

^{*1} This research is part of the Flexigas project (www.flexigas.nl).

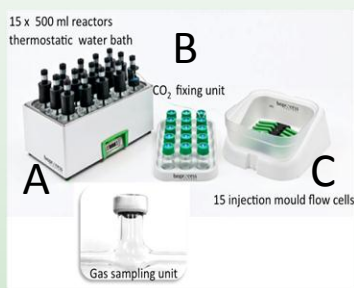
In addition, Gert Hofstede is supported by a Hanze educational grant (HG Opleidingsfonds).

Introduction:

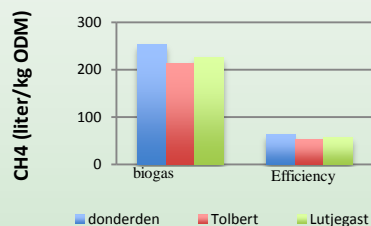
In the field 'renewable energy resources' is formation of biogas an important option. Biogas can be produced from biomass in a multistep process called anaerobic digestion (AD) and is usually performed in large digesters. Anaerobic digestion of biomass is mediated by various groups of microorganisms, which live in complex community structures. However, there is still limited knowledge on the relationships between the type of biomass and operational process parameters. This relates to the changes within the microbial community structure and the resulting overall biogas production efficiency. Opening this microbial black box could lead to a better understanding of on-going microbial processes, resulting in higher biogas yields and overall process efficiencies.

Strategy:

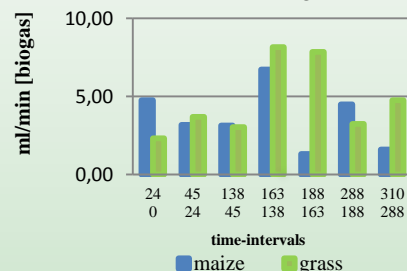
We have studied the effect of different types of digestate on the biogas production of maize-silage (total amount and production rate) -figure (2)-, using the Automated Methane Production Test System II (AMPTSII) -figure (1)-. Biogas production efficiencies of two types of biomass (maize-silage and grass) were also measured with the same, standard type of digestate as inoculum -figure (3)-. The continuous production of biogas from maize-silage was studied in two Infors bioreactors (10 litres), which simulate farm-scale type of digesters -figure 4-. Different stirring regimes (50 and 150 rpm) demonstrated that biogas production was affected -figure 5-, probably due to an effect on the microbial community -figure 6-.

**Figure 1: AMPTSII for biomethane potential assays.**

The AMPTSII can be divided into three units: A, B and C. Unit A is the sample incubation unit, with controlled stirring possibilities and a thermostatic water bath. The gas released from unit A is measured using a wet flow-measuring device with a multi-flow cell arrangement. This measuring device works according to the principles of liquid displacement; a digital pulse is generated when a defined volume of gas flows through the unit.

Biogas production from maize biomass with different digestates**Figure 2**

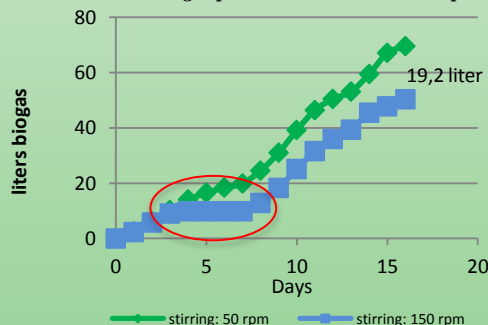
Conclusion: There is no significant difference between the biogas production with the digestates from Donderden, Tolbert and Lutjegast (ANOVA, one-tail, $p=0.05$).

Biogas from different biomasses maize and grass**Figure 3**

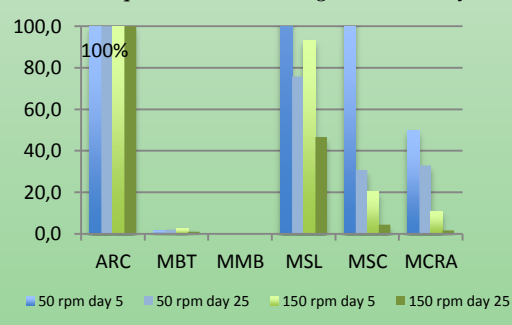
Conclusion: Maize degraded more rapidly than grass does. It seems that maize is easier to break down through microorganisms present in the digestate.

**Figure 4: Two Infors 13 liter Lab-scale Bioreactors:**

Both bioreactors are a continuous stirring type reactor (CSTR) with a reactor volume of 10 litres. The bioreactors are fed on daily basis with ($\approx 7\%$ ODM maize slurry) with an OLR of $1,25 \text{ kg ODM} \cdot \text{m}^{-3} \cdot \text{day}^{-1}$. The hydraulic retention time was kept at 40 days. The default process temperature was 35°C . The content of the reactor was stirred at 50 rpm.

Biogas production at 50 and 150 rpm**Figure 5**

Conclusion: Different stirring regimes (50 and 150 rpm) demonstrated that biogas production was increased at 50 rpm, probably due to an effect on the microbial community. It also seems that there was a temporary stop in the biogas production at day 5 at 150 rpm. Then the biogas-production turns back to normal. The production rate is almost the same given the slope of the line.

qPCR on the methanogenic community**Figure 6**

Conclusion: The purpose of this experiment is to investigate whether there were changes in microbial community under the influence of a higher stirring speed into Methanobacteriales (MBT), Methanomicrobiales (MMB), Methanosarcinales (MSL), Methanosarcinaceae (MSC), and the gene *mcrA*. It is clear to see that MSL, MSC, and MCRA demonstrate a stronger decrease at 150 rpm compared to 50 rpm. Only a small part of the Archaea contains the *mcrA* gene.

Conclusion:

The AMPTSII and the Infors Bioreactors are successfully implemented to investigate biogas (methane) potential and the microbial communities. In the future, molecular diagnostic tools will likely be used as indicator for biogas production, efficiency and flexibility with respect to the type of biomass used.

