



## Original Research Article

### Co-digestion of waste activated sludge and silaged mix of chicken litter and fodder beet

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## ABSTRACT

### Keywords

Co-digestion;  
substrate;  
sludge;  
silage;  
inoculum;  
biogas;  
methane

In order to determine the yield of methane in a Co-degradation study with different substrates. The study involved the following substrates : WAS – only; WAS+silage 2:1; WAS+silage 1:1; WAS+silage 1:2. Studied is the contents of the macro and micronutrient in the tested substrates and biogas yield after methane fermentation. It was found that major disadvantage of the BMP test is the fact that it does not provide short-term results because of it's duration, methane yield during a shorter period could be predicted by evaluating the reaction rate provided by the rate constant.

## Introduction

The activated sludge process is the most widely used biological process for domestic wastewater treatment. During aerobic biological treatment, organic pollutants are mineralized into carbon dioxide and water with the generation of excess microbial biomass commonly known as waste activated sludge (WAS). The amount of WAS generated is significant because biomass yield in aerobic biological treatment is 0.4 gVSS/gCOD.

The expense for of excess sludge treatment, handling and disposal approximates 60%

of the total expense wastewater treatment operating costs (Egemen *et al.*, 2001). Despite that wastewater treatment facilities are subject to increasing legal and social constraints, land application is widely adopted practice for sludge disposal.

Although sludge is rich in nutrients, it is not yet generally accepted for use as a fertilizer for agricultural purposes. The resistances from the farming industry concerns are based mostly on fear of pathogenic microorganisms, heavy metals and other presumably toxic compounds. The anaerobic digestion is widely used

biological method for sludge stabilization, which not only reduces the quantity of sludge to be disposed off, but also produces valuable methane gas, high quality biosolids for land application, and as a carbon source for denitrification.

In medium and large wastewater treatment plants anaerobic digestion is generally applied to mixture of primary and secondary (waste-activated) sludge, but despite the primary sludge is an easily digestible substrate, the waste-activated sludge (WAS) is known to be only about 30-45 % digestible in conventional anaerobic digester. Therefore treatment of exclusively WAS is more difficult and the anaerobic stabilization is a slow process (Lafitte-Trouque and Forster, 2002), (Harrison, S.T.L., 1991), (Chynoweth, D., 1993).

The specific biogas production determined on the destroyed volatile matter when treating the waste activated sludge is in the range 0.6–0.8 m<sup>3</sup>/kg VSS<sub>destroyed</sub> rather than a typical value of some 1m<sup>3</sup>/kg VSS<sub>destroyed</sub> observed when digesting mixed sludge (primary and secondary sludges), (Metcalf & Eddy Inc, 1991).

Lots of studies have considered various pretreatments to improve the conversion of sewage sludge to biogas. These pretreatments may be classified into thermal, biological, chemical or mechanical treatments. Among all of them, thermal and ultrasonic treatments are the most applied, specially at full scale plants (Chauzy, J. *et al.*, 2007; Kepp *et al.*, 2000; Neis *et al.*, 2007).

Another option to increase biogas production from a WAS digester is co-digestion with residues which present high methane potential.

Biogas production using energy crops, as main feedstock or as co-substrate, is attracting increasing attention. Often silaging is used to avoid the effect of seasonal availability of some of them. Fodder beets are one of the highest yielding forage crop. Due to the low dirt tare and high yield of highly digestible dry matter, fodder beets are also very well suited as substrate for anaerobic digestion (Weiland, 2010).

The changes in wet weight and total solids (TS) during ensiling are small and the loss of energy negligible. Despite that methane yields related to wet weight and to volatile solids (VS) are not significantly different before and after ensiling, the lower fatty acids (acetic acid, lactic acid ...) and alcohols formed by ensiling are easily degradable components and are responsible for a very fast rate of digestion of beet silage (Kreuger *et al.*, 2011).

Other potential substrat for co-digestion is farm manure. Anaerobic digestion of chicken litter has been widely studied (Bujoczek *et al.*, 2000). Main problems with the use of chicken litter is that, the high total nitrogen concentration and the break down of the proteins during anaerobic digestion, result in an ammonia inhibition of the process Co-digestion with a carbon-rich substrates improves carbon/nitrogen ratio, avoiding ammonia inhibition and enhancing the final methane yield (Angelidaki and Ahring, 1993).

The abundant information concerning biogas production using specific substrates is quite unsuitable when mixed substrates are treated. Direct information on anaerobic mineralization of any mixed substrate could be obtained by using the biochemical methane potential (BMP) assay. The method was developed and

improved by Owens et al. in 1979 and 1993.

The biochemical methane potential (BMP) test used to determine methane potential is a batch procedure carried out over a period of time sufficient for the available carbon in the test substrate to be converted to biogas (Angelidaki *et al.*, 2009; Chynoweth *et al.*, 1993; Owen *et al.*, 1979; Owens and Chynoweth, 1993). The protocol for this assay was designed to assure that the degradation of the compound is not limited by nutrients, inoculum, substrate toxicity, pH, oxygen toxicity or substrate overloading.

The heavy pollution load of waste activated sludge and chicken litter as well as the excellent harvest of fodder beets are the reasons behind the current research based on BMP assay.

## **Materials and Methods**

### **Experimental set-up**

The standard approach of a BMP assay is to incubate the substrates, under batch conditions, with an anaerobic inoculum and measure the methane generation (VDI 4630, 2006).

BMP tests were carried in triplicate on the three substrates against duplicate blanks consisting of inoculum only to account for the endogenous biogas produced from the inoculum. The inoculum used was digestate from a mesophilic anaerobic digester treating municipal wastewater. The test was carried out in glass reactors each with a total volume of 1.8 litres and working volume of 1.5 litres. Each reactor contains inoculum and substrates according Table 1. All reactors were supplemented with 10 mL macronutrients,

8 mL micronutrients and 50 mL buffer - see Table 2. Finally, the the headspace was flushed with nitrogen.

The reactors were maintained at a temperature of 37 +/- 0.5 °C in a thermostatically-controlled incubator and mixed manually twice a day. Biogas generated was collected in glass cylinders holding approximately 1.2 litres, filled with a 75 % saturated solution of sodium chloride acidified to pH 2 to reduce carbon dioxide and methane solubility. Gas production was measured daily by the displacement of this solution. Samples of biogas were taken from the collection cylinder and analysed for methane content using absorption of CO<sub>2</sub> by concentrated KON solution.

### **Seeding sludge and digestion substrates**

The inoculum in this study was collected from an anaerobic digester treating municipal wastewater. It was maintained at 38°C for 40 days in order to reduce the endogenous biogas production and to achieve enrichment of methanogenic organisms.

At the end of this period the inoculum had the following characteristics: 2,51% TS, 1,48% VS and pH = 7.8.

The main substrate used in the study was waste sludge after the secondary treatment, collected from the municipal wastewater treatment plant, city of Stara Zagora. The sludge had the following characteristics: 2.95% TS, 2.61% VS and pH = 7.1. It was used without any dilution or separation.

In this study we used silage containing 40% chicken litter and 60% fodder beets. The mix has been ensiled in for 60 days.

Five grab samples from the silage were diluted with water, treated with high speed blender and passed through 2 mm sieve. The final silage sample had the following characteristics: 6.83% TS, 5.74% VS and pH = 5.6

### Analytical procedures

The characteristics of the inoculum and the different substrates were analyzed to determine the total solids (TS) and volatile solids (VS). Both parameters were determined following APHA standard methods (APHA, 1995).

Biogas volume and methane content of biogas were measured according liquid displacement and CO<sub>2</sub> stripping techniques (VDI 4630, 2006).

### Results and Discussion

The results obtained during the BMP assay of the four substrates (Table.1) are presented in Table 3. For technical reasons all assays were terminated after forty days, despite that some of the samples were still producing gas.

There are a number of ways to interpret results of a BMP assay. The most common interpretation is Specific Methane Yield or the volume of CH<sub>4</sub> produced per mass of VS added.

The specific methane yields for the four substrates in the conditions of the assay are as follows:

WAS only - 0.184 LCH<sub>4</sub>  
g<sup>-1</sup> VS

WAS + silage 2 : 1 - 0.265 LCH<sub>4</sub>  
g<sup>-1</sup> VS

WAS + silage 1 : 1 - 0.301 LCH<sub>4</sub>  
g<sup>-1</sup> VS

WAS + silage 1 : 2 - 0.295 LCH<sub>4</sub>  
g<sup>-1</sup> VS

The results of the BMP assay for the four substrates, presented as cumulative methane yield in Figure 1, illustrate a considerable deferens not only in the methane production potential but also in the rate of the process. It can be calculated by applying the first-order kinetic model, which is a simple and useful model that has been frequently applied to anaerobic digestion systems.

The basic equation is:  $dS/dt = -k \cdot S$  where  $k$  is the first-order kinetic constant (time<sup>-1</sup>),  $t$  is the digestion time and  $S$  represents the biodegradable substrate concentration. As  $S$  is a difficult parameter to measure, it is preferable to derive the model by using the measurement of gas, which is much easier to determine.  $B = B_0 \cdot [1 - \exp(-k \cdot t)]$  (8) where  $B$  (mLCH<sub>4</sub> g<sup>-1</sup> VS) is the cumulative methane yield for a specific time period,  $B_0$  (mLCH<sub>4</sub> g<sup>-1</sup> VS) is the maximum methane yield of the substrate,  $k$  (days<sup>-1</sup>) is the first-order rate constant and  $t$  (d) is the time (Veeken and Hamelers, 1999).

Despite that major disadvantage of the BMP test is the fact that it does not provide short-term results because of its duration, methane yield during a shorter period could be predicted by evaluating the reaction rate provided by the rate constant.

**Table.1** Compositions of substrates

Experiment number	Inoculum %	Waste sludge %	Silage %	Total VS gL
1	10	90	–	24.97
2	10	60	30	34.36
3	10	45	45	39.07
4	10	30	60	43.75

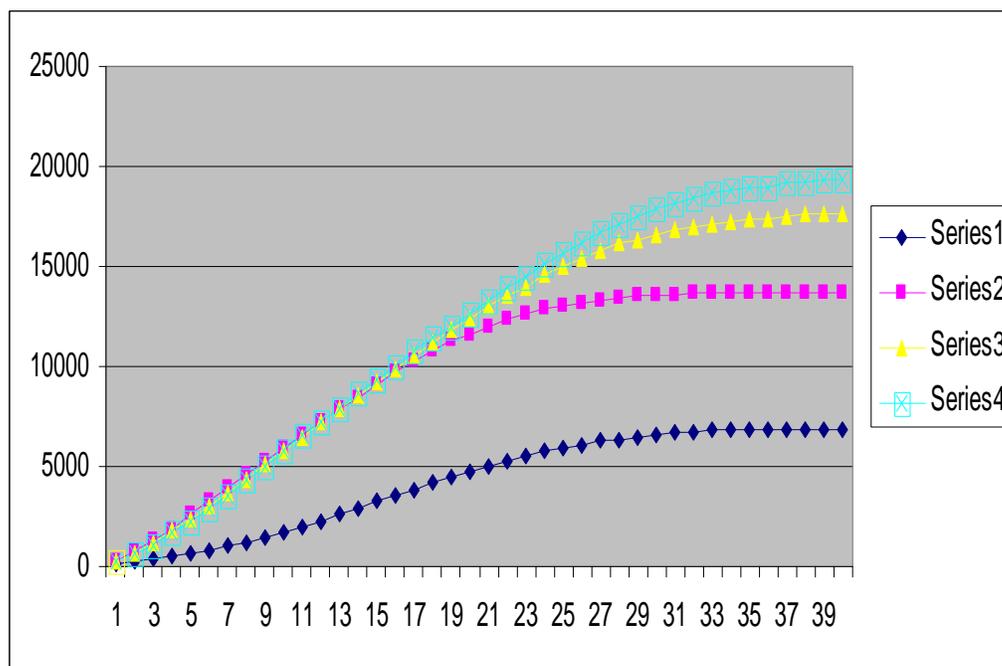
**Table.2** Content of macro and micro nutrients in the tested substrates

<b>Macronutrients</b>		
NH <sub>4</sub> Cl	g/L	26.6
KH <sub>2</sub> PO <sub>4</sub>	g/L	10
MgCl <sub>2</sub> , 6H <sub>2</sub> O	g/L	6
CaCl <sub>2</sub> , 2H <sub>2</sub> O	g/L	3
<b>Micronutrients</b>		
FeCl <sub>2</sub> , 4H <sub>2</sub> O	g/L	2
CoCl <sub>2</sub> , 6H <sub>2</sub> O	g/L	0.5
MnCl <sub>2</sub> , 4H <sub>2</sub> O	g/L	0.1
NiCl <sub>2</sub> , 6H <sub>2</sub> O	g/L	0.1
ZnCl <sub>2</sub>	g/L	0.05
H <sub>3</sub> BO <sub>3</sub>	g/L	0.05
Na <sub>2</sub> SeO <sub>3</sub>	g/L	0.05
CuCl <sub>2</sub> , 2H <sub>2</sub> O	g/L	0.04
Na <sub>2</sub> MoO <sub>4</sub> , 2H <sub>2</sub> O	g/L	0.01
<b>Buffer</b>		
NaHCO <sub>3</sub>	g/L	50

**Table.3** Biogas yield in the tested substrates

Experiment	1		2		3		4	
	Biogs ml	CH4 %						
<b>Day</b>								
1	87		300		241		211	
2	113		505		425		487	
3	147		548		556		385	
4	159		550		569		596	
5	124	52	671	53	559	52	587	52
6	215		650		647		608	
7	198		678		689		697	
8	203		680		708		735	
9	218		650		714		733	
10	256	54	704	53	658	53	758	53
11	279		668		732		741	
12	287		651		714		732	
13	316		650		704		685	
14	326		552		685		711	
15	348	53	604	52	664	54	701	54
16	314		612		617		671	
17	286		547		628		688	
18	314		511		610		659	
19	276		562		658		623	
20	251	53	315	55	610	52	657	53
21	259		408		589		662	
22	268		410		510		612	
23	256		265		512		584	
24	245		201		564		602	
25	213	53	149	53	483	54	558	53
26	157		154		411		522	
27	146		152		382		485	
28	112		112		324		465	
29	127		65		150		348	
30	92	54	72	55	248	54	385	55
31	78		21		221		295	
32	52		8		149		251	
33	82		11		159		210	
34	36		3		111		183	
35	14	54	0	55	132	56	152	59
36	17		0		98		33	
37	3		0		83		143	
38	0		0		56		78	
39	0		0		12		72	
40	0		0		31		53	

**Figure.1** Methane production at various substrates



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### References

Angelidaki, M. Alves, D. Bolzonella et al., "Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays," *Water Science and Technology*, vol. 59, no. 5, pp. 927–934, 2009

Angelidaki, I., Ahring, B.K. 1993. Thermophilic anaerobic digestion of livestock waste: the effect of ammonia.

*Appl. Microbiol. Biotechnol.* 38, 560±564.

APHA. 1995. Standard methods. 19th Edition. American Public Health Association, Washington, DC.

Bujoczek, G.; Oleszkiewicz, J; Sparling, R and S. Cenkowski. 2000. High Solid Anaerobic Digestion of Chicken Manure. *J. agric. Engng Res.* 76, 51-60

Chauzy, J., D. Cretenot, A. Bausseon and S. Deleris, "Anaerobic digestion enhanced by thermal hydrolysis: First reference BIOTHELYS® at Saumur, France", in *Facing sludge diversities: challenges, risks and opportunities*, Turkey: Antalya, 2007, pp. 141-148.

Chynoweth, D. 1993. Biochemical methane potential of biomass and waste feedstocks. *Biomass And Bioenergy.* 5 (1), 95-111

Chynoweth, D. P., C. E. Turick, J. M. Owens, D. E. Jerger, and M. W. Peck, "Biochemical methane potential of biomass and waste feedstocks," *Biomass and Bioenergy*, vol. 5, no. 1, pp. 95–111, 1993.

- Egemen, E., J. Corpening and N. Nirmalakhandan, Evaluation of an ozonation system for reduced waste sludge generation. *Water Science & Technology* Vol 44 No 2-3 pp 445–452 © IWA Publishing 2001
- Harrison, S.T.L. 1991. Bacterial cell disruption: a key unit operation in the recovery of intracellular products. *Biotechnology Advances*, 9 (2), 217-240.
- Kepp, U., I. Machenbach, N. Weisz, and O.E. Solheim, “Enhanced stabilisation of sewage sludge through thermal hydrolysis - three years of experience with full scale plant”. *Water Science and Technology*, vol. 42(9), pp. 89-96, 2000.
- Kreuger et al. 2011. Ensiling of crops for biogas production: effects on methane yield and total solids determination. *Biotechnology for Biofuels*, 4:44
- Lafitte-Trouque, S. and Forster, C.F. 2002. The use of ultrasound and  $\gamma$ -irradiation as pre-treatments for the anaerobic digestion of waste activated sludge at mesophilic and thermophilic temperatures. *Bioresource Technology*, 84, 113–118.
- Metcalf & Eddy Inc. Wastewater engineering: treatment, disposal, reuse, Int. ed. New York: McGraw-Hill; 1991.
- Neis, U., K. Nickel and A. Lunden, “Improving anaerobic and aerobic degradation by ultrasonic disintegration of biomass”, in *Facing Sludge Diversities: Challenges, Risks and Opportunities*, Turkey: Antalya, 2007, pp. 125-132.
- Owen, W. F., Stuckey, D. C., Healy, J. B., Jr., Young, L. Y. and McCarty, P. L. 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Research*, 13, pp. 485-492.
- Owens, J.M. and D.P. Chynoweth, "Biochemical Methane Potential of Municipal Solid Waste (MSW) Components", *Water Science and Technology*, Vol. 27, No. 2, pp. 1-14, 1993
- VDI 4630, 2006. Fermentation of organic materials. Characterisation of the substrates, sampling, collection of material data, fermentation tests. Verein Deutscher Ingenieure (Ed.), VDI-Handbuch Energietechnik
- VDI 4630, 2006. Fermentation of organic materials - Characterisation of the substrate, sampling, collection of material data, fermentation tests. STANDARD published 04/01/2006 by Verband Deutscher Ingenieure / Association of German Engineers
- Veeken A and Hamelers B, Effect of temperature on hydrolysis rates of selected biowaste components. *Bioresource Technol* 69:249–254(1999).
- Weiland P., 2010. Biogas production: current state and perspectives. *Appl Microbiol Biotechnol*. 85:849-860.