CIE4485 Laboratory Experiment:

SMA Test

Course:	CIE4485 Wastewater Treatment
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Lab

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1 Introduction

Interest in renewable energy sources has grown dramatically during the recent decades. Biogas produced by anaerobic digestion (AD) processes is a renewable energy source and can be used for the generation of electricity, heat and even vehicle fuel. Apart from the energy production point of view, AD is also seen as an effective biotechnological method for COD removal of organic waste and wastewater. Current national environmental regulations and other policies governing land use and waste disposal have increased interest in anaerobic digestion. The EU countries have agreed on a directive requiring that the amount of biodegradable organic waste deposited in landfills to be decreased by 65% by 2016 (CD 1999/31/EC). These environmental and political pressures have increased the need for harmonisation of the anaerobic biodegradation assays.

The AD is characterized by a sequence of metabolic processes carried out by specific microorganisms. The complex organic compounds are firstly hydrolyzed by fermentative bacteria, and the by-products from this process (sugars, amino acids, monoglycerides, long-chain fatty acids, among others) are turned into smaller organic acids (or volatile fatty acids) by acidogenic microorganisms. The products from acidogenesis (for instance, propionic and butyric acids) can be converted into acetate and hydrogen by the acetogenic populations, which have the role of converting longer chain organic acids into substrates for the methanogenesis step. The last biological phase of the anaerobic conversion is performed by methanogenic archaea, which transform acetate, hydrogen and carbon dioxide, formate and other one-carbon compounds into methane (*Aquino and Chernicharo 2005*). The conversion processes are schematically represented in Figure 1.

In this complex interaction amongst the anaerobic microorganisms, the methanogenic community is usually regarded as the most sensitive to the environmental and operational conditions undergone by the treatment system (*Silveira et al. 2000*). So, monitoring the methane-producing microorganisms is important to determine the capability of anaerobic biomass in treating certain types of effluents or wastes; and the measurement of the maximum methane production rate has been a useful tool for this. Such quantification is normally assessed by the well-known **Specific Methanogenic Activity** (**SMA**) test, which evaluates the anaerobic sludge capability to convert an organic substrate into methane, which escapes easily to the gas phase, reducing the chemical oxygen demand (COD) in the liquid phase.

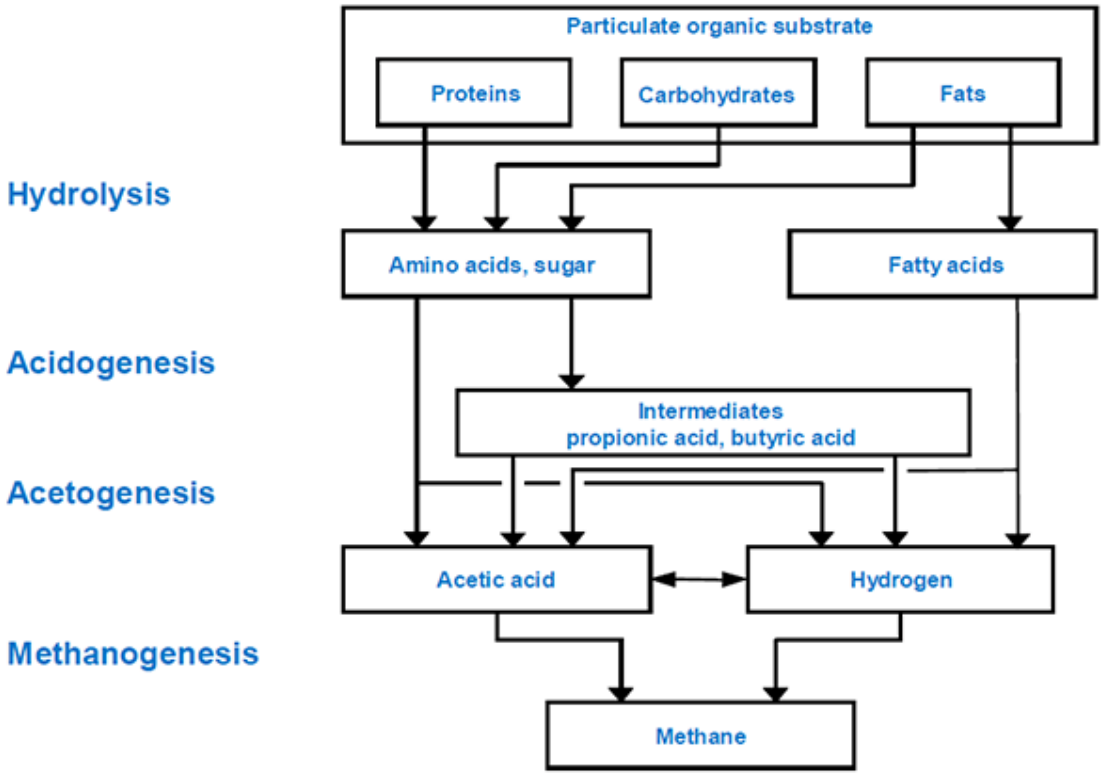

Figure 1 Process steps of the anaerobic digestion

The SMA test is used to calculate the maximum methanogenic potential, which represents the maximum capacity of a reactor, operating under ideal conditions, to convert volatile fatty acids into methane. Besides being a parameter for evaluation of the efficiency of the treatment, the SMA is also used to evaluate the sludge activity during different operational steps of an anaerobic system, to select an adapted sludge as inoculum, to follow the changes in sludge activities due to a possible build-up of inert materials, to estimate maximum applicable loading rate to certain sludge or to evaluate batch kinetic parameters.

In this laboratory experiment you will learn to determine the specific methanogenic activity (SMA) of anaerobic sludge taken from the anaerobic digesters of DSM, Delft. You will use an Automatic Methane Potential Test System (AMPTS) to perform this activity test. With the data you can calculate the allowed loading rate of the EGSB reactors at DSM and reason about the rate determining steps in the degradation of different waste streams.

2 Experiment

This experiment will be performed using the AMPTS II (figure 2), which an analytical device developed for on-line measurements of (ultra low) bio-methane flows produced from the anaerobic digestion of any biological degradable substrate at laboratory scale. The experimental set-up consists of three different elements; a waterbath with 15 slots for reaction vessels (bottles of 500 mL each). Anaerobic digestion can be performed by two temperature ranges (mesophillic and thermophillic), which can be set by the temperature of the waterbath. In this experiment mesophillic conditions will be used. A mechanical stirring device can be mounted on the bottles to mix the content during the test.
The biogas that is produced will flow to a wash bottle that is connected to the reaction vessels. The wash bottle is filled with a caustic solution (3M NaOH, with a colour indicator). *Why is the produced gas washed with this solution? And what is the colour indicator used for?*
Methane is flowing from the wash bottles to the flow cell array unit, to measure the volume of the produced biogas. Each click of the gas collectors represents 10 mL gas produced. A flow between 0.01 and 20 L per day can be measured with this unit. The cumulative number of clicks given is logged in a data file and automatically recalculated to the produced methane in NmL per time unit.

Figure 2 Experimental set-up (AMPTSII)

3. Procedure

The experiment consists of two different phases, since it needs several days to produce a good biogas production curve from which the SMA can be determined. Therefore, on day 1, the incubation vessels need to be prepared and mounted into the AMPTS. After 3 days, the data can be collected and the set-up should be cleaned.

DAY 1

3.1 Preparation and initial measurements

Stock Solutions

Phosphate buffer (already prepared)

- Stock solution "a" *0,2 M* $K_2HPO_4.3H_2O$ = 45,65 g/L
- Stock solution *"b" 0,2* M $NaH_2P0_4.2H_20$ = 31,20 g/L

Macronutrients (already prepared)

- NH_4 Cl 170 g/L
- $CaCl_2.2H_2O$ 8 g/L
- $MgSO_4.7H_2O$ 9 g/L

Micronutrients (already prepared)

- $FeCl_3.4H_2O$ 2 g/L
- $CoCl_2.6H_2O$ 2 g/L
- $MnCl_2.4H_2O$ 0,5 g/L
- $CuCl_2.2H_2O$ 30 mg/L
- $ZnCl_2$ 50 mg/L
- HBO_3 50 mg/L
- $(NH_4)_6Mo_7O_2.4H_2O$ 90 mg/L
- $Na_2SeO_3.5H_2O$ 100 mg/L
- $NiCl_2.6H_2O$ 50 mg/L
- EDTA 1 g/L
- HCl 36% 1 mL/L
- Resazurine 0,5 g/L
- Yeast extract 2 g/L

Inoculum

The inoculum is typical anaerobic sludge from a full scale biogas plant (DSM - EGSB). Write down the total suspended solids (TSS) and total volatile suspended solids (VSS) of the sludge, provided by your supervisor.

Substrates

As substrate for aceticlastic methanogenesis activity, sodium acetate-3hydrate salt ($NaC_2H_3O_2$-$3H_2O$) (PM=136.02 g/mol) is suggested to be used.

COD value: 1 mol $NaC_2H_3O_2$-$3H_2O$ ~ 2 mol O_2

Calculate (check with your supervisor if the calculation is right) and prepare 1 Liter solution of this salt (*substrate* solution) to a concentration of 2.0 g COD/L, including the stock solutions:

- Phosphate buffer:
  50 ml per liter substrate medium to obtain a 10 mM phosphate buffer at pH=7
  (use a measuring cylinder or balance)
- Macronutrients: dose 6 mL per L substrate medium (use a pipette)
- Micronutrients: dose 6 mL per L substrate medium (use a pipette)

Measure the COD concentration of the substrate solution.

For the blanks, prepare the same solution but without substrate (*medium* solution). If you do this experiment with more groups, only one group needs to make this medium solution, since data of the blank can be shared.

3.2 Preparation of the reaction vessels

Use triplicates for both blank and sample:

- 3 reactors as *blanks* (inoculum + only media solution): if you perform the experiment with more groups, only one group needs to prepare the blank bottles (another group than prepared the media solution)
- 3 reactors for *sample* (inoculum + substrate solution)

Inoculum to substrate ratio of 2:1 (based on mg/L VSS and mg/L COD) is normally used in the SMA test.
Choose the total volume of liquid that is suitable for the reaction vessels (400 mL should be a proper value). Then, calculate the amounts of inoculum and substrate in each reactor according to the following expressions:

$$\frac{V_{sub} \times COD_{sub}}{V_{sludge} \times VS_{sludge}} = 0.5$$

$$V_{sub} + V_{sludge} = 0.4$$

3.2 Start-up of the experiment

1. Mark all reactors and their lids with number, so you are able to recognise your bottles.
2. Fill the reactors with the inoculum and substrate as calculated above.
3. Check and write down the pH in the bottle (kept as initial pH and to compare with the ending pH on day 2).
4. Apply silicone to the rubber lid (will make it smoother to put it into the bottle) and tighten the aluminium screw cap carefully (see instructions in the AMPTS manual and ask your supervisor).
5. Fasten the stirring stick to the motor.
6. If not already done, mount a short piece of tygon tubing on one of the metal tubes in the lid and place a tube stopper on it.
7. Place all reactors in the water bath (run at 35^{o}C) and connect to the CO_2-fixing bottles to the flow cells.
8. Connect the contacts for the stirring to the DAQ-box.
9. Flush all reactors with N_2 approximately 2 minutes, using the extra inlet in the lid, to achieve anaerobic conditions.
10. Empty each flow cell.
11. The supervisor will show how the temperature is selected (and fills the waterbath to an appropriate level if necessary) and he/she will start the data logging program and explain you how this should be done.

DAY 2

3.3 End of the experiment

According to schedule you will finish your experiment on Thursday or Monday following your first day. To end the experiment you should perform the following steps:

1. Turn off the heated water bath and disconnect the contacts for the stirring.
2. Disconnect the reactors one by one from the CO_2-fixing bottles and immediately measure the pH in each reactor (as the ending pH).
3. Disconnect the CO_2-fixing bottles.
4. The waste sludge will be collected and disposed by the supervisor.
5. Wash the reactors and other equipment and clean the set-up and its surroundings.
6. Extract your data from the laptop (bring a USB-stick).

6. Elaboration

The results are strongly dependent of the sludge quality. Although much effort has been put in maintaining a good sludge quality, success is not guaranteed.

- Plot the cumulative methane production with the time (NmL) of each reactor (medium and blank reactors).
- Calculate the slope of the curve of cumulative methane production with time (NL/d) of each reactor (the most linear part).
- Calculate average and standard deviation of the triplicates and comment on your result.
- Subtract the average slope of the blanks from the average slope of the sample.
- Change the results to the unit of g COD_{CH4}/(gVSS·d) (ask you supervisor how to). This value represents the SMA of the tested sludge.
- Discuss the importance of the blank reactors in the SMA test of a given sludge.
- Compare your results with the ones obtained by other students and discuss if (and why) there is any difference among them.
- Revise some references and discuss if the tested sludge shows a high/low SMA value and why this could be the case.
- Express the loading rate (kgCOD/m^3/day) that the operator of DSM can apply to the EGSB reactors on site, if biomass concentration in the reactor is 40 kg VSS/m^3 and the COD removal efficiency is 80% and the energy that can be generated with the produced biogas.

7. Further reading

Henze, M. (2008), Biological wastewater treatment: Prnciples, Modeling and Design, IWA Publishing, 511 p., ISBN 1843391880. Chapter 16