Anaerobic degradation of straw by lignocellulose degrading bacteria

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Abstract

One of the most promising technologies in renewable energy production is the anaerobic digestion which can be an alternative of other conventional fuels like coal, wood, petroleum and oil shale, because it produces a high energy potential biogas.

Here, the anaerobic degradation was realized through utilizing bacterial anaerobic cultures, prelevated from Lake Hévíz (Hungary). This is a natural environment in which anaerobic degradation occurs. In the present study these samples were incubated at 55°C, and the degradation of straw was observed by the measurement of gas volume. The results indicate that there was significant gas production, especially in the first 15 days of incubation.

Keywords: anaerobic digestion; cumulative gas production; lignocellulosic biomass

Introduction

Anaerobic digestion (AD) is a biochemical process that operates without free oxygen [1] using bacterial fermentation and results in a biogas containing mostly methane (CH_4, 60%) and carbon dioxide (CO_2, 40%) [2].

Biogas from biomass like energy crops, residues and wastes, is a versatile renewable energy source. It can replace fossil fuels in power and heat generation and natural gas in the production of chemicals. Additionally it can also serve as a gaseous fuel [3].

Because lignocellulosic biomass has a very compact crystalline structure [4] and because lignin physically shields the cellulose and hemicelluloses parts, this material is very resistant to anaerobic digestion [5]. Because straw has a high Carbon / Nitrogen (C/N) ratio and low levels of trace elements it limits the activity and growth of microbes. In order to stimulate the anaerobic digestion, different pre-treatment methods can be used [6, 7].

Straw is one of the major crop residues in Europe that could be used for the production of biogas [8]. It has a high lignocellulose proportion but it can be hardly degraded microbiologically under anoxic conditions in engineered systems [9].

The aim of this study is to evaluate from quantitative point of view the gas production from anaerobic digestion using straw as biomass and sediments from the Hévíz Lake (Hungary) as source of active bacteria.

Material and Method

The degradation of cellulose was followed by the measurement of gas. The basic principle of the experiment was that gas production was measured three times per week.

In our case, the anaerobic degradation was realized through utilizing bacterial anaerobic cultures, prelevated from natural conditions and measured three times per week, according to the scheme of experiment presented in **Figure 1.**

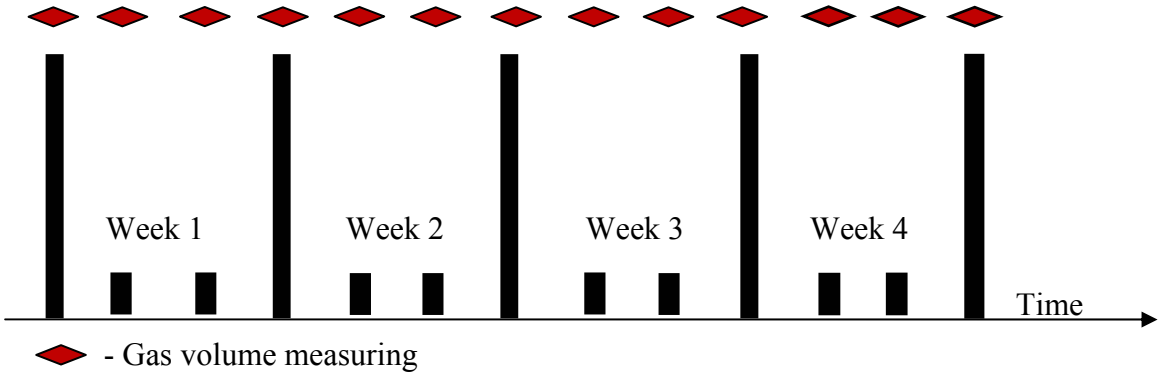

Figure 1. *Experiment design*

In Figure 1 we can see the scheme of experiment, but usually the gas measurement was carried out at an interval of 2-3 days.

During the degradation of straw the amount of produced gas was measured with a liquid column counter (**Figure 2**). The device measuring principle is based on the movement of a liquid containing 50 g/l NaCl and 1.25 g/l citric acid, in 250 ml of deionized water ISO/DIN 14853, 1997.

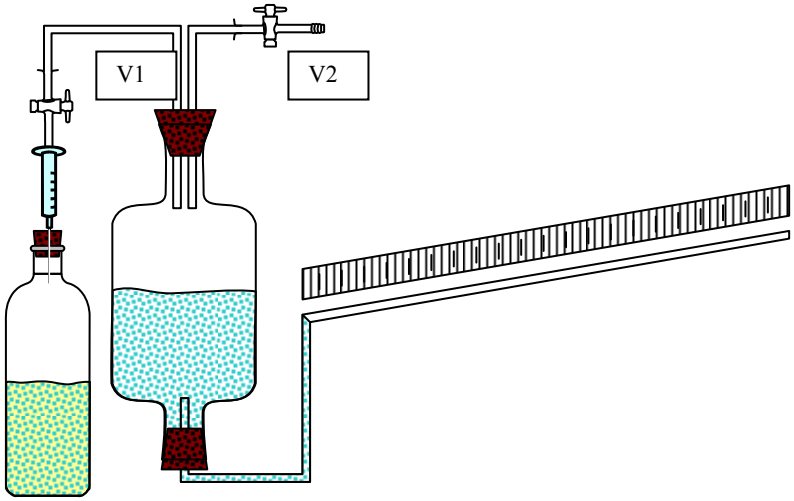

Figure 2. *Liquid column counter*

This U-shaped tube is half-full of liquid, and the difference in the height of the fluid is proportional to the pressure difference. Valve 1 adjusts the gas intake from the culture bottle and the valve 2 is for the gas outlet. The steps of measuring are shown in **Table 1.**

Through the measuring of gas everything was made under sterile condition (clean bench) and everything was sterilized in the autoclave.

Table 1. *Method of measuring the volume of gas*

Stages	Using	Description
Sterilization	Ethylic alcohol lighter	To sterilize the cap of the bottle it is given a little ethanol and then it is lit by the igniter
Connection	Needle Liquid column counter	The culture bottle from which the measurement was made was connected through a cannula to the counter valve. This was opened while valve 2 was closed, in this way the gas pressure was measured from the bottle. This can be seen by the movement of the liquid in the U shaped reservoir, and thus a change at the liquid level was experienced.
Reading the amount of gas in cm		Valve 1 was closed and the volume of liquid displaced was read on a scale in centimeters (centimeters into the water column)
Evacuation of gas from the counter		With the help of valve 2 the gas was discharged from the device and the liquid flowed back into the tank. This process was repeated until the liquid level has not changed in the horizontal U tube. This way in the culture bottle ambient pressure dominated once again.

The gas volume is determined based on the fact that in the 20 cm column is a volume of 3 ml liquid, with the equation:

$$V[ml] = y_1 + \frac{3}{20} x_1 \tag{1}$$

where V [ml] – Raw gas volume, x_1 [cm] – the measured quantity of gas, y_1 [ml] – the amount of gas removed for other experiments.

The next step is the calculation of the normalization factor through the conversion to normal conditions:

$$F_N = \left(p_u - 10^{7.19621 - \frac{1730.63}{233.426 + t_u}} \right) \cdot \frac{273.15}{101.325 \cdot (273.15 + t_u)} \tag{2}$$

where p_u [kPa] – Ambient pressure, T_u [K] – Ambient temperature, t_u [°C] – Ambient temperature, F_N [ml] – Normalization factor.

The standard atmospheric pressure (101.325 kPa), the standard temperature (273.15 K) and the removal of the water vapor are effectuated with the Antoine equation [10].

It is normalized the quantity of liquid remaining in the bottle. Every week 1ml of liquid was extracted for other measurements:

$$V_{N_n} = V_N \cdot V / l_1 \tag{3}$$

where l_1 [ml] – the amount of liquid in the bottle at the time of measurement, V [ml] – Raw gas volume, V_N [ml] – Standard volume

Each week subsequent calculations were made based on the amount of liquid that remained in the culture bottle at the moment of measurement. The standard gas volume is measured corresponding to 1ml of liquid from the culture bottle.

The cumulative gas production was calculated for the four cultures, from two sampling sites, and negative controls. Negative controls are made for control, to see that without the microorganisms from the Lake Hévíz, it produces no gas.

For this study samples were prelevated from Hévíz Lake, Hungary, Europe's largest thermal lake [11] with the characteristics [12] presented in **Table 2**.

Table 2. *Characteristics of thermal Lake Hévíz*

Characteristics	Value	M. U.
Geographical coordinates (GPS)	46.7680 N; 17.2487 E	°
Lake surface	47500	m^2
Maximum depth	38	m
Flow	1584	m^3/h
Water outlet	Hévíz channel	-
Components	S, Ra, CO, Ca, Mg	-
Water temperature in summer	33 – 36	°C
Water temperature in winter	23 – 25	°C

The samples were prelevated on 18th of April 2011, when water temperature was 29 °C and the pH was between 7.1 and 7.2.

Samples were taken from two areas of the Hévíz Lake (**Table 3**) and were enriched anaerobically in a complex cultivation medium (DSMZ 640) containing straw as carbon source.

Table 3. *Characteristics of sampling site*

Name of cultures	Depths of sampling	Sampling conditions	Date of sampling
Typha	0.5 m	Temperature 29 °C pH 7.1 – 7.2	18.04.2011
Sediment	2 m		

The Typha cultures were prelevated from the sediment of the littoral zone vegetated with reeds, and the Sediment cultures were prelevated from the middle of the lake.

The medium DSMZ 640 was prepared at the Helmholtz Center for Environmental Research, Department of Microbiology-UFZ in Leipzig. To remove the oxygen the media was boiled in the microwave and cooled down with nitrogen. In the anaerobic box it was measured and closed with butyl robber stopper and for sterilizing it was autoclaved (30 min, 121°C, and 2 bars). This medium was used in a modified formula (**Table 4**) as in our experiment we wanted to use straw instead of cellulose.

Table 4. *Caldicellulosiruptor Medium DSMZ 640*

Components	DSMZ 640 (Standard)	DSMZ 640 (Modified)	U.M.
Distilled water	1000.00	1000.00	ml
NH_4Cl	0.90	0.90	g
NaCl	0.90	0.90	g
$MgCl_2 \cdot 6H_2O$	0.40	0.40	g
KH_2PO_4	0.75	0.75	g
K_2HPO_4	1.50	1.50	g
Peptone	0	1.00	g
Trypticase	2.00	0	g
Yeast extract	1.00	0.50	g
Trace element solution SL-10 (DSM 320)	1.00	1.00	ml
$FeCl_3 \cdot 6H_2O$	2.50	2.50	mg
Cellobiose or cellulose	1.00	0	g
Straw	0	0.25	g
NaOH	0	0.50	g
Cystein – $HCl \cdot H_2O$	0.75	0.75	g
Resazurin	0.50	0.50	mg

Cysteine was added separately to the media because Cysteine cannot be autoclaved, so it was added at the end. Cysteine was added as a source of sulfur and seeks to reduce the traces of oxygen. Sodium hydroxide is added to adjust the pH to 7.2 – 7.3, because the medium at first has an acidic pH around 4.0 - 5.0. These

two compounds are added separately to the media.

After the shredded wheat straw was measured and prepared with 5 ml of tap water one day earlier, the second day it is filtered closed with black rubber stoppers and aluminium rings. The bottles are placed in an autoclave at 121°C, and 2 bars for 30 min. for sterilization. After which the necessary amount of media is introduced in the culture bottles and inoculated with a known amount of microorganisms.

Results and Discussions

After the volume of gas was measured the data which resulted in cm, from the measurement with the liquid counter are shown in **Table 5.**

Table 5. *Data resulted from measurements*

Date	**17.10**	**19.10**	**22.10**	**24.10**	**26.10**	**29.10**	**01.11**	**02.11**	**05.11**	**07.11**	**09.11**	**12.11**	**14.11**	**16.11**
Day	0	2	5	7	9	12	15	16	19	21	23	26	28	30
Temperature [°C]	20.4	22.1	24.2	21.9	22.8	22.7	22.5	20.8	22.6	22.5	23.2	23.9	23.6	22.4
Pressure [kPa]	99.56	99.67	100.46	100.21	99.47	99.83	97.26	98.06	98.06	99.86	99.86	100.32	101.3	100.27
GC measurement				1			1			1			1	1
Liquid removal				1			1			1			1	1
V liquid	25	25	25	25	24	24	24	23	23	23	22	22	22	21
Negative control 1	0	24	3	1	0	0	3	0	0	0	0	0	0	0
Negative control 2	0	16	4	2	0	0	2	0	0	0	0	0	0	0
Typha A	0	53	26	26	25	28	21	17	12	1	0	0	0	0
Typha B	0	30	28	25	5	3	12	1	1	0	0	0	0	0
Sediment A	0	29	31	34	25	22	19	0	8	0	0	0	0	0
Sediment B	0	58	94	27	5	1	23	1	2	0	0	0	0	0

In this Table the ambient temperature and the ambient pressure are also introduced, because at further calculations (**Table 7**) they are going to be needed.

After the value of the gas is known, this has to be converted to ml gas with Formula 2. The results of the conversion are shown in **Table 6.**

Table 6. *Conversion of gas from cm to ml*

Date	**17.10**	**19.10**	**22.10**	**24.10**	**26.1**	**29.10**	**1.11**	**2.11**	**5.11**	**7.11**	**9.11**	**12.11**	**14.11**	**16.11**
Day	0	2	5	7	9	12	15	16	19	21	23	26	28	30
Negative control 1	0	3.6	0.45	1.15	0	0	1.45	0	0	0	0	0	0	0
Negative control 2	0	2.4	0.6	1.3	0	0	1.3	0	0	0	0	0	0	0
Typha A	0	7.95	3.9	4.9	3.75	4.2	4.15	2.55	1.8	1.15	0	0	0	0
Typha B	0	4.5	4.2	4.75	0.75	0.45	2.8	0.15	0.15	0	0	0	0	0
Sediment A	0	4.35	4.65	6.1	3.75	3.3	3.85	0	1.2	0	0	0	0	0
Sediment B	0	8.7	14.1	5.05	0.75	0.15	4.45	0.15	0.3	0	0	0	0	0

This was calculated assuming that in 20 cm of liquid is 3 ml of gas.

Before the volume of the gas is measured the ambient pressure and temperature has to be converted to normal conditions (**Table 7**).

Table 7. *Calculation of standard volume*

Day	**0**	**2**	**5**	**7**	**9**	**12**	**15**	**16**	**19**	**21**	**23**	**26**	**28**	**30**
Normalization constant	0	0.89	0.88	0.89	0.88	0.88	0.86	0.88	0.87	0.89	0.88	0.88	0.89	0.89

With the results in **Table 7** the volume of gas is normalised and also divided with the quantity of liquid in the bottle (**Table 8**).

Table 8. *Correction for the remaining liquid in the bottle*

Day	0	2	5	7	9	12	15	16	19	21	23	26	28	30
Negative control 1	0	0.13	0.02	0.04	0	0	0.05	0	0	0	0	0	0	0
Negative control 2	0	0.09	0.02	0.05	0	0	0.05	0	0	0	0	0	0	0
Typha A	0	0.28	0.14	0.17	0.14	0.15	0.15	0.10	0.07	0.04	0	0	0	0
Typha B	0	0.16	0.15	0.17	0.03	0.02	0.10	0.01	0.01	0	0	0	0	0
Sediment A	0	0.15	0.16	0.22	0.14	0.12	0.14	0	0.05	0	0	0	0	0
Sediment B	0	0.31	0.50	0.18	0.03	0.01	0.16	0.01	0.01	0	0	0	0	0

These corrections were made because every week 1 ml of gas and 1 ml of liquid was removed from the bottles. After all the corrections and normalizations, the cumulative gas volume was calculated, by making a sum of the daily results which is also shown in **Figure 4**.

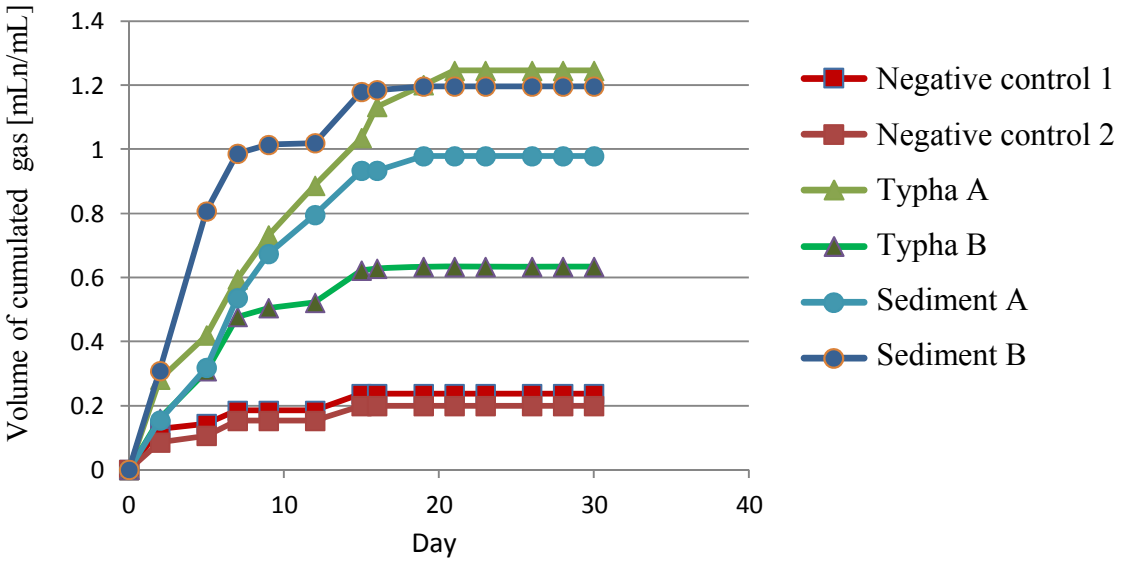

Figure 4. *Volume of cumulated gas*

In the Negative controls compared to the beginning of the incubation almost no change could be observed. The medium was clear and the wheat straw was still in its initial state. The slight gas productions in the Negative controls were around 0.1-0.2 mL/ml until day 10, which then no longer increased, where mLn/mL is the ratio between normalized gas volume, based on temperature and pressure.

The mixed cultures, however produced over the entire course of the experiment almost continuously gas. The biggest increase in gas production showed the culture Typha A and Sediment B. Typha B produced a much lower amount of gas than the other three cultures.

Conclusions

From the prelevated samples in Hungary (Hévíz Lake) different microbial communities were enriched and used in anaerobic digestion of straw. The obtained results indicate that there gas production from the cultures is significant and it is justified to continue different qualitative and quantitative studies concerning composition, energy capacity and other useful parameters for future industrial use.

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