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Dynamic Modeling of Anaerobic Degradation in Batch Operation for Gelatine, Sucrose, Rapeseed oil and their Mixture

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Abstract—**Anaerobic digestion of biomass represent a complex process with many unknown parameters and often not well defined experimental conditions. To overcome these problems a simple dynamic model was formulated that should allow to foresee the dynamics of anaerobic fermentation by adjusting three master substrates (proteins, carbohydrates and lipids). The model describes also the dynamics of intermediate products involved in digestion that represent volatile fatty acids and long chain fatty acids since both could take part in inhibition of the process. The model was calibrated and validated using four sets of anaerobic digestion experiments: three mono-fermentation trials with either gelatine, sucrose and rapeseed oil and a forth approach with a mixture of all of them. The identification of the parameters was based on the experimental data sets using the least squares method. The parameterized model accurately reproduced the anaerobic digestion of the mixture of the substrates for the volume of biogas and methane, the volumetric flow rate of biogas, the volumetric concentration of methane and the total chemical oxygen demand during 28 days.**

***Index Terms*—Anaerobic digestion, gelatine, rapeseed oil, sucrose**

I. INTRODUCTION

The world's fossil fuel reserves are getting depleted and, environmental and economic concerns can represent prominent reasons for the restricted use of natural sources in the near future. For European countries the development of substituting energy sources has become vitally important considering the dependency on energy imports on the one side and the growing energy demand on the other side [23]. In this context anaerobic biomass digestion can be considered as one of the most promising and feasible alternative among the existing renewable energy sources [1]. Anaerobic fermentation of biological wastes provides biogas which can be used similarly as conventional natural gas for heating and electricity generation [7]. The process of biogas production is quite complex implying the simultaneous performance of physical, chemical and biological reactions catalyzed by a consortium of various bacteria and additionally influenced by seasonal changes and daily feeding [8]. Economic benefit of the biogas production process depends mainly on substrate prices, and the digestion stability, which, in turn, are influenced by the chemical composition of the feedstock. In operating plants, the feedstock quantity and quality may vary from day to day. This affects bacterial growth, and, therefore, biogas composition and methane yield. Consequently, successful feedstock combinations require a method to foresee the process outcome when new input waste material is introduced. Mathematical modeling represents a quite attractive method for studying and improving the biogas process dynamics [28].

Modeling of anaerobic digestion (AD) has been widely developed since the early seventies. The first generation models were simple and classified as exclusive models, which described only the limiting step e.g. methanogenesis [15], [18], [27]. The second type model, named minimalist model, possesses a minimum number of process steps with a specific purpose [2]. Inclusive models describe all process steps and components found in the anaerobic digestion, e. g. Anaerobic Digestion Model no. 1 (ADM1) [3] with 19 reactions, eight bacterial groups, and detailed description of pH and temperature changes. ADM1 requires the simultaneous solution of mass balance equations for each individual substrate and type of bacteria. Such approach is rather complex containing many unknown parameters. A biogas production model developed by Bernard et al. [4] and improved by Blesgen [5] and Blesgen and Hass [6] was reformulated and simplified in order to reduce the amount of unknowns and assumptions. A general idea of the study to define experimental parameters was to use proteins (gelatine), carbohydrates (sucrose) and lipids (rapeseed oil) as basic master biomass to mimic the properties of any organic substrate as a linear combination of different biomass (e.g. domestic and industrial wastes, silage, leftovers, manure, agricultural residues and food industry waste). The model can be used as a tool for the measure of certain parameters describing the anaerobic digestion of certain master biomass and predicting the process dynamics of a mixture of them. From the model's prediction one can judge the process state and study the influence of the proportion of master substrates in organic waste and their quantity on the process. Such an approach can be used for the increase of the stability of biogas production and could potentially improve the operation of biogas plants.

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The aim of this study was to develop a relatively simple model which is supposed to represent accurately the following key process variables such as the volume of biogas and methane, the volumetric flow rate of biogas, the volumetric dynamics of methane concentration and the total chemical oxygen demand (COD). For the estimation of parameters it was decided to conduct the AD experiments with the most abundant representative compounds of proteins, carbohydrates and lipids: sucrose, gelatine and rapeseed oil, respectively. The experimental conditions referred to the German Standard Procedure VDI 4630 for standardized batch trials [13]. Furthermore, the individual investigation of the dynamics of the degradation of the master biomass and their arbitrary mixture were studied.

II. A DYNAMICAL MODEL OF ANAEROBIC DIGESTION

Numerical modeling is a tool for investigation of the static or dynamic processes without conducting or reducing the number of experiments. The qualitative and quantitative variations of the substrate input and also the process conditions changes (pH, T) are difficult to test experimentally due to the long-term running of the experiments. At that point mathematical models came into use. However, at present, one should accept that it is still not possible to adopt a general mathematical model applicable under all circumstances and completely representing the overall process of biogas production with all reactions and all parameters of the process. There are three basic requirements for the formulated model: cause-effect, relative simplicity, and predictive capability [9]. The proposed model represents a reformulated version of the biogas model of Bernard [4] and a version of Blesgen [5] and Blesgen and Hass [6]. It was translated from Fortran 95 to C++ language and Microsoft Visual Studio 8.0 was used to calculate numeric model solutions. The model has an improved structural performance with a minimized number of unknowns and assumptions. This means, the reduced model has some limitations: pH dynamics, temperature and accumulation of ammonia are not included. The comparative characteristics between the biogas model of Blesgen [5] and Blesgen and Hass [6] and the proposed model are presented in the Table 1.

Table 1 Comparative characteristics of the model of Blesgen [5] and Blesgen and Hass [6] and the model used in this study

Properties	The model of Blesgen	Proposed model
Structure	Four sub-model: biological, physicochemical reactor and plant	Single model
Phases of CH_4 release	Liquid and gaseous	Gaseous
Inhibition	Temperature, pH, VFA	LCFA, VFA
Total organic carbon	Fractionation into HCO_3^-, CO_3^{2-}, CO_2	No speciation
Products	CH_4, CO_2, biomass, heat	CH_4, CO_2, biomass
Parameters	Mass balance is not implied	Mass balance is implied

The model describes three steps of anaerobic digestion: hydrolysis, acidogenesis, and methanogenesis. Hydrolysis is generally declared as one of the limiting steps of anaerobic digestion. It is described by the first order reaction kinetics. The mathematical model has a three-step structure. In the first step (hydrolysis) the primary organic compounds (*Cp*, *Pp*, and *Lp*: *primary* carbohydrates, proteins and lipids, respectively) are hydrolyzed into simple accessible mono-/oligomers (C_S, P_S and L_S: carbohydrates, proteins and lipids), as substrates for the acidogenic bacterial group. Acid forming bacteria (*Xaci*) produce CO_2 (total inorganic carbon: *TIC*) and volatile fatty acids (*VFA*). Finally, methanogenic bacteria (*Xmeth*) convert VFA (*VFA*) into methane (*Me*) and total inorganic carbon or carbon dioxide (*TIC*). The basic structure is shown in Table 2. The model accounts for 10 biochemical reactions associated to two bacterial populations (acidogens and methanogens). The kinetics is described according to the Monod function. Bacterial growth rate is taken as proportional to substrate uptake.

Hydrolysis rate constants are determined by using the first order kinetic model (1):

$$r_{hyd}S = k_{hyd\,S} \cdot S_p \quad (1)$$

where *S* – *C* (carbohydrates), *P* (proteins), *L* (lipids).

There are 11 differential equations describing the AD: Dynamical change of acidogenic (2) and methanogenic (3) bacteria ($kg \cdot s^{-1}$):

$$\frac{dX_{aci}}{dt} = Y_{XC} \cdot \mu_C + Y_{XP} \cdot \mu_P + Y_{XL} \cdot \mu_L \quad (2)$$

$$\frac{dX_{meth}}{dt} = Y_{XVFA} \cdot \mu_{VFA} \quad (3)$$

Disintegration of *primary* substrates: *S* - carbohydrates (*C*) (4), proteins (*P*) (5) and lipids (*L*) (6) ($kg \cdot s^{-1}$):

$$\frac{dSp}{dt} = -r_{hyd}\,S \quad (4-6)$$

Hydrolysis of simple accessible mono-/oligomers: *S* - carbohydrates (C) (7), proteins (P) (8) and lipids(L) (9) ($kg \cdot s^{-1}$):

$$\frac{dSs}{dt} = r_{hyd}\,S \cdot r_{XS} \quad (7-9)$$

Dynamical change of an intermediate product - volatile fatty acids ($mmol \cdot s^{-1}$) (10):

Table 2 Biochemical rate coefficients and kinetic rate equations for carbohydrates, proteins and lipids

Rate	X_{aci}	X_{meth}	C_p	P_p	L_p	C_s	P_s	L_s	*VFA*	*TIC*	*Me*
r_{hydC} [a]			-1								
r_{hydP} [b]				-1							
r_{hydL} [c]					-1						
r_{XC} [d]	Y_{XC}					-1			$(1\text{-}Y_{XC}) \cdot U_C$	$(1\text{-}Y_{XC}) \cdot (1-U_C)$	
r_{XP} [e]	Y_{XP}						-1		$(1\text{-}Y_{XP}) \cdot U_P$	$(1\text{-}Y_{XP}) \cdot (1-U_P)$	
r_{XL} [f]	Y_{XL}							-1	$(1\text{-}Y_{XL}) \cdot U_L$	$(1\text{-}Y_{XL}) \cdot (1-U_L)$	
r_{VFA} [g]		Y_{XVFA}							-1	$(1\text{-}Y_{XVFA}) \cdot (1-v_{VFA})$	$(1-Y_{XVFA}) \cdot v_{VFA}$

$$\frac{d_{VFA}}{dt} = (1-Y_{XC}) \cdot U_C \cdot \mu_C + (1-Y_{XP}) \cdot U_P \cdot \mu_P + (1-Y_{XL}) \cdot U_L \cdot \mu_L - \mu_{VFA} \quad (10)$$

Change of biogas volume production is integrated from the biogas flow rate ($m^3 \cdot s^{-1}$):

$$\frac{dVBG}{dt} = \dot{q}_{outGa} \quad (11)$$

There are 5 algebraic equation calculating the inorganic carbon rate (mmol $\cdot s^{-1}$) (12), the molar release of CO_2 (13) and CH_4 (14) (mol $\cdot s^{-1}$), volumetric concentrations of CO_2 (15) and CH_4 (Vol.-%) (16), and biogas flow rate (17) ($m^3 \cdot s^{-1}$):

$$rTIC = (1-Y_{XC}) \cdot (1-U_C) \cdot \mu_C + (1-Y_{XP}) \cdot (1-U_P) \cdot \mu_P + (1-Y_{XL}) \cdot (1-U_L) \cdot \mu_L + (1-Y_{XVFA}) \cdot (1-v_{VFA}) \cdot \mu_{VFA} \quad (12)$$

$$\dot{n}_{CO2} = \frac{rTIC}{MWCO2} \cdot V_{liq} \quad (13)$$

$$\dot{n}_{CH4} = \frac{(1-Y_{XVFA}) \cdot v_{VFA} \cdot r_{VFA}}{MWCO2} \cdot V_{liq} \quad (14)$$

$$y_{CO2} = \frac{\dot{n}_{CO2}}{\dot{n}_{CO2} + \dot{n}_{CH4}} \cdot 100\% \quad (15)$$

$$y_{CH4} = 1.0 - y_{CO2} \quad (16)$$

$$\dot{q}_{outGa} = (\dot{n}_{CO2} + \dot{n}_{CH4}) \cdot R \cdot \frac{TGas}{P_{outGa}} \quad (17)$$

For acidogens (18-20) and methanogens (21) Monod-type kinetics for growth is considered and the inhibition by long chain fatty acids is introduced:

$$\mu_P = \mu_P^{max} \cdot X_{aci} \cdot \frac{P_s}{P_s + K_{P_s}} \quad (18)$$

$$\mu_C = \mu_C^{max} \cdot X_{aci} \cdot \frac{C_s}{C_s + K_{C_s}} \quad (19)$$

$$\mu_L = \mu_L^{max} \cdot X_{aci} \cdot \frac{Ip_L}{Ip_L + L} \cdot \frac{L_s}{L_s + K_{L_s}} \quad (20)$$

$$\mu_{VFA} = \mu_{VFA}^{max} * X_{meth} * \frac{Ip_L}{Ip_L + L_s} * \frac{VFA}{VFA + K_{VFA}} \quad (21)$$

where μ_P^{max}, μ_C^{max}, μ_L^{max} and μ_{VFA}^{max} are the maximum bacterial growth rates on proteins (P) carbohydrates (C), proteins (P) and lipids (L), K_{P_s}, K_{C_s}, K_{L_s} and K_{VFA} are the half-saturation constants associated with the substrate, *IpL* is the inhibition coefficient.

III. Estimation of Parameters

To find the best agreement between simulated and experimental data, an appropriate criterion must be selected for the optimal solution of the model parameter identification. The values for the kinetic coefficients of the first-order rate of hydrolysis were based on previous studies (Table 3).

Estimation and model calibration of the parameters was performed on the basis of least squares procedure by measuring the deviation between the model and real system outputs. The solution of the system can be presented as (22):

$$\Psi(\theta) = \sum_{i=1}^{N} \left(\frac{y_{exp}(\theta) - y_{sim(\theta)}}{\sigma_t}\right)^2 \quad (22)$$

where $\Psi(\theta)$ is the objective function, y_{exp} are the collected measurements, y_{sim} are the model - predicted outputs, θ represents the parameters to be determined and N is the number of measurements. When the errors of the measurements do not have a constant standard deviation, then it is generally required to introduce weighting factors (σ_t), leading to a weighted least-square criterion [9]. The

Table 3 Literature overview of hydrolysis constant

Substrate	K*hyd* [day^{-1}]	Reference
Carbohydrates	0.5 - 2.0 (at 35ºC)	Garcia-Heras [12]
	0.041 - 0.13	Gujer and Zehnder [16]
	0.25 vary within (100%)	Batstone et al. [3]
Lipids	0.1 - 0.7 (at 35ºC)	Garcia-Heras [12]
	0.08 - 0.4	Gujer and Zehnder [16]
	0.1 vary within (300%)	Batstone et al. [3]
Proteins	0.25 -0.8 (at 35ºC)	Garcia-Heras [12]
	0.02 - 0.03	Gujer and Zehnder [16]
	0.2 vary within (100%)	Batstone et al. [3]
Gelatine	0.27 ± 0.13	Raposo et al.[25]
	0.65	Flotats et al. [11]

calculation of the most probable parameter [19] was achieved by the Numeric's library Minuit. The software allows the sharing of any subset of the model parameters to

minimize the sum of squares. In addition, the identification space of the model parameters can be limited individually for each parameter. The robustness of the parameter estimation resulted from the possibility to restrict the identification space, thus, it excluded critical parameter values that caused numerical instability [9].

IV. Materials and Methods

A. Equipment and measurements

Triplicate batch experiments were conducted in glass flasks (1,000 mL). The digesters were manually mixed several times per day and maintained at a constant temperature of 38±0.2°C controlled by a thermostat (Haake DC 30/K10) in a water bath. After filling with the substrate and inoculum, the bottle was flushed with 100% N_2 gas for 2 min at 2 bars. The discharge of biogas occurred through a port in the fermenter cap. The outlet tube was connected to a CO_2 capture unit (filled with 3M NaOH) when methane recordings were needed. In the case of the recording of the biogas production, the sodium hydroxide unit was omitted. Generated methane and biogas passed through a condensate trap for vapor removal and were recorded by a gas volume sensor (gasUino). The gasUino device [10] is a gas volume counter based on the low-cost gas sensor developed by Liu et al. [24] where the recordings are adapted to standard conditions. A 75% NaCl solution (pH 2) served as a sealing liquid for decreasing the gas solubility. Finally, the biogas was collected in biogas bags. The total methane and biogas volumes were estimated by subtracting the volume of the average blank samples respectively. The data acquisition (date, time, temperature, pressure and amount of clicks made by gas counter) was developed in processing and stored in text files separated by commas. LabVIEW VI automatically corrected biogas and CH_4 volumes to standard conditions, reproduced it on the screen and saved the data in a MySQL database [10]. For the estimation of the biogas production the volume of the NaOH solution should be neglected. Blanks without substrate were maintained as control to measure biogas and methane production from the sludge.

The following parameters were determined from the substrates: total solids (TS) and volatile solids (VS) of the substrates were measured by drying and calcinating the samples at 105°C and 550°C, respectively, for 24 h (P300, Nabertherm), and Total COD (Hach-Lange, Germany) was determined of samples taken on daily basis. The pH was measured at the beginning, and at the end of the experiments.

B. Inoculum and substrates characteristics

The inoculum was a seeding sludge blend originating from a wastewater treatment plant (Farge, Bremen, Germany), a pig and cattle manure digestion plant (Ritterhude, Lower Saxony, Germany) and sludge from corn and silage digesting plant (Osterholz-Scharmbeck, Germany). In order to reduce the endogenous methane production by the inoculum, the sludge was pre-incubated at 38±0.2°C during one week. Hydraulic retention time for each experiment was defined by VDI protocol which equaled 28 days.

Table 4 Characterization of the inoculum and substrates used

Description	COD [g L^{-1}]
Inoculum $_{Gelatine}$	25.600
Inoculum $_{Rapeseed\ oil}$	25.586
Inoculum $_{Sucrose}$	25.400
Inoculum $_{Mixture}$	23.690
Gelatine	11.440
Sucrose	15.316
Rapeseed oil	15.420
Mixture of three substrates	14.466

Three different single substrates were tested in batch mono-digestions and finally their mixture: sucrose (Nordzucker AG), gelatine (Backfee) and rapeseed oil (EUCO GmbH). The concentration of the substrates was defined according VDI 4630 (2006). The concentration of different substrates was: sucrose -16.0 g L^{-1}, gelatine -15.8 g L^{-1}, rapeseed oil – 8.2 ml L^{-1}, and for the mixture sucrose -5 g L^{-1}, gelatine - 6 g L^{-1}, rapeseed oil -3 g L^{-1}, in total -14g L^{-1}. Table 4 summarizes the characteristics of the used substrates and the inoculum.

V. Results and Discussion

A. Calibration of the experimental data of the anaerobic digestion with sucrose

Table sugar (sucrose) is a disaccharide consisting of two hexoses, glucose and fructose. Although sucrose is soluble in water, it is too complex to enter the cell for some bacterial strains. First, sucrose must be hydrolyzed to glucose and fructose which after hydrolysis can enter the bacterial cell and be degraded. Hydrolysis of table sugar is achieved through exoenzymes. Once hydrolyzed, glucose and fructose enter the cell, where they are degraded by endoenzymes [14], and subsequently fermented into VFA and CO_2. VFA are further converted by acetogenic bacteria into acetate and H_2/CO_2. Finally, methanogenic bacteria convert acetate and H_2 into methane. Fig.1 shows the data from the AD of sucrose. Initially, 16 g of sucrose were added to the inoculum sludge. The biogas process production stopped after 16th day. In total, during 28 days of experiment 9.2 L of biogas corresponding to 4.96 L of The biogas flow rate was quite high at the first day and reached 0.145 L h^{-1}. Starting from the second day till the fifth it increased from 0.01 L h^{-1} to 0.071 L h^{-1}. Subsequently, it dropped down and reached at day 10 0.002 L h^{-1}. Afterwards, the flow rate increased slightly until 0.007 L h^{-1} and then stopped at day 16. COD_{Tot} decreased from 15.31 to 0.04 g$CODL^{-1}$ during 16 days.methane were produced. The volumetric concentrations of methane and carbon dioxide were calculated from the measured corresponding volumes as well as the biogas flow rate. The minimum methane concentration was reached at day 9 and showed 50.18Vol.-%. Subsequently, it increased and reached 53.62 Vol.-%.

The simulations showed initially a discrepancy between the experimentally measured biogas and methane volumes. The simulated biogas and methane produced volumes were by 397 mL and 313 mL less, respectively, as compared to the experimental data. The methane volumetric concentration was kept at 52.67 Vol.-% of total biogas which was within the experimental range. The

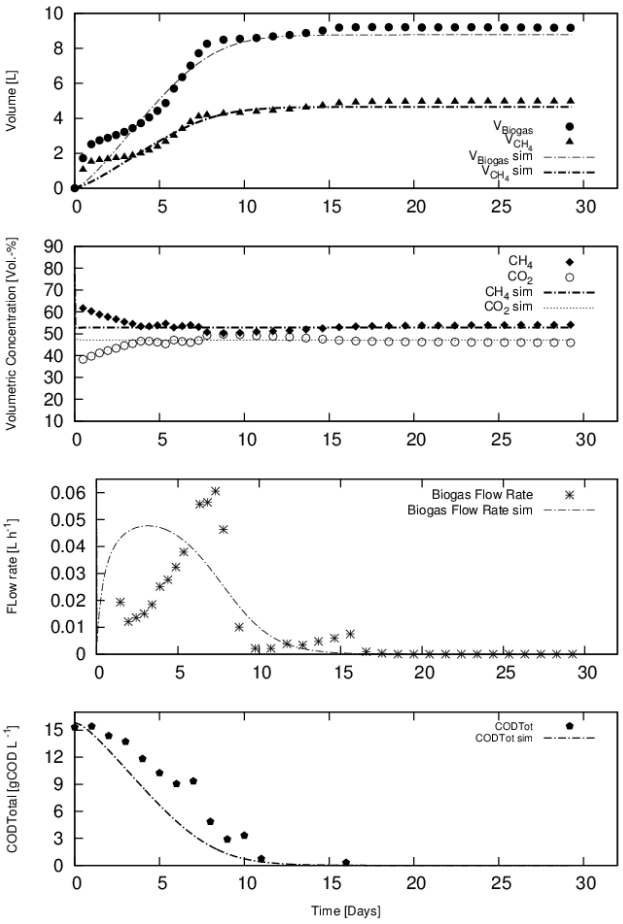

Fig. 1 Experimental and simulation data of anaerobic mono-digestion in batch of 16 g L $^{-1}$ sucrose. It shows the dynamics of the biogas and methane volume production, volumetric concentration of CH_4 and CO_2, biogas flow rate and COD_{Tot}

model proposed the biogas flow rate with a shift to the very beginning of the batch experiment. In terms of COD_{Tot} the simulated version showed a somewhat faster degradation as compared to the measured values. Generally, the simulations followed the key dynamics of the sucrose AD dynamics.

B. Calibration of the experimental data of the anaerobic digestion with gelatine

Proteins are complex, high molecular-weight compounds and are degraded more slowly than carbohydrates. It is a complex process starting with hydrolyzation by proteolytic enzymes e.g. protease and peptidase, into peptides and amino acids which are then acidified into VFA, H_2, NH_3 and S. The initial step of fermentation, hydrolysis, is rate-limiting and the overall proteins degradation is a slow process. Fig. 2 shows the experimental results from the AD of gelatine. Gelatine in a mass of 15.8 g L^{-1} was added into the sludge. After 28 days 7.19 L of biogas and 3.93 L methane were produced. The volumetric methane concentration in total biogas was fluctuating between 53.1 to 54.5 Vol.-%. The biogas flow rate was increasing within the 6 days and reached its maximum at 0.035 L h^{1}. COD_{Tot} was depleting from 15.96 to 0.97 gCOD L^{-1} during 16 days.

The simulated data was in a good agreement with the corresponding experimental measurements: in particular for the biogas and methane volume production and biogas flow rate and, for the and for methane about 225 mL. Simulated COD_{Tot} decrease was slightly faster than the experimental results.

C. Calibration of the experimental data of the anaerobic digestion with rapeseed oil

Lipids are attractive substrates for anaerobic digestion and co-fermentation due to their high putative methane yield. However, the digestion of lipid matter can cause some problems. In anaerobic environments lipids are hydrolyzed by lipases to glycerol and long-chain fatty acids (LCFA). Many researches consider LCFA degradation as a ‘‘limiting step’’ for a number of reasons: formation of floating scum which causes limiting bioavailability and becomes toxic for acetogenic and methanogenic bacteria [22, 29]. Bacterial degradation of LCFAs begins with adsorption of LCFA by the cell and this can lead to growth inhibition depending on type of bacteria, and size, concentration and saturation degree of LCFA [26]. Fig. 3 shows the experimental results from the AD of 8 mL L^{-1} rapeseed oil. After day 25 the biogas

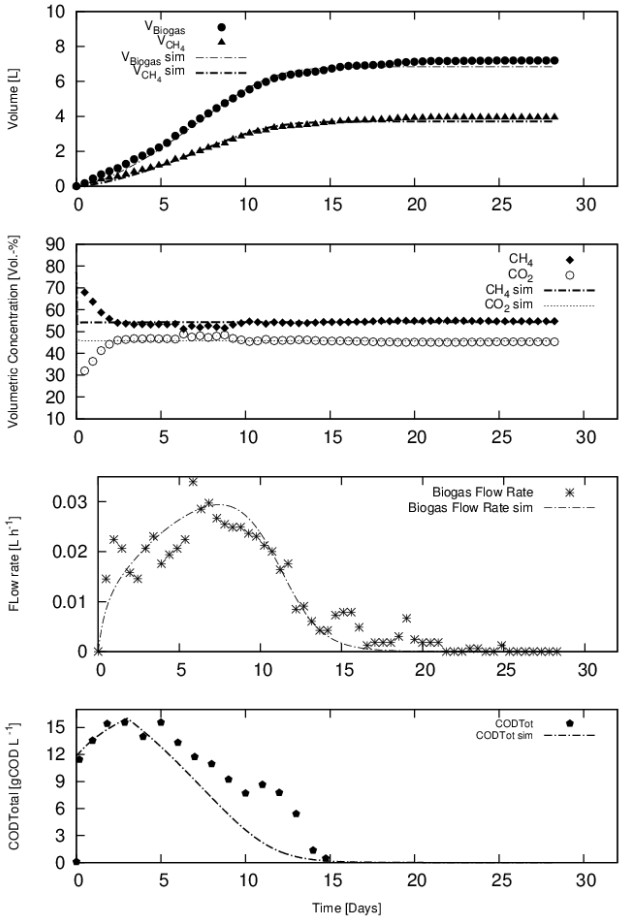

Fig. 2 Experimental data and simulation of anaerobic mono-digestion in batch of 15.8 g L^{-1} gelatine. There different panels show biogas and methane volume production, volumetric concentration of CH_4 and CO_2, biogas flow rate and COD_{Tot}. Results for blank biogas and methane formation were subtracted

production was stopped. The total amount of produced biogas and methane was 6.9 L and 5.22 L, respectively. From the data it was possible to judge that the hydrolytic step took nearly 5 days. The inhibition of biogas production mediated by LCFA through hydrolysis lasted until day 18. Hypothetically, LCFA concentration became favorable after bacterial growth and consequently also for biogas production. The volumetric methane concentration in biogas was increasing till day 10 and was subsequently constant at 75.4 Vol.-%. The first 15 days the biogas flow rate ranged between 0.006 - 0.002 L h^{-1}. Five days later the flow strongly increased and reached its maximum at 0.045 L h^{-1} and dropped then. Within the first five days COD_{Tot} was increasing from 10.63 to 20.43 g COD L^{-1} . It was further decreasing until day 26 of the experiment.

Initially, it was challenging to describe the inhibition of biogas production by LCFA which caused delay in biogas and CH_4 production. The introduction of an inhibition factor brought positive results and the simulated data matched the experimental data (Fig. 3). However, there was some mismatches observed at the beginning in the graph regarding the volumetric concentrations and some regarding the biogas flow rate. Generally, the simulations followed the obtained dynamics of the rapeseed oil AD.

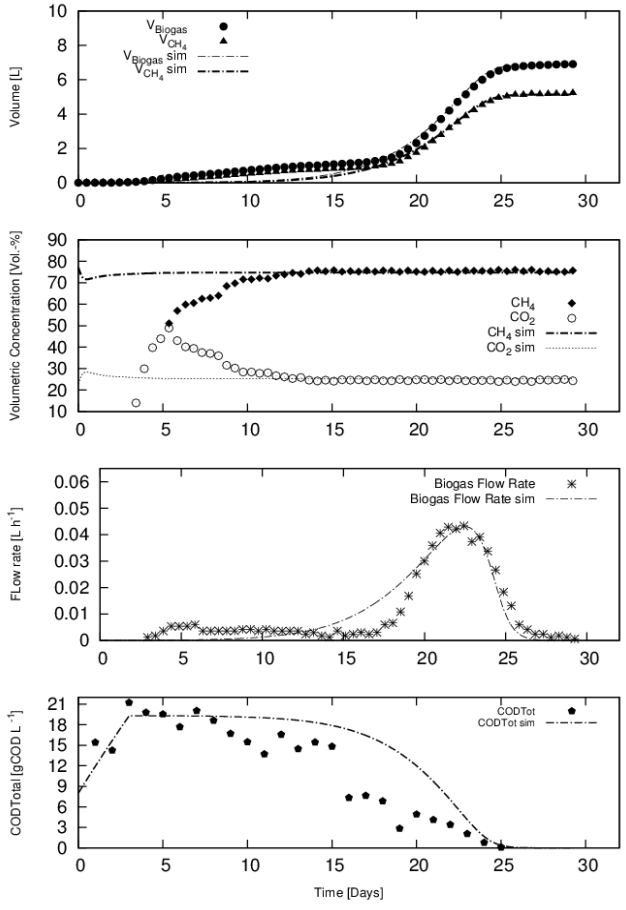

Fig. 3 Experimental data and simulation of anaerobic mono-digestion in batch of 8 ml L^{-1} rapeseed. Biogas and methane volume production, volumetric concentration of CH_4 and CO_2, biogas flow rate and COD_{Tot} are displayed. Blank biogas and methane formation were subtracted

D. Calibration of the experimental data of the anaerobic digestion with sucrose, gelatine and rapeseed oil

For the final experiment using the mixture of all three substrates it was decided to take the arbitrary substrate mixture with 5 g L^{-1} sucrose, 6 g L^{1} gelatine, 3 ml L^{-1} rapeseed oil, in total a substrate concentration of 14 g L^{-1} (Fig. 4). The maximum biogas production was achieved after 25 days which was also measured earlier [20]. Within 28 days of AD, 8.14 L biogas and 4.62 L methane were produced. The hydrolysis took nearly 6 days. It is assumed that the inhibition of LCFA slowed down the AD by inhibiting acetogens and methanogens but not in such an aggressive manner as compared to mono-AD of rapeseed oil. The mean volumetric methane concentration was

Table 5 Kinetic parameters used in the model for AD of mixture: gelatine, sucrose, rapeseed oil

Para-meter	Definition	Value	Unit
$k_{hyd\,C}$	Hydrolysis constant for carbohydrates	$7.9 \cdot 10^{-6}$	s^{-1}
μ_C^{max}	Maximum uptake rate for carbohydrates	$4.2 \cdot 10^{-6}$	s^{-1}
K_{C_s}	Half-saturation constant carbohydrates	6.5	$kg \cdot m^{-3}$
Y_{XC}	Yield factor for primary carbohydrates degradation	0.22	$kg \cdot kg^{-1}$
U_C	Yield factor for VFA production from carbohydrates	0.65	$kg \cdot kg^{-1}$
$k_{hyd\,P}$	Hydrolysis constant for proteins	$5.1 \cdot 10^{-6}$	s^{-1}
μ_P^{max}	Maximum uptake rate for proteins	$3.3 \cdot 10^{-6}$	s^{-1}
K_{P_s}	Half-saturation constant proteins	5.0	$kg \cdot m^{-3}$
Y_{XP}	Yield factor for primary proteins degradation	0.50	$kg \cdot kg^{-1}$
U_P	Yield factor for VFA production from protein	0.68	$kg \cdot kg^{-1}$
$k_{hyd\,L}$	Hydrolysis constant for lipids	$4.56 \cdot 10^{-6}$	s^{-1}
μ_L^{max}	Maximum uptake e rate for lipids	$5.6 \cdot 10^{-6}$	s^{-1}
K_{L_s}	Half-saturation constant lipids	3.2	$kg \cdot m^{-3}$
Y_{XL}	Yield factor primary lipids degradation	0.55	$kg \cdot kg^{-1}$
U_L	Yield factor VFA production from lipids	0.96	$kg \cdot kg^{-1}$
μ_{VFA}^{max}	Maximum uptake rate for VFA	$8.20 \cdot 10^{-6}$	s^{-1}
K_{VFA}	Half-saturation constant VFA	0.01	$kg \cdot m^{-3}$
Y_{XVFA}	Yield factor VFA degradation	0.35	$kg \cdot kg^{-1}$
v_{VFA}	Yield factor for CH_4 production from VFA	0.552	$mol \cdot kg^{-1}$
Ip_L	Inhibition coefficient	0.05	$mol \cdot kg^{-1}$
VR	Volume of the reactor	1.1^{-2}	m^3
TR	Temperature in the reactor	311.0	K

constant at 58.8 Vol.-%. Starting from the day 6, the biogas flow rate was smoothly increasing and reached its maximum at 0.025 L h^{-1} on day 17, after which it decreased and production completely stopped at day 26. Direct model validation was tested on the AD of the chosen substrates mixture. The set of chosen kinetic parameters is given in Table 5.

The parameterized model followed well the progress of the experimental data; however, the volumetric concentration of CH_4 was lower in the experiment during days 15-23. The experimental results during the next five days were in good agreement with the simulated dynamics of the CH_4 concentration. Besides, there is slight difference in volume production of methane during the days 7-17. Afterwards complete agreement between simulated and measured data was observed. The predicted probity of the proposed mathematical model compared to the observed experimental data was about 20% in most cases or showed complete agreement, suggesting that the model can be used for the relatively accurate prediction of the dynamics of AD. This applies also to the kinetic coefficients concerning the bacterial activity, which limit the AD.

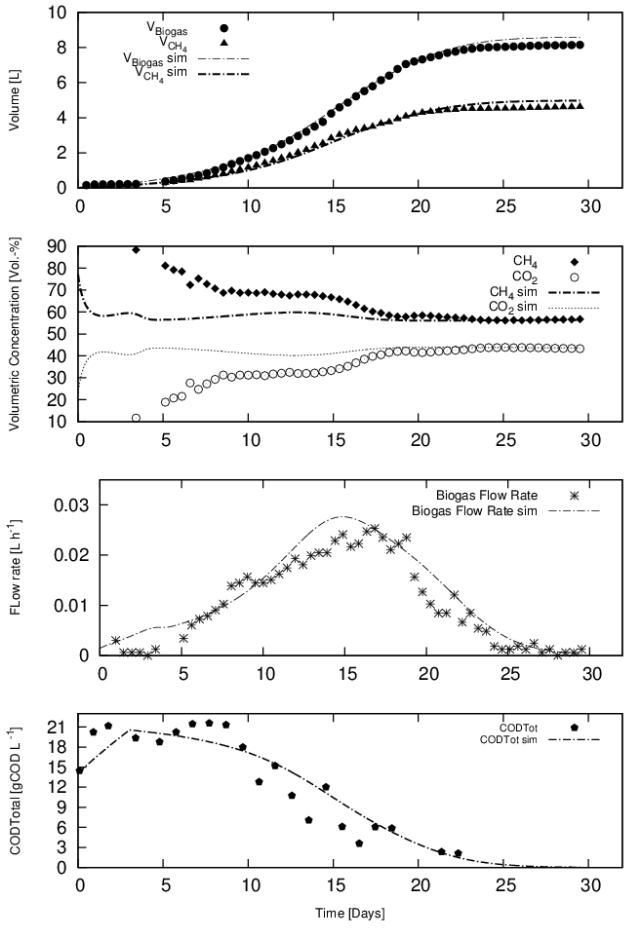

Fig. 4 Comparison of simulated and experimental results of AD in batch of mixed substrates (5 g L^{-1}sucrose, 6 g L^{-1} gelatine, 3 ml L^{-1} rapeseed oil, in total -14 g L^{-1}). The volume of biogas and methane, the volumetric concentration of CH_4 and CO_2 and, the biogas flow rate are shown. Volumes of blank biogas and methane formation were subtracted. The kinetic parameters used in the model are given in the Table 5.

The theoretical methane and biogas yield were calculated based on the following formula (23):

$$V_{BG\,Tot,mix} = \frac{V_{BG\,Tot,\,S}}{m_S} \cdot m_{S,mix} + \frac{V_{BG\,Tot,G}}{m_G} \cdot m_{G,mix} + \frac{V_{BG\,Tot,R}}{m_R} \cdot m_{R,mix} \quad (23)$$

where *BG Tot* (L) or CH_4 *Tot* is biogas (or CH_4) total (L), *m* - mass of the substrate (VS added), *S*, *G*, *R* and *mix* - sucrose, gelatine, rapeseed oil and mixture, respectively.

The total theoretical biogas production is equal to 8.228 L while VCH_4 was 4.74 L. Comparing the experimental yield with the theoretical volumes in CH_4 (53 mL per g VS) and in biogas (80 mL per g VS) there is a difference of yield. The summary of the total methane and biogas volume is shown in Fig. 5. There could be several reasons why the measured biogas is less than the theoretically predicted potential: the bacterial population of the inoculum was not initially diverse as compared to the sludge used for the mono - fermentations. One more reason could be that more organic material was consumed to build up the bacterial biomass (about 5-10% of substrate) during AD [1]. Another reason could be that some part of the substrate was not accessible for the microorganisms.

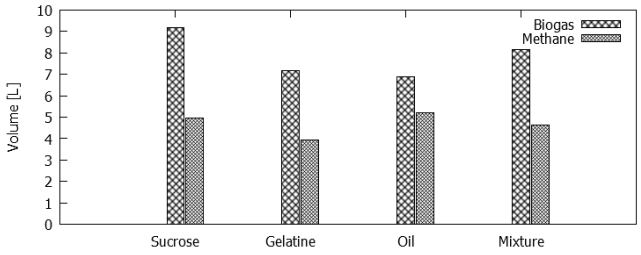

Fig. 5 Cumulative biogas and methane, with average CH4 %, produced in AD batch tests

In earlier studies it has been reported that biochemical methane potential (BMP) of rapeseed oil showed 704±13 ml CH_4 per g VS [21]. The present experiments yielded 655 ml CH_4 per g VS. However, the results of Hansen et al. [17] corresponded to a higher production of 800-900 ml CH_4 per g VS. BMP tests with gelatine were carried out by Hansen et al. [17] and 100-150 mL CH_4 per g VS were produced which is lower compared to 205 mL CH_4 /g VS obtained here. The methane yield produced from AD of sucrose varied in between 240 and 360 mL CH_4 per g VS [21]. The present mean yield of CH_4 matched published results and was equal to 310 mL CH_4 per g VS.

VI. Conclusion

A three-step model was suggested to describe AD for sucrose, gelatine and rapeseed oil. The inhibition by LCFA of acetogenic and methanogenic microorganisms caused a decrease of hydrolysis rate and slower biogas production was accurately described by the model. The additional degree of reduction compared to the model of Blesgen is due to the aim to make the estimation of model parameters from experimental data simpler. Parameterization became easy to handle and the validation of the calibrated model satisfy our intention to predict the biogas dynamics only by

adjustment of three master substrates (proteins, carbohydrates and lipids). The model can be applied as a teaching tool for students or young specialists to study an influence of initial inputs on the efficiency of the biogas process generation.

APPENDIX

Abbreviations

AD: anaerobic digestion
ADM1: Anaerobic Digestion Model no. 1
COD_{Tot}: total chemical oxygen demand [kg COD·m^{-3}]
LCFA: long - chain fatty acids
oTS: organic total solids [g·L^{-1}]
TS: total solids [g·L^{-1}]
VS: volatile solids [g·L^{-1}]
Cp: *primary* carbohydrates [kg·m^{-3}]
Pp: primary proteins [kg·m^{-3}]
Lp: *primary* lipids [kg·m^{-3}]
C_S: accessible carbohydrates [kg·m^{-3}]
P_S: accessible proteins [kg·m^{-3}]
L_S: accessible lipids [kg·m^{-3}]
Xaci: acid forming bacteria [kg·m^{-3}]
Xmeth: methanogenic bacteria [kg·m^{-3}]
TIC: total inorganic carbon [mol·s^{-1}]
VFA: volatile fatty acids [mol·s^{-1}]
Me: methane
Y_{XC} : yield factor for primary carbohydrates degradation [kg·kg^{-1}]
Y_{XP} : yield factor for primary proteins degradation [kg·kg^{-1}]
Y_{XL} : yield factor primary lipids degradation [kg·kg^{-1}]
Y_{XVFA} : yield factor VFA degradation [kg·kg^{-1}]
U_C: yield factor for VFA production from carbohydrates [kg·kg^{-1}]
U_P: yield factor for VFA production from protein [kg·kg^{-1}]
U_L: yield factor VFA production from lipids [kg·kg^{-1}]
v_{VFA}: yield factor for CH_4 production from VFA [mol·kg^{-1}]
$k_{hyd\,C}$: hydrolysis constant for carbohydrates [s^{-1}]
$k_{hyd\,P}$: hydrolysis constant for proteins [s^{-1}]
$k_{hyd\,L}$: hydrolysis constant for lipids [s^{-1}]
μ_C^{max}: maximum uptake rate for carbohydrates [s^{-1}]
μ_P^{max}: maximum uptake rate for proteins [s^{-1}]
μ_L^{max}: maximum uptake rate for lipids [s^{-1}]
μ_{VFA}^{max}: maximum uptake rate for VFA [s^{-1}]
μ_C: rate of acidogens production on carbohydrates [kg·m^{-3}·s^{-1}]
μ_P: rate of acidogens production on proteins [kg m^{-3}·s^{-1}]
μ_L: rate of acidogens production on lipids [kg·m^{-3}·s^{-1}]
μ_P: rate of methanogens production on VFA [kg·m^{-3}·s^{-1}]
K_{C_s}: half-saturation constant carbohydrates [kg·m^{-3}]
K_{P_s}: half-saturation constant proteins [kg·m^{-3}]
K_{L_s}: half-saturation constant lipids [kg·m^{-3}]
K_{VFA}: half-saturation constant VFA [kg·m^{-3}]
Ip_L: inhibition coefficient [mol·kg^{-1}]
V_{liq}: volume reactor [m^3]
TR: temperature in the reactor [K]
$MWCO2$: molecular weight of carbon dioxide [kg·mol^{-1}]
R: the ideal gas constant [J·mol^{-1}·K^{-1}]
$TGas$: temperature of gas [K]
P_{outGa}: the pressure of the gas [Pa]
r_{hydC}: rate describing the hydrolysis of carbohydrates
r_{hydP}: rate describing the hydrolysis of proteins
r_{hydL}: rate describing the hydrolysis of lipids
r_{XC}: rate describing the acidogenesis carbohydrates
r_{XP}: rate describing the acidogenesis proteins
r_{XL}: rate describing the acidogenesis lipids
r_{VFA} : rate describing methanogenesis

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REFERENCES

[1] Agency for Renewable Resources. (2013). Basic data bioenergy. 3rd edn. Rostock Available: http://mediathek.fnr.de/media/downloadable/files/samples/b/a/basisdaten_9x16_2013_web_neu2.pdf, accessed 2014-08-19 14:20.

[2] J. D. Bastone. (2006). Mathematical modelling of anaerobic reactors treating domestic wastewater: Rational criteria for model use. *Envir. Sci and Biotechnol.* 5. pp. 57-71.

[3] J. D. Bastone, J. Keller, I. Angelidaki, S. V. Kalyuzhnyi, S.G. Pavlostathis, A. Rozzi, W. T. M. Sanders, H. Siegrist, V. A. Vavilin. (2002, January). The IWA Anaerobic Digestion Model No 1 (ADM1). *Water Sci and Technol*. 45(10). pp. 65-73.

[4] O. Bernard, Z. Hadj-Sadok, D. Dochain, A. Genovesi, J.-P. Steyer. (2001, October). Dynamical model development and parameter identification for an anaerobic wastewater treatment process. *Biotechnol Bioeng.* 75(4). pp. 424-438.

[5] A. Blesgen. (2009) PhD Thesis, Faculty of Chemistry and Biology, University of Bremen, Bremen, Germany

[6] A. Blesgen, V. C. Hass. (2010, March). Efficient biogas production through process simulation. *Energy Fuels* 24(9). pp. 4721-4727.

[7] B. Cécile, F. Fabien, G. Benoit, M. Bruno. (2010, May). Biofuels, greenhouse gases and climate change. A review *Agron Sustain Dev*. 3(1). pp. 1-79.

[8] D. Deublein, and A. Steinhauser, Biogas from waste and renewable sources, Wiley-VCH, Weinheim, 2008, ch. 6-18.

[9] A. Donoso-Bravo, J. Mailier, C. Martin, J. Rodrıguez, C. A. Aceves-Lara, A. V. Wouwer. (2011, November). Model selection, identification and validation in anaerobic digestion: A review. *Water Res.* 45(17). pp. 5347-5364.

[10] H. M. Falk. (2011, Decemeber). Monitoring the anaerobic digestion process. PhD Thesis, Jacobs University Bremen, Germany

[11] X. Flotats, J. Palatsi, B. Fernández, M. A. Colomer, J. Illa. (2010, March). Identifying anaerobic digestion models using simultaneous batch experiments. *Environ Eng and Manag J*. 9(3). pp. 313-318.

[12] J. Garcia-Heras. (2003, March). Reactor sizing, process kinetics and modelling of anaerobic digestion of complex wastes. In: Mata-Alvarez, J.(Ed.), Biomethanization of the organic Fraction of Municipal Organic Solid waters. *Water Sci Technol*. 40. pp. 339-346.

[13] German Engineers Association (2006) VDI 4630: Fermentation of organic materials - Characterization of the substrate, sampling, collection of material data, fermentation tests. VDI Handbook Energietech. Berlin: Beuth Verlag GmbH

[14] M. Gerardi, "The microbiology of anaerobic digesters," John Wiley& Sons Inc, Hoboken, New Jersey, 2003, pp.

[15] S. P. Graef, J.F. Andrews. (1974, April). Stability and control of anaerobic digestion. *J WPCF*. 46(4). pp. 667-682.

[16] W. Gujer, A. J. B. Zehnder. (1983). Conversion processes in anaerobic digestion. *Water Sci. Technol*. 15(8). pp. 127–167.

[17] T. L. Hansen, J. E. Schmidt, I. Angelidaki, E. Marca, J. L.C. Jansen, H. Mosbaek, T.H. Christensen. (2004, January). Method for determination of methane potentials of solid organic waste. *Waste Manag.* 24(4). pp. 393–400.

[18] D.T. Hill, C. L. Barth. (1977, October). A dynamic model for simulation of animal waste digestion. *J WPCF*. 49(10). pp. 2129-2143.

[19] F. James, "Statistical methods in experimental physics," 2nd edn. World Scientific, London, 2006, pp.127-139.

[20] P. G. Kougias, K. Boe, P. Tsapekos, I. Angelidaki. (2013, February). Foam suppression in overloaded manure-based biogas reactors using antifoaming agents. *Bioresour Technol*. 153. pp. 198-205.

[21] L. Matsakas, U. Rova, and P. Christakopoulos. (2014, August). Evaluation of dried sweet sorghum stalks as raw material for methane production. *BioMed Res Int.* Available: http://www.hindawi.com/journals/bmri/2014/731731/abs/.

[22] H.B. Nielsen, I. Angelidaki. (2003, February). Co-digestion of manure and organic waste at centralized biogas plants: process imbalances and limitations. *Appl Biochem Biotechnol*. 109(1-3). pp.95-105.

[23] M. Lebuhn, B. Munk, M. Effenberger. (2014, May). Agricultural biogas production in Germany -from practice to microbiology basics. *Energy Sustain and Soc*. 4(10). Available: http://link.springer.com.

[24] J. Liu, G. Olsson, B. Mattiasson. (2004, July). Control of an anaerobic reactor towards maximum biogas production. *Water Sci Technol.* 50(11). pp. 189-198.

[25] F. Raposo, V. Fernandez-Cegri, M. A. De la Rubia, R. Borja, F. Beline, C. Cavinato, G. Demirer, et al. (2011, July). Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. *J Chem Technol Biotechnol.* 86(8). pp. 1088–1098.

[26] E. Salminen, J. Rintala. (2002, May). Anaerobic digestion of organic solid poultry slaughterhouse waste – a review. *Bioresour Technol* 83(1). pp. 13–26.

[27] P.H. Smith, F.M. Bordeaux, M. Goto, A. Shiralipour, A. Wilke, J. F. Andrews, S. Ide, M.W. Barnett (1988a, February). Biological production of methane from biomass. In: Smith WH, Frank JR (Eds.) *Methane from biomass. A treatment approach.* Elsevier, London, pp.291- 334.

[28] C. Wolf, S. McLoone, M. Bongards. (2009, March). Biogas plant control and optimization using computational intelligence methods. *Automatisierungstech* 57. pp. 638-649.

[29] J. C. Ye Chen Jay, and K.S. Creamer. (2008, July). Inhibition of anaerobic digestion process: A review. *Bioresour Technol*. 99(10). pp. 4044–4064.

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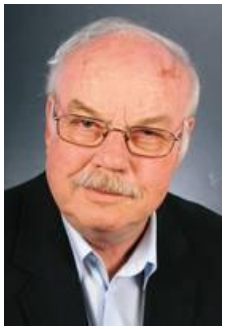

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