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## **The role of inoculum on biomethane potential tests: influence on biogas yield and kinetic parameters**

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### Presentation outline

1. Introduction
2. Influence of inoculum origin
  - Materials and methods
  - Influence on biomethane potential (BMP) batch-test results
  - Influence on kinetic parameters of biogas formation
3. Is there a priming-effect? – Interactions between inoculum and substrate
  - Experimental setup
  - Results and conclusions
4. Conclusions

### Background and motivation

#### **BMP batch-tests: well established method for**

- characterizing new feedstock materials for anaerobic digestion (AD)
- comparing different qualities of feedstock (e.g. in order to evaluate the effect of different pre-treatment)

#### **Current lab practice:**

- no standard equipment (digester volume, measurement of gas volume, measurement of gas composition)
- no international standard protocol
- German directive VDI 4630 : approach to test routine standardization

#### **Aim of work:** elucidate the role of inoculum in terms of

- influence on BMP batch-test results
- Influence on kinetic parameters of biogas formation
- interaction between inoculum and substrate (“priming –effect”)

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### Batch-test-assays



- Large lab digesters : 30 L
- Testing protocol according to German directive VDI 4630
- S/I- ratio = 0,4 (VS based)
- Digestion at 38 °C (heated chamber)
- Biogas is captured in gas bags
- Biogas volume and composition is measured daily
- Experiment is skipped when daily increase of gas production < 1% .

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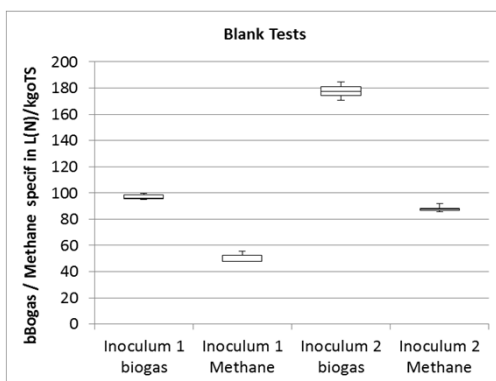
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Batch-test-assays

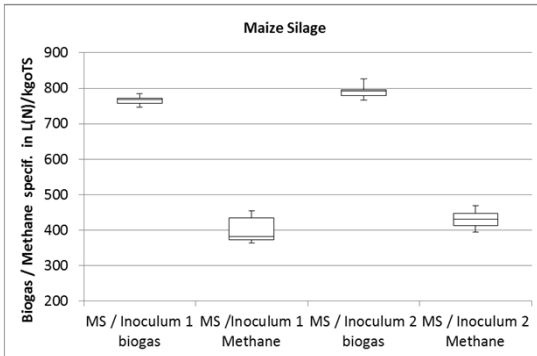
- **2 inocula**
  - In. 1 : sewage sludge / anaerobic wastewater treatment
  - In. 2 : digester sludge / agricultural biogas plant
- **3 different substrates**
  - maize silage
  - poultry manure
  - cellulose
- n=5
- S/I = 0,4 (on VS basis)

parameter	unit	Inoculum 1	Inoculum 2	maize silage	poultry manure	cellulose
TS		2,2	4,8	30,2	78,3	92,1
VS	% OS	1,4	3,3	29,2	45,5	92,0
XA		0,8	1,5	1,0	32,8	--
pH		7,5	7,8	3,6	--	--
VFA/TAC		0,08	0,12	--	--	--

Batch-test-assays  
compare of inocula: blank tests



Batch-test-assays  
maize silage

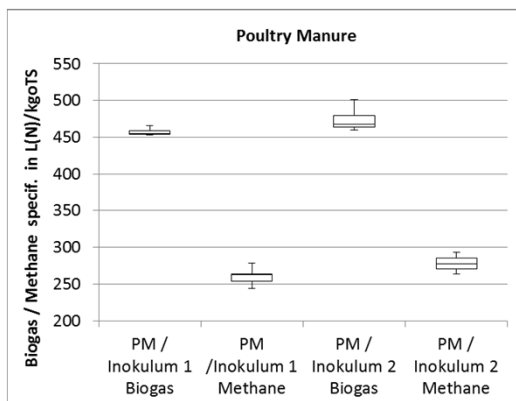


		Inoculum 1	Inoculum 2
mean biogas	L(N) / kg <sub>VS</sub>	770,6	782,0
RSD		2,1%	2,2%
mean methane	L(N) / kg <sub>VS</sub>	399,1	429,6
RSD		8,9%	5,5%

t-test / homogeneity of means

p=	0,493 (biogas)
	0,251 (Methane)

Batch-test-assays  
poultry manure

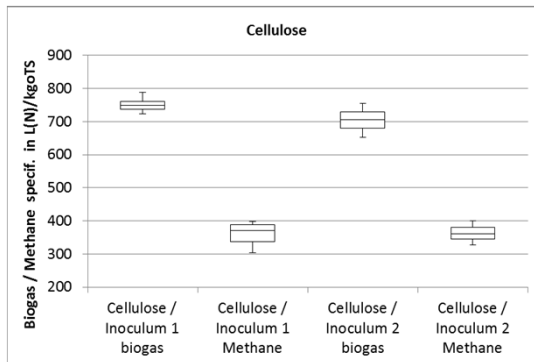


		Inoculum 1	Inoculum 2
mean biogas	L(N) / kg <sub>VS</sub>	457,8	466,4
RSD		0,8%	2,4%
mean methane	L(N) / kg <sub>VS</sub>	257,1	277,1
RSD		3,5%	3,1%

t-test / homogeneity of means

p=	0,171 (biogas)
	0,125 (Methane)

Batch-test-assays  
cellulose



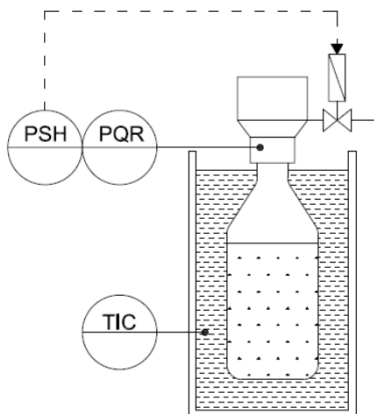
		Inoculum 1	Inoculum 2
mean biogas	L(N) / kg <sub>VS</sub>	747,9	702,2
RSD		2,6%	4,5%
mean methane	L(N) / kg <sub>VS</sub>	363,2	362,6
RSD		6,6%	6,8%

t-test / homogeneity of means

p=	0,105 (biogas)
	0,967 (Methane)

Influence on kinetic parameters

ANKOM Gas Production System



- 500 ml vessels
- Pressure increase in head space is measured
- Maximum pressure of 50 mbar is assured by pressure relief valve
- Changes in ambient pressure are considered automatically
- Temporal resolution: 30 min.

Kinetic tests

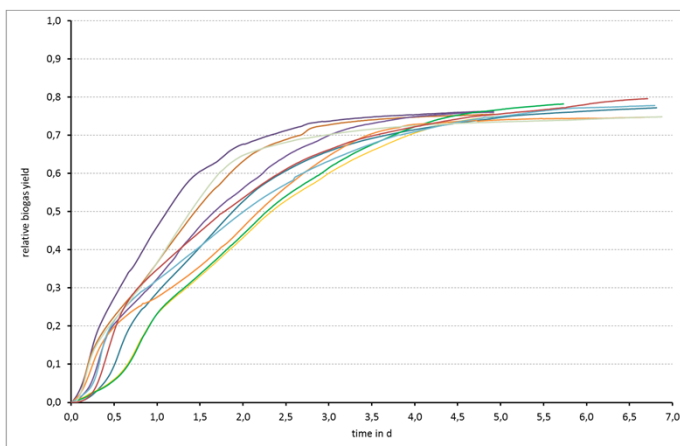
**Synthetic standard substrate (synsub):** mixture of

- Cellulose
  - Starch
  - Glucose
  - Urea
  - Phosphate
- } Fixed mixture: fully degradable, reproducible, free of process inhibitors or promoters, well known maximum biogas yield

Previous research project: synthetic standard substrate was used with incula from > 90 biogas digesters, with the same experimental equipment and protocol:

- n=3
- S/I = 0,3 (VS-based)
- 38 °C
- daily mixing with magnetic stirrer
- relative biogas yield: referred to maximum yield (BUSWELL)

Kinetic tests



kinetic tests: Relative biogas yield of synthetic standard substrate, using inocula from 10 different biogas digesters.

### Batch-Test-Assays

- **Inoculum 1 / 2**: huge difference in properties (TS,VS) and background biogas and methane yield.
- **Maize silage, poultry manure, cellulose**: divergent means of biogas and methane yields between both inocula, however no statistic significance.
- Relative standard deviation was comparatively high in few cases, particularly for methane yields.
- **Overall result**: no statistical evidence was found that origin of inoculum has influenced the test results.

### Kinetic Tests

- origin and type of inoculum have massive influence on kinetic parameters of biogas formation, ultimate biogas yield however is hardly affected.

### Background

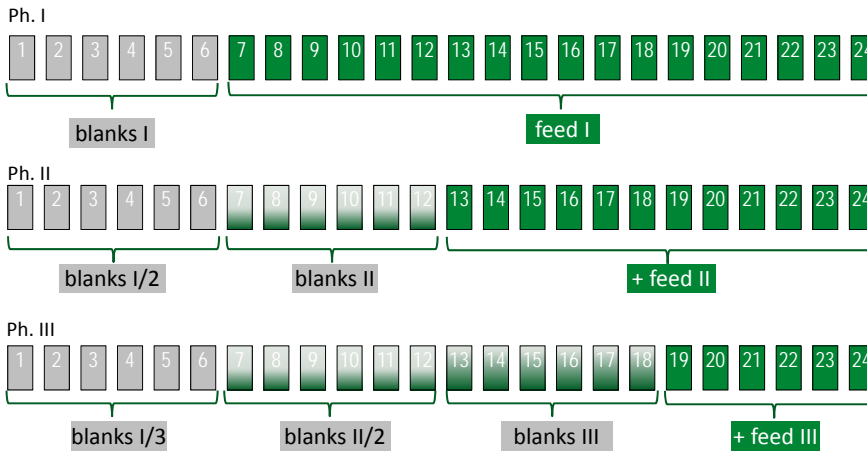
Key assumption of BMP-tests : background biogas production by the inoculum and the gas production from the substrate do not interfere.



Blank tests allow to account for the background biogas and Methane production of inoculum

Experimental works (e.g. HEUWINKEL et. al. 2009) however suggested that the inoculum is generating more biogas in the presence of a substrate than measured from the blank tests. ("priming-effect")

Experimental setup



Experimental setup

Priming-effect would cause misleading measurements due to

- underestimation of the background biogas production by the inoculum
- hence overestimation of specific biogas yield of the substrate



**This effect should gradually decline in a sequenced fed-batch !  
Expected experimental result:**

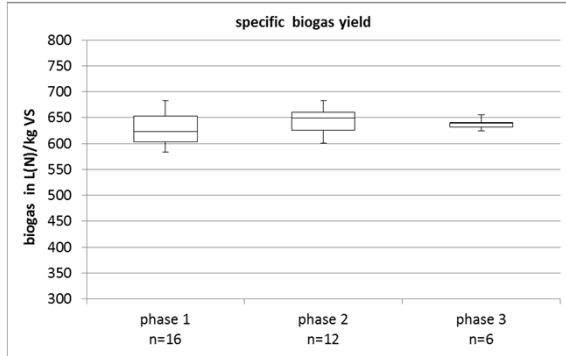


**gradually decrease in specific biogas yield of substrate**



Results

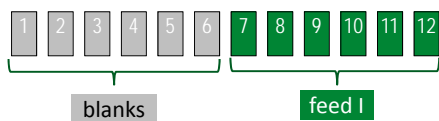
Phase 1 ⇒ Phase 2 ⇒ Phase 3



		phase 1	phase 2	phase 3	ANOVA / homogeneity of means	
mean biogas	L(N) / kg <sub>VS</sub>	626,2	645,6	634,6	test variable	biogas yield
RSD		4,7%	3,0%	1,3%	levels	ph1 / ph2 / h3
					p-value	0,253

Experimental setup (2)

Ph. 1



Ph. 1+2

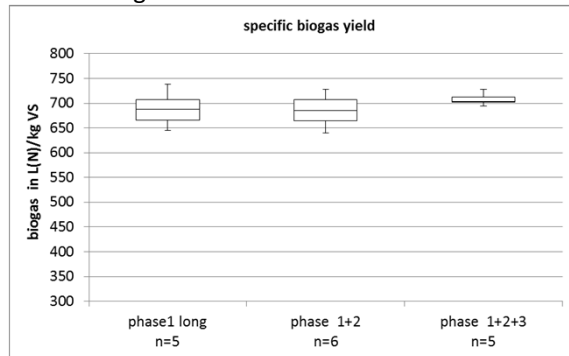


Ph. 1+2+3



## Results

Phase 1 long  $\Rightarrow$  Phase 1+2  $\Rightarrow$  Phase 1+2+3



		phase 1	phase 1+2	phase 1+2+3	ANOVA / homogeneity of means	
mean biogas	L(N) / kg <sub>VS</sub>	685,8	686,5	698,7	test variable	biogas yield
RSD		3,8%	3,9%	2,4%	levels	ph1 / ph 1+2 / ph1+2+3
					p-value	0,773

## Summary

### Batch-test-assays

- Divergent biogas and methane yields for all substrates with both inocula, however results are not statistic significant.
- BMP test results from different labs using different inocula are not necessarily incomparable.
- Kinetic parameters of biogas formation are however massively influenced by type and origin of inoculum.

### Sequenced-fed-batch

- No evidence was found that there is a significant priming-effect.
- Blank tests are an appropriate method to account for the background biogas and Methane production by the inoculum.

Thank you for your kind attention.

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