Determination of the hydrolysis constant using Anaerobic Batch Tests

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Abstract
Hydrolysis is the rate limiting step for particulate/solid substrates under anaerobic digestion. The first order kinetic model is commonly applied to describe this step and it can be determined by anaerobic batch test. In this paper we assess three issues with respect to defining the hydrolysis rate constant; source of inoculum, influence of substrate to inoculum ratio and the pre-incubation time of the inoculum. Tests were carried out with five different inoculums that showed different hydrolytic activity under the same test conditions. Sludge from MWWTP showed the highest activity (0.95 d^{-1}) and sludge from co-digestion plant the lowest (0.29 d^{-1}). 7 days pre-incubation of sludge reduced the hydrolytic activity by ca. 30%. Increasing the substrate to inoculum ratio lead to an increase in hydrolytic activity until the optimum ratio, and then it decreased. Under real substrate to inoculum ratio conditions maintained in large scale biogas plants, the measured hydrolysis rate constants were 0.32, 0.49, 0.41 and 0.17 d^{-1} for dog food, cacao shells, CCM and rye respectively. The parameter can be used for anaerobic reactor design and as initial values for modelling.

Keywords
Anaerobic digestion, ABT, VDI 4630, Hydrolysis rate constant

INTRODUCTION

Anaerobic digestion is a biochemical process for the treatment of organic substrates such as domestic and industrial wastewaters, manure and solids like energy crops, agricultural residues and food wastes. The process can be modelled based on the rate-limiting step. Depending on the characteristics of the substrates is either hydrolysis or methanogenesis the rate limiting step. When the substrate is in particulate/solid form, hydrolysis is normally considered as the rate limiting step. The first order model is commonly applied to describe the progress of the hydrolysis, where the constant k is also known as the hydrolysis rate constant k_{hyd}. This is also applied in ADM1 for disintegration and for hydrolysis. The hydrolysis rate depends on the characteristics of used substrates as well as on the inoculum and the operating conditions. Considering the essentials of the anaerobic fermentation, the anaerobic batch test- ABT (comparable to BMP assay) is a relatively simple and reliable method to obtain this parameter. The Information provided by ABT is valuable for evaluating potential substrates and for sludge characterisation. Literature related to ABT assays is extensive and particularly increased over recent years. Several methods have been utilized for measuring methane potentials and activity, but there is still no international standard protocol. In this paper we assess three issues with respect to hydrolysis rate constant k_{hyd}:

(a) source of inoculum (Comparing different anaerobic sludge regarding its hydrolytic activity)- test a
(b) influence of substrate to sludge ratio (gCOD/gVS)- test b
(c) influence of the duration of pre-incubation of inoculum- test c
(d) and also define the k_{hyd} for different substrates- CCM (corn cob mix), rye, cacao shells and dog food, including the influence of substrate to inoculum ratio on the results- test d

The evaluated data will be compared with literature.

MATERIAL and METHODS

ABT Test- The ABT tests are based on end product (biogas) measurement according to German standard VDI 4630. The gas volume measurement occurs with pressure transducers according to DIN EN ISO 11734 (**Figure 1**).

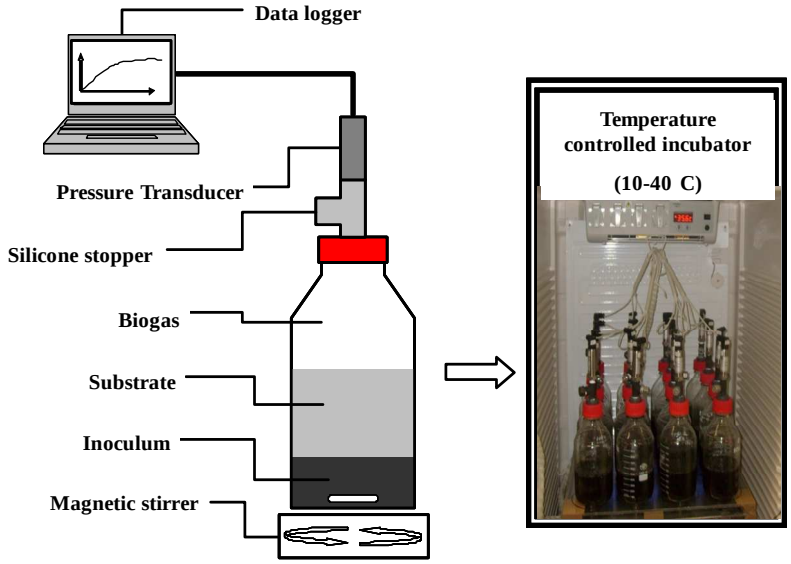

Figure 1. Anaerobic batch test apparatus in accordance with DIN EN ISO 11734

Tests were performed in 1000 mL glass vials with approx. 300 mL working volume. After filling with substrate and inoculum, the oxygen containing air in the bottles were flushed out with 100 % N_2 gas for 0.5 min at 2 bars. The bottles were than sealed with gas-tight caps and pressure transducers devices respectively, and stored in temperature-controlled incubators at 37 °C. Bottles were mixed by inverting once per day for granular sludge and continuously by magnetic stirrer for floccular sludge. Blanks without addition of substrate were maintained as control to measure the biogas production from the inoculum. The amount of inoculum was identical in all bottles for each test. The pH was recorded before and after the tests. All tests were carried out in triplicates.
In order to determine the rate of degradation as exactly as possible the increase in pressure is recorded continuously. Accumulated gas production was calculated from the pressure increase in the headspace volume (ca. 700 mL) and the dissolved gas content in the liquid phase was calculated according to Henry´s Law and expressed under standard conditions (0 °C, 1.013 bar). The net gas production was obtained by deducting gas production of the blanks. The biogas quality (CH_4, CO_2 and H_2) was determined via GC for several times during the test.

Substrates

Hydrolysis rate were determined for: corn-cob-mix (CCM), dog food, rye and cacao shells. The soluble part of the substrates, which do not need to be hydrolysed, was assumed to be negligible. Microcrystalline cellulose was added as sole carbon source in the other batch tests (a, b and c).
Table 1 summarizes the characteristics of used substrates.

Table 1. Characterization of substrates used

	TS	VS	VS/TS
	[g/L]	[g/L]	[%]
Corn-cob-mix	566.8	489.9	86.4
Rye	886.5	767.4	86.6
Dog food	800.9	721.2	90.1
Cacao shells	917.1	843.9	92.0
Microcrystalline cellulose	965.7	921.2	95.4

Inoculum

Depending on the aim of the tests, inocula were collected from five different anaerobic reactors. **Table 2** summarizes the main characteristics of used inocula.

Table 2. Characteristics of the inocula

Origin/Source	pH	TS	VS	VS/TS
		[g/L]	[g/L]	[%]
Biogas Plant-energy crop	7.65	48.0	35.3	73.5
Biogas Plant-co digestion	8.16	29.3	17.2	58.5
MWWTP	7.60	26.8	17.8	66.6
Soft drink industry- Biobed, only pellets	6.97	143.9	123.1	85.5
Distillery- IC, only pellets	7.05	72.4	63.6	87.8
Distillery- IC	7.13	57.1	42.3	74.1

Calculation of Hydrolysis Rate Constants

Hydrolysis rate constants were determined by using first order kinetic model:

$$dS/dt = -k_{hyd} \cdot S_t \quad (1)$$

difficult to measure, it is preferable to derive the model by using the measurement of produced gas out of the substrate, as an indirect method, which is much easier to determine:

$$\ln \frac{B_0}{B_0 - B_t} = k_{hyd} \cdot t \quad (2)$$

where, B_t is the cumulative biogas yield (mL Biogas/g VS) for time t and B_0 is the ultimate biogas yield of the substrate. The model is usually used to determine the extent and rate of biodegradation. The model defines substrate utilization rate as function of substrate concentration only. Many aspects such as microbial growth and decay or concentration are not considered. Equation 2 will produce a linear curve when the degradation kinetics is first order; the slope of the curve represents the hydrolysis rate constant k_{hyd}.

RESULTS and DISCUSSION

Comparing different anaerobic sludge regarding its hydrolytic activity

The hydrolytic activities, or rather cellulose activities, for five different inocula were compared. The tests were performed under same conditions. The sludge loading rate was 0.5 g $COD_{Substrate}$/g $VS_{Inoculum}$ for all runs. The highest hydrolytic activity was found for MWWTP-Sludge and the lowest for co-digestion biogas plant. This measurement method depends directly on biogas formation and the activity of methanogens (SMA). The methane content of biogas was ca. the same for all runs and as expected around 50%. That indicates that the methanogens were not inhibited for this SLR.

Table 3 Comparison of hydrolytic activities from different inocula

Sludge	k_{hyd}	lag phase	Methane content
	[d^{-1}]	[h]	[%]
BP-energy crop	0.35	54	52
BP-co digestion	0.29	80	52
MWWTP	0.95	53	53
Soft drink industry- Biobed, only pellets	0.83	97	55
Distillery- IC, only pellets	0.60	126	52

Influence of the substrate/sludge ratio on hydrolytic activity

Substrate to inoculum ratio or sludge loading rate is also an important factor for microbiological activities. **Figure 2** shows the changes of k_{hyd} for inocula from MWTP and distillery. The lag phase for MWTP-sludge was 50, 34, 0 and 33 h respectively. The lag-phase was same (28 h) for all loading rates for distillery sludge. The methane content from biogas was same for all runs, which indicates there is no inhibition on methanogens.

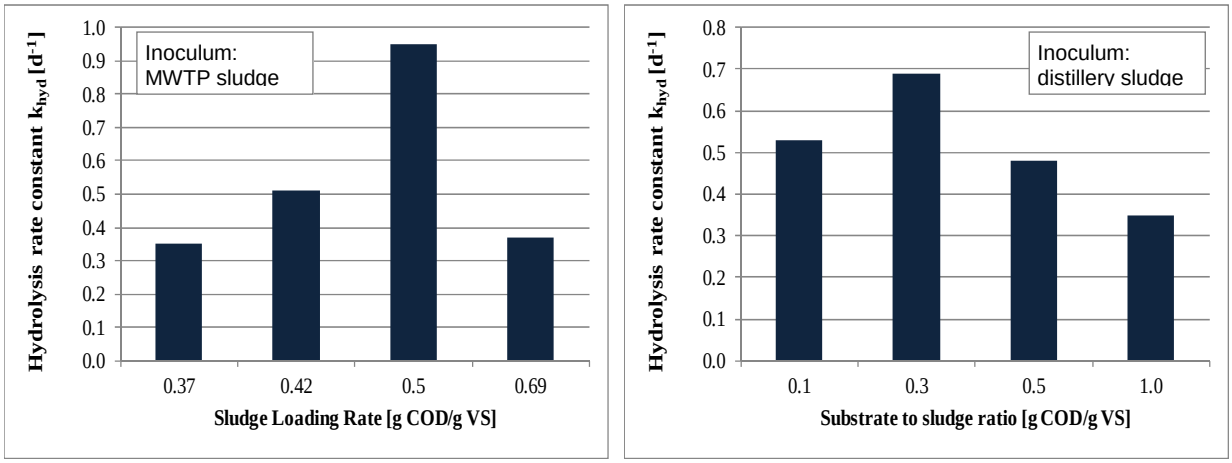

Figure 2. Influence of SLR on hydrolytic activity for MWTP-sludge (left) and distillery sludge (right)

Influence of the sludge pre incubation on hydrolytic activity

The inoculum should be pre-incubated (degassed) for one week or longer according to VDI 4630. To find out the influence of this pre-incubation a BMP test was carried out with relatively "fresh" and pre-incubated sludge from a co-digestion biogas plant. The hydrolysis rate constant k_{hyd} for 1 day pre-incubated inoculum was 0.83 d^{-1} and drops after 7 days pre-incubation to 0.57 d^{-1}. The SLR (0.06 g COD/g VS) was also the same for both runs and the biogas plant is also normally loaded with this SLR- value. The TS and VS concentration and VS/TS ratio from sludge did not change significantly between 1d and 7d pre-incubation. It is preferable to take fresh inoculum for measuring its actual activity.

Determining the hydrolytic activity for different substrates

The hydrolysis rate constants for dog food and cacao shells were carried out for different SLRs. The sludge from a co-digestion biogas plant fed with the above mentioned substrates was chosen as inoculum and pre-incubated for 7 days. The hydrolysis rate constants were also defined for CCM and rye. The sludge from an energy-crop biogas plant was used as inoculum and the test was carried out for the same SLR for both substrates. The pre-incubation time of this inoculum was 14 d instead of 7d, due to the gas production of the sludge itself after 7d.

Table **4** summarizes the results from these tests.

Table 4 Hydrolysis rate constants for dog food, cacao shells, CCM and rye

		Dog food		Cacao shells		CCM	Rye
SLR	[g COD/g VS]	0.08	0.42	0.09	0.66	0.16	0.16
$k_{hyd.}$	[d-1]	0.32	0.21	0.49	0.17	0.41	0.17

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