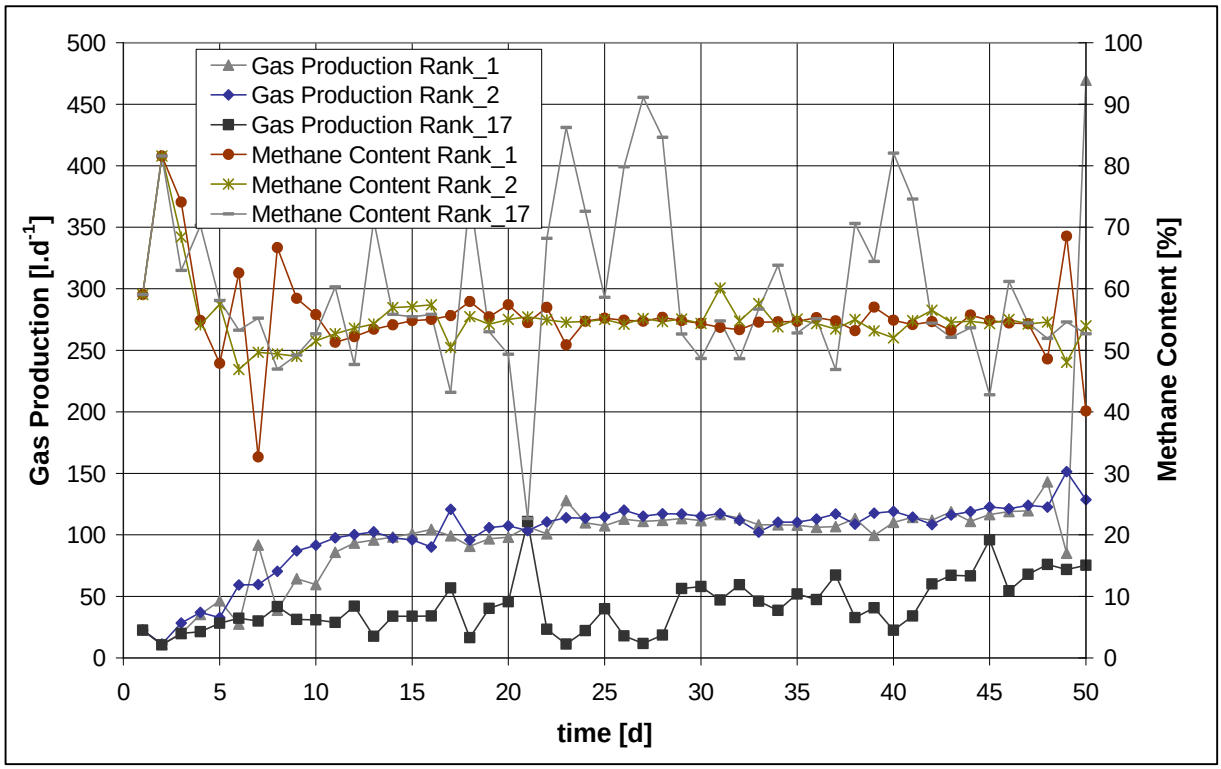

Figure 4.5.8: Result of the comparison of different combinations of membership functions F_DSS_090806

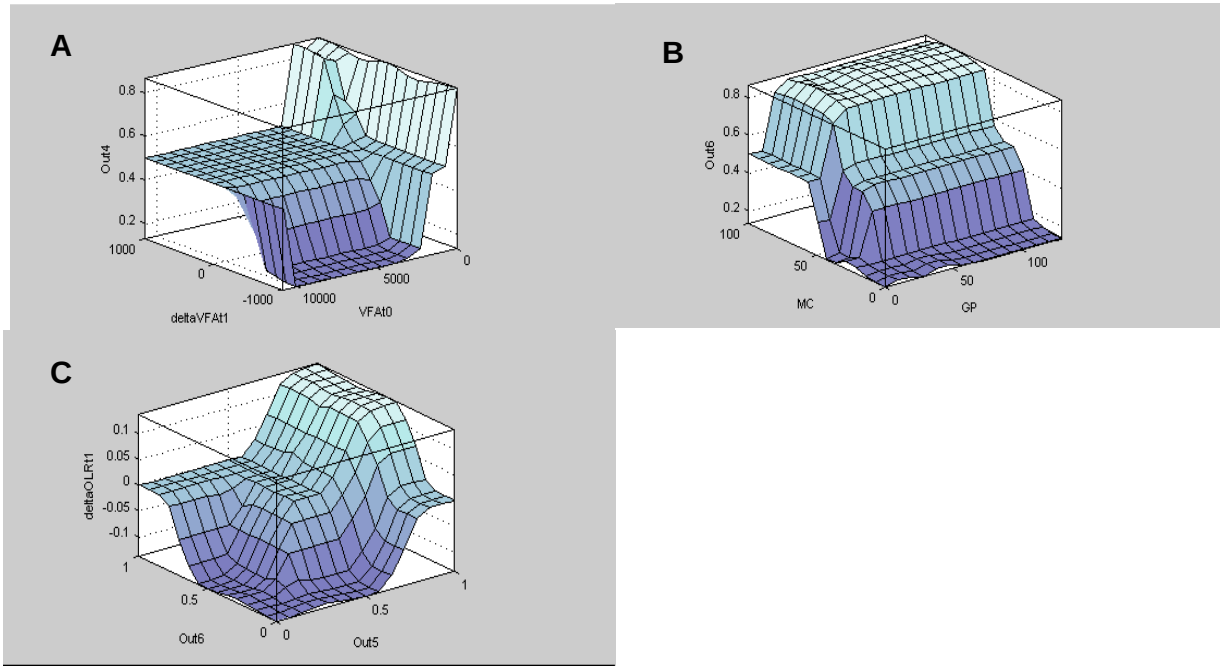

Figure 4.5.9: Structure of the best combination of the membership functions of F_DSS_090806; A) Membership function for the VFA concentration, B) membership functions for gas production and methane content and C) membership function for OLR.

Fuzzy Tool 4: F_DSS_101006

F_DSS_101006 (Figure 4.5.10) uses the same blocks as F_DSS_051006, however the structure is a bit more complicated. The tool is comprised of membership functions in all 5 blocks. The input in this case, like in F_DSS_051006, is the VFA concentration of the current day and the difference between the VFA concentration of the current day and the day before, the propionic acid of the current day and the difference between the propionic acid of the current day and the day before, also the gas production of the current day and the methane content of the current day. The output, is as in all previous tools, the organic loading rate of the next day and the feed volume.

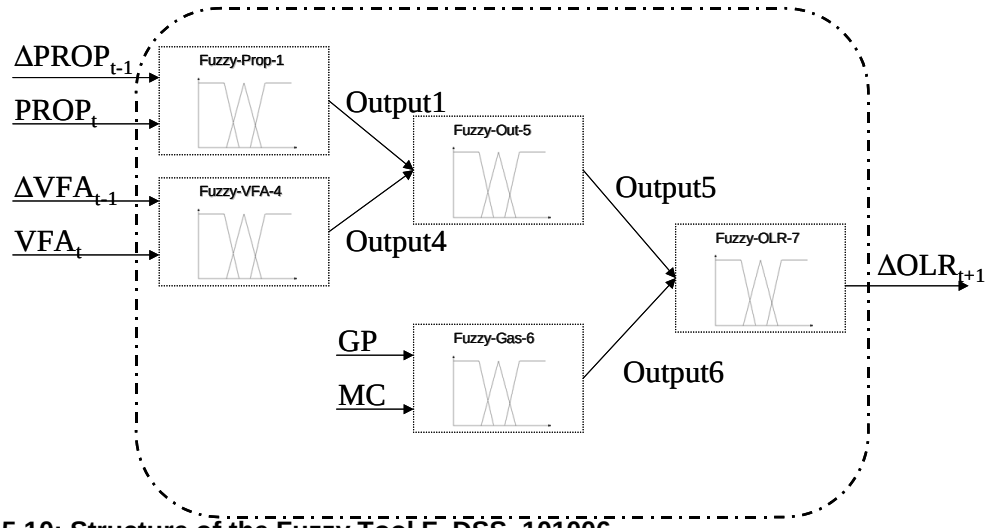

Figure 4.5.10: Structure of the Fuzzy Tool F_DSS_101006

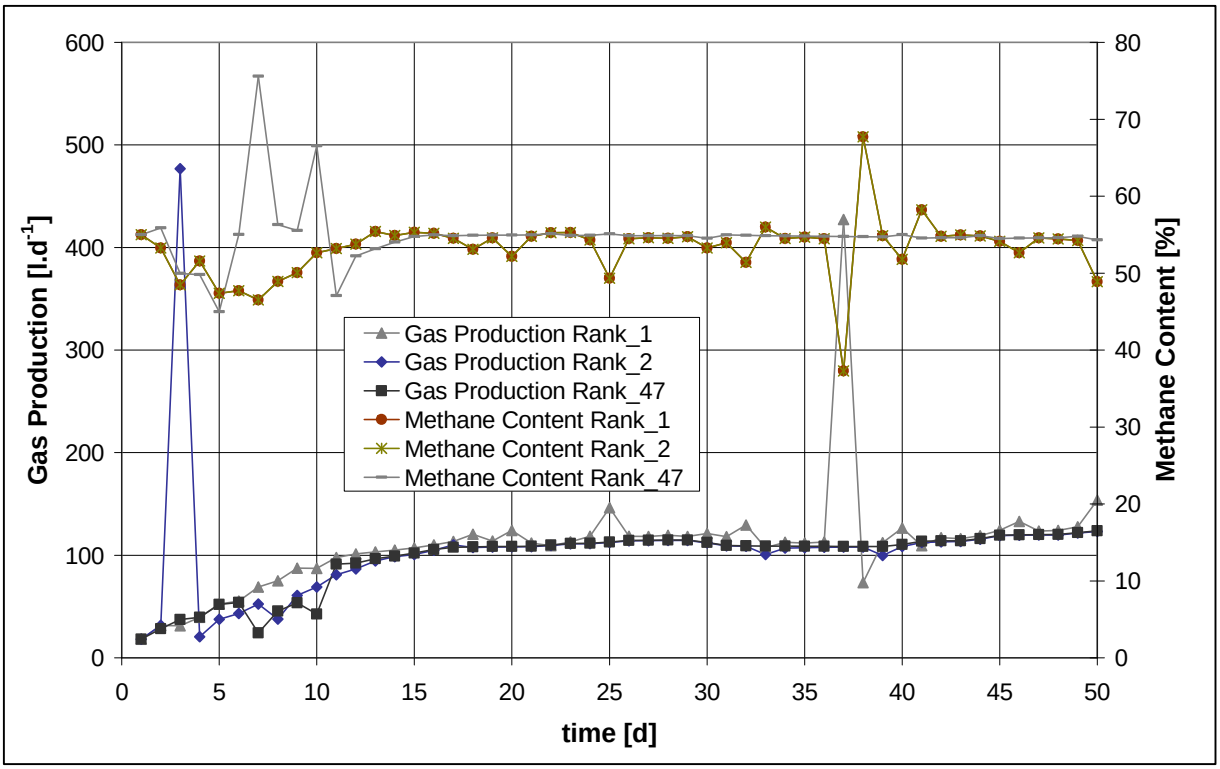

Figure 4.5.11: Result of the comparison of different combinations of membership functions F_DSS_101006

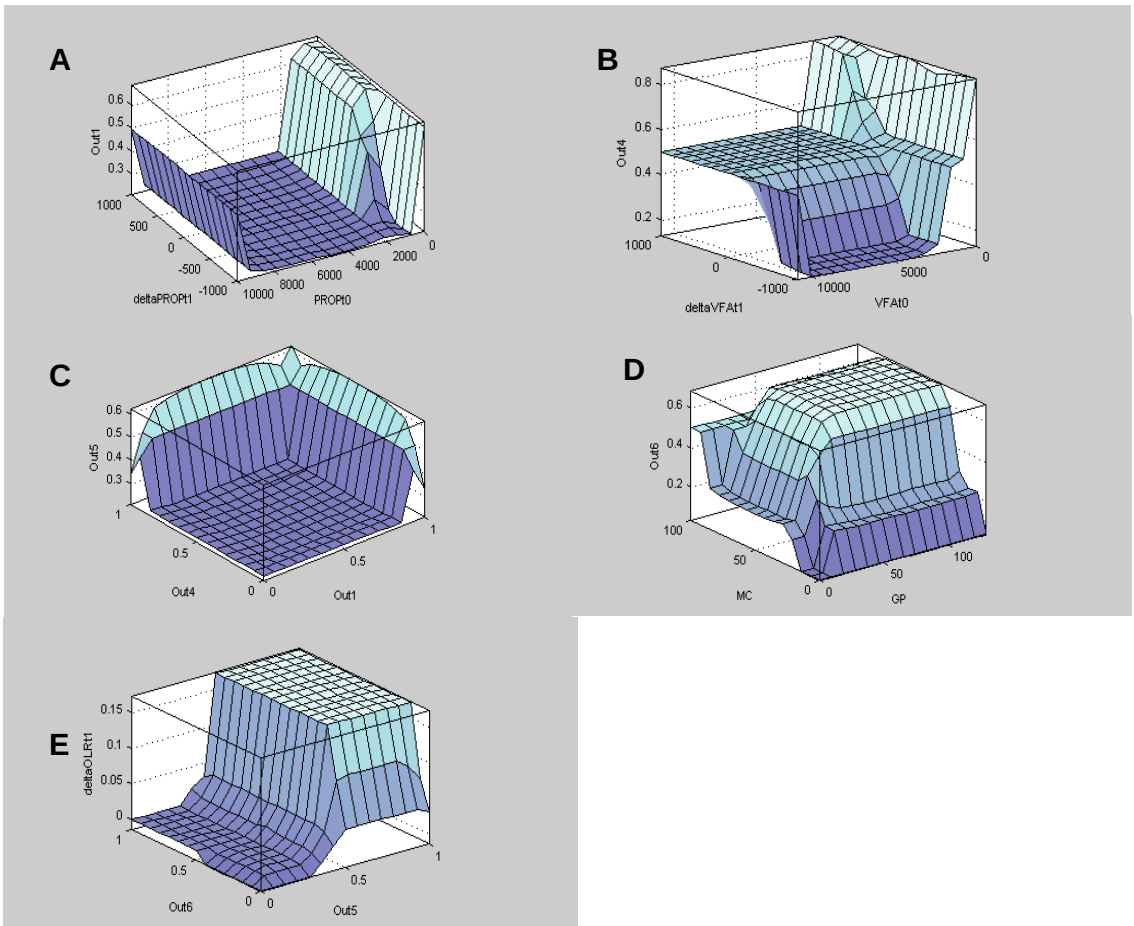

Figure 4.5.12: Structure of the best combination of the membership functions of F_DSS_101006; A) Membership function for the propionic acid concentration, B) membership function for the VFA concentration, D) membership functions for gas production and methane content and E) membership function for OLR.

In this case 2820 different combinations of membership functions were compared to each other (Figure 4.5.11) to find the best combination of membership functions (Figure 4.5.12).

Fuzzy Tool 5: F_DSS_181006b

Measurements are always a cost factor, therefore the attempt was made to simplify the control tool as much as possible. Thus three input tools were developed consisting of only one fuzzy block of membership functions with only two input variables. For these tools 180 combinations of membership functions were compared to each other, to find the best structure. The first tool is F_DSS_191006b (Figure 4.5.13). For this tool the concentration of the propionic acid of the current day and the methane content of the current day are used as input variables. Figure 4.5.14 shows the results of this comparison and Figure 4.5.15 the best structure of the membership functions.

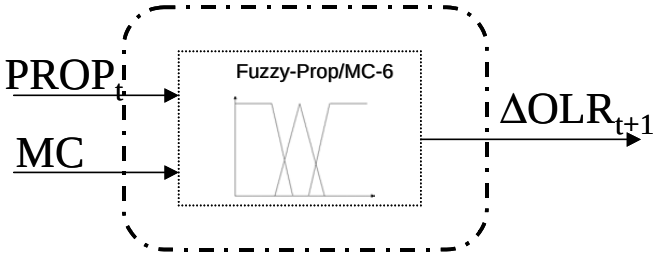

Figure 4.5.13: Structure of the Fuzzy Tool F_DSS_181006b

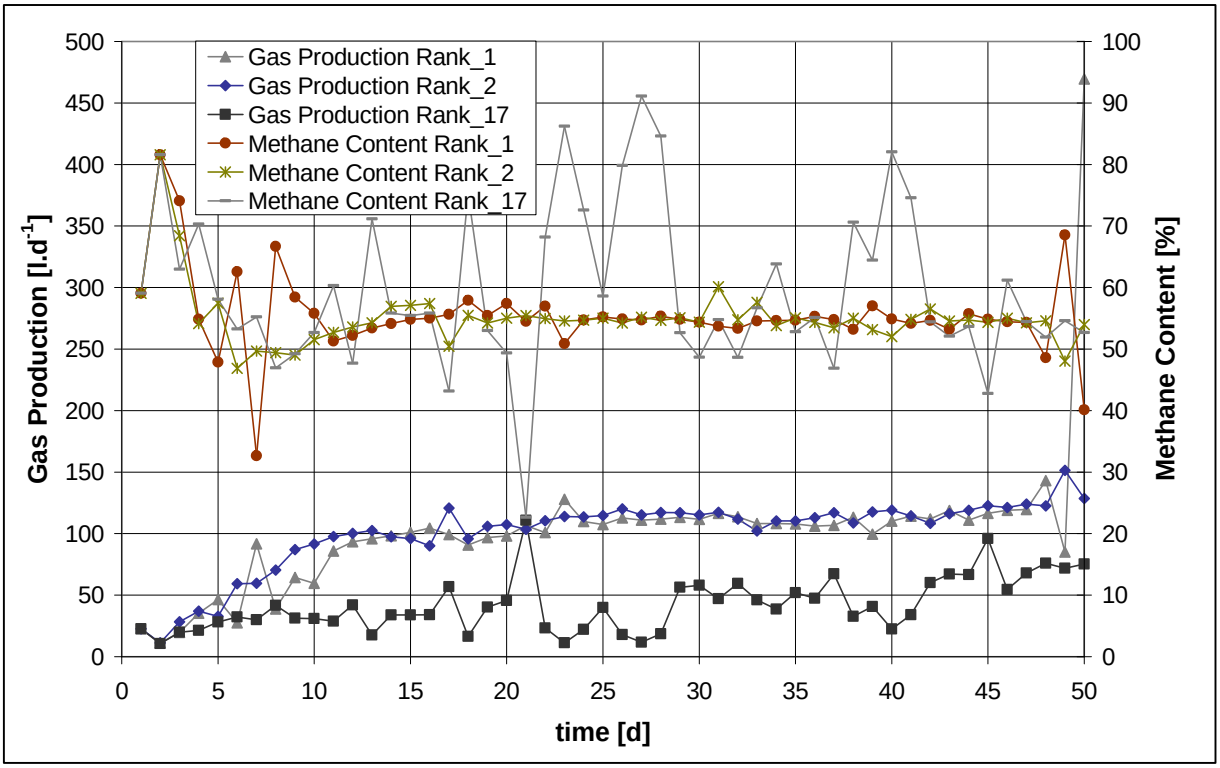

Figure 4.5.14: Result of the comparison of different combinations of membership functions F_DSS_181006b

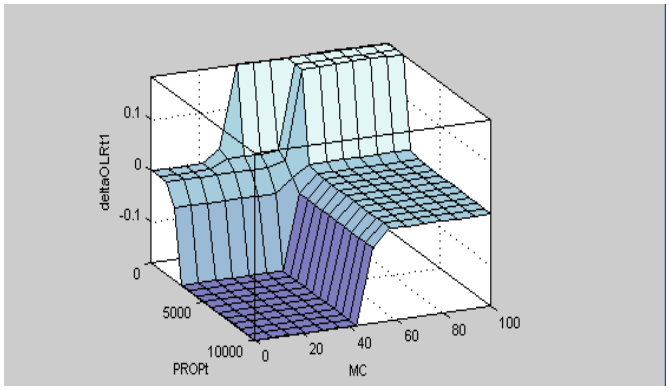

Figure 4.5.15: Structure of the best combination of the membership functions of F_DSS_181006b - Membership function for the propionic acid concentration and methane content

Fuzzy Tool 6: F_DSS_191006

The second of the simple fuzzy tools uses the pH of the current day and the methane content of the current day (Figure 4.5.16).

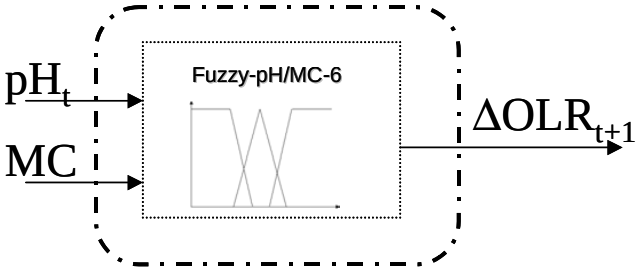

Figure 4.5.16: Structure of the Fuzzy Tool F_DSS_191006

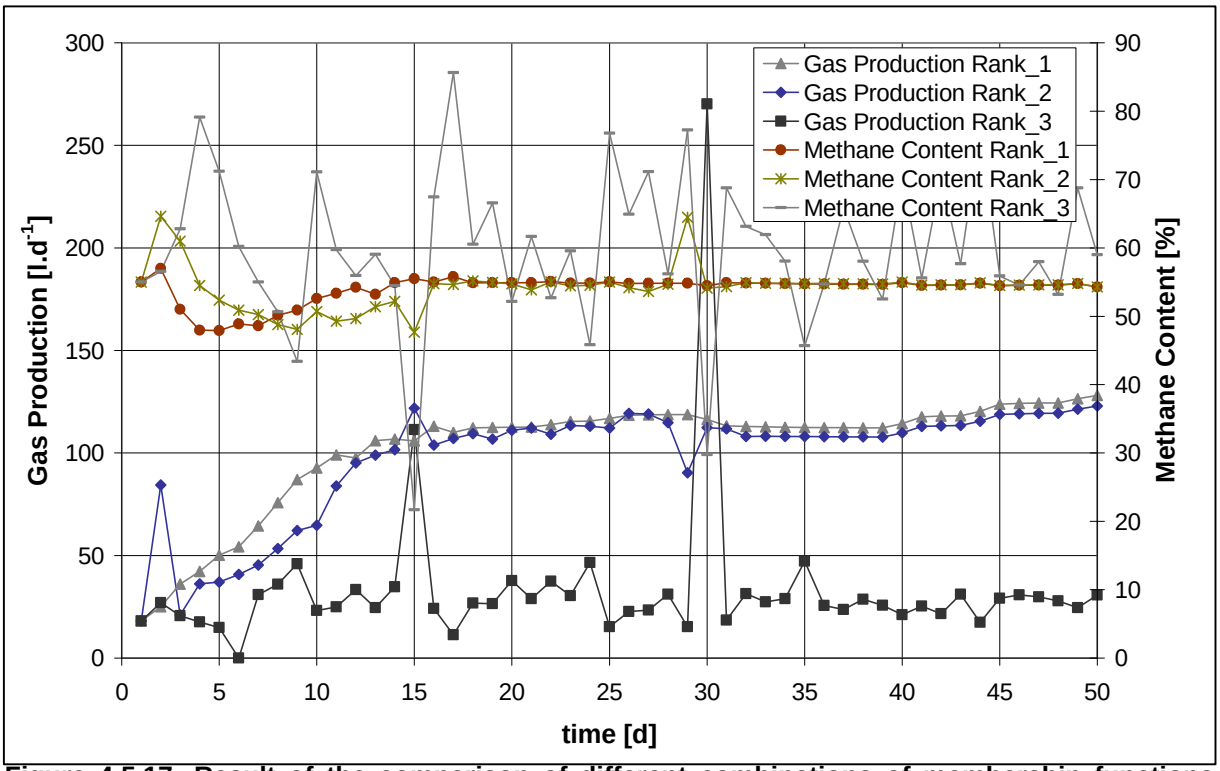

Figure 4.5.17: Result of the comparison of different combinations of membership functions F_DSS_191006

Normally the optimum pH for the overall AD process is between 6.8 and 7.2 (Gerardi 2003). The advantage of using the pH as the input for the fuzzy tool is that online sensors are cheap. Moreover the pH is normally a standard measurement in technical biogas plants. Yet, using the pH as a variable has the disadvantage that it can only serve as an indicator for what has already happened in the reactor.

Figure 4.5.17 shows the results of the comparison of the different membership functions. In the figure the gas production and the methane content of the combination of membership functions of the first three ranks is shown. The best membership function is shown in Figure 4.5.18.

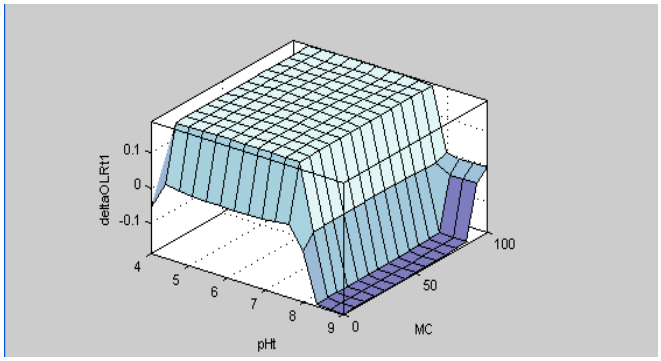

Figure 4.5.18: Structure of the best combination of the membership functions of F_DSS_191006 - Membership function for the pH and the methane content

Fuzzy Tool 7: F_DSS_191006b

The input of this Tool is the VFA concentration and the methane content of the current day. The output is the organic loading rate of the next day and the feed volume.

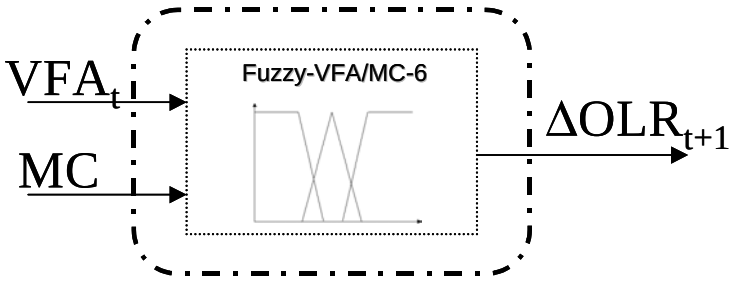

Figure 4.5.19: Structure of the Fuzzy Tool F_DSS_191006b

As for F_DSS_181006 and F_DSS_191006 180 combinations membership are tested for F_DSS_191006b (Figure 4.5.20). The best membership function is shown in Figure 4.5.21.

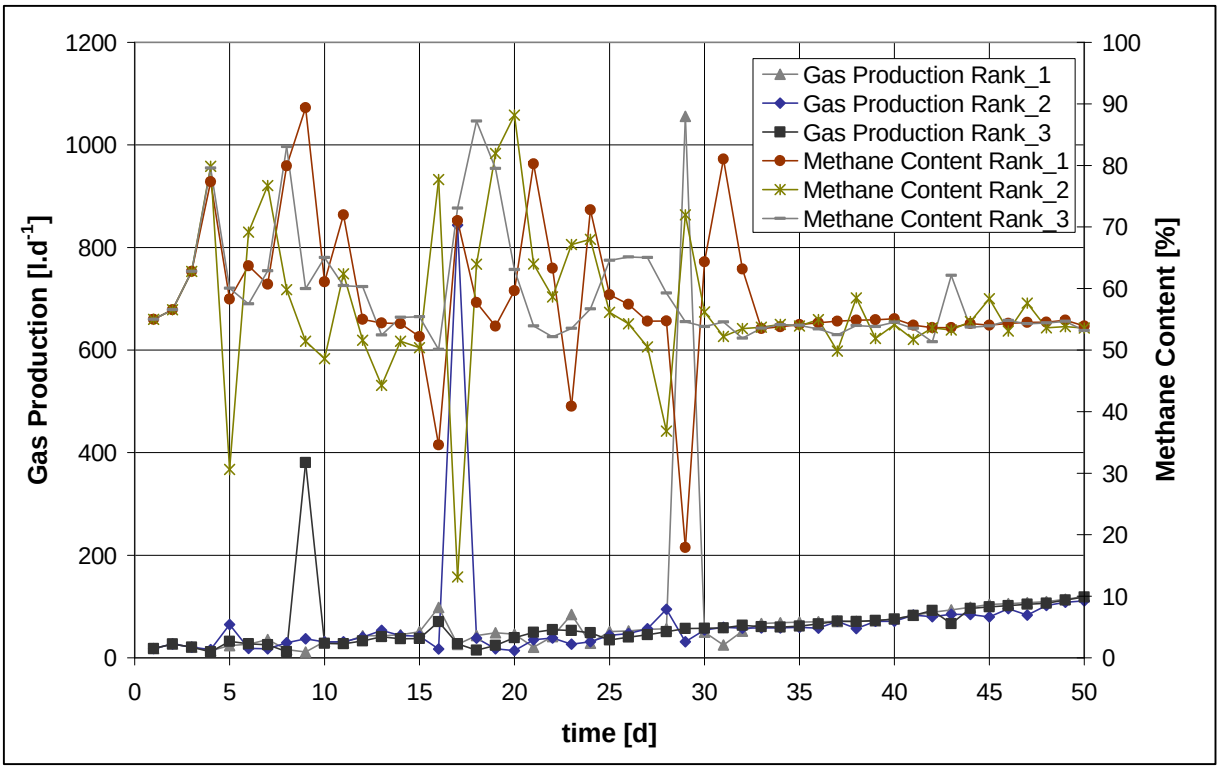

Figure 4.5.20: Result of the comparison of different combinations of membership functions F_DSS_191006b

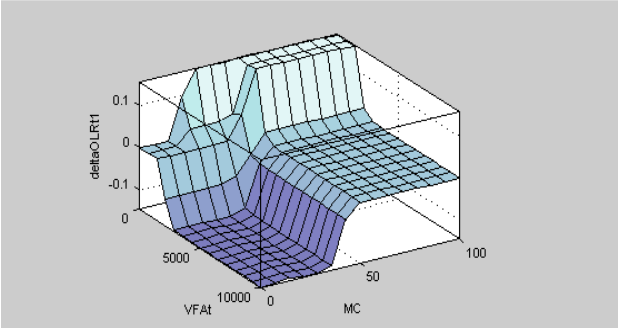

Figure 4.5.21: Structure of the best combination of the membership functions of F_DSS_191006b - Membership function for the VFA concentration and the methane content

Fuzzy Tool 8: F_DSS_191006c

The last fuzzy tool F_DSS_191006c is the most complicated tool of all the tools developed.

In this tool not only are the organic acids considered, but also the pH of the reactor. The input of this tool was the concentration of the propionic acid of the current day and the day before, the pH of the current day and the day before and the gas production of the current day and the methane content of the current day, as well. The output is the organic loading rate of the next day and the feed volume.

Figure 4.5.23 shows the results of the comparison of the different membership function for the tool F_DSS_191006c. In the figure the gas production and the methane content of the combination of membership functions of rank 1, rank 2 and rank 47 are shown. The membership functions which were finally chosen are shown in Figure 4.5.24.

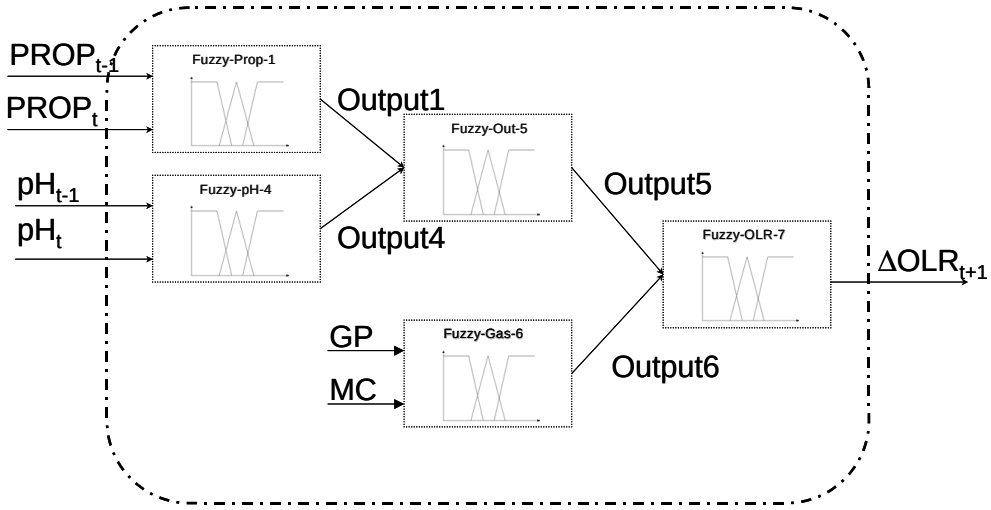

Figure 4.5.22: Structure of the Fuzzy Tool F_DSS_191006c

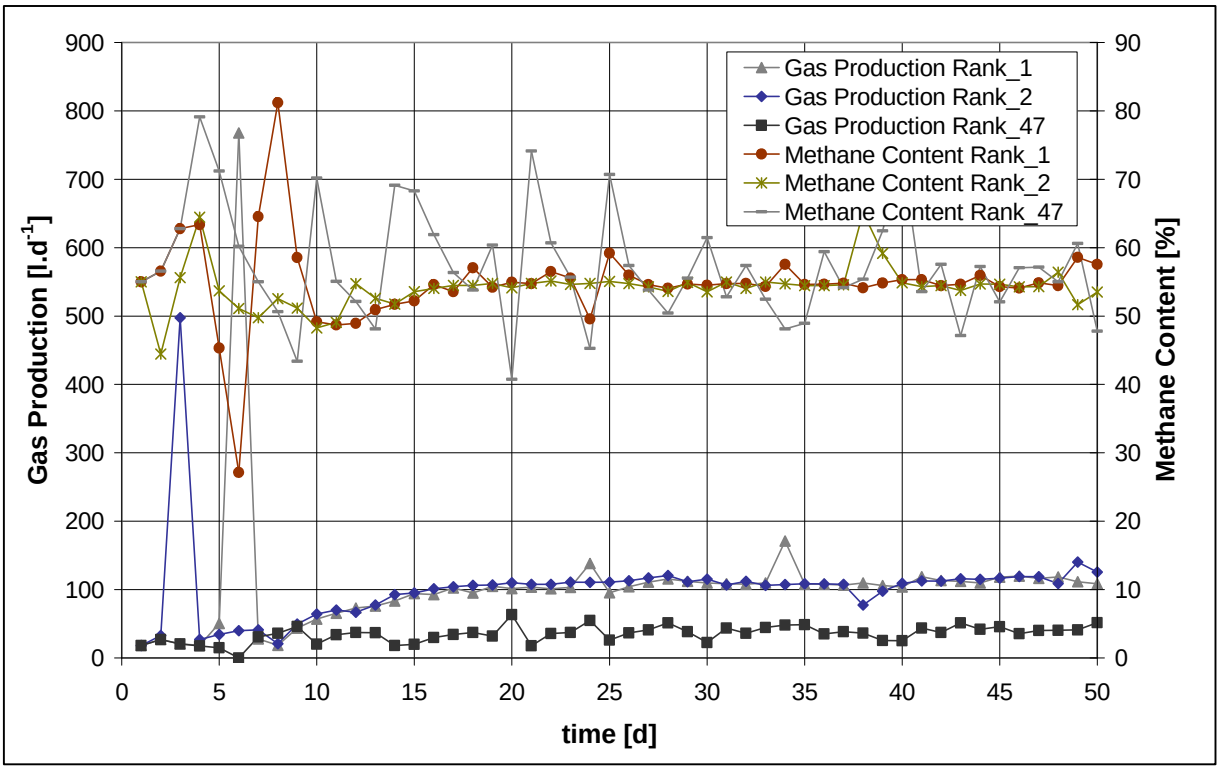

Figure 4.5.23: Result of the comparison of different combinations of membership functions F_DSS_191006c

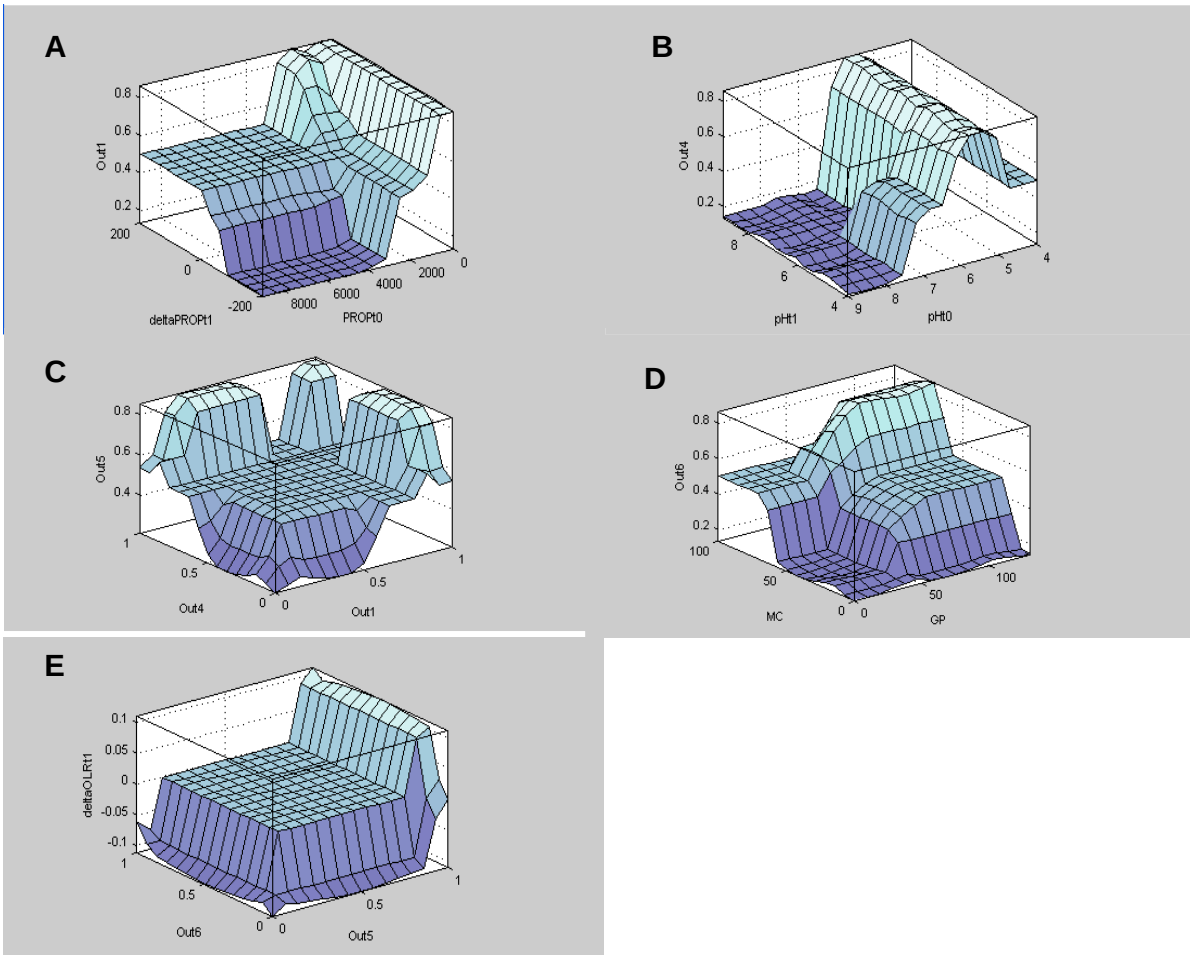

Figure 4.5.24: Structure of the best combination of the membership functions of F_DSS_101006; A) Membership function for the propionic acid concentration, B) membership function for the VFA concentration, D) membership functions for gas production and methane content and E) membership function for OLR

Comparison of Fuzzy Tool 1 to 8

After finding the best combinations of membership functions for the tools developed, all eight tools were compared to each other to find the best structure for the fuzzy based DSS (Table 4.5.1). This ranking of the different fuzzy tools' structures is done in the same way as the search for the best combination of the membership of the single fuzzy tools.

Hereby the tools using the propionic acid concentration, the VFA concentration or pH, the gas production and the methane content or only the propionic acid concentration, the gas production and the methane content (F_DSS_051006b, F_DSS_101008 and F_DSS_191906c) were found to be the tools with the best structures. They were followed by the simple tool using only the propionic acid and the methane content as input variables (F_DSS_181006b). The tool using the VFA concentration, the gas production and the methane content (F_DSS_090806) was found to be at rank 5. The worst tools are the two other simple tools using either the VFA concentration (F_DSS_191006b) or the pH (F_DSS_191006) and the methane content and the tool (F_DSS_051006) using the same input variables as F_DSS_101006 (propionic acid,

VFA concentration, gas production and methane content), however using another combination than in F_DSS_101006.

Table 4.5.1: Sensitivity coefficients of the simplified Model ADMML

#	Fuzzy Tool Name	Points
2	F_DSS_051006b	2.510E-16
4	F_DSS_101006	2.486E-16
8	F_DSS_191006c	2.468E-16
5	F_DSS_181006b	2.460E-16
3	F_DSS_090806	2.440E-16
6	F_DSS_191006	2.438E-16
1	F_DSS_051006	2.397E-16
7	F_DSS_191006b	2.338E-16

4.5.2 Testing of the Fuzzy Tool in the laboratory reactors

In order to ensure functionality and usefulness the DSS has to be evaluated. An evaluation for this kind of DSS is difficult, due to the fact that it depends on a lot of criteria and parameters. The evaluation of two tools (F_DSS_191006b and F_DSS_191006c) was done by testing the functionality and efficiency in the laboratory reactors.

Firstly the F_DSS_191006b was tested in the mesophilic reactor for 22 days (Figure 4.5.25 and Figure 4.5.26). Figure 4.5.25 shows the gas production and the methane content of the biogas produced. Moreover the organic loading rate calculated and the current organic loading rate are shown. The organic loading rate was hereby increased up to 5.91 $kg.m^{-3}.d^{-1}$. Figure 4.5.26 shows the VFA concentration and the pH.

This test, however, was only partly successful – the gas production and methane content increase slightly in the beginning, however after two weeks the VFA concentration starts to increase and the gas production and methane content decreases. The tool decreased the OLR, however the increase of the VFA was not reversed. After 22 days the test was stopped. This test showed that F_DSS_191006b has some weaknesses, which is consistent with the comparison done before of the fuzzy tools developed – where this tool was determined to be the worst of all eight tools.

The second fuzzy tool that was tested was F_DSS_191006c. This control tool was tested in the thermophilic reactor system for 16 days. F_DSS_191006c showed better results than the previously tested tool, which is again in accordance with the ranking of the tools performed earlier.

In this case the methane content is decreased significantly ($P = 0.001$) in the beginning, but stabilised around 50 % Methane during the test period. Yet, the gas production rate increased significantly ($P = 0.025$) in the same period (Figure 4.5.27) and the volatile fatty acid concentration decreased (Figure 4.5.28). The OLR was rather low at the beginning, as the VFA concentration was about 4000 $mg.l^{-1}$, but the OLR was increased to 2.5 $kg.m^{-3}.d^{-1}$ and the VFA concentration decreased to 778.5 $mg.l^{-1}$ during the test.

These results indicate that the tool was able to stabilise the process and increase the methane production slightly from 0.46 $m^3_{Biogas}m^{-3}_{Reactor}{}^{-1}$ to 0.68 $m^3_{Biogas}m^{-3}_{Reactor}{}^{-1}$.

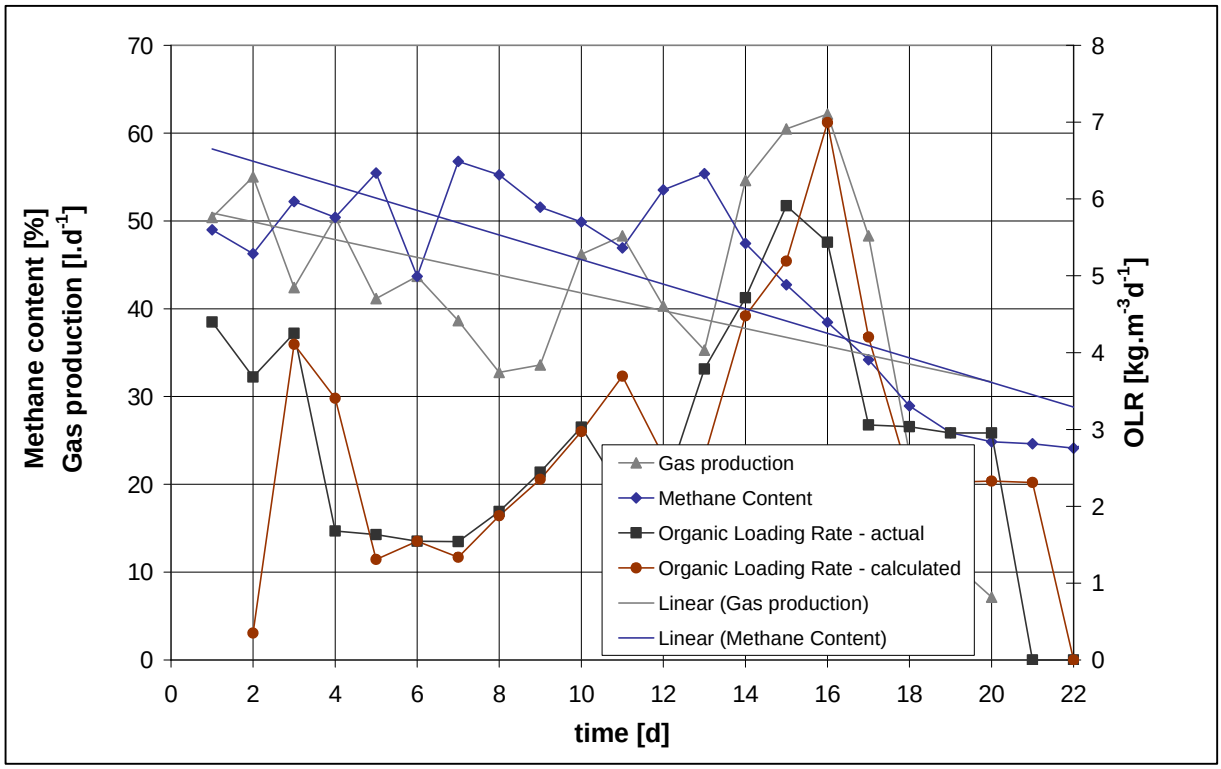

Figure 4.5.25: Test of the Fuzzy control tool (F_DSS_192006b) in the mesophilic reactor system – showing the gas production, methane content and the actual and calculated OLR

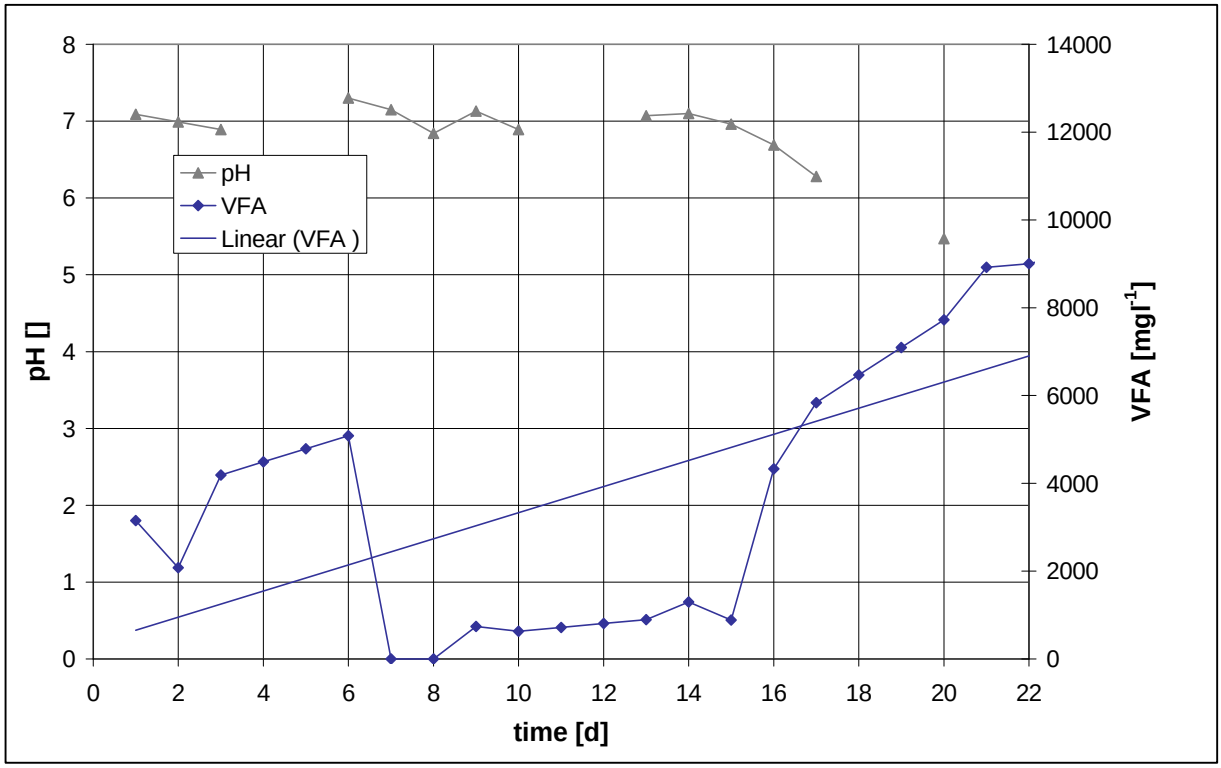

Figure 4.5.26: Test of the Fuzzy control tool (F_DSS_192006b) in the mesophilic reactor system – showing the VFA concentration and pH

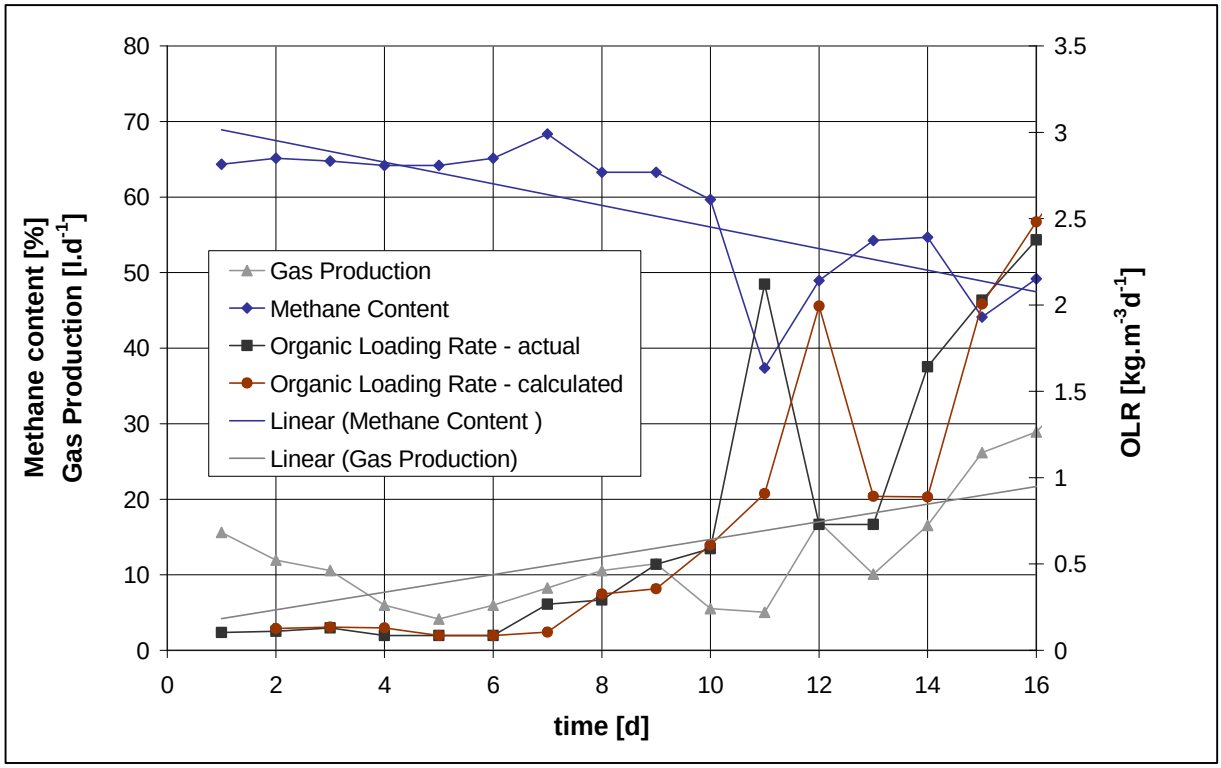

Figure 4.5.27: Test of the Fuzzy control tool (F_DSS_192006c) in the thermophilic reactor system – showing the gas production, methane content and the actual and calculated organic loading rates.

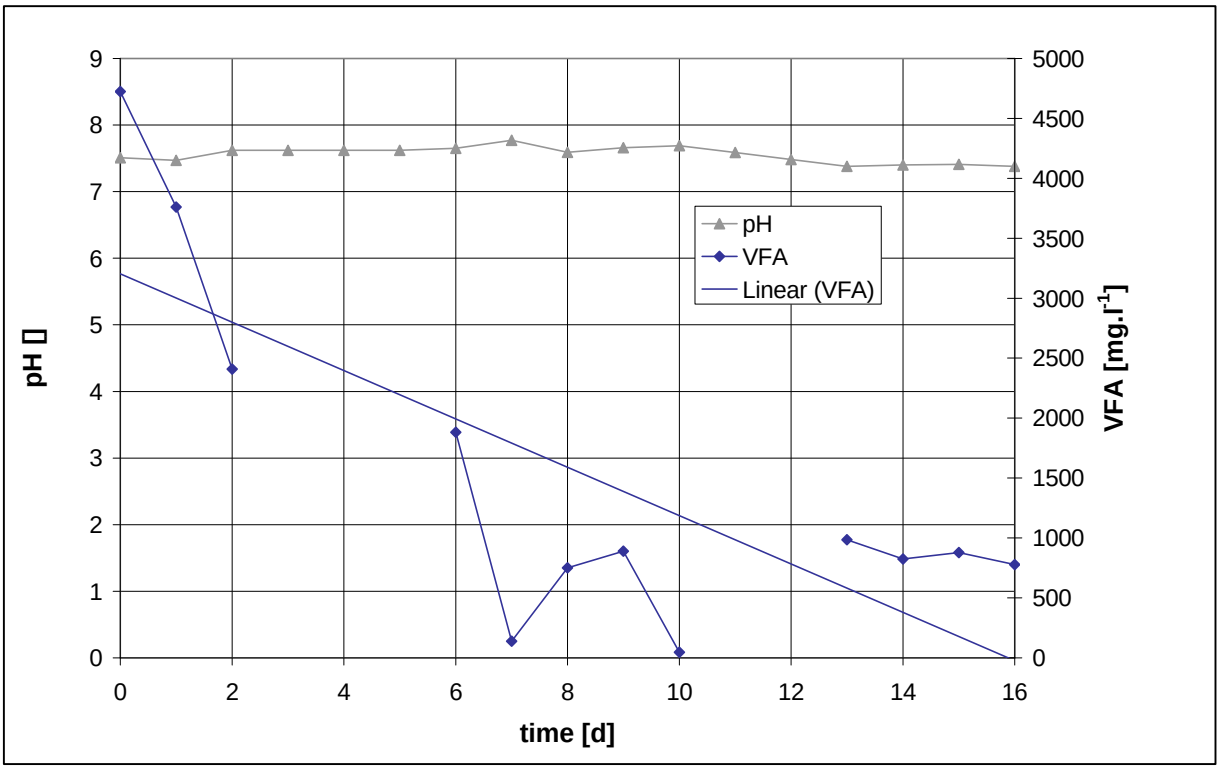

Figure 4.5.28: Test of the Fuzzy control tool (F_DSS_192006c) in the thermophilic reactor system – showing the VFA concentration and pH.

It was not possible in the frame of this study to test more of the fuzzy control tools. Therefore more tests are necessary.

Further evaluation of the final chosen fuzzy controller has to include a test in a technical scale reactor. Apart from that, one should evaluate, using a questionnaire, whether it is user-friendly, whether the tutorials and training units are adequate, also if the users like the appearance of the DSS. Furthermore whether the outcome of the DSS is understandable and can be directly implemented within the plant. Moreover one should evaluate whether the DSS influences the structure of the organisation, the people's positions, or the information flow. Also, how heavy side effects are, such as cost factors and training.

4.5.3 Fuzzy Controller

To provide a user friendly HMI (human machine interface) the fuzzy based DSS was written in the graphic programming system LABVIEW®, with an implementation of the Fuzzy algorithm as MATLAB® script and the Fuzzy Logic Toolbox, compiled in MATLAB® executable (Figure 4.5.29 and Figure 4.5.30).

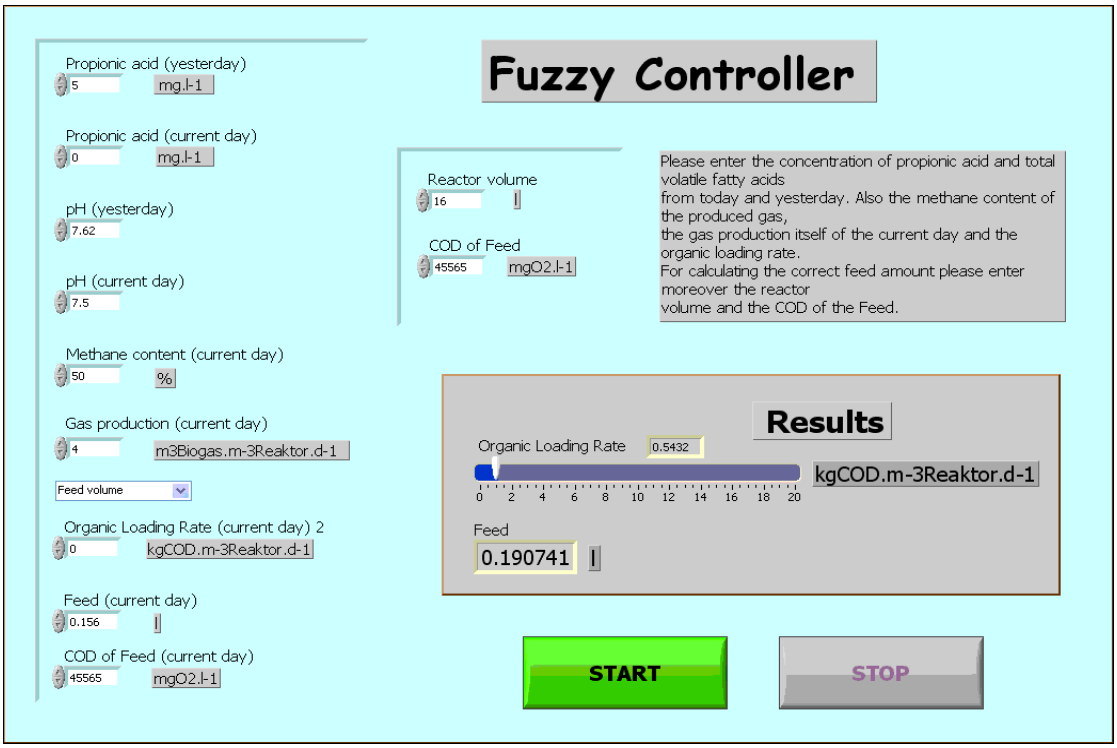

Figure 4.5.29: Screenshot of the Fuzzy Logic based Decision Support System

The implementation of such a system in technical plants is difficult, mostly to the scepticism of the plant operators, which is understandable due to a certain risk. To achieve a broad acceptance the DSS should be cheap, easy to use, which means that the user interface should be clearly arranged and self-explanatory. Also it should appeal to the users. A corresponding tutorial should be comprehensive and provide a lot of examples.

Yet, an implementation of such a control tool will be very complicated for existing biogas plants; therefore a possible implementation and the type of control tool should already be

in mind during the planning phase of any biogas plant, as improved measurement equipment and the feasibility for sampling are necessary.

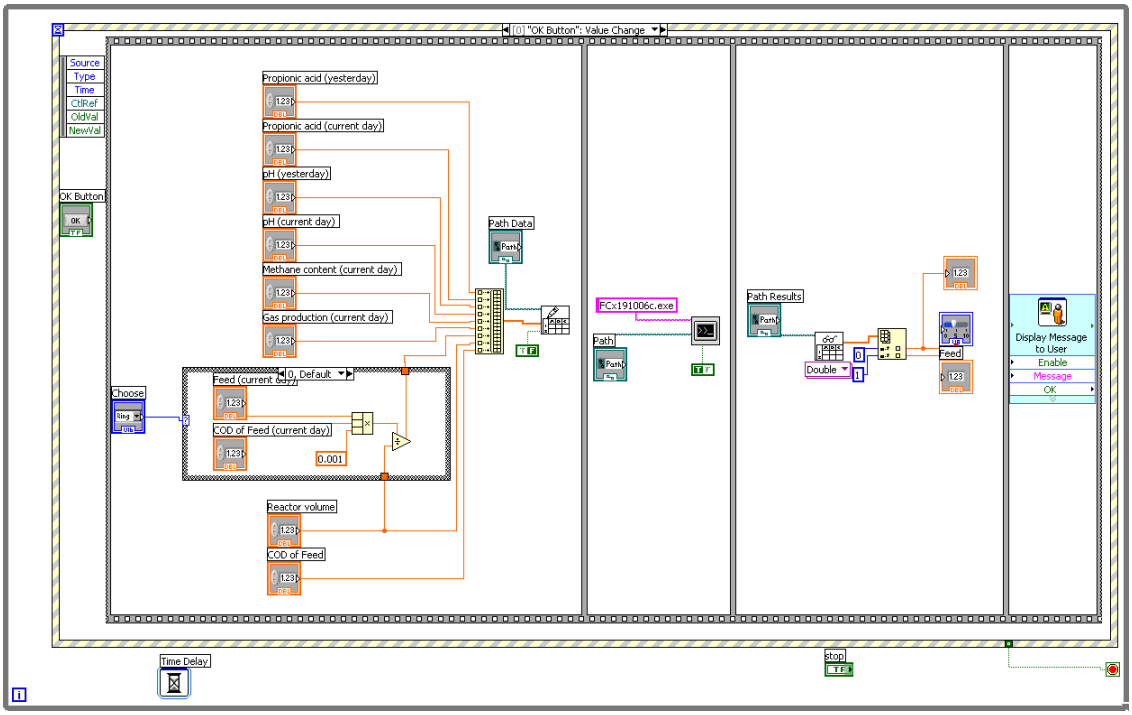

Figure 4.5.30: Labview Structure of the Fuzzy Logic based Decision Support System

5 Conclusion

The objective of this study is the optimisation of the biogas process using enhanced process control. This advanced process control is achieved developing a Decision Support System (DSS). To obtain more knowledge of the process and to support the development of the DSS the AD process was modelled using a modified version of the Anaerobic Digestion Model No.1 (Batstone *et al*, 2002).

In order to obtain data for model validation and calibration and also to glean kinetic data, four completely stirred tank reactors (CSTR) were operated at 35°C (mesophilic) (FM1, FM3) and at 60°C (thermophilic) (FM2, FM4). Three different substrates were used in single crops experiments: Maize silage (only corn, "*Maize*"), whole crop corn silage ("*GPS*") and sunflower press residues ("*Sunflower*").
The influence of the hydraulic retention time was tested, since it the HRT is one of the most important factors for the control of the process (De la Rubia *et al*. 2006). Additionally the influence of digesting different substrates (*Maize*, *GPS* and *Sunflower*) and the influence of different temperature ranges (mesophilic and thermophilic) were examined.
The optimal HRT is found to lie around 30 d. The gas and methane production is highest at lower HRTs, however at an HRT < 15 d a wash out of the biomass is observed, moreover the COD removal is lower and fluctuates more at lower HRTs.
Comparing *GPS* with *Maize* in the thermophilic reactor system, it can be found that *GPS* is more efficient than Maize as substrate. As the gas production and methane production are higher using *GPS* and considering additionally the harvest per hectare it is much more economical to use the whole plant. Yet some disadvantages have to be considered, as well. Higher VFA concentrations, sulphate and H_2S concentrations are found for the *GPS*, which can easily lead to reactor failure if the process is not controlled properly.
In addition *Maize* was compared with *Sunflower* in the mesophilic reactor system. *Maize* was found here to have advantages over *Sunflower* as substrate – for the gas production no difference was found, however the methane production is higher when using *Maize*. Moreover the high ammonia nitrogen values and the high H_2S concentrations can lead to fatal reactor failure if the reactor is not adapted to the *Sunflower* substrate. However if sunflower press residues are digested it is advisable to mix them with substrates of low protein content, for example potatoes or Sudan grass.
Comparing these three substrates, *GPS* is the one which should be preferred. It has also the advantage of being a crop grown in Central Europe since long time.
Furthermore the optimum temperature range was researched. No general optimum was found. In the thermophilic temperature range the gas and methane production is also significantly higher than in the mesophilic temperature range, however the VFA concentration is significantly higher in the thermophilic range. Yet the choice of temperature depends on several criteria: one important criteria is the bio-climatic

conditions (Bouallagui *et al*. 2004) – which has a great influence on the energy balance of the reactor. The substrate used has a great impact on the optimum temperature, as well, especially the sulphate and ammonia content of the substrate.
One can therefore conclude that in Central Europe the mesophilic temperature range is in advantage over the thermophilic temperature range, due to the bio-climatic conditions. Yet, due to improved insulation and a change in substrate (more energy crops), self-heating of reactors (Lindorfer, 2007) occurs, which leads to larger number of reactors operating in the lower limit of the thermophilic temperature range.

To get now more insight into the AD process, the process was modelled using the ADM1 (Batstone *et al*., 2002). As mentioned previously, before a model can be used for any application it has to be calibrated, in the sense that the parameter values are chosen in such a way that best possible conformity between the model predictions and the data measured can be obtained (Madsen 2000).
Before the model can be calibrated several questions have to be answered and problems have to be solved: Which variables have to be measured and how can the variables required be measured? How can the data measured be further processed, due to measurement errors, missing data or experimental problems. Which parameter should be calibrated up front?
In order to determine experimentally all state variables required for the model (input, initial) a lot of measurements are necessary. It is practically impossible in an industrial plant to measure all parameters and variables required, even in laboratory experiments the determination of all variables is a difficult task. However the widespread utilization of the ADM1 stands or falls with an easy application of the model (Kleerebezem and Van Loosdrecht 2004).
Therefore a compilation of different measurement methods was done, comprising most of the variables needed.
Yet, further research should focus on new measurement methods, as there is generally a lack on suitable online measurements for biogas plants. This makes the application of any AD model in a industrial plant a difficult task.
To compensate for measurements missing the initial conditions for the ADM1 were estimated using a “mass_balance”. This estimation is especially applied in the Virtual Laboratory 1.2. The basis of this “mass_balance” is the ADM1 model itself, where the calculation of the initial conditions occurs iteratively. The estimation of the methane production was done according to Baserga (1998)[22] using the protein, fat and carbohydrate content in the feed and the reactor sludge.
Some differences can be found, in particular the propionic acid and acetic acid concentrations are overestimated. This makes it clear that some further developments are still contingently necessary, which were not possible within the framework of this study. Especially as some simplifications are made in the mass balance: In this way the

[22] Baserga, U., 1998, Landwirtschaftliche Co-Vergärungsanlagen, FAT-Berichte Nr. 12

pH (pH = 7) is taken as constant, because the pH calculation in the program was too unstable. The same is true for the calculation of some bacteria concentrations (X_{aa}, X_{pro} and X_{H2}). A further difficulty is the nitrogen balance.
Yet, the results of the mass balance for nearly all state variables were in the same range as the state variables measured or otherwise determined for the initial conditions and therefore within an acceptable range. These results indicate that the mass balance is definitely suitable for a first estimation of the initial conditions.
However, this “mass_balance” is suitable alternative, but real measurements should be preferred. Thus one can conclude that without an improvement in measurement methods for example volatile , no widespread application of the ADM1 or other AD models will be possible and modelling of the AD process will always stick to laboratory scale reactors.

Due to the complexity of the ADM1 and in order to glean more information for the model calibration and to make a pre-selection of parameters a sensitivity analysis was done to find the most sensitive parameter. Which makes a sensitivity analysis to an important task, which should be carried out during calibration process, especially to avoid unnecessary laboratory experiments.
For the original ADM1 (Batstone *et al*, 2002) the parameters with the most influence according to the sensitivity analysis carried out are the hydrolysis rate of the proteins ($k_{hyd,pr}$), the hydrolysis rate of the carbohydrates ($k_{hyd,ch}$) and the hydrolysis rate of the lipids ($k_{hyd,li}$). Also influential are the disintegration rate (k_{dis}) and the substrate uptake rate of amino acids ($k_{m,aa}$).
This leads to the conclusion that especially the particulates and their compositions and concentrations have great influence on the whole model and should be therefore carefully estimated during the calibration process.

There are now different possibilities for finding the optimum parameter: determining the parameter in laboratory experiments, using literature data or automatic calibration algorithms. The application of automatic calibration algorithms is especially interesting for simplified AD models that are identifiable. Automatic control algorithms are applicable for the ADM1 if the model is divided in smaller sub-models, which are again identifiable.
In this study now three different automatic control algorithms were adapted for use with AD models:

- a Genetic algorithm,
- Simulated Annealing and
- a Luus-Jaakola algorithm.

Unfortunately it was only possible to test these control algorithms cursorily within the framework of the project. For practical application further tests and possibly some improvements are still necessary (e.g. adaptation of optimisation parameters).
The development of more than one optimisation algorithm was necessary as in a lot of practical applications many local minima exist, this also applies for the ADM1. The global optimum, is therefore difficult to determine, a cross-check with different optimisation procedures is important (Bojkov and Luus 1992).

But this cross-checking of different algorithms could be a possibility to use the calibration algorithms to the determine the parameter of the ADM1, without parting the model and ignoring its identifiable. Especially when this cross-checking is used with random start values for the first parameter guess and extended validation of the model.

By far the majority of the existing anaerobic digestion models are developed or implemented for the use of wastewater or sludge as substrate. AD models especially for the use of energy crops as substrate were not found in literature. The original ADM1 does not specify all mechanisms of anaerobic digestion, either.
Energy crops, such as rye, triticale, sunflower…, have a high content of cellulose and hemicellulose. In order to compensate for the slower degradation of this material a second hydrolysis rate for slow degradable carbohydrates was added.
Yet no real difference can be found between the original and the adapted model. This indicates that the original model was not structurally wrong for this application – except for the problem in predicting VFA values, which is true for both models. However, the adapted model is a better illustration of the reality.
Generally it was found that the model predicts the data measured accurately enough to predict the rough development of the data and the correct range of the data, however not precisely enough to predict the “correct” value and thus the model is not suitable for a Decision Support System (DSS).
Moreover for practical application in industrial plants the large amount of data needed in the ADM1 is a core issue – as only a small number of values are normally measured in industrial plants. Sure it is useful to calibrate the model using a laboratory reactor and it is possible to estimate most of the values, for example using the “mass_balance”. Yet, the more values are estimated the more imprecise is the prediction, as changes in the input and the initial conditions have a great impact on the model output. Which leads again to before mentioned need of sophisticated, cheap and easy handling measurement technologies and methods for biogas plants. Surely better results could be achieved if more state variable could have been measured during the study.
A further core issue is also that the adaptation to high ammonium and ammonia values can not be modelled and that apparently the partly high VFA concentrations cannot be modelled. Thus further improvements in model would be necessary, however this was not possible in the framework of this study.

As an alternative to a model based DSS, a Fuzzy Logic based DSS was developed. Eight tools were developed, differing in their structure. These tools were tested with the ADM1 (Batstone *et al*. 2002) and compared with a composite programming based ranking method, not only to find the best structure, but also to find a possibility for supporting experts through the development of a fuzzy tool.
To ensure functionality and usefulness the DSS has to be evaluated. An evaluation for this kind of DSS is difficult, due to the fact that it depends on a lot of criteria and parameters. The evaluation of two tools (F_DSS_191006b and F_DSS_191006c) was carried out by showing their functionality and efficiency in the laboratory reactors.

First the F_DSS_191006b was tested in the mesophilic reactor for 22 days. This test showed that F_DSS_191006b has some weaknesses, which is consistent with the comparison done previously of the fuzzy tools developed – where this tool was determined to be the worst of all eight tools.
F_DSS_191006c was tested in the thermophilic reactor system for 16 days. In this case the methane content decreased significantly (P = 0.001) in the beginning, but stabilised around 50 % Methane during the test period. Yet, the gas production rate increased significantly (P = 0.025) over the same period and the volatile fatty acid concentration decreased. F_DSS_191006c showed better results than the tool tested previously, which is again in accordance with the previous ranking of the tools.
It was not possible in the frame of this study to test more of the fuzzy control tools. Thus more test are necessary, before the best tool can be identified.
Yet, these results lead to the conclusion that the method used - combining the ADM1 with a composite programming based ranking method – is suited to supporting the development of a proper Fuzzy Logic based DSS.

Work pursued should concentrate on the testing of the automatic control algorithm and the use of this algorithm to calibrate the ADM1 and thus improve the performance of the model further. Furthermore on improved measurement methods, for example VFAs, H_2,..
Moreover to show the advantages of using a DSS further evaluation of the final chosen fuzzy controller has to include a test in a technical scale reactor.

6 Summary

The biological conversion of crops and agro-wastes in the absence oxygen (anaerobic digestion) can be used to produce a sustainable fuel. Converting the formed biogas via gas engines yields electrical energy and heat. However technical biogas plants often have to face severe problems: Low methane values in the biogas (approximately 50%) and low overall biogas production, instable process, reactor overload and process failure, low efficiency and high operational costs, self-heating in the plants and odour problems, which results often in a low economical efficiency. To make the biogas process more economically attractive, the process has to be optimised.

Thus main goal of the so-called CROPGEN project (Renewable energy from crops and agrowastes, from March 2004 to June 2007) is the integration of energy crops as an economically attractive energy source into the existing energy infrastructure. This integration as sustainable fuel source will be achieved by the optimisation of the biogas process. This optimisation is achieved by new reactor designs, determination methane yields, the optimisation of storage and pre-treatment and the identification of process using a Decision Support Systems (DSS).

This study was accomplished within the framework of the CROPGEN project and encompasses the modelling of the AD process using energy crops as substrate and the development of a DSS for optimisation. The main task to prove was that the AD model used can predict the digestion of energy crops accurately enough.

The majority of the AD model were developed for AD processes using wastewater, sewage sludge or manure as substrate, but hardly any model developments or applications for AD processes using energy crops as substrate exist in literature.

Thus one objective of this study is the extension and adaptation of an existing AD model for the use of energy crops as substrate, here the ADM1 (Batstone *et al.* 2002) was used as a basis for further development.

A further goal was the development of a “Virtual Laboratory” (VL) for data processing and the simulation of the AD process. The adjusted model is designed to be implemented in this VL. The VL should primary help to provide a better understanding of the AD process.

Moreover an existing Decision Support System (DSS) based on Fuzzy Logic was further developed to assist in operational control for optimisation. The DSS is used to identify process-control strategies in order to yield an optimised biogas production and a high methane content.

6.1 Anaerobic digestion of energy crops

To obtain data for model validation and calibration and also to obtain kinetic data, four completely stirred tank reactors (CSTR), as described before, later noted as FM1 to FM4 were operated at 35°C (mesophilic) (FM1, FM3) and at 60°C (thermophilic) (FM2, FM4). Whereby three different substrates were used in single crops experiments: Maize silage

(only corn, "*Maize*"), whole crop corn silage ("*GPS*") and sunflower press residues ("*Sunflower*").
The main focus was to reach an optimal methane production in a stable reactor. Thus it was necessary to push the envelope of stable conditions.
For a better understanding of the reactor behaviour and to find optimal conditions, the influence of the HRT, the substrates and temperature of the anaerobic digestion of energy crops in CSTRs were examined.

6.1.1 Influence of the hydraulic retention time (HRT) and OLR

The hydraulic retention time is one of the most important factors for the control of the process (De la Rubia *et al.* 2006).
For lower HRTs (< 20 days) the data had a high fluctuation range, which could be a result of the partly wash-out of the biomass at HRT lower < 15 days and/or inhibitory effects.
For the gas and methane production per volume of reactor in the thermophilic reactor digesting *GPS* the gas production declines by -0.06 $m^3_{Biogas}.m^{-3}_{Reactor}.d^{-1}$ per 10 d HRT respectively by -0.04 $m^3_{Methane}.m^{-3}_{Reactor}.d^{-1}$ per 10 d HRT. Using *Maize* as substrate in the same temperature range the gas production sinks by -0.03 $m^3_{Methane}.m^{-3}_{Reactor}.d^{-1}$ per 10 d HRT and methane production per volume of reactor by -0.02 $m^3_{Methane}.m^{-3}_{Reactor}.d^{-1}$ per 10 d HRT. In the mesophilic temperature using the same substrate the gas production demounts by -0.02 $m^3_{Biogas}.m^{-3}_{Reactor}.d^{-1}$ per 10 d HRT and the methane production falls by –0.01 $m^3_{Methane}.m^{-3}_{Reactor}.d^{-1}$ per 10 d HRT. In the mesophilic reactor system using *Sunflower* as substrate the methane production lessens by -0.13 $m^3_{Methane}.m^{-3}_{Reactor}.d^{-1}$ per 10 d HRT.
The pH significantly increases with higher HRTs. So the pH increases by 0.045 per 10 d HRT using *Maize* as substrate in the thermophilic temperature range. In the mesophilic reactor using *Maize* the pH only rises by 0.036 per 10 d. The highest slope was found in the reactor using *GPS* as substrate (thermophilic range), here the pH rises by 0.055 per 10 d HRT. If *Sunflower* is used as substrate in at 35 ± 1 °C no significant change in the pH is found, which seems to be a result of the high buffer capacity in this system due to the high TAN concentration.
It is interesting that with higher HRTs the acetic acid, the propionic acid and the VFA increase significantly, as well. In the thermophilic system the acetic acid rises by 0.036 $g.l^{-1}$ per 10 d HRT (*Maize* as substrate), the acetic acid increases a bit slower (0.033 $g.l^{-1}$/10 d) in the mesophilic reactor system using *Maize* as substrate. Digesting *GPS* the acetic acid is increasing by 0.042 $g.l^{-1}$ per 10 d HRT. Using *Sunflower* as substrate no significant influence of the HRT on the acetic acid, propionic acid and VFA concentration is found. The propionic acid increases faster is in the mesophilic system (0.079 $g.l^{-1}$/10 d HRT) than in the thermophilic (0.063 $g.l^{-1}$/10 d HRT) reactor using *Maize*. However the average and maximum propionic acid concentration is higher in the thermophilic reactor system. For the systems using *GPS* and *Sunflower* no significant

influence of the HRTs on the propionic acid is detected. The VFA concentration rises by 0.084 $g.l^{-1}$ (thermophilic) and 0.056 $g.l^{-1}$ (mesophilic) using *Maize* as substrate and by 0.184 $g.l^{-1}$ using *GPS* as substrate.
For the mesophilic reactor using *Sunflower* the COD removal rate also increases with the HRTs, yet this correlation is not significant. An interesting effect can be observed, as the influence of the HRT on the COD removal efficiency can be separated in two parts – for a HRT < 40 d the efficiency is lower and fluctuates a lot and for a HRT of 40 to 100 d HRT the COD is higher and fluctuates less.

Summarizing the above discussed knowledge about influence of the HRT in anaerobic systems it can be concluded that the optimal HRT lies around 30 d. The gas and methane production is highest at lower HRTs, however a at HRT < 15 d a wash out of the biomass is observed, moreover is the COD removal lower and fluctuates more at lower HRTs.

6.1.2 Comparison of different substrates

Three different substrates, as mentioned before, were compared in single crops experiments: Maize silage (only corn, "*Maize*"), whole crop corn silage ("*GPS*") and sunflower press residues ("*Sunflower*").

Comparison between *Maize* and *GPS* in the thermophilic reactor system

In the thermophilic reactor FM2 *Maize* and *GPS* were compared. The pH, the biogas production, the methane production, the acetate concentration, the total volatile fatty acid concentration (VFA), the degraded COD, the volatile suspended solids, the total nitrogen concentration (TAN), sulphate concentration and the H_2S concentration.
To be comparable all variables were based on the degraded COD and statistical outliers were removed, to get typical reactor values. "Wrong" data from reactor failure, due to broken tubes, leaking reactor, … was removed manually. The first 50 days were also removed, as this data was designated as the "start-up" of the reactor.
The pH is significantly higher using *GPS* as substrate. The gas production and methane production (per COD) is on average lower for *Maize* compared to using *GPS* as feed.
The acetic acid, the propionic acid and the VFA are lower for *Maize*, hereby the difference is significant for acetic and propionic acid.
The VSS and TOC concentration are significantly lower in respect to the TOC concentration using *Maize* as substrate, as well. This can be a result of on the one side undegraded material from former experiments remaining in the reactor or higher biomass formed, as no new inoculum was used to if a new substrate was tested.
TAN concentration is significantly higher if *Maize* is used as feed, whereby the sulphate concentration in the reactor and the H_2S concentration in the biogas are higher for the second case, where *GPS* is used as feed.

These results indicate that it is more efficient to use *GPS* as substrate and not *Maize*. First of all the gas production and methane production are higher and considering additionally the harvest per hectare is it much more economical to use the whole plant. However some disadvantages have to be considered, as well. Higher VFA concentrations are found for the *GPS*, which can easily lead to reactor failure if the process is not controlled properly. Efficient digestion with low volatile fatty acids leads to a higher methane content in the biogas (Farhan *et al.* 1997).
Yet, the TAN concentration is higher for the *Maize*, due to the higher protein content, which can lead on the one hand to dangerous NH_3 concentrations in the reactor and on the other hand stabilize the reactor pH.
The Sulphate and H_2S concentration is higher using *GPS* as feed. This can lead to toxic concentrations in the reactor and therefore to an inhibition of the process (toxic effects appear from 30 $mg.l^{-1}$ (Bischofsberger *et al.* 2005)). Increasing the process temperature can decrease inhibitory effects (Bischofsberger *et al.* 2005), because higher temperature means higher solubility of the H_2S in the sludge. Yet, a high H_2S content in the biogas is a problem for CHP or gas turbines. A removal of the H_2S from the gas is thus absolutely necessary.

Comparison between *Maize* and *Sunflower* in the mesophilic reactor system

In the mesophilic reactor FM3 *Maize* and *Sunflower* were compared. The procedure was the same as for the comparison of *Maize* and *GPS*.

Comparing now the different substrate, it can be observed that the gas production when digesting *Maize* respectively *Sunflower* show no significant difference, however the methane production is significantly higher when digesting *Maize*.
No significant difference could be found for the fatty acid concentration. The main different was the higher pH, TAN, NH3 and H_2S concentration, if *Sunflower* was used as feed, which is a result of the high protein content in the sunflower press residues.

Large increases in the ammonia nitrogen can cause inhibition of hydrogen and gas production (Sterling Jr. *et al.* 2001). The very high ammonia values for *Sunflower* (up to 4900 $mg_{NH4}l^{-1}$ and 260 $mg_{NH3}l^{-1}$) (NH_3 is inhibiting from 100 – 200 $g_N.l^{-1}$ (Henze and Harremoes 1983), however, is only in part a problem as the reactor got used to it. As adaptation can increase the ammonia tolerance of the micro-organisms (Sung and Liu 2003). On the other hand increases in already high TAN concentration can stabilise the pH in the reactor. Nevertheless attention has to be paid to this high ammonia and ammonium concentrations during reactor operation. A further problem is the high H_2S concentration when digesting *Sunflower*.
This comparison shows that *Maize* as substrate has an advantage over *Sunflower* – however no yields per hectare are considered in this comparison. Yet if sunflower press residues are digested it is advisable to mix with substrates of low protein content, for example potatoes or Sudan grass.

6.1.3 Mesophilic vs. thermophilic

To find the optimum temperature range, two mesophilic reactors (FM1 and FM3), operating at 35 °C (± 1 °C), were compared with the two thermophilic reactors (FM2 and FM4), operating at 60 °C (± 1 °C). The procedure was the same as before for the comparison of the different substrates.
The gas production and the methane production are significantly higher in the thermophilic reactors.
The pH showed no significant difference. Nevertheless, on average the pH in the mesophilic reactors was lower than in the thermophilic reactors.
The VFA concentration is, on average, in both cases nearly the same, but shows a small but nevertheless a significant difference. While, the acetic concentration is higher in the mesophilic reactor than in the thermophilic reactor on the other hand the propionic acid concentration in the thermophilic reactor is significantly higher than in the mesophilic reactor.
The VSS and TOC concentrations were higher in the mesophilic reactor compared to the reactors with higher temperatures. The H_2S concentration in the gas phase is significantly higher in the thermophilic reactor than in the mesophilic, which can be explained by the lower solubility of the H_2S in the sludge at higher temperature.
The COD removal is higher in the thermophilic reactor compared to the mesophilic reactor (Kiyohara *et al.* 2000).

These results again confirm the higher gas yield operating the plant in the thermophilic temperature range. Kim and co-workers (2006), for example, found the optimum temperature to be at 50 °C. Whereas Ahring (1994) detected 60 °C as the optimum temperature to digest manure, but recommended that the reactor be operated at under 60 °C to ensure that fluctuation in temperature have no disastrous effect on the microbial activity. Varel *et al.* (1980) suggested that there is no advantage in increasing the fermenting temperature of waste from 50 to 60 °C.
Generally it can be said, AD in the thermophilic temperature range is more efficient in terms of COD removal and methane production than operation in the mesophilic temperature range (Gavala *et al.* 2003). Moreover the thermophilic process has the benefit that, to a great extent, it destroys pathogens (El-Mashad *et al.* 2004). A further advantage is the less viscous sludge in the thermophilic reactor compared to the mesophilic reactor, therefore less energy is needed for stirring.
However, more problematic is the higher propionic acid concentration, which supports the argument of the instable process in the thermophilic temperature range. Nevertheless with adequate reactor control this is not a real problem. El-Mashard and co-workers (2004) also concluded from their results that a stable digester operation under thermophilic conditions is very possible.

The main disadvantage of reactor operation in the thermophilic range is the high energy consumption required to heat up the reactor. But if self heating occurs there is no need to

heat the reactor. On the other hand the reactor has to be cooled down to keep the process within the mesophilic temperature range. But taking the results observed as well as the literature it can be concluded that it make no sense to waste energy in order to keep the process within the mesophilic temperature range. Yet, one should ensure that there are no abrupt changes in the temperature, as variations in the temperature can dramatically affect the performance of the AD process (Leitao *et al.* 2006).

The choice of temperature depends on several criteria: one criteria is the bio-climatic conditions (Bouallagui *et al.* 2004) – which have an important influence on the energy balance of the reactor. The substrate used also has a great impact on the optimum temperature, especially its sulphate and ammonia content.

6.2 Calibration of an AD model

Before a model can be used for any application it has to be calibrated, in the sense that the parameter values are chosen in such a way that best possible conformity between the model predictions and the data measured data can be obtained (Madsen 2000).
Model calibration can be done in different ways either manual (trial and error), following a calibration protocol or using automatic optimisation and calibration tools (Madsen 2000).
Most of the models which describe such complicate dynamic processes show a high degree of uncertainty, as the knowledge of the process is incomplete and the kinetic parameters are not normally known (Kremling and Saez-Rodriguez 2007).
This study endeavoured to address comprehensively all issues of the calibration problem, however the calibration of such a model entails a lot of different questions and problems, which makes it impossible to deal with all issues in detail within the framework of this study.

6.2.1 Suggested Measurements for the calibration of the ADM1

In order to determine experimentally all state variables required for the model (input, initial) a lot of measurements are necessary. But to determine the waste (resp. crop) composition from standard measurements as required for the ADM1 is not an easy task (Kleerebezem and Van Loosdrecht 2004). The same is true for the reactor conditions. In particular the intermediate products and the bacteria concentrations are difficultly to estimate.
Table 6.2.1 show the measurement methods used in this study to estimate the input variables and initial conditions and give suggestions for alternative methods respectively further analytical methods.

Table 6.2.1: Parameter measured for the estimation of the "Input"/"Initial Condition" in the model

Measured variable	ADM1 state variable	Measurement method
Acetic Acid, Propionic Acid, VFA	S_{pro}, S_{ac}, S_{va}, S_{bu}, S_{va-}, S_{bu-}, S_{pro-} and S_{ac-}	HPLC, FTIR, GC, test kit
COD, $COD_{soluble}$	ADM1 variables are based on COD	standard method (DEV S9), test kit
gas production	$S_{CH4,gas}$, $S_{CO2,gas}$, $S_{H2,gas}$, S_{CH4}, S_{CO2}, S_{H2},	flow meter, displacement method
gas components (CH_4, CO_2, H2, (H_2S))	($S_{H2S,gas}$ and S_{H2S})	infrared sensors, dräger tubes, GC
VSS, SS	X_{xc}, X_I, X_{su}, X_{aa}, X_{fa}, X_{c4}, X_{pro}, X_{ac} and X_{H2}	standard method (DEV H1, DIN 38414-10)
Glucose	S_{su}	enzymatic tests, HPLC, titration, polarimetry
Amino Acids	S_{aa}	Ninhydrin method, HPLC
Lipids	X_{li}	solvent extraction
Carbohydrates	X_{CH}	HPLC, Anthrone method
Protein	X_{pr}	Kjeldahl, Lowry, Bradford
NH_4^+, NH_3	S_{IN}, S_{NH3}	single measurement rod
Total Nitrogen		Kjeldahl, test kit
Total Alkalinity, Partial Alkalinity	S_{IC}	standard method (DIN EN ISO 9963-1)
Total Carbon, Total Organic Carbon		TC Analyser
pH	S_{H+}	pH electrode, standard method (DIN 19265, DIN 19268)
Sulphate	S_{S2-}, S_{SO42-}, S_{HS-}	Standard method (ISO/DIS 10304), test kit
Sulphide		Standard method (DIN 38405-26)

6.2.2 Mass balance of the AD process

A mass balance was used to estimate the initial conditions for the ADM1.
The basis of this mass balance is the ADM1 model itself, where the calculation of the initial conditions happens iteratively. For these calculations a MATLB®-Script was written ("mass_balance").
The estimation of the methane production was done according to Baserga (1998)[23] using the protein, fat and carbohydrate content in the feed and the reactor sludge.
Complex particulates (X_c), carbohydrates (X_{ch}), proteins (X_{pr}), lipids (X_{li}), particulate inerts (X_I), soluble inerts (S_I), soluble sugar (S_{su}), soluble amino acids (S_{aa}), soluble LCFA (S_{fa}), valeric acid (S_{va}), butyric acid (S_{bu}), propionic acid (S_{pro}), acetic acid (S_{ac}), hydrogen (S_{H2}), methane (S_{CH4}), sugar degraders (X_{su}), amino acid degraders (X_{aa}), LCFA using bacteria (X_{fa}), valeric acid and butyric acid using bacteria (X_{c4}), propionic acid degrading bacteria (X_{pro}), acetate degraders (X_{ac}) and hydrogen using bacteria (X_{H2}) are estimated using a stoechiometric mass balance and parts of the ADM1.

Some differences can be found; especially the propionic acid and acetic acid concentrations are overestimated. This makes it clear that some further developments which were not possible within the framework of this study are still contingently necessary. Especially as some simplifications are made in the "mass_balance": thus the pH (pH = 7) is taken as constant, because the pH calculation in the program was too unstable. The same is true for the calculation of some bacteria concentrations (X_{aa}, X_{pro} and X_{H2}). A further difficulty is the nitrogen balance.
Yet, the results of the "mass_balance" for nearly all state variable lay in the same range as the state variables for the initial conditions measured or otherwise determined and therefore within a acceptable range. These results indicate that the "mass_balance" is definitely suitable for a first estimation of the initial conditions.

6.2.3 Sensitivity analysis

In order to obtain more information for the model calibration a sensitivity analysis was done to find the most sensitive parameter.
Similarly to DePauw (2005), a pre-selection of parameters which have to be calibrated was carried out in order to reduce the calculation effort: it will be not necessary to calibrate stoechiometric parameters (as they are well known), influent composition parameters, and temperature correction factors (De Pauw 2005).

[23] Baserga, U., 1998, Landwirtschaftliche Co-Vergärungsanlagen, FAT-Berichte Nr. 12

Table 6.2.2: Results of the sensitivity analysis of the ADM1 (Parameter ranking according to the calculated Sensitivity coefficients). Furthermore: comparison with the literature data: Most sensitive parameter of the ADM1 found by Wett *et al.*, 2007 and Batstone *et al.*, 2002 (shown as circles (without ranking)).

#	Parameter	Senstivity Coefficient	Wett et al. 2007	Batstone et al., 2002	#	Parameter	Senstivity Coefficient	Wett et al. 2007	Batstone et al., 2002
1	$k_{hyd,pr}$	1.12E-02		o	20	$K_{S,aa}$	1.27E-10		
2	$k_{hyd,ch}$	1.12E-02		o	21	$K_{S,pro}$	6.30E-11		
3	$k_{hyd,li}$	1.11E-02			22	$K_{S,H2}$	4.92E-11		
4	k_{dis}	9.82E-03	o	o	23	$k_{m,fa}$	4.75E-11		
5	$k_{m,aa}$	1.54E-03			24	$k_{m,H2}$	3.61E-11		
6	$k_{dec,pro}$	1.29E-03			25	$K_{S,su}$	3.12E-11	o	
7	$k_{dec,su}$	1.11E-03	o		26	$K_{S,fa}$	3.03E-11		
8	$k_{dec,ac}$	1.08E-03			27	$k_{m,c4}$	2.45E-11		
9	$k_{dec,c4}$	1.07E-03			28	Y_{fa}	2.18E-11		
10	$k_{dec,fa}$	9.45E-04			29	$K_{S,IN}$	1.66E-11		
11	$k_{dec,h2}$	9.23E-04			30	$K_{S,ac}$	1.36E-11		o
12	$k_{dec,aa}$	9.09E-04			31	Y_{c4}	1.32E-11		
13	$k_{m,su}$	1.97E-07			32	Y_{pro}	1.10E-11		
14	$K_{I,H2,pro}$	1.80E-08			33	$K_{S,c4}$	8.22E-12		
15	Y_{su}	9.52E-09			34	Y_{H2}	8.18E-12		
16	Y_{aa}	8.09E-09			35	Y_{ac}	6.66E-12		
17	$K_{I,H2,c4}$	7.70E-09			36	$K_{I,NH3,ac}$	6.21E-12		
18	$K_{I,H2,fa}$	1.70E-09			37	$k_{m,ac}$	3.21E-12		o
19	$k_{m,pro}$	9.49E-10							

The parameter with the most influence on the original ADM1 according to the sensitivity analysis carried out is the hydrolysis rate of the proteins ($k_{hyd,pr}$), also the hydrolysis rate of the carbohydrates ($k_{hyd,ch}$) and the hydrolysis rate of the lipids ($k_{hyd,li}$). Further the disintegration rate (k_{dis}) and the substrate uptake rate of amino acids ($k_{m,aa}$).

6.2.4 Parameter estimation

Parameters have to be chosen in such a way that the likelihood that measured data can be predicted by the model (Olsson and Newell 1999). Yet, most of the parameters suggested in the ADM1 are not fully suitable for energy crops. Therefore a literature search for appropriate parameters was done for the most important and sensitive parameter.
As there is generally a great lack of specific data on energy crops digestion in literature (Lindorfer 2007) and the values found in the literature have a great range of margin. The parameters were also experimentally estimated (by other project partners, too) for different crops, as well, particularly maize silage.

During the project hydrolysis rates for energy crops and other substrates were determined in batch experiments over a temperature range from 35 °C to 55 °C by project partners, such as hydrolysis rates for bracken, buckwheat, carrots, hay, Jerusalem artichoke, knotweed, lawn, lupine, maize silage, oil seed rape, sweet clover and more.

Table 6.2.3: Hydrolysis rates of different substrate determined during the CROPGEN project from project partners

Substrate	$k_{hyd(first\ order)}$ [d^{-1}]	Temperature [°C]	Reference
Braken	1.81E-01	35(+-2)	Publication in preparation. Results Environmental Technology dpt. Wageningen University. Pabon, van Lier et al. 2007
Carrot	1.00E+00	35(+-2)	Publication in preparation. Results Environmental Technology dpt. Wageningen University. Pabon, van Lier et al. 2007
Grass hay 2 since day 0	1.90E-02		Lehtomäki et al., 2005
Jerusalem artichoke 1 since day 0[b]	2.10E-02		Lehtomäki et al., 2005
Knotweed 1	5.60E-02		Lehtomäki et al., 2005
Lawn since day 49[a]	9.40E-02		Lehtomäki et al., 2005
Lupine 1 since day 25[a]	6.70E-02		Lehtomäki et al., 2005
Maize silage	2.70E-01	35(+-2)	Publication in preparation. Results Environmental Technology dpt. Wageningen University. Pabon, van Lier et al. 2007
Marrow kale 1since day 0	9.00E-03		Lehtomäki et al., 2005
Mix Market waste (fruit+vegetable)	2.10E-01	37	Bolzonella
Mixed Agro-wastes	2.80E-01		Bolzonella
Mixed market waste and sewage sludge	3.05E+00	55	UNIVE-DSA, Cavinato
Nettle 2 since day 0	3.00E-02		Lehtomäki et al., 2005
Oil seed rape	1.61E-01	35(+-2)	Publication in preparation. Results Environmental Technology dpt. Wageningen University. Pabon, van Lier et al. 2007
Red clover 1 since day 10	3.90E-02		Lehtomäki et al., 2005
Reed canary grass 1	3.90E-02		Lehtomäki et al., 2005
Rhubarb 1 since day 0	3.40E-02		Lehtomäki et al., 2005
Sewage Sludge + Market waste (1305.7 $g_{COD}d^{-1}$)	4.28E+00		Pavan
Spartina-Cordgrass	1.67E-01	35(+-2)	Publication in preparation. Results Environmental Technology dpt. Wageningen University. Pabon, van Lier et al. 2007
Straw of oats since day 17	4.30E-02		Lehtomäki et al., 2005
Tops of sugar beet since day 0	1.90E-02		Lehtomäki et al., 2005
Triticale	1.21E-01	35(+-2)	Publication in preparation. Results Environmental Technology dpt. Wageningen University. Pabon, van Lier et al. 2007
Vetch 2 since day 12	4.70E-02		Lehtomäki et al., 2005
Yellow lupin	3.18E-01	35(+-2)	Publication in preparation. Results Environmental Technology dpt. Wageningen University. Pabon, van Lier et al. 2007

6.2.5 Calibration algorithms

The application of an automatic calibration algorithm is especially interesting for simplified AD models that are identifiable. Automatic control algorithms are applicable for the ADM1 if the model is divided in smaller sub-models, which again are identifiable.
In this study now three different automatic control algorithms were adapted for the use with AD models:

- a Genetic algorithm,
- Simulated Annealing and
- a Luus-Jaakola algorithm.

All three algorithms were implemented in script-form in MATLAB®.

Genetic Algorithm (GA)

Genetic Algorithms (GA) are loosely based on the mechanics of natural selection and genetics (Laquerbe *et al.* 2001; Alcock and Burrage 2004; Mwembeshi *et al.* 2004; De Pauw 2005; Coleman and Block 2006) Convergence to the exact minima cannot be guaranteed, but it is a robust method for lot of objective functions to find at least a “near-optimum” (Alcock and Burrage 2004). In the GA a potential solution is called individual or chromosome – each is represented by a sequence of “genes” and ranked corresponding to their objective function value (= fitness) (Laquerbe *et al.* 2001; Alcock and Burrage 2004; Mwembeshi *et al.* 2004). A population is then a set of chromosomes with their fitness values (Alcock and Burrage 2004). The GA is iteratively improving the fitness either by reproduction, cross-over or mutation (Laquerbe *et al.* 2001; Alcock and Burrage 2004).

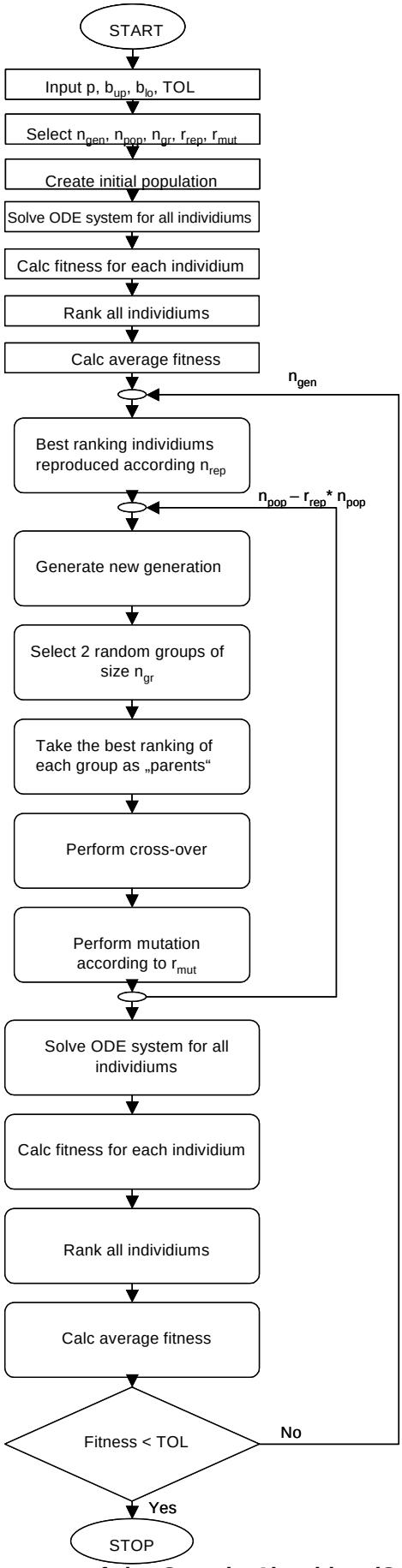

Figure 6.2.1: Adapted structure of the Genetic Algorithm (GA) optimisation algorithm for the use with AD models

Simulated Annealing (SA)

A further optimisation technique is the simulated annealing (SA) procedure. SA is a heuristic method (Schramm *et al.* 2004) and was first introduced by Kirkpatrick and co-workers in 1983[24] (Lee *et al.* 2008) and copies the annealing of solid, where the reorder of the crystals follows the laws of probability (Laquerbe *et al.* 2001; Kaczmarski and Antos 2006).

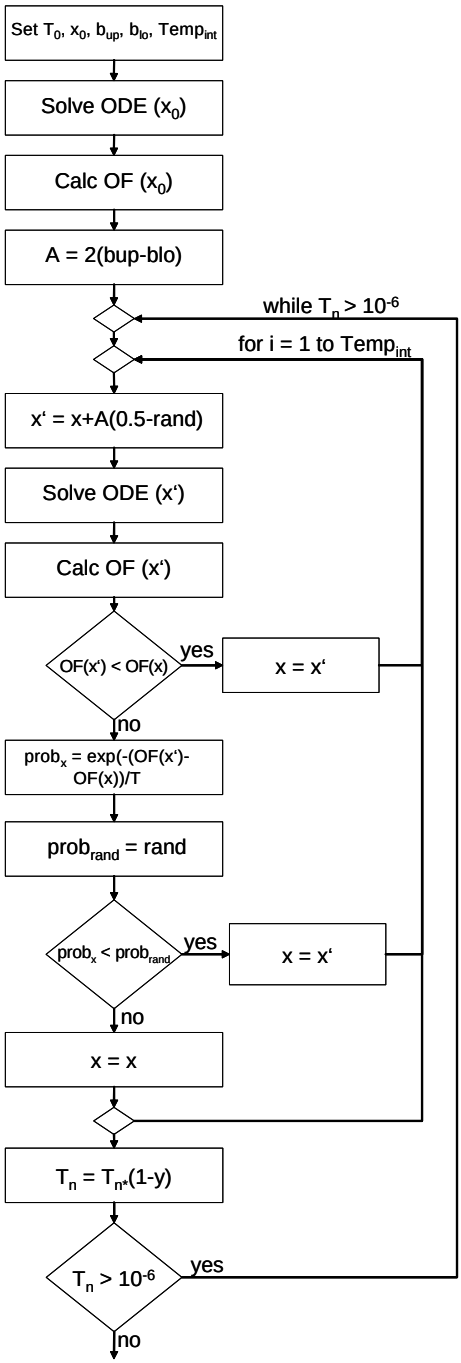

Figure 6.2.2: Adapted structure of the Simulated Annealing (SA) optimisation algorithm for the use with AD models

[24] Kirkpatrick, S, Gelatt, C.D. and Vecchi, M.P., 1983, Optimization by Simulated Annealing, Science, 220, 4598, 671-680

The aim is to reach the atomic configuration, which minimizes the internal energy (Laquerbe *et al.* 2001; Kaczmarski and Antos 2006).

Luus-Jaakola Algorithm (LJ)

The LJ method is an optimisation method using random search points and region reduction (Peng Lee *et al.* 1999) and was introduced by Luus and Jaakola (1973). With the LJ method even optimisation problems with multiple local minima could be solved (Linga *et al.* 2006).

Unfortunately it was only possible to test these control algorithms cursorily within the framework of the project. For practical application further test and possibility some improvements are still necessary (e.g. adaptation of optimisation parameters).

The development of more than one optimisation algorithm was necessary as in a lot of practical applications many local minima exists, this applies also for the ADM1. The global optimum, is therefore difficult to determine, a cross-checking with different optimisation procedures is important (Bojkov and Luus 1992).

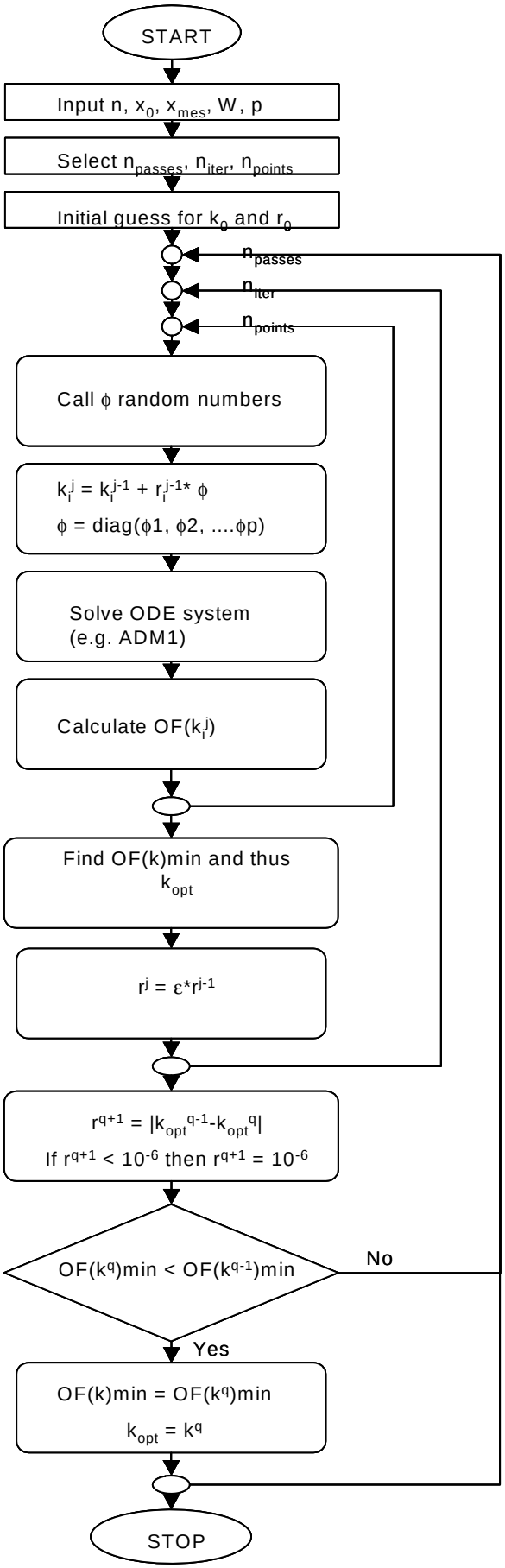

Figure 6.2.3: Adapted structure of the LJ optimization procedure according to Linga *et al.* (2006) for the use with AD models

6.3 AD models for energy crop digestion

By far the majority of the existing anaerobic digestion models are developed or implemented for the use with wastewater or sludge as substrate. AD models especially for the use of energy crops as substrate were not found in literature. Therefore the ADM1 was adapted to model the biogas process using crops as feed input. For the use in the Virtual Laboratory the sulphate reduction process was added, as well. Furthermore a simpler model, based on the ADM1 (Batstone *et al.* 2002) and the AD model by Marsili-Libelli and Beni (1996), was developed for the implementation in a model based decision support system.

6.3.1 Adaptation of the ADM1

The original ADM1 does not specify all mechanisms of anaerobic digestion, for instance solid precipitation, homoacteogenesis, glucose alternative products, sulphate reduction and sulphide inhibition, nitrate, weak acid and base inhibition, LCFA inhibition and acetate oxidation (Batstone *et al.* 2002), but encourages the extension and development of it (Strik 2004).

Addition of a second hydrolysis rate

Energy crops, such as rye, triticale, sunflower…, have a high content on cellulose and Hemicellulose. A second hydrolysis rate for slow degradable carbohydrates was added in order to compensate for the slower degradation of this material (Figure 6.3.1).

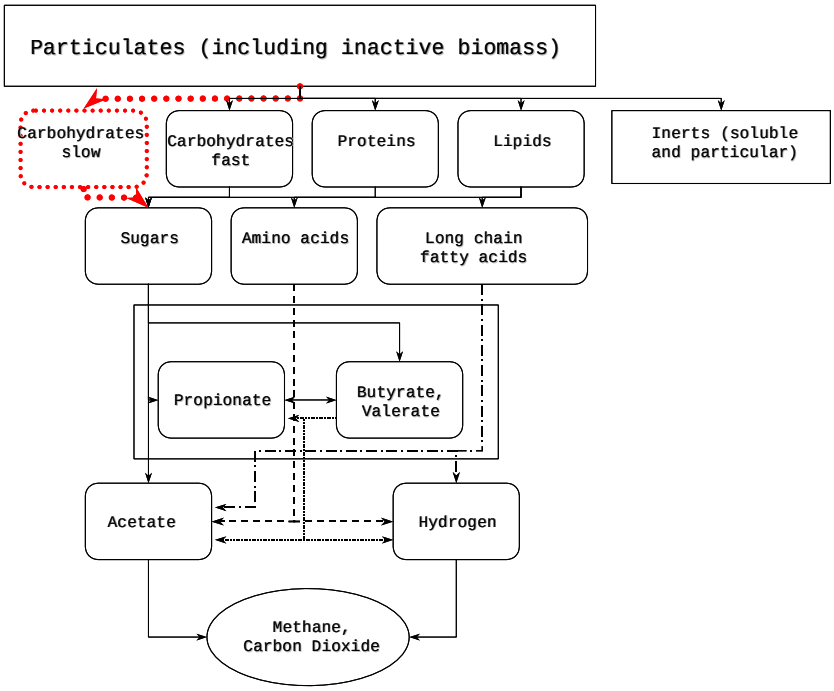

Figure 6.3.1: Schema of the biochemical processes of the adapted model. ..... Shows the changes compared to the original model

This small adaptation was encouraged by a comparison of the adapted model and the original model using the original parameters, suggested by the IWA Task group for mathematical modelling of anaerobic digestion (Batstone *et al.* 2002). It was be observed that using the adapted model better results can be achieved compared to the original model using different statistical parameters such as the determination coefficient (r^2), the index of agreement (d) and the Ratio of means (R_{Mean}). Looking at the methane production and COD reduction – for the original model a value for $r^2_{CH4,original}$ = 0.19 and $d_{CH4,original}$ = 0.60 can be found, though for the adapted model the determination coefficient $r^2_{CH4,adapted}$ = 0.40 and $d_{CH4,adapted}$ = 0.62. For the COD reduction $r^2_{COD,original}$ = 0.06 and $d_{COD,original}$ = 0.06 and $r^2_{COD,adapted}$ = 0.62 and $d_{COD,adapted}$ = 0.88. Whereby a correlation coefficient of 1 would describe an ideal model (Elias *et al.* 2006).

In this study it was established that a value > 0.6 indicates an accurate model, in accordance with Elias *et al.* (2006). Even though the determination coefficient is rather low in both cases, the index of agreement indicates a good model, as the values of d > 0.6. Values of d between 0.5 and 0.6 would be within an acceptable range. A value of < 0.3 (absolute) for the Ratio of means (R_{Mean}) indicates that the model predicts the observation with acceptable accuracy (Elias *et al.* 2006).

For the parameter calibration, literature data, data from our own experiments and from experiments performed by project partners were used (Table 6.3.1).

The adapted and calibrated model predicts most of the values accurately enough (Table 6.3.2). The COD reduction is predicted very well by the model (r^2 = 0.57, d = 0.85 and R_{Mean} = 0.03). The low determination coefficient are a result of the large amount of data, in similar studies a data range of 66 days on average (Angelidaki *et al.* 1993; Christ *et al.* 2000; Kalyuzhnyi *et al.* 2000; Seok and Komisar 2002; Siegrist *et al.* 2002; Aceves-Lara *et al.* 2005; Bernard *et al.* 2005; Flotats *et al.* 2006) is shown. If only part of the data is examined higher determination coefficients can be found, for example for the final period (day 501 – 555) an r^2 = 0.92 can be found for the COD reduction. Yet, as biogas plants are planned and constructed as long term processes, it seemed more reasonable to make also a long term simulation. The prediction of the gas and methane production as well as the pH was done accurately. The negative value of R_{Mean} of the acetate, propionate and VFA concentration, and the COD reduction signifies that the values measured are underestimated in the model (Elias *et al.* 2006). It seems that the model cannot handle the high VFA concentration which appeared in this study, which leads to an underprediction of these values – the addition of a new inhibition function could improve the performance of the model. Yet, the implementation of a new inhibition function was not possible within the framework of this study.

Table 6.3.1: Original and calibrated parameters in the adapted ADM1

Parameter		original values	adapted values
$f_{XI,Xc}$	[]	2.50E-01	3.00E-01
$f_{li,xc}$	[]	2.50E-01	2.00E-01
N_{xc}	[]	2.00E-03	2.69E-03
N_i	[$kmol_N kg_{COD}^{-1}$]	2.00E-03	4.29E-03
k_{dis}	[d^{-1}]	5.00E-01	4.10E-01
$k_{m,aa}$	[d^{-1}]	5.00E+01	3.00E+01
$k_{m,c4}$	[d^{-1}]	2.00E+01	2.20E+01
$k_{A,B,va}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
$k_{A,B,bu}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
$k_{A,B,pro}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
$k_{A,B,ac}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
$k_{A,B,co2}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
$k_{A,B,IN}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
C_{xc}	[$kmol_C kg_{COD}^{-1}$]	3.00E-02	2.79E-02
$k_{hyd,ch,s}$	[d^{-1}]	1.20E+00	8.50E-01

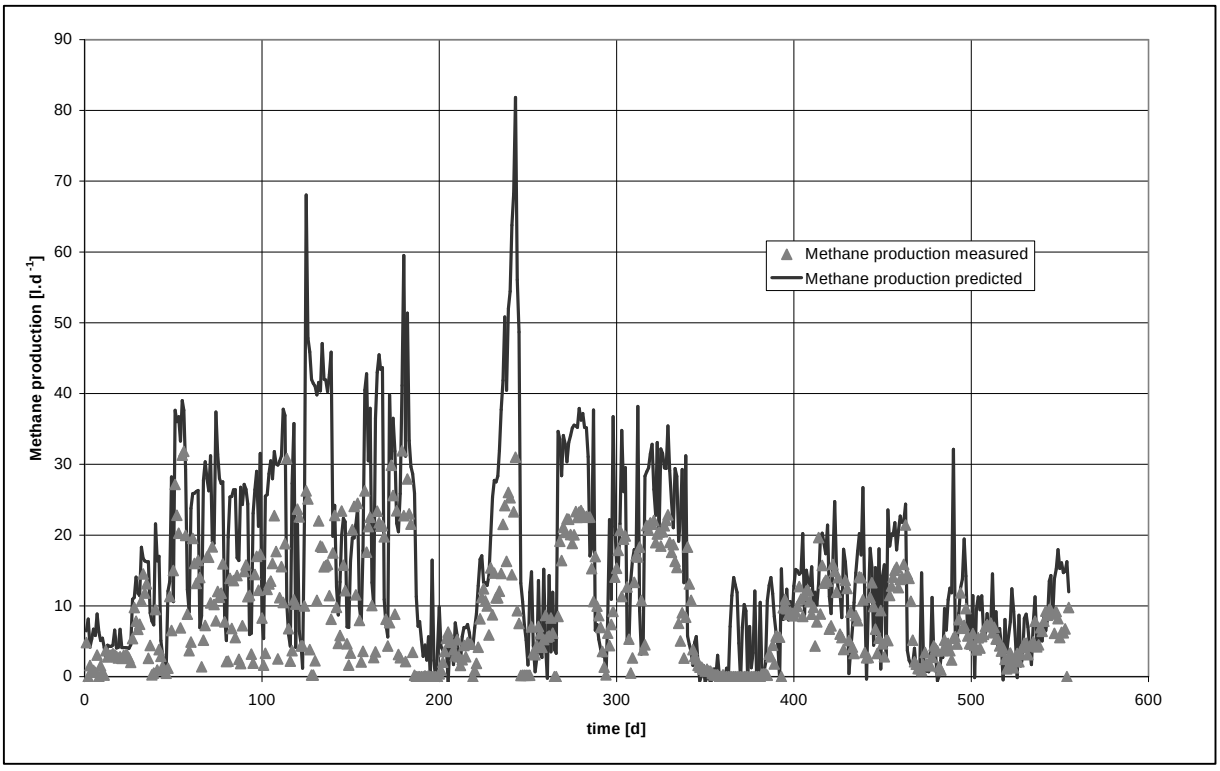

Figure 6.3.2: Methane production predicted using the calibrated adapted model

Table 6.3.2: Values of the statistical indicators r^2, d and R_{Mean} for the adapted model using the calibrated parameter (green = good, yellow = okay)

	r^2	d	R_{Mean}	
Gas production	**0.48**	**0.70**	0.73	okay
Methane production	**0.48**	**0.68**	0.84	okay
Acetate Concentration	0.00	0.36	-0.46	bad
Propionate Concentration	0.03	0.43	-0.99	bad
VFA	0.01	0.40	-0.77	bad
pH	**0.43**	**0.72**	**0.03**	good
COD Reduction	**0.57**	**0.85**	**0.03**	good

The results of the calibrated adapted model were compared with results from the calibrated original model (Table 6.3.3 and Table 6.3.4). In the original model sixteen parameters were adapted for this study. Whereby $N_{xc,}$ N_I, C_{xc}, and $k_{A,B}$ were adapted according to Rosen and Jeppson (2006), in the same way as for the adapted model. All other parameter were calibrated by trial and error. Whereby the hydrolysis rates had to be chosen as 1 d^{-1} instead of 10 d^{-1} (suggested by Batsone and co-workers (2002)), to reach reasonable results.

Comparing the adapted and the original calibration it was found that rather similar results are achieved using the calibrated original model.

The gas and methane production was predicted less adequately using the adapted model (Table 6.3.2 and Table 6.3.4). On the other hand the pH and the COD reduction were predicted slightly better using the adapted model compared to the original model. The VFA could not be predicted better in the original model than in the adapted model.

Table 6.3.3: Calibrated and original parameters in the original ADM1

Parameter		original values	adapted values
$f_{XI,Xc}$	[]	2.50E-01	3.00E-01
$f_{li,xc}$	[]	2.50E-01	2.00E-01
Nxc	[]	2.00E-03	2.69E-03
Ni	[$kmol_N kg_{COD}^{-1}$]	2.00E-03	4.29E-03
k_{dis}	[d^{-1}]	5.00E-01	3.00E-01
$k_{hyd,ch}$	[d^{-1}]	1.00E+01	1.00E+00
$k_{hyd,pr}$	[d^{-1}]	1.00E+01	1.00E+00
$k_{hyd,li}$	[d^{-1}]	1.00E+01	1.00E+00
$k_{m,ac}$	[d^{-1}]	6.00E+00	8.00E+00
$k_{A,B,va}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
$k_{A,B,bu}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
$k_{A,B,pro}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
$k_{A,B,ac}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
$k_{A,B,co2}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
$k_{A,B,IN}$	[$M^{-1}d^{-1}$]	1.00E+08	1.00E+10
C_{xc}	[$kmol_C kg_{COD}^{-1}$]	3.00E-02	2.79E-02

Table 6.3.4: Values of the statistical indicators: square of the correlation coefficient (r^2), index of agreement (d) and Ratio of means (RMean) for the original model using measured values to estimate the initial conditions (case 1) (green = good, yellow = okay)

	r^2	d	R_{Mean}	
Gas production	**0.51**	**0.74**	0.67	okay
Methane production	**0.51**	**0.70**	0.81	okay
Acetate Concentration	0.00	0.40	-0.76	bad
Propionate Concentration	0.03	0.43	-0.99	bad
VFA	0.01	0.42	-0.90	bad
pH	**0.42**	**0.70**	**0.03**	good
COD Reduction	**0.52**	**0.84**	**-0.01**	good

Addition of the Sulphate reduction process according to Federovich (2003) and according to Batstone (2006)

In particular when substrates with high protein content are used e.g. sunflower press residues high amounts of sulphate are found in the liquid and gas phase. These high levels of sulphate and sulphide results in different problems. First of all, sulphate reducing bacteria (SRB) compete with the methanogenic bacteria for the same substrate. The first inhibition effects have to be taken into account beginning at an H_2S concentration of 30 $mg.l^{-1}$. An H_2S content of more than 10 % in the biogas disrupted the acetate production (Bischofsberger *et al.* 2005). An elimination of the H_2S is not only important for successful operation of the AD process, but also to prevent problems of corrosion in the plant or minimize SO_2 emissions during the combustion of the biogas (Bischofsberger *et al.* 2005).

Thus the ADM1 augmented by the sulphate reduction process was added into the Virtual Laboratory 1.2 in two versions: the sulphate reduction process according to Federovich *et al.* (2003), already described in (Strik 2004), and the extension with the SR according to Batstone (2006).

6.4 Virtual Laboratory (VL)

One output of this study was the development a so-called “Virtual Laboratory” (VL), which was one of the goals of the CROPGEN project, for data processing and interpretation as well as mathematical model formulation of the biogas process. Two different VLs were developed. The basis of the VLs is the ADM1 (Batstone *et al.* 2002). The VL is conceived as a software training tool to provide users with more detailed insight into the AD process.

The VLs are written in the graphic programming system LABVIEW®, with an implementation of the ADM1 as MATLAB® script, compiled in MATLAB® executable. The MATLAB® code for the VLs was written, based on an existing MATLAB®-SIMULINK® file of the original ADM1 (Rosen and Jeppsson 2002). The LABVIEW® program serves as user interface.

6.5 Decision Support System based on Fuzzy Control

In order to make the biogas process economically attractive, the process has to be optimised. There are different ways to improve biogas production. One way is an advanced process control. Advanced process control can be realized using a decision support system (DSS). "*DSS are computer-based systems used to assist and aid decision makers in their decision making processes*" (Bogardi 2004)
For DSS based on complex mathematical models (e.g ADM1), a significant problem is the amount of data required, which is often a problem for technical biogas plants. This is due to the fact that there is a lack of online sensors available and existing sensors need extensive maintenance (Olsson and Newell 1999). Moreover operators are in the most cases not sufficiently educated to deal with the instrumentation and control adequately (Olsson and Newell 1999). One possibility is to use a simpler model as basis for the DSS, which was shown before. Another alternative is a Fuzzy based control tool.

6.5.1 Comparison of different Fuzzy Tools using the ADM1 and a composite programming based ranking method

A Fuzzy Logic Tool, which has been designed during another EU project (AMONCO project), was now enhanced to improve the control performance. This DSS is thought to identify process control strategies to yield a high methane content final product.
Seven different tools were developed. These tools and the original developed tool were tested with the ADM1 (Batstone *et al.* 2002) and compared with a developed composite programming based ranking method, not only to find the best structure, but also to find a possibility to supporting experts in the development of Fuzzy Logic tool.
The gas production, the methane content, the concentration of the acetic acid, the concentration of the propionic acid, the total concentration of volatile fatty acids, the COD reduction and the pH were chosen as appraisal factors.

Comparison of Fuzzy Tools 1 to 8

After finding the best combinations of membership functions for the tools developed, all eight tools were compared to each other to find the best structure for the fuzzy based DSS (Table 6.5.1). This ranking of the different fuzzy tools' structures is done in the same way as the search for the best combination of the membership of the single fuzzy tools.
Hereby the tools using the propionic acid concentration, the VFA concentration or pH, the gas production and the methane content or only the propionic acid concentration, the gas production and the methane content (F_DSS_051006b, F_DSS_101008 and F_DSS_191906c) were found to be the tools with the best structure. They were followed by the simple tool using only the propionic acid and the methane content as input variables (F_DSS_181006b). The tool using the VFA concentration, the gas production and the methane content (F_DSS_090806) was found to be on rank 5. The worst tools

are the two other simple tools using either the VFA concentration (F_DSS_191006b) or the pH (F_DSS_191006) and the methane content and the tool (F_DSS_051006) using the same input variables as F_DSS_101006 (propionic acid, VFA concentration, gas production and methane content), however using a other combination as in F_DSS_101006.

Table 6.5.1: Sensitivity coefficients of the simplified Model ADMML

#	Fuzzy Tool Name	Points
2	F_DSS_051006b	2.510E-16
4	F_DSS_101006	2.486E-16
8	F_DSS_191006c	2.468E-16
5	F_DSS_181006b	2.460E-16
3	F_DSS_090806	2.440E-16
6	F_DSS_191006	2.438E-16
1	F_DSS_051006	2.397E-16
7	F_DSS_191006b	2.338E-16

6.5.2 Testing of the Fuzzy Tool in the laboratory reactors

To prove the suitability of the tools, they were tested in laboratory experiments. The first Fuzzy tool tested (F_DSS_191006b) was only partly successful (data not shown). The second Fuzzy tool (F_DSS_191006c) was tested in the thermophilic reactor system.

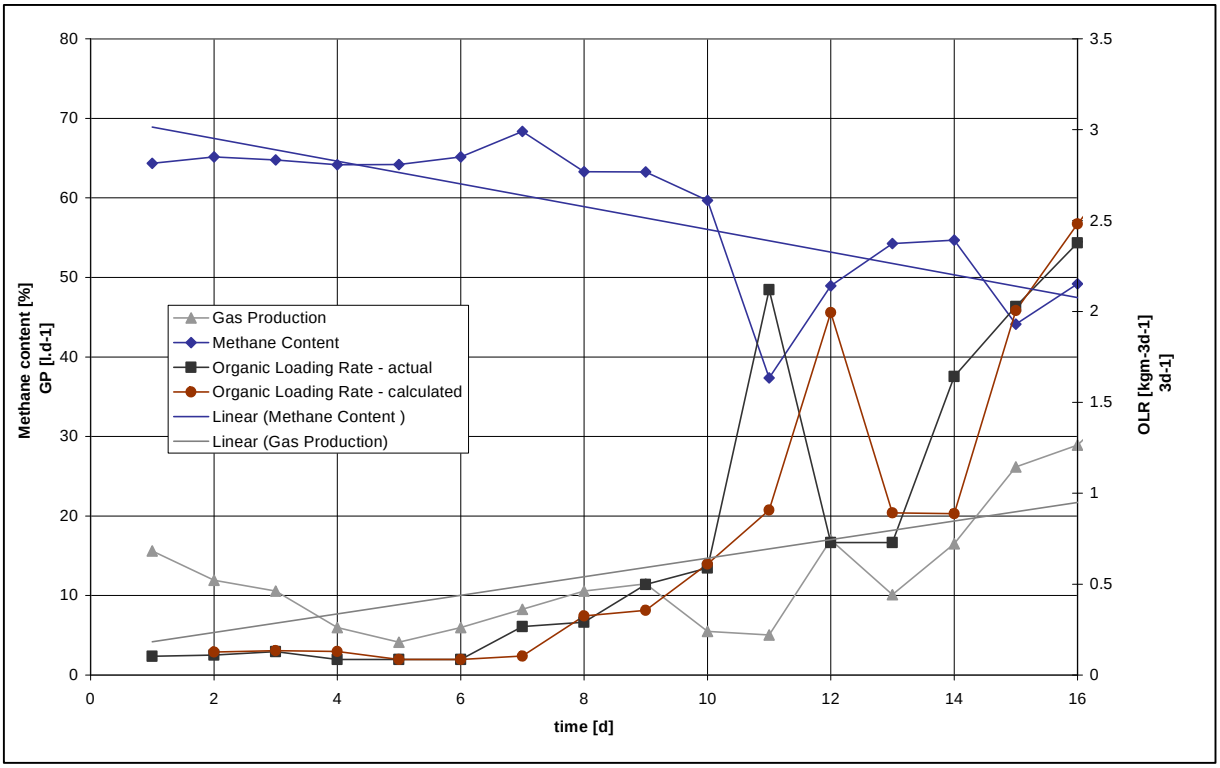

Figure 6.5.1: Test of the Fuzzy control tool (F_DSS_192006c) in the thermophilic reactor system – showing the gas production, methane content and the actual and calculated organic loading rate.

In this case the methane content is significantly decreased in the beginning, but stabilised at around 50 % Methane during the test period. Yet, the gas production rate

increased over the same period (Figure 6.5.1) and the volatile fatty acid concentration decreased. The OLR was rather low in the beginning, as the VFA concentration was about 4000 $mg.l^{-1}$, but the OLR was increased to 2.5 $kg.m^{-3}.d^{-1}$ and the VFA concentration decreased to 778.5 $mg.l^{-1}$ during the test. These results indicate that the tool was able to stabilise the process and increase the methane production slightly from 0.46 $m^3_{Biogas}m^{-3}_{Reactor}{}^{-1}$ to 0.68 $m^3_{Biogas}m^{-3}_{Reactor}{}^{-1}$.

6.5.3 Fuzzy Controller

To provide a user friendly HMI (human machine interface) the fuzzy based DSS was written in the graphic programming system LABVIEW®, with an implementation of the Fuzzy algorithm as MATLAB® script and the Fuzzy Logic Toolbox, compiled in MATLAB® executable.

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