Deutsches Biomasseforschungszentrum
gemeinnützige GmbH

DBFZ

Analysis in a biogas laboratory
沼气的实验室分析
- parameter, significance, methods -
K. Strach 参数，权重（重要性）和方法




Content

- Introduction 简介
- Characterisization of substrates 基底特性描述
- Anaerobic digestion test 间歇性发酵实验 Batch test 连续性发酵实验
- Parameter: DM, ODM, VFA, Ammonium nitrogen, gas volume, gas composition, trace elements,
- 参数：干物料、有机干物料、缓冲容量、铵态氢 、氮元素、气体体积、气体组成、微量元素

Introduction 简介

Making analysis – but why?
为什么要做实验室分析？

To control and optimize the biogas process

为了控制和优化沼气生产过程

To estimate the suitability of substrates.

为了测试底物的适用性

To avoid inhibition of the process.

为了避免沼气生产过程中的抑制作用

Characterization of substrates 基底特性评价

Different substrates are suitable for anaerobic 适用于厌氧发酵的各类底物：

- Agricultural residues 农业废弃物
- Energy crops 能源作物
- Residues from indursties, biowaste 工业废物，生物废料

The substrates have different properties. With the aid of characterization you can estimate their suitability for the biogas process.

.. 基底原料有不同的特性，通过表征可以判断它们是否适用于沼气的生产

Parameter 参数:

- Dry matter/water content 干物料/水含量
- part of organics 有机物比例
- Combounds: proteines, fats, carbohydrates 组成： 蛋白质、油脂、碳水化合物
- Biogas-/methane potential 沼气/甲烷含量
- macronutrients und micronutrients 大分子养分和小分子养分
- Inhibitors 抑制剂

Characterization of substrates 基底特性评价

Sample taking ..

Some questions must be answered before 采样前须思考:

- Aim and motivation of the investigation 实验的目的和原因
- Derivation of the material 材料的来源
- Expected spectrum of components 物质的预期范围
- Required safety precautions 安全要求

In a protocoll for samle taking must be written 采样报告里须写明:

- Local terms (i. e. container, tubes, heaps) 本地条件（容器、管路、堆垛）
- Description of consistency and homogeneity 浓度和均匀性的描述
- Determination of volume/mass 体积和质量的测量
- Number of samples (mixed sample, total sample) 采样的数量 （混合样品、总样品）
- Method of sample taking 获取样品的方法
- Preservation 保存
- packaging and distribution 包装和分配

Characterization of substrates 基底特性评价

Sample taking 取样

To achieve good values it is necessary to investigate representative samples 为了取得可靠的结果必须获取具有代表性的样品

The parameters must not change by sample taking, conservation or preparation 必须根据样品的采集、保存和制备来确定需要的参数

Influencing factors of a representative sample:
选择具有代表性的样品需要考虑的因素：

- Location: Tubes, tanks, mucks 位置： 管路、容器、废物
- Time: seasons, time of day 时间： 季节、一天中的时间
- Variety of the sample: liquid, solid, homogeneity
  样品的种类：液体、固体、均匀与否

Anaerobic digestion tests 厌氧发酵实验

Batch digestion test according to VDI 4630

间歇式发酵实验用批处理过程（VDI 4630）

To determine the biogas/methane production of substrates or degistates

确定沼气/生物甲烷生产的底物或沼渣

Parameters: dry matter, biogas/methane production

参数：干物料、沼气/生物甲烷生产

Gadgets: reactors, eudiometer (barrier liquid), heating, gas bag, gadget for gas measurement, barometer, thermometer

设备及材料：反应器、量气管（隔离液体）、加热装置、集气袋、气体测量工具、压力计、温度计

barrier liquid 隔离液体:

- Sulfuric acid, Sodiumsulfate, Methyl orange硫酸、硫酸钠、甲基橙

Deionized water 去离子水

Substances 物质:

3 to 6 gVS substrate 基底

250 ml active seeding sludge营养液

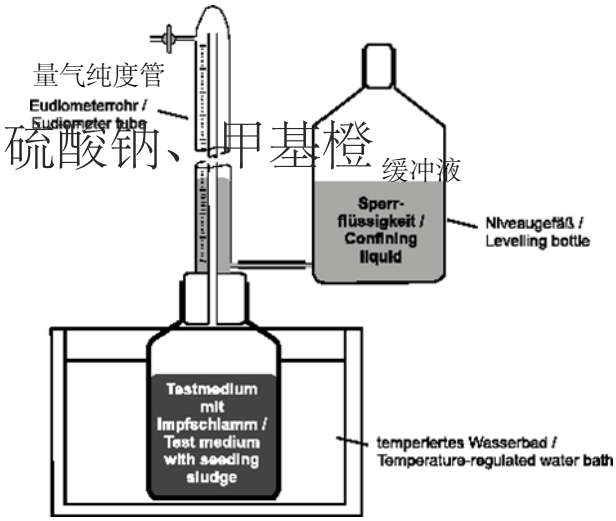

Fig. 1: Batchtest

Anaerobic digestion tests 厌氧发酵测试

Batch digestion test according to VDI 4630
间歇式发酵实验用批处理过程（VDI 4630）

To start 开始:

Every sample is determined three times每个样品要被确定三次:

- a blank sample 空白样品→ only 250 ml seeding sludge只有250 mL营养液
- reference → with 1g micro crystalline cellulose reference substance and 250 ml seeding sludge对照组：250 mL营养液和1 g标准细胞质
- 1 gVS of each sample in a sample bottle and mix this with 250 ml with seeding sludge 每件样品各取1 gVS放入样品瓶内，同250 mL营养液混合
- record the pH-value of each sample记录每个样品的ph值.
- put the sample bottles into a water bath 水浴
- flush the headspace upside the samples with nitrogen用氮气置换样品上部的空间
- quickly connect the eudiometer pipe with the sample bottle 将量气管和样品迅速链接

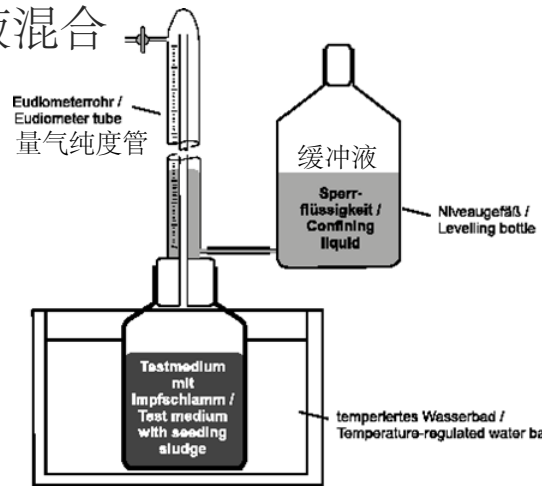

Fig. 2: Batchtest

Anaerobic digestion tests 厌氧发酵测试

Batch digestion test according to VDI 4630
间歇式发酵实验用批处理过程（VDI 4630）

open the plug above at the eudiometer and lift up the supply bottle – this will equal both liquid levels at "0"

打开量气管顶部的塞子，拎起供应瓶，使两者液面同为“0”

Pull the plug when both liquids show "0" 当两者液面同为“0”时，拔下塞子。

Daily 每日

- Meter reading 读表→ liquid level of pipe and supply bottle must be the same管和供应瓶子内的液面必须相同
- Record air pressure, room temperature and bath temperature
  记录大气压强、室温和样品温度

Anaerobic digestion tests 厌氧发酵测试

Batch digestion test according to VDI 4630
间歇式发酵实验用批处理过程（VDI 4630）

Gas analysis 气体分析

- When the gas volume has almost entirely replaced the liquid in the eudiometer pipe 当气体完全置换了量气管中的液体时
- Connect a gas bag with the pipe → open supply line 将气球安装在接口
- open the plug above at the eudiometer and lift up the supply bottle –this will equal both liquid levels at "0"
  打开量气管顶部的塞子，拎起供应瓶，使两者液面同为“0”
- Pull the plug when both liquids show "0"
  当两者液面同为“0”时，拔下塞子。
- Close supply line 关闭供应线
- Gas measurement from the gas bag从气囊中测量气体

eudiometer 是一个测量气体体积变化的装置，以上部分是具体使用方法的描述。

Anaerobic digestion tests 厌氧发酵测试

Batch digestion test according to VDI 4630

间歇式发酵实验用批处理过程（VDI 4630）

（沼气一直在产出，每天都会有一个日产量，最开始的时候沼气池能产生很多气体，总产量也会越来越多。当日产量达到目前总产量1%的时候说明沼气池能够产出的气体很少，沼气池已经没有以前产气多，需要对沼气生产重新调整）

Finish完成:

abort criterion reached → when the daily biogas rate is equivalent to only 1 % of the total volume of biogas produced up to that time

中止—— 当沼气的日产率达到总产量的1%的时候

measure again the gas composition and the pH-value after disconnecting the eudiometer 移除量气管之后再次测量气体组成和ph值

Evaluation评价:

calculate the normal volume of the fermentation gas

计算出发酵气体的正常体积

obtained the dry gas volume得到的干气体积

pu	ambient pressure [kPa] 大气压力
Tu	temperature of the fermentation gas [° C] 发酵温度（一般高于室温）
VGas	gas volume [ml] 气体体积

$$V_N[ml] = \frac{\left(pu[kPa] - 10^{\overbrace{7{,}19621 - \frac{1730{,}63}{233{,}426 + Tu[°C]}}^{[kPa]}} \cdot 273{,}15K\right)}{101{,}325kPa \cdot (273{,}15 + Tu[°C])K} \cdot V_{Gas}[ml]$$

（kPa是压力的单位， K是温度的单位）

Anaerobic digestion tests 厌氧发酵测试

Continuous test according to VDI 4630
连续发酵实验（VDI 4630）

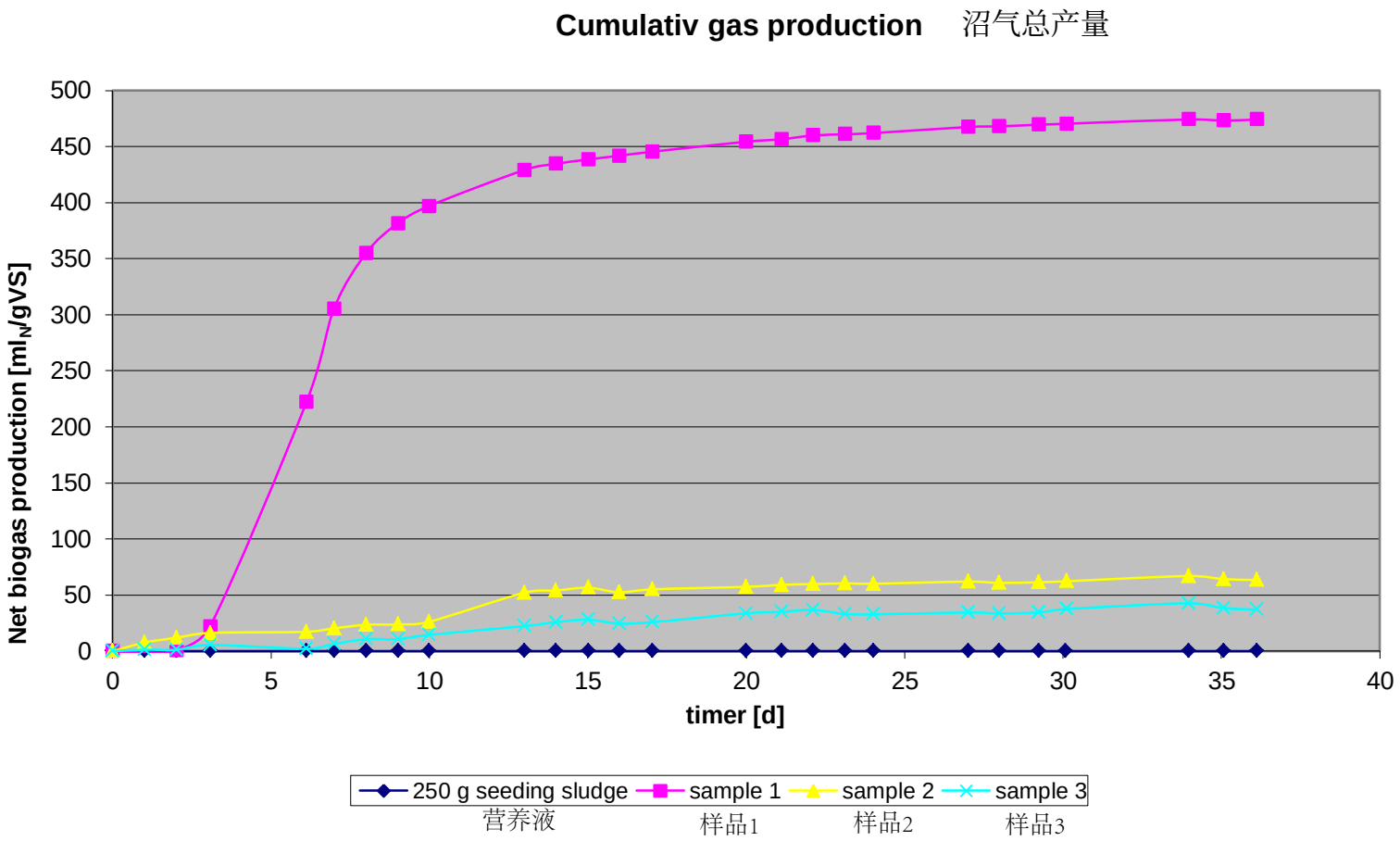

Fig. 3: Typical gas formation curves

Anaerobic digestion tests 厌氧发酵测试

Continuous test according to VDI 4630
连续发酵实验 （VDI 4630）

Correction of biogas composition沼气组分的修正

$$c_{CH4korr.}[Vol-\%] = 100[Vol-\%] \cdot \frac{c_{CH4}[Vol-\%]}{c_{CH4}[Vol-\%] + c_{CO2}[Vol-\%] + c_{H2S}[Vol-\%]}$$

c_{CH4} concentration of methane甲烷浓度 [vol-%]

c_{CO2} concentration of carbon dioxide二氧化碳[vol-%]

c_{H2S} concentration of hydrogen suphide硫化氢 [vol-%]

The net gas normal volume of the substrate is obtained as the difference between the normal volume of the dry gas in the test less the normal volume of the dry gas from the seeding sludge.
原料的标准产气潜力量为测试获得的标准干气总体积减去接种物的标准干气体积

Parameter 参数

pH value值

pH optimum for methanogenic archea 6,5 – 8 产甲烷菌的最适宜ph是6.5-8

Dimension: -

Gadgets设备及材料：pH electrode, pH meter, buffer solution (pH 4/7), deionized water, 3M KCl ph电极，ph计，缓冲溶液（控制ph的），去离子水， 3 mol/L 氯化钾

How to方法:

- Clean the electrode from KCl solution 用氯化钾溶液清洗电极
- Put the elektrode into the sample 将电极放入样品
- Get the value 读数
- Clean the electrode 清洗电极
- Put it back in KCl solution 将电极放回氯化钾溶液

Calibration before use - weekly每周都要校准电极

Source of pictue: ats online shop

Parameter 参数

pH value值

pH value depends on　ph值取决于

- Temperature　温度
- Oxygen　氧气含量（因为是厌氧细菌）

→ Measurement immediately after sample taking　提取样品之后马上测量ph

→ Not much stirring　不需要搅拌

Samples with high dry matter: 当样品中有很干燥的物质

- Dissolve 10 g of the sample in 100 ml of water　把10g这样的样品放入100mL水中
- Shake it for ten minutes　搅拌十分钟

Parameter 参数

Dry matter 干物料

Dry matter干物料 (or total solids或干质含量 –TS)

Concentration of solids in the sample 样品中干物料的浓度

Includes organic substances 有机物

Influences the technology of the biogas plant影响沼气厂的技术：：

Dry Matter 干物料	Substrates基底	Technology 技术
Low低 < 6% 干物料小于6%时相当于废水	wastewater废水	Upstream anaerobic sludge blanket reactor, fixed bed reactor
Medium中 < 30%	Manure, co-fermentation of energy crops 粪污、能源作物的共同发酵	Wet digestion: CSTR 湿法发酵
High高 > 30%	Energy crops, biowaste 能源作物、生物废料	Dry digestion: plug flow reactor 干法发酵

Parameter 参数

Dry matter 干物料

Dimension单位 %

Method方法: DIN 12880 – drying at 105° C until constant weight 在105度干燥，直到重量不再减少

Gadgets设备及材料: Drying cupboard, scales, crucibles, tongs, desiccator
具有干燥功能的储物室 刻度尺 坩埚 钳子 干燥器

How to: put 5 -10g of the sample in a crucible → in the drying cupboard 方法：将5到10克样品放入坩埚，接着放入储物室 for 24h 24小时

Calculation计算:

$$TS = 100 \cdot \frac{m_3 - m_1}{m_2 - m_1}$$

TS	dry matter干物料 [%]
m1	Mass of the empty crucible空坩埚质量 [g]
m2	Mass of the crucible 坩埚质量+ mass of the sample样品质量 [g]
m3	Mass of the crucible after drying干燥后坩埚质量 [g]

Parameter 参数

Organic dry matter有机干物料

Organic dry matter (or volatile solids – VS)（VS= 可挥发性的固体物质，主要指有机酸或者脂肪酸）

Concentration of organic substances related to the dry matter (TS干物料)

（TS=trockene Substanzen 干物料)

To calculate the specific gas production

沼气日产量的计算

specific gas production, specific 是指单位时间或者单位距离或者单位热量，这里可能是时间单位日

Typical values参考值:

Energy crops能源作物	90 – 95 %TS
Manures粪污	57 – 80 %TS
Biowaste生物废料	40 – 78 %TS

Parameter 参数

Organic dry matter有机干物料

Dimension单位: %TS

Method方法: DIN 12879 – annealing at 220° C for 30 min and at 550° C for 2 h 2小时

设备及材料: annealing furnace, scales, crucibles, tongs, desiccator
退火炉 刻度尺 坩埚 钳子 干燥器

How to: after drying put the sample in the annealing furnace → start
方法: 干燥后将样品放入退火炉中——开始

programm

Calculation计算:

$$VS = 100 \cdot \frac{m_3 - m_4}{(m_3 - m_1)}$$

VS	volatile solid [%TS] 挥发性固体物质
m1	Mass of the empty crucible空坩埚质量 [g]
m2	Mass of the crucible 坩埚质量+ mass of the sample 样品质量[g]
m3	Mass of the crucible after drying 干燥后坩埚质量 [g]
m4	Mass of the crucible after annealing 退火后坩埚质量[g]

Parameter 参数

Volatile organic acids挥发性有机酸 (VOA)

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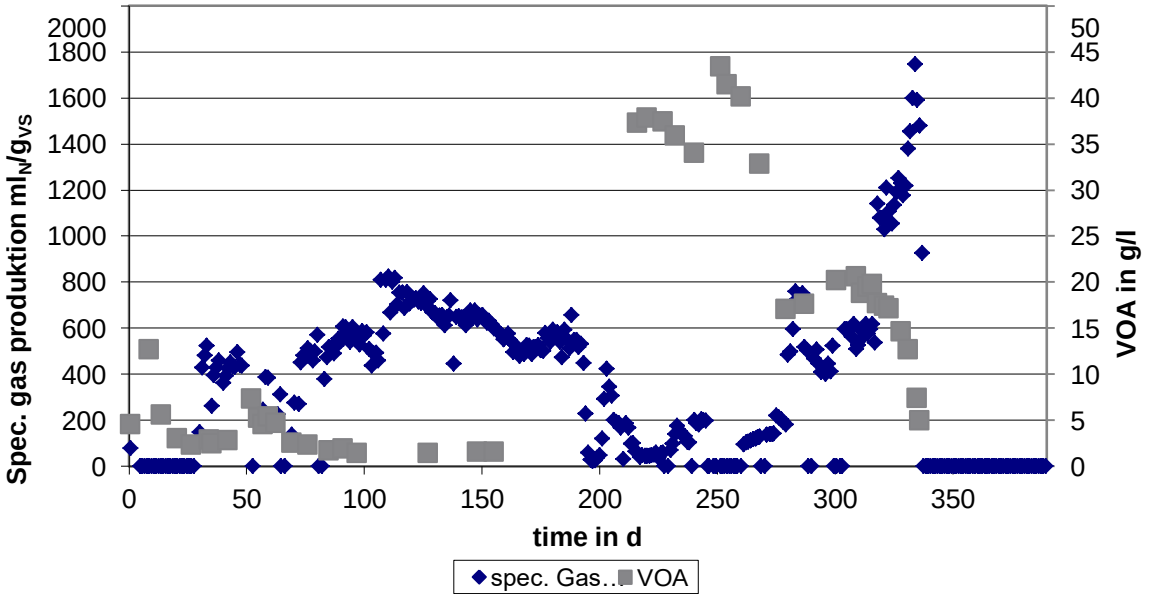
VOA and VOA/TAC

- Shows the stability of the biogas process显示沼气过程的稳定性
- VOA – sum parameter of volatile fatty acids
  VS的主要参数——可挥发性有机酸的重量
- VOA/TAC – dimension of the buffering capacity缓冲能力（保持ph稳定的能力)
  ≤ 0,3 gVOA/gCaCO3



Parameter 参数

Volatile organic acids挥发性有机酸 (VOA)

Dimension: VOA in g/l, VOA/TAC in gVOA/gCaCO3

Method方法: VOA according to Kapp, VOA/TAC according to FAL

- samples must be centrifuged for 25 min. at 20 000 xg
  样品须经离心25分钟
- the liquid phase for titration to pH values 5,0, 4,4, 4,3 and 4,0
  滴定液体至PH值5.0、4.4、4.3和4.0

Gadgets设备及材料:burette, beaker, magnetic stirrer, stirring bar, pH meter with electrode
滴定管、烧杯、磁力搅拌器、搅拌棒、pH计电极

Substances物质: sulfuric acid, deionized water硫酸、去离子水

Parameter 参数

Volatile organic acids 挥发性有机酸(VOA)

How to方法:

- samples must be centrifuged for 25 min. at 20 000 xg 样品须经离心25分钟
- fill the burette with滴定管中滴入0,2 N H2SO4
- 20 ml centrifugate will be saved by a 20ml pipette and given into a 100ml beaker
  离心20毫升, 用移液枪将20毫升液体转移到100毫升的烧杯中
- Fix the pH-electrode at the support rod
  将PH电极固定在支撑棒上
- fix stirrer between 100 to 250 revs/min
  将搅拌棒设定为每分钟100至250转之间
- Drop by drop you will now add the 0,2 N H2SO4 until pH values 5,0, 4,4, 4,3 and 4,0
  一滴一滴添加0.2M H2SO4直到PH值达到5.0, 4.4, 4.3和4.0
- Write down the single needs of acid cumulative记录每个阶段所用酸的用量

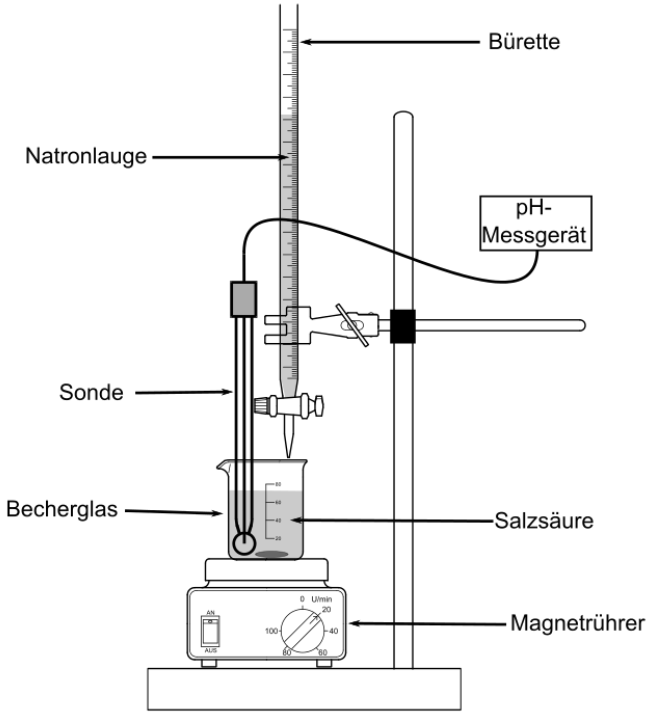


Fig 4.: Gadget for Titration

Lit.: Wikipedia – Sä ure- Base - Titration

Parameter 参数

Volatile organic acids 挥发性有机酸(VOA)

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Evaluation 评价VOA:

$$VOA = 131340 \cdot (V_{pH4,00} - V_{pH5,00}) \cdot \frac{c_{H2SO4} \cdot 2}{V_{Probe}} - 3{,}08 \cdot \overbrace{V_{pH4,30} \cdot \frac{N_{H2SO4}}{V_{Probe}} \cdot 1000}^{Ks_{4,3}\ [mmol/l]} - 10,$$

VOA	Concentration of valtile organic acids [mg/l] 可挥发性有机酸的浓度
VpH4,00	Volume of acid up to pH酸体积 = 4,00 [ml]
VpH4,30	Volume of acid up to pH酸体积 = 4,30 [ml]
VpH5,00	Volume of acid up to pH酸体积 = 5,00 [ml]
VProbe	Volume of the sample样品体积 [ml]
cH2SO4	Concentration of the acid酸浓度 [mol/l]
Ks4,3	Alcalinity 碱度4,3 [mmol/l] (Normenausschuss Wasserwesen; DIN 38409-7; 2005; Beuth Verlag)

Parameter 参数

Volatile organic acids 挥发性有机酸(VOA)

Evaluation VOA/TAC:

$$VOA/TAC = \frac{((V_{pH4,4} - V_{pH5,0}) \cdot \frac{20}{V_{Probe}} \cdot \frac{N_{acid}}{0,1} \cdot 1,66 - 0,15) \cdot 500 \cdot V_{Probe}}{0,5 \cdot N_{Acid} \cdot V_{pH5,0} \cdot M_{CaCO3} \cdot 1000}$$

VOA/TAC	relation between volatile fatty acid and buffering capacity related to Calziumcartbonat [gVOA/gCaCO3] 可挥发性脂肪酸（有机酸）与缓冲能力的关系
VpH4,4	Volume of acid up to pH酸体积 = 4,00 [ml]
VpH5,0	Volume of acid up to pH 酸体积= 5,00 [ml
VProbe	Volume of the sample样品体积 [ml]
NAcid	Normality of the Acid [mol/l]
MCaCO3	Molar mass of calcium carbonate碳酸钙的摩尔质量 [g/mol]

Parameter 参数

Gas composition气体组成

Important parameter with regard to重要参数

- Use it in a combined heat or power unit在热电联产机组中使用
- Stability of the biogas process沼气过程中的稳定性

Biogas: CH4, CO2, trace gases: O2, H2S, H2, N2, CO, NH3

沼气的主要成分，甲烷和二氧化碳。 少量成分：氧气，硫化氢，氢气，氮气，一氧化碳，氨气

Depends on the components of the substrate, additives, technology

根据底物的成分，添加剂的种类和设备

Components of substrate基底成分	Theoretical composition理论组成 CH4	CO2
Carbohydrates	50%	50%
Fats	72%	28%
Proteines	60%	40%

Literature: VDI 4630

Parameter 参数

Gas composition气体组成

Measurement of gas quality 沼气质量好坏的测量

- Mobile gadgets （随身的、手持的）便利的装置
- Fix installed analyzers 内置检测器
- Gas chromatography 气相色谱

Fig. 5: Mobile gadgets

Fig. 6: fix installed gadget

Parameter 参数

Nutrients营养成分

Elementary composition: 元素组成

Gives evidence to 确保

- Macronutrient supply (C:N-ratio, sulfur, phosphor)
  确保氮含量，硫，磷
- Absence of micronutrients (Fe, Ni, Co, Mo, W, Se…)
- Inhibition because of heavy metals (Pb, Hg, Cd, Cr…)
  重金属抑制剂（铅，汞，镉，铬）

	Recommended concentration*	Grain stillage	Jatropha press cake**	Sugar beet	Maize silage	Rye corn	Cow slurry
Nickel	**4–30**	0.90	<3.00	1.41	2.67	2.13	7.60
Cobalt	**0.4–10**	0.20	<3.00	0.04	0.01	n.d.	1.88
Molybdenum	**0.05–16**	0.80	0.35	0.55	1.09	1.4	2.79
Iron	**750–5000**	70.00	280.00	49.40	87.20	64.97	1072.00
Manganese	**100–1500**	54.00	87.00	28.00	20.40	23.47	337.00
Copper	**10–80**	7.90	27.00	4.07	4.67	4.04	63.20
Selenium	**0.05–4**	n.a.	0.31	1.37	2.02	1.50	0.63
Tungsten	**0.1–30**	n.a.	<0.34	n.d.	n.d.	n.d.	0.40
Zinc	**30–400**	60.00	64.00	n.a.	n.a.	n.a.	319.00

n.d.: not detected; n.a.: not analyzed
* According to (Oechsner et al. 2008; EP EP 1 997 901 A2)
** According to (Schmidt 2011, Waste Manag Res. 2011 Nov;29(11):1171-6.)

Parameter 参数

Ammonium 氨NH4

氨——在分解含氮的物质时产生氨

Ammonia → is built by degradation of substances that contain nitrogen

Ammonium → Ammonia dissociated in water

铵离子——氨溶解于水中产生铵离子

$$NH_3 + H^+ \leftrightarrows NH_4^+$$

Equilibrium depends on pH value

and temperature

溶解平衡取决于ph和温度

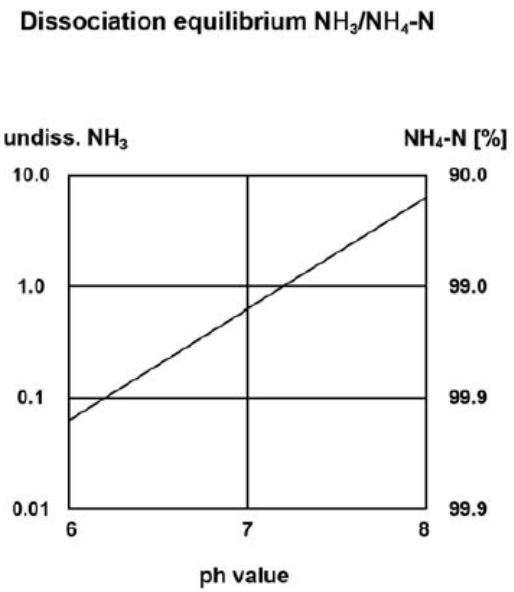


Fig. 7: Equlibrium of NH4/NH3 depending of temperature

Literature: FNR, Guide to Biogas – from podution to use, 2012

Parameter 参数

Ammonium氨 NH4

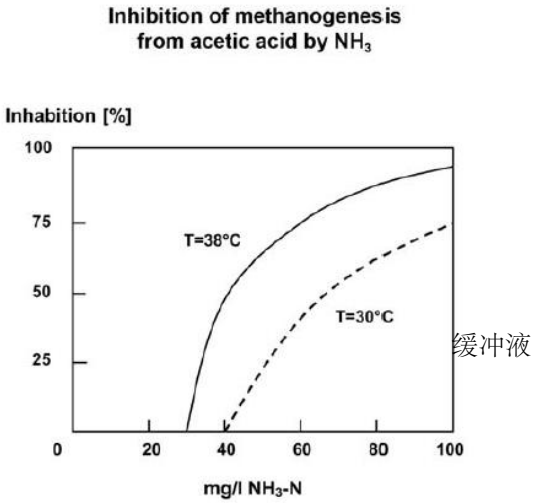
Nitrogen氮:

- is necessary for building cell structure 氮是细胞生长所必须的元素
- a necessary nutrient必需的营养物质
- can cause inhibition effects on methanogenesis

  过高浓度的氨或者铵离子会抑制产甲烷菌的细胞生长

Photometric methods: 光学测量

- According to Neßler （测量方法的发明者）
- Indophenol blue method （测量方法的名字）



缓冲液

Fig. 8: Effect of inhibition of NH4/NH3 depending of temperature

Literature: FNR, Guide to Biogas – from podution to use, 2012

Parameter 参数

Elution洗提

to dissolve away substances out of the sample with deionized water用去离子水溶解掉样品中的物质

Used when用于以下情况

- the sample has a high TS样品干物料高
- Photometric method 光学测量
- HPLC 高效液相色谱法

How to方法:

- put 10% of the water volume in a flask (20 g sample + 200 ml deionized water)10%的水放入烧瓶（20g样品+200ml去离子水）
- Shake it for 24h in the overhead shaker晃动24h
- Filter and use the eluent for analyzes过滤，并用洗脱液分析

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Biogas process