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Impact on Biogas Production by Using Inoculums with the Relevant Substrate

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Abstract

Biomass is one of the renewable sources of bioenergy, capable of contributing significantly to the future global energy supply [18]. The anaerobic digestion process involving biological conversions in which a consortium of interdependent organisms responsible for the degradation of complex organic matter [2]. It is important to evaluate appropriate techniques and / or technologies for effective energy recovery from waste. The effect of inoculums on the substrate ratio (I / S) in biogas production was investigated by changing the amount of substrate added to the inoculums. Process for the "production" of inoculate, the experiment was selected case, 14 days, 50°C, and constituted BATCH system, chicken waste, cattle waste, corn silage and corn (corn – without milled). Biogas production was the final step of the experiment. The "Batch Constant Temperature System" system used ration: 25gr. inoculums: 0,5gr. substrate. In the environmental experiment, the time period was 69 days (i.e. about 9 weeks). From this, it was noted that, despite the prolonged time, the maximum reached value was 68.33% CH_4, while in laboratory conditions was ~70% CH_4. The maximum value of biomethane produced reached for 56 days, while in laboratory conditions it reaches for 25-28 days.

Keywords: Biomass, inoculums, biomethane, substrate, biogas.

1. Introduction

The apparent growth of the biogas industry in the world over recent years has started an intensive search for new streams of organic waste to serve as a substrate for anaerobic digestion [1]. Biomass is one of the renewable bioenergy sources capable of contributing significantly to the future global energy supply [18]. However, anaerobic waste / material waste from agricultural crops, livestock production and agro-processing industries can be a simple, efficient and low-cost conversion technology [12]. The anaerobic digestion process involves biological conversions in which a consortium of the inter-microorganisms is responsible for the degradation of complex organic matter [2]. The guidelines for the definition of BMP in the pooled analysis are proposed, including characterization of the substrate, inoculums and its activity, experimental procedure, collection, interpretation and reporting of data [2]. They suggested that the inoculums was "fresh", homogeneous, filtered and pre-incubated, have a wide microbial variety to provide a sufficient level of hydrolytic and methanogenic activity and be tested to model substrates, such as cellulose and acetic acid [3]. Raposo and colleagues reviewed the factors that influence the performance of anaerobic fluid analysis and showed that although the experimental conditions of lecture analysis are synchronized, a certain degree of variability in results always remains due to the biological nature of the test systems [16]. This biological change can be assigned to the origin of the inoculums as it comes with a different microbial population, leading to changes in initial activity and adaptation of the substrates [19]. The influence of the source of inoculation on methane production has recently been demonstrated by [4] for Turkish manure and by Elbeshbisy and colleagues for food debris and

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primary sludge and in both cases methane depended on inoculums samples that were chosen. However, this impact was not related to the characteristics of the microbial community in the inoculums [11]. It is important to evaluate appropriate techniques and / or technologies for effective energy recovery from waste [11]. Anaerobic digestion is considered as the best treatment method for the fractional organic fraction [11]. The anaerobic digestion process consists of hydrolysis, acidgenesis, acetogenesis and phases of methane-genesis, and each stage is a function of the metabolic state of different microorganisms [15]. For the optimization of the anaerobic digestion process, selection of the inoculation source and the inoculums ratio to the substrate are important operational parameters for the assessment of anaerobic biodegradability of organic waste [17]. [5] also investigated the effect of inoculums on substrate ratio (I/S) in biogas production by changing the amount of substrate added to the inoculums. Different types of substrate represent different types and degrees of limitations in optimum anaerobic digestion performance that can be met by various pre-treatment mechanisms. Most importantly, potential mechanical problems must be treated by homogenization and unwanted components, as generally found in food residues classified by source from households, should be separated [14]. The pre-treatment effects on biodegradability and degradation rates can be better evaluated by BMP tests (biochemical potential of methane), provided the test conditions are appropriate and the test constraints are properly considered. On the other hand, the results from trials allow estimates of methane yields in the practical systems and physicochemical properties of the digestate but are inevitably related to the conditions of the specific test process. Thus, results from many experimental conditions, possibly strengthened by computer simulations, are needed for generalizations of pre-treatment effects on anaerobic digestion performance. Pretreatment has the potential to significantly improve anaerobic digestion systems, but their implementation should be guided by the potential of actual substrate improvement and be evaluated in their specific context of design and process conditions [14].

2. Materials and Methods

The experiment was developed nearby the cowsheds complex at E.D.E, Agricultural University of Tirana. Relevant reports were determined. The experiment, developed for biogas generation, is initially based on the production of inoculums by the mixing of various residues: (beef residue, chicken residue, corn silage, and grain unrefined corn). The report of these elements is presented in Table 1 below:

Table 1: Composition in percent and in liter of inoculums components

	In percentage (%)	In litres (lt)
CORN SILAGE	45,00	24,30
CORN-WITHOUT MILLED	7,00	3,78
CATTLE RESIDUE	30,00	16.20
CHICKEN WASTE	18,00	9,72

Based on Table 2, inoculate is produced in three types of temperatures: 20°C, 37°C, 50°C, with time periods: 14 days, 20 days and 14 days. All three ways are effective, which was previously proven at the State Institute of Agricultural Engineering and Bioenergy (740), Stuttgart, Germany. Reasons for implementation in three types of temperatures were given for the rapid activation of microbial activity, for producing biogas at the respective temperatures. Theprocess of "inoculation" production, in our experiment, was selected the third case.

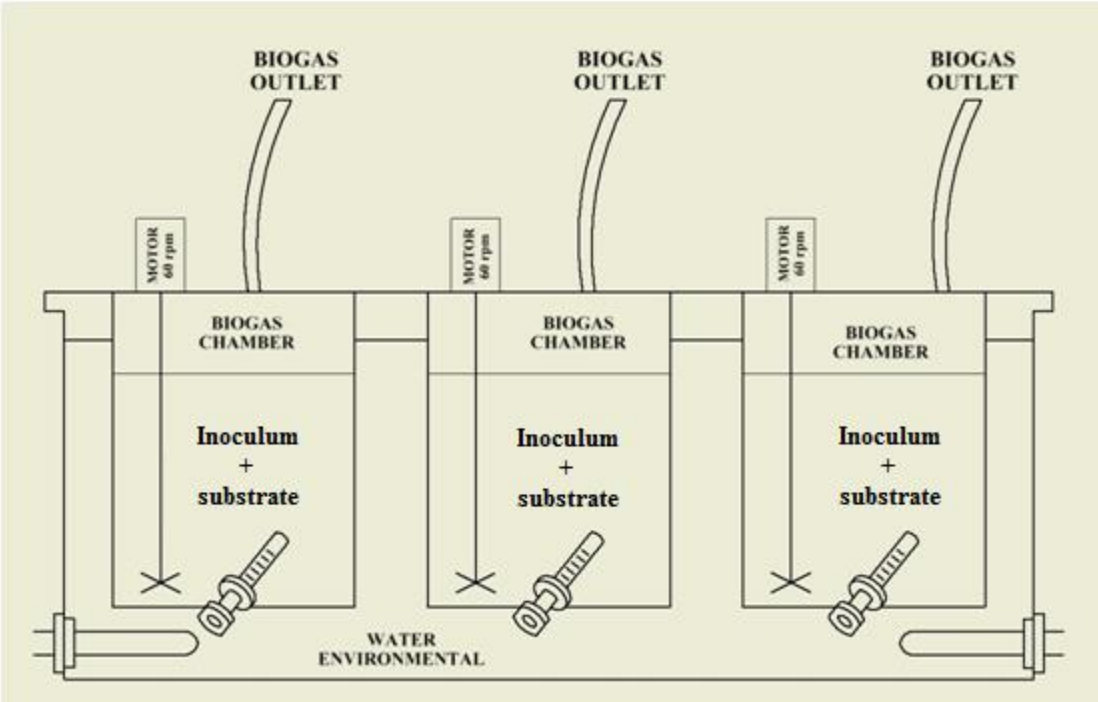


Figure 1: Batch Constant Temperature System (BCTS)

So, 14 days, 50°C, the BATCH system and composition: chicken waste, cattle residue, corn silage and maize (corn-without milled). The experiment was performed at Batch Constant Temperature System device (showed at figure 1), which is part of Experimental Didactic Economy (E.D.E), Agricultural University of Tirana. The analysis of the inoculums used was performed on the basis of its constituent elements. This situation was reflected in Tables 3 and 4. From these analyzes we estimate lost moisture, mineral matter and organic matter.

Table 2: Data on inoculums production process

		TEMPERATURE (°C)		
TIME (day)		20	37	50
	14	Cattle residue, corn (without milled), corn silage, concentrate corn.		
	20		Cattle residue, corn (without milled), corn silage, concentrate corn.	
	14			Chicken waste, cattle residues, corn silage, corn (without milled).
	Note: All three processes are developed in the BATCH system device (not in continuous).			

The measuring vessel is scaled up to 2 liters (≈ 2 kg). The experiment time was 35 days. The time of our experiment is in the months of July-August 2017. A very important process is the pretreatment of agro-industrial waste. This process was carried out for the pruning of weeds, various stones that may be present, etc. Also, this process helped to mix and homogenize all four types of waste entering the digestive process. Pretreatment time, realized with a mixing device (1400 rpm) was 4 minutes. Mixing process inside the digester was realized by an electromotor connected to a shaft, where at the end of it was mounted a cross-shaped cross-section. The time set for mixing was accomplished with the help of a device called "Timer", i.e. 15 minutes / 2 hours. The temperature, as an important factor for our experiment, is constant, i.e. 50°C (according to Table 2).

Biogas production was the final step of the experiment. The duration of the experiment extension was 69 days. The "Batch Constant Temperature System" device was also used for the production of biogas, in the ratio 25gr inoculums: 0.5gr substrate. This function under the complete insulated system, when it is mounted the necessary equipment such as thermostat, 60 rpm electric motor and biogas plastic pipe line [6]. The substrate used was "Concentrated Feed". Concentrated Feed was practically used at E.D.E for cattle feed.

Table 3: Analysis of inoculum component (chicken wastes)

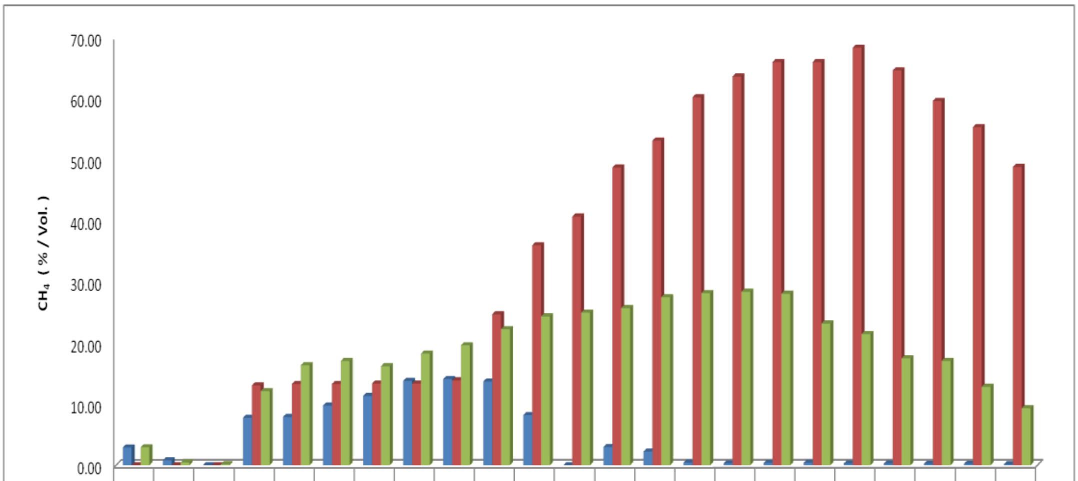
	Sample of dry matter and Lost of moisture (Chicken wastes)					
	Weight of initial sample (gr)	Weight of sample after drying (gr)	Initial dry matter (%)	Average of Initial dry matter (%)	Lost of moisture (%)	Average of lost of moisture (%)
Sample 1	112.50	15.23	17.13		82.87	
Sample 2	142.90	45.60	31.91	30.06	68.09	69.94
Sample 3	148.00	27.80	41.14		58.86	
	Mineral matter and Organic matter (Chicken waste)					
	Weight of empty vessel (gr)	Weight of initial sample (gr)	Weight after burn + weight of vessel (gr)	Weight after burn (net) (gr)	Mineral matter (%)	Organic matter (%)
Sample 1	36.60	10.33	37.34	0.74	10.52	89.48
Sample 2	40.89	11.21	40.96	0.07	0.62	99.38
Sample 3	22.29	5.70	23.30	1.01	17.71	82.29
				AVERAGE:	9.62	90.38

Table 4: Analysis of inoculums components (cattle residues)

	Sample of dry matter and Lost of moisture (Cattle residues)					
	Weight of initial sample (gr)	Weight of sample after drying (gr)	Initial dry matter (%)	Average of Initial dry matter (%)	Lost of moisture (%)	Average of lost of moisture (%)
Sample 1	139.15	25.05	18.00		82.00	
Sample 2	131.45	23.50	17.88	17.97	82.12	82.03
Sample 3	127.00	22.90	18.03		81.97	
	Mineral matter and Organic matter (Cattle residue)					
	Weight of empty vessel (gr)	Weight of initial sample (gr)	Weight after burn + weight of vessel (gr)	Weight after burn (net) (gr)	Mineral matter (%)	Organic matter (%)
Sample 1	36.60	7.03	37.34	0.74	10.52	89.49
Sample 2	40.89	7.42	41.71	0.83	11.15	88.85
Sample 3	22.29	4.79	22.80	0.51	10.59	89.41
				AVERAGE:	10.75	89.25

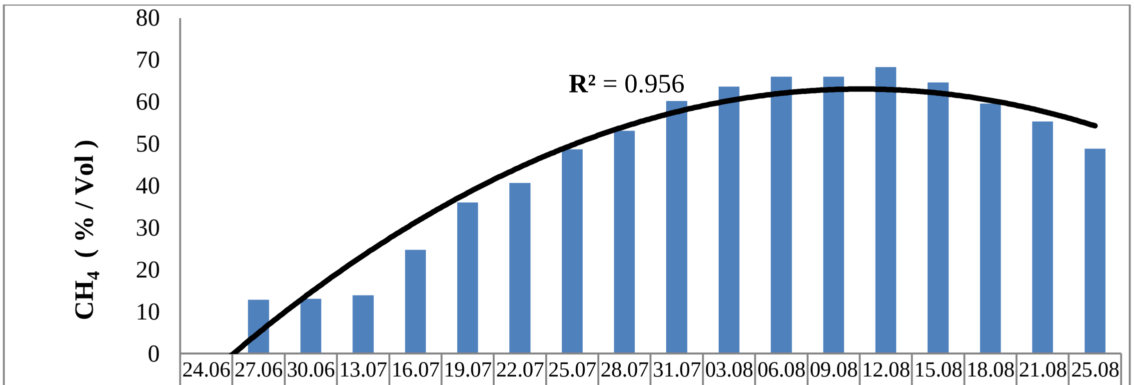
3. Results and Discussions

Monitoring in careful way of organic and mineral substances of cattle residue and chicken waste, as selected compound for inoculum, was very necessary. This was noticed in their analysis. The values shown in Tables 3 and 4 give a clear picture that this large amount of organic matter compared to the mineral content makes us realize that they are the basis for the production of biogas required. In this situation situation, in "help" comes another factor. It is the temperature. Its temperature as a factor affects the activation or deactivation of microorganisms. Seeing that the thermophilic temperature lies in the range of 37°C - 55°C, then the created environment gives us a great deal of help by activating the microorganisms and holding the constant temperature. The contents of the miniphase 1, 2 and 3: Inoculum + Concentrated Food. From this created situation and with this induced temperature (50°C), suitable for the activation of microorganisms for the digestion and formation of biogas, we have obtained these percentages / volume of all components as shown in Figure 1. From the values of measured indicates that the CH_4 production trend is increasing whereas the opposite occurs with oxygen O_2. Knowing that the environment is anaerobic, so in the absence of oxygen, microorganisms inside the digester, "consume" oxygen by decreasing to the lowest possible value and producing or releasing CH_4 by increasing the value from the minimum of 0 %/vol to ~70 %/vol. A great „help" gave inoculum. In Chart 2 we see a total decrease or total prohibition of biogas production at the beginning of the process. The main reason for the fall is often acidification (obtaining yellow mixing color inside the diester). The time period of the laboratory experiment compared to the environmental one shows its changes. Theoretically, the time period is 35 days, with a constant temperature of 50°C. If we take a little more careful look at the environmental experiment, we will notice that the time period is 69 days (i.e. about 9 weeks). The reason for the length of our experiment is to reach the goal of maximizing the experimental value in laboratory conditions. From this it was noted that, despite the prolonged time, the maximum value reached 68.33% CH_4, while in laboratory conditions it is 70% CH_4. The maximum amount of methane produced reached 12.08.2017, i.e. 56 days, while in laboratory conditions it reaches 25-28 days. At the temperature of the mesophilic process, this percentage reached 57% and duration 35 days [7]. The inoculum joint together with the substrate used at 50°C showed a higher percentage of methane, accelerating the development of metonoforming bacterial reactions compared to composition of cattle residue with water. This showed the difference in the duration of methane production as well as the maximum benefit in % / volume of methane produced. This makes us understand that the definition of working conditions and the strict application of them will give us the expected result (theoretically on paper). The change of approximately 2% is considered to be environmental losses, thermal insulation of the system as well as minor technical problems. Figure 2 shows that the values approximate to the polynomial regression line. This shows that the truthfulness of these values reaches the maximum, which is also seen in the regression line that is R^2 = 0.956. By experimenting with three digesters, digester no.2 gave the best result, compared to the first two. Chart 3 gives a clear view of the percentage concentration of CH_4 in function of the mixed substrates using concentrated food as a distinguishing factor. Mixed substrates can be classified as categorical factors with three repetitions. From Figure 3 it is shown that Group 2 has the highest concentration of CH_4 with media about 27% and maximum value about 45%. Digester no.2 represents the largest concentration of CH_4 in value and this is seen from the position of the median. It is exactly digester no.2 (Group 2) the main indicator of the maximum biogas values produced by this experiment.



	18.06.17	21.06.17	24.06.17	30.06.17	03.07.17	05.07.17	07.07.17	10.07.17	13.07.17	16.07.17	19.07.17	22.07.17	25.07.17	28.07.17	31.07.17	03.08.17	06.08.17	09.08.17	12.08.17	15.08.17	18.08.17	21.08.17	25.08.17
Digester 1	2.90	0.80	0.00	7.77	7.90	9.77	11.33	13.80	14.10	13.70	8.20	0.00	2.97	2.23	0.43	0.30	0.37	0.40	0.27	0.27	0.23	0.23	0.17
Digester 2	0.00	0.00	0.00	13.07	13.30	13.30	13.37	13.37	13.90	24.70	36.00	40.70	48.73	53.13	60.23	63.63	66.00	66.00	68.33	64.63	59.63	55.33	48.83
Digester 3	2.93	0.47	0.17	12.13	16.37	17.07	16.20	18.27	19.63	22.27	24.37	24.97	25.73	27.50	28.17	28.40	28.07	23.20	21.47	17.50	17.07	12.83	9.33

Figure 1: Progress in biogas production in three digesters



	24.06.17	27.06.17	30.06.17	13.07.17	16.07.17	19.07.17	22.07.17	25.07.17	28.07.17	31.07.17	03.08.17	06.08.17	09.08.17	12.08.17	15.08.17	18.08.17	21.08.17	25.08.17
Digester no.2	0	12.8	13.07	13.9	24.7	36	40.7	48.73	53.13	60.23	63.63	66	66	68.33	64.63	59.63	55.33	48.83

Figure 2: Trend line and regression line at experimental data

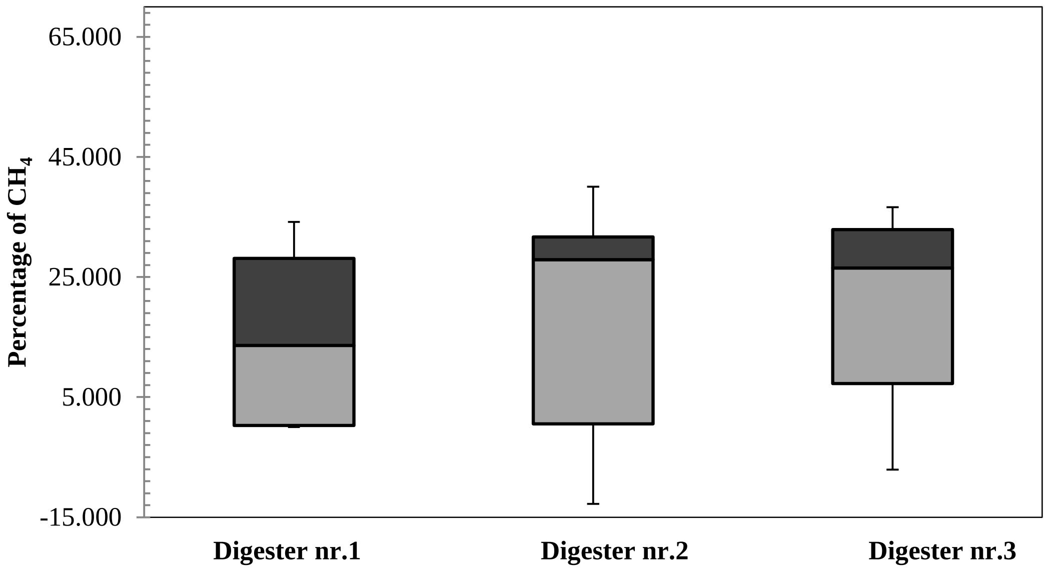


Figure 3: Concentration of CH_4 in function of environ. experimental repetitions

4. Conclusions

The pre-processing process by means of a mixing machine was indeed valid. Homogenization of the residue provides good inoculums formation. Also, the mixing process lead to the non-separation of the mixing layer into the mixer. This helped to develop the best chemical-organic reactions. Of great importance is the filling material (types of waste) used for digestion. Initiating the process through the inoculum produced gives a greater guarantee for the production of biogas required. The maximum value of biomethane produced reached 12.08.2017, i.e. 56 days, while in laboratory conditions it reaches in 25-28 days. So the definition of working conditions and the strict application of them will give us the expected result (theoretically on paper). The 68.33% CH_4 result from the environmental experiment compared to the 70% CH_4 laboratory showed us that the opportunity to benefit bioenergy is far greater and very cheap in price.

5. References

1. Appels, L., Lauwers, J., Degreve, J., Helsen, L., Lievens, B.,Willems, K., et al. (2011) **Anaerobic digestion in global bioenergy production: potential and research challenges.** Renew Sustain Energy Rev 15: 4295–4301.
2. Angenent, L.T., Karim, K., Al-Dahhan, M.H., and Domiguez-Espinosa, R. (2004) **Production of bioenergy and biochemicals from industrial and agricultural wastewater.** Trends Biotechnol 22: 477–485.
3. Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., et al. (2009) **Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays.** Water Sci Technol 59: 927–934.
4. Chamy, R., and Ramos, C. (2011) **Factors in the determination of methanogenic potential of manure.** Bioresour Technol 102: 7673–7677.
5. Chynoweth D.P., Turick C.E., Owen J.M., Jerger D.E. (1993): **Biochemical methane potential of biomass and waste feedstocks. Biomass and Bioenergy**, 5: 95–111.
6. Demollari E., Jojic E., Vorpsi V., Dodona E., Sallaku E (2017). **Temperature and Stirring Effect of Biogas Production from Two Different Systems.** American Journal of Energy Engineering. Vol. 5, No. 2, 2017, pp. 6-10. doi: 10.11648/j.ajee.20170502.11.
7. Demollari E., Jojic E., Vorpsi V., Sallaku E., Dodona E., Ciftja F. (December 2014). **Assessment of the digestate over some indication of the process of anaerobic digestion for biogas production.** International Conference “Green Energy and Environmental Sciences in Albania”.
8. Elbeshbishy, E., Nakhla, G., and Hafez, H. (2012) **Biochemical methane potential (BMP) of food waste and primary sludge: influence of inoculum pre-incubation and inoculums source.** Bioresour Technol 110: 18–25.
9. Feng L., Li Y., Chen C., Liu X., Xiao X., Ma X., Zhang R., He Y., Liu G. (2013): **Biochemical Methane potential (BPM) of vinegar residue and the influence of feed to inoculum ratios on biogas production.** Bioresources, 8: 2487–2498.
10. Gough, H.L., Nelsen, D., Muller, C., and Ferguson, J. (2013) **Enhanced methane generation during theromophilic co-digestion of confectionary waste and grease-trap fats and oils with municipal wastewater sludge.** Water Environ Res 85: 175–183.
11. Kalloum slimane, Salem Fathya, Kouki Assia and Mokaddem Hamza, **“Influence of inoculums/substrate ratios (ISRs) on the mesophilic anaerobic digestion of slaughterhouse waste in batch mode: Process stability and biogas production”**, Energy Procedia 50 (2014) 57 – 63.
12. Lawal A.A., Dzivama A.U., Wasinda M.K. (2016): **Effect of inoculum to substrate ratio on biogas production of sheep paunch manure.** Res. Agr. Eng., 62: 8–14.
13. Labatut R.A., Scott N.R. (2008): **Experimental and predicted methane yields from the anaerobic co-digestion of animal manure with complex organic substrates.** In: Proceedings of the American Society of Agricultural and Biological Engineering. St. Joseph.
14. My Carlsson, **doctoral thesis, ISBN 978-91-7583-377-4** Luleå University of Technology, pdf version.

15. Oliveira SVWB, Moraes EM, Adorno MAT, Varesche MBA, Foresti E, Zaiat M. **Formaldehyde degradation in an anaerobic packed-bed bioreactor.** Water Res.2004; 38:1685–1694.

16. Raposo, F., De la Rubia, M.A., Fernandez-Cegri, V., and Borja, R. (2012) **Anaerobic digestion of solid organic sub- strates in batch mode: an overview relating to methane yields and experimental procedures.** Renew Sustain Energy Rev 16: 861–877.

17. Sri BalaKameswari K, Chitra K, Porselvam S, Thanasekaran K. **Optimization of inoculum to substrate ratio for bio-energy generation in codigestion of tannery solid wastes.** Clean Techn. Environ. Policy 2010; 12:517–524

18. WEC (2004): Renewable energy projects handbook. London, World Energy Council.

19. Wittebolle, L., Verstraete, W., and Boon, N. (2009) **The inoculums effect on the ammonia-oxidizing bacterial communities in parallel sequential batch reactors.** Water Res 43: 4149–4158.