7. Conference contributions

Application of a lab-scale multiple fermenter system

Poster presentation

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Abstract

Here we show a relatively easy and cheap application of an anaerobic fermenter system in lab-scale where six 0.25-litre fermenters can be operated anaerobically simultaneously (Figure 1). They are connected to a water bath with a pumping and heating unit that maintains the temperature of every fermenter via a water jacket constant. The gas quantification was done by water displacement via a gas lock bottle which simulates an open fermentation system where only marginal overpressure might be built up. Gas-tight butyl rubber stoppers applied to the fermenters allow an easy gas sampling. Additionally a port for liquid sampling and feeding is applied to every fermenter too.

One of the main reasons in building up this fermenter system was that due to the long incubation periods usually only few parallels can be investigated. Our fermenter system enables to run six parallels at the same time so that uncertainties due to the individual variations are reduced. Based on this system even a higher number of parallels could be applied.

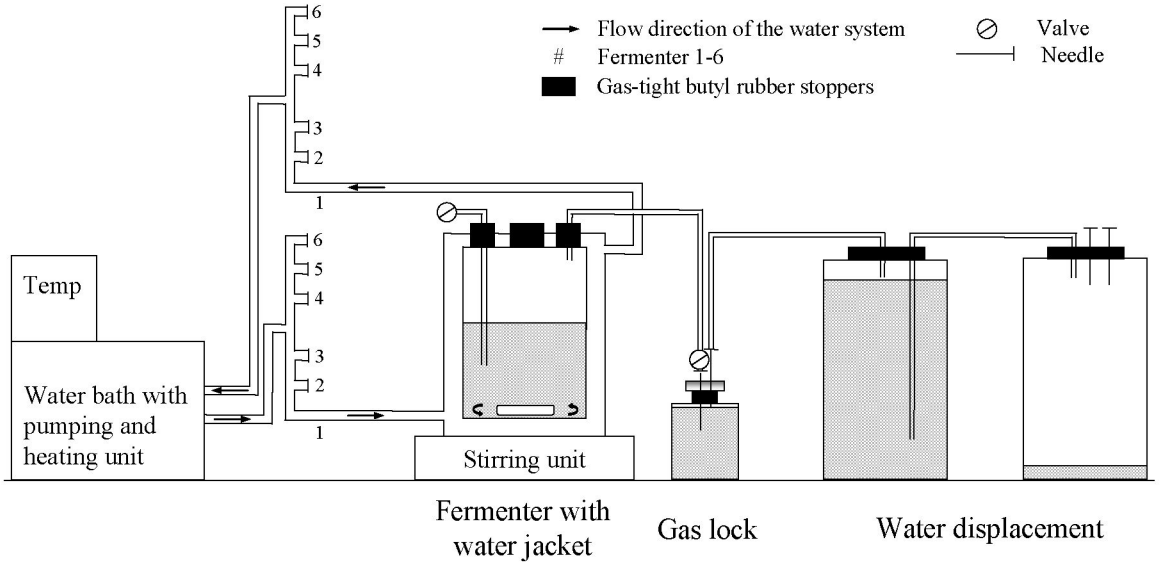

Figure 1: Schematic assembly of the presented multiple fermenter system where a representative of six fermenters is shown in detail.

This fermenter system was used for several experiments with microorganisms isolated out of the fermenter sludge of a 750,000-litre anaerobic reactor. Among others the mode of operation, the effects of stirring and the addition of different buffers were investigated. Within the poster we are going to present the results of these investigations where especially the stirring and addition of buffers showed significant influence on the methane and total biogas production rate.

Effects of Agitation on Methanogenesis during Anaerobic Digestion

Poster presentation

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Abstract

Background:

The anaerobic digestion of organic waste has gained increasing popularity during the last few decades. Its main advantage to conventional composting is the lucrative production of biogas (methane, CH_4), which can be used as motor fuel, to feed to the public gas distribution system, or to produce electricity or heat. Besides, there are also some drawbacks like high maintenance costs, low process stability and high complexity. Thus, it is important to recognize process failures as soon as possible, which should be assured by an adequate monitoring of several process parameters. However, a fundamental knowledge of the physiology of the relevant microorganisms involved is still lacking. The mode of mixing and agitation is an important parameter during the digestion process. Therefore, the effects of different agitation speeds of the incubation bottles were investigated on the production of CH_4 and its intermediates.

Methods:

The media were prepared anaerobically and fermenter sludge of a thermophilic anaerobic plug-flow reactor located in Roppen (Tyrol) with an operating volume of 750 m^3 was used for inoculation. The main soluble intermediates were determined with HPLC, the gaseous compounds of the biogas were analyzed with GC, and the gas production was determined via a manometer. The variations were run in quintuplicates for 77 days.

Results:

The variation without agitation showed a significant lower methane production than agitation with 150 rpm, although a further increase inhibited methanogenesis, assumably due to disaggregating microbial communities. Interestingly, after the first day of incubation, the H_2 production increased with increasing the agitation. That is directed to the microbial activity, since an abiotic effect due to an enhanced evolution of the produced gases through agitation could be discriminated.

Conclusion:

The results that will be presented help to better understand the still so-called “Black Box” of anaerobic digestion of organic waste.

Factors affecting methane production from biogas plant sludge

Oral presentation

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Abstract

The complete anaerobic degradation of organic waste is a complex process where several trophic levels are involved. In the first hydrolytic step polymers are cleaved to monomers. These are further degraded to short organic acids like acetate, and H_2 and CO_2, which are the main substrates for methane-producing archaea [1]. Therefore, a well working degradation process requires a reduction of the intermediates in each trophic level. The accumulation of a single intermediate might lead to an end product inhibition because of thermodynamic unfeasible degradation pathway(s), and subsequently to a digestion failure accompanied by a stop in the lucrative production of biogas [2].

Several important process parameters have to be monitored during anaerobic digestion including the pH value, volatile fatty acid (VFA) concentrations, gas quality and quantity. Furthermore, also the concentration of ammonium/ammonia, the organic loading rate and hydraulic retention time are of interest. On the other side, the temperature and the mode of stirring and mixing are generally kept constant. Among these parameters the pH value plays a central role because it affects the process directly and indirectly. Directly, because in the first step of the anaerobic degradation hydrolytic microorganisms favour an acidic pH but methanogens at the end of the degradation cascade prefer a neutral up to slightly alkaline pH. In a well working digester both reactions run concurrently and thus no significant accumulations occur. Indirectly, because the pH changes the ratio of the dissociated and undissociated species of a VFA and NH_4^+/NH_3, which might have toxic effects on the microorganisms because their dissociated species are able to penetrate the membrane and cause irreversible damages like changing the intracellular pH [3].

The temperature influences several parameters significantly, and it is known that a thermophilic digestion is more vulnerable to digestion failures than a mesophilic digestion, however, the theoretical methane output is higher during thermophilic treatments [4;5].

The concentrations of VFA are very important because they represent like H_2 the bottleneck of the complete cascade. Elevated concentrations might be an indicator for a stressed process or an already starting process failure. Usually the easiest although unsatisfying state of the art is to reduce the organic load, which might be followed by the addition of bicarbonate to buffer the system. The latter is the case because an accumulation of VFAs generally has the additional drawback in the acidification of the system, and thus the pH drops below the favoured range for methanogenesis [6].

Lately another strategy to combat a process failure was published [2] where an acetate-degrading enrichment culture was added to a digester with highly accumulated VFAs, and within a few days the digester stabilised and showed a distinct VFA reduction and methane production.

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Effects of different antibiotics and BES on the methane production from volatile fatty acids

Poster presentation

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Abstract

The complete anaerobic degradation of organic waste requires a well working cascade of different trophic levels. Complex substrates are degraded to monomers, which are further degraded to short organic acids like acetate, and H_2 and CO_2. These intermediates are the main substrates for methane-producing archaea. Within a biogas plant sludge a multitude of different microorganisms are present, which are only cultivable under difficult and laborious circumstances. To gain insight into their complex interactions the effects of several antibiotics on the degradation of volatile fatty acids were investigated. Lately, the influences of antibiotics on the methanization process received increased attention especially from cow and pig manure.

In the present study eleven antibiotics were investigated: chloramphenicol, penicillin G, tetracycline, streptomycin sulfate, neomycin sulfate, rifampicin, kanamycin sulfate, ampicillin, gentamicin sulfate, bacitracin, D-cycloserine. In addition controls were run where distilled water and ethanol (because of alcohol soluble antibiotics) were supplemented instead of antibiotic stock solutions. During the incubation for 77 days the biogas production and the gas quality was monitored. At day 77 the samples were investigated concerning the removal efficiency of short volatile fatty acids (formate, acetate, propionate and butyrate; each initially added at 10 mM). As inoculum 1:5 diluted fermenter sludge of a thermophilic biogas plant with an operating volume of 750,000 litre was used.

The samples where chloramphenicol and tetracycline were added showed a distinct initial methane production and a high accumulation of acetate (>100 mM) followed by a drop in pH to about 5.5. However, this was directed to the degradation of ethanol (alcohol soluble antibiotics) because at the controls similar courses were observed. Afterwards no remarkable methane production was detectable.

The supplementation of the water soluble antibiotics led to a lower methane production compared to the controls. The most distinct inhibition of the methanogenesis could be observed by streptomycin, gentamicin and neomycin sulfate, which act as inhibitors of the protein synthesis.

In addition, the effects of different concentrations of 2-Bromo-1-ethanesulfonic acid (BES), which is a coenzyme M analogue and thus inhibits the last step of methanogenesis, was determined and compared with the antibiotics.

Adapted Inocula as Tool for Combating Heavy Acetate Loads during Start-up of an Anaerobic Digestion

Poster presentation

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Abstract

Background:

The anaerobic digestion of organic waste involves different trophic levels for a complete degradation and subsequent biogas production. In the last step strict anaerobic methanogens produce methane either by an acetoclastic or hydrogenotrophic pathway. The degradation of acetate presents a bottleneck of a digestion, mostly dependent on the capacity (abundance and activity) of key microorganisms. Based upon results of a full-scale anaerobic digester for several years, it was detected that high acetate concentrations were detrimental to the overall digestion performance especially during the start-up. Therefore, the aim of the investigation was to adapt and enrich microorganisms to combat this problem in lab-scale.

Methods:

The medium was prepared anaerobically and fermenter sludge of a thermophilic anaerobic plug-flow reactor located in Roppen (Tyrol) with an operating volume of 750 m^3 was used for inoculation and enrichments. The main soluble intermediates were determined with HPLC, the gaseous compounds of the biogas were analyzed with GC, and the gas production was determined via a manometer. Real-time PCR was performed for relevant methanogenic groups. The engaged microorganisms were directly inoculated into a nutrient solution containing 150 mM acetate for 1, 2, 4 or 6 weeks, or a stepwise adaptation to increasing acetate concentrations (50, 100 and 150 mM) took place. The subsequent evaluation was run in triplicates for 77 days.

Results:

The stepwise adaptation for 4 and 6 weeks resulted in inocula, leading to a significant improved start-up compared to controls and shock variants. Confirmed by molecular approaches dominant methanogens were *Methanoculleus* sp. and *Methanosarcina* sp. Dominant bacteria were different representatives of *Clostridium* sp., which might have had a crucial role in acetate degradation.

Conclusion:

These results point to the possibility to facilitate the start-up under high acetate concentrations during anaerobic digestion by addition of specific adapted inocula.

Monitoring microbial communities and process parameters affected by antibiotics and BES during thermophilic anaerobic digestion

Poster presentation

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Abstract

The anaerobic digestion represents an economical and ecological reasonable way for treating organic waste; however, the overall performance of biogas production processes still needs to be optimized. Furthermore, the so-called 'black-box' system anaerobic digestion involves several trophic levels, which include up to hundreds of different microorganisms, whereof most are not detectable[1]. Nevertheless, efforts are made to get knowledge of the key microorganisms mainly carrying the whole process[2,3]. Because of microbial interactions such as syntrophy or competition, the complex community is inevitably vulnerable towards deteriorated conditions, potentially leading to accumulation of intermediates if a single key organism is inhibited. Thus, it results in a suboptimal process or even complete digestion failure.

In the present study diluted fermenter sludge of a biogas plant with a 900,000-litre reactor was used as inoculum. In preliminary experiments the inoculum was screened on its susceptibility to separately applied antibiotics, which were compared with the methanogenic inhibitor 2-bromethanesulfonic acid (BES). Based on the volatile fatty acid degradation course and methane production, only the protein synthesis interfering antibiotics, i.e. neomycin, gentamicin and rifampicin, showed auspicious effects. Therefore, a closer look was drawn to these three antibiotics during the degradation of acetic, propionic and butyric acid separately. With denaturing gradient gelelectrophoresis gels the effects on the microbial community was followed and further investigated towards sequencing approaches. Interestingly, all three antibiotics inhibited the degradation of propionic and butyric acid, whereas only neomycin and BES inhibited the degradation, and thus methanogenesis, from acetic acid.

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Conference contributions as a co-author

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Wagner, A.O., **Lins, P.**, Reitschuler, C. & Illmer, P. (2011). Effect of different acetate:propionate ratios (0.25-4) on the methane production of a batch culture. 1st International Conference on Biogas Microbiology, Leipzig (Germany), 14.09.-16.09.11, poster presentation.

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Reitschuler, C., **Lins, P.** & Illmer, P. (2014). Cultivability of Unusual and Undescribed Non-Extremophilic Archaea from Alpine Moonmilk. 9th International Symposium on Subsurface Microbiology (ISSM 2014), Monterey (USA), 05.10.-10.10.14, poster presentation.