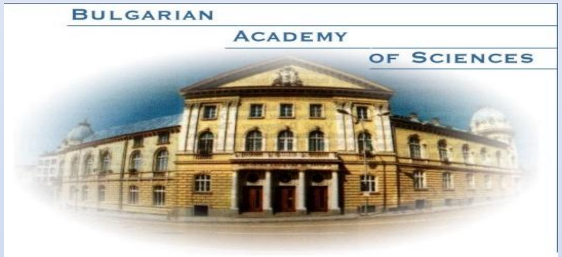

Anaerobic co-digestion of different mixtures of organic wastes: an overview of our own studies

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I. INTRODUCTION

Anaerobic digestion (AD) of organic wastes is a very attractive biotechnology during last years mainly in the field of the **renewable energy sources and biofuels.** The increased interest of many European countries in this biotechnology is due to the following advantages:

1. It solves **environmental problems**;
2. It solves **energy problems**;
3. The **digestate is natural manure** replacing the high energy consuming fertilizers and it can be used in **biological agriculture**.
4. Volatile fatty acids (VFAs), formed as **intermediate products**, are important precursors for various industries as mixed or purified chemicals **with high market value**.

Normally, AD processes are designed for a single substrate. **Anaerobic co-digestion** (AcoD) can be considered as the instantaneous digestion of two or more substrate and co-substrate mixtures. The AcoD technology employs different feed wastes for **increasing methane generation** and makes the **process more stable**.

The AD is a **very complicated process** consisting of the following stages: **hydrolysis, acidogenesis, acetogenesis, and methanogenesis**. Usually, in AD processes a single type of substrate is applied. However, mono-digestion (i.e., anaerobic digestion using one feedstock) suffers from challenges associated with feedstock characteristics. **Co-digestion** using multiple feedstock **provides the potential to overcome these limitations**.

Many researchers have been eager to investigate co-digestion using various mixtures of industrial, farming, agricultural, and municipal waste materials.

During **the last two decades** our team (in the frame of The Stephan Angeloff Institute of Microbiology - Bulgarian Academy of Sciences) performed a lot of experiments of AcoD of different organic wastes - activated sludge, cattle dung, milk whey, etc.

The aim of this paper is to deliver an overview of our own studies in the field of the AcoD of organic wastes and to summarize the obtained results.

II. MATERIALS AND METHODS

Substrates. For the purpose of the studies presented in this article, the following materials (wastes) were used as substrates (separately and in mixtures): activated sludge (AS) from the Sofia Municipal Wastewater Treatment Plant; cattle manure (CM); milk whey (MW), pig manure (PM), wheat straw (WS) and a specific mixture of wasted fruits and vegetables (WFV).

Laboratory equipment and experimental design feature. For the experimental studies conducted during two decade laboratory scale CSTRs equipped with automation control of the temperature regime (34÷37 ± 0.5°C for mesophilic and 54÷55 ± 0.5°C for thermophilic conditions) were used. The working volumes of CSTRs were in a wide range (1-15 dm^3). Some experiments were conducted in our pilot scale bioreactor with working volume of 80 dm^3, equipped with automation control and monitoring system.

Inoculum: For initiate the anaerobic digestion processes an inoculum from a working methanogenic process was used. Usually it was added in amount of 5 ÷ 10% (v/v) of the working volume of the bioreactor in use.

Analysis: Dry matter (DM) and Volatile solids (VS) was performed by a standard gravimetric procedure. The difference between TS and ash content shows the amount of VS in the sample.

pH was measured with Seibold pH-meter Type G 104 equipped with Ingold 465 combined pH-electrode.

Chemical oxygen demand (COD) was determined by means of the Open Reflux Method according to the APHA Standard Methods of Examination of Water and Wastewater.

Biological oxygen demand (BOD5) was determined by standard method and specialized device for dissolved oxygen concentration measurement.

The **volatile fatty acids** (VFA) and the ratio VA / alkalinity were determined with **gas-chromatograph**.

To measure the **obtained biogas volume** (Q) every bioreactor was provided with **gas-holder** based on water-displacement method or **gas-meter**.

The **content of methane** in the obtained biogas was measured using gas analyzer model X-am 7000 of the Dräger company, equipped with infrared detector for CH_4 or automatic sensors.

III. AcoD OF A MIXTURES OF TWO SUBSTRATES

Activated sludge - Cattle manure – milk (dairy) whey

Table 1. The average characteristics of the substrates used

Substrate	Dry weight [%]	$COD_{soluble}$ [g/L]	COD_{total} [g/L]
Cattle manure	6.86	18.0	69.6
Milk whay	4.19	48.8	64.8
Activated sludge	2.45	11.6	37.8

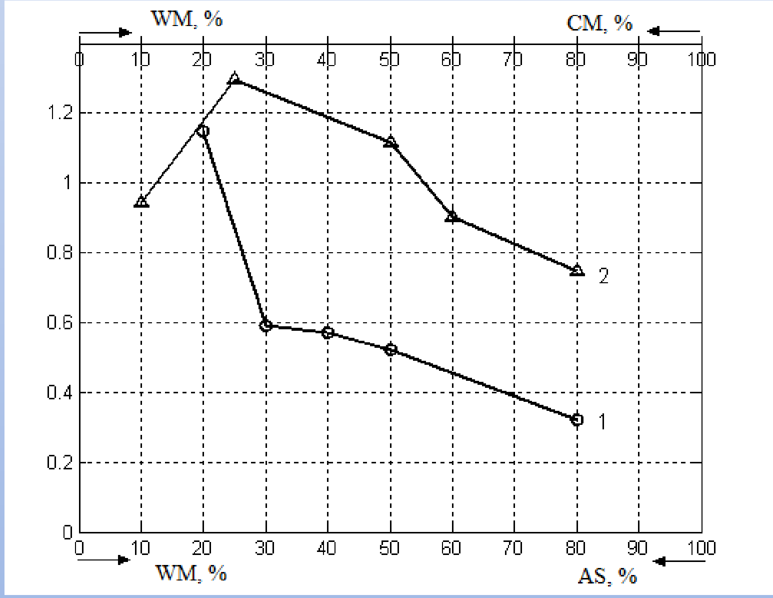

Fig. 1. Biogas production with two-components mixtures (line 1 – mixture „activated sludge (AS)” - milk whey (WM)”, line 2 – mixture “cattle manure (CM) – milk whey (WM)”)

Table 4. Different ratios cattle dung/milk whey

Cattle dung / milk whey	Q, L d^{-1} L^{-1}	VFA, g L^{-1}	COD, gO_2 L^{-1}	pH
80:20	0.6	5.5	20.3	6.7
60:40	0.9	3.0	16.0	7.0
50:50	1.3	4.7	11.6	6.7
25:75	1.4	4.1	5.8	7.1
10:90	1.1	10.5	7.1	7.1

Industrial alcohol production waste – cattle manure

Laboratory experiment with AcoD of a mixture of **waste from industrial alcohol production and cattle dung** are performed as well. The addition of 20 % waste from industrial alcohol production causes unstable increase in the biogas yield. A higher increase in biogas yield is observable at content of waste of **40% in the mixture**. At this ratio waste from industrial alcohol production/cattle dung the biogas yield is more stable as well. Above this content of waste, the biogas yield decreases and the process becomes more unstable.

Cattle manure – poultry litter

Four experiments were performed with continuous AcoD (D = 0.05 day^{-1}) of mixtures of cattle manure (CM) and poultry litter (PL) in BR of working volume of 2 L (**mesophilic conditions**) with a dry weight of the input mixture of about 7% - one control experiment with cow dung only and three with mixtures of different ratios (PL:CD = 20:80; 50:50 and 80:20).

Table 5. Biogas yield in different ratio of PL and CD

Ratio PL:CD	$Q_{average}$ [L_{biogas}/dm^3]
20:80	0.510 (from 27 days in steady state mode)
50:50	0.900 (from 31 days in steady state mode)
80:20	0.890 (from 35 days in steady state mode)

The resulting biogas had the following average composition CH_4 = 63.8 %; CO_2 = 35.6 %, H_2 = 0.56 %.

Wasted fruits and vegetables - Pig manure

Comparative studies of AcoD with mixtures of pig manure (PM) and a specific mixture of waste fruits and vegetables (WFV) were used as single substrates and in a mixture at various ratios in mesophilic and thermophilic conditions are performed. The mixture of WFV was with a constant ratio of 40% waste potatoes (WP), 20% waste tomatoes (WT), 20% waste cucumbers (WC) and 20% waste apples (WA).

Table 6. Some results in AcoD of mixtures of PM and WFV in mesophilic conditions

Substrate	Organic loading rate [kg organic dry matter/m^3. day]	Daily Biogas yield [L/ kg organic dry matter.day]	Content of CH_4 [%]	Daily CH_4 yield [L/ kg organic dry matter.day]	Degree of biodegradation [%]
PM, only	0. 446	0. 67	62	0.42	72.3
90%PM+10 WFV	0.500	0.726	61	0.44	50.3
70%+30% WFV	0.466	1.090	60	0.65	60.3
50%PM+50%WFV	0.970	0.536	59	0.32	70.6
25%PM+75%WFV	1.290	0.580	58	0.34	83.1
WFV, only	1.15	0.91	57	0.52	78.9

The degree of biodegradation (DBD) was calculated according to :

$$DBD = \frac{VS_i - VS_e}{VS_i} . 100, [\%] \qquad [2]$$

where VS_i and VS_e, [g/L] are volatile solids, per 1 L of the working volume, of the influent and of the effluent, respectively.

Obviously, the ratio of 70% PM+30% WFV is optimal in regards of biogas and methane yields.

The experiments were carried out in a 100-L pilot-scale continuously stirred ABR with a working volume of 80 L in mesophilic conditions (34 ± 0.5°C). The ABR was operated in semi-continuous mode. Different view of the pilot-scale ABR is shown on Fig. 3.

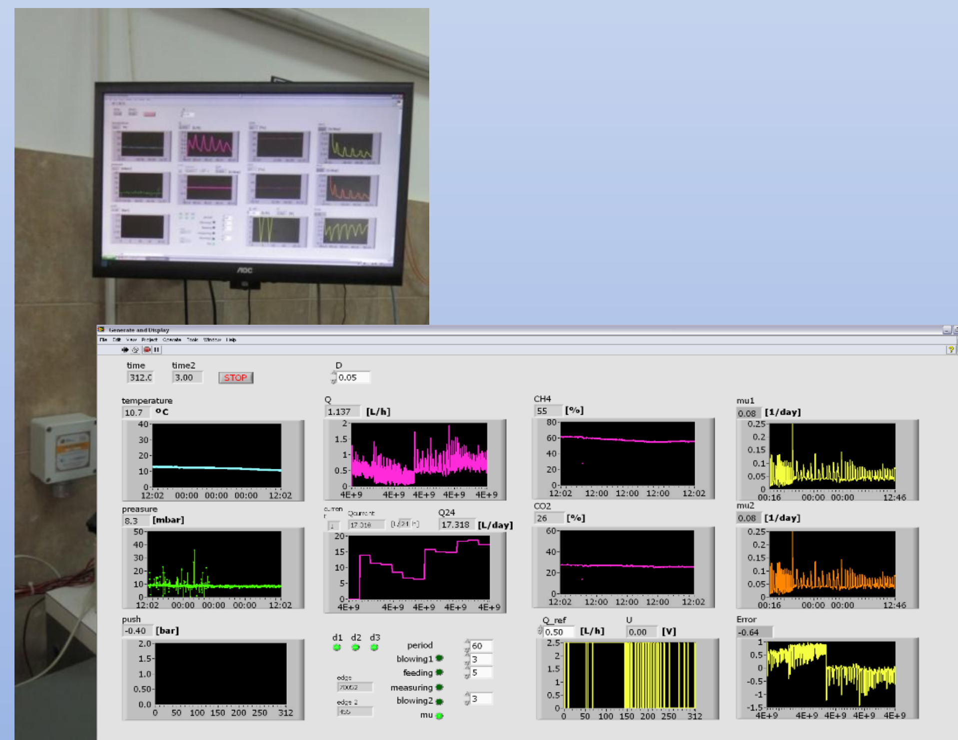

Fig. 3. Different view of the pilot-scale ABR with automatic control system:

The yields of biogas and methane in **the thermophilic mode of operation** are comparable to those in the mesophilic mode, but in the thermophilic mode of operation a more complete degradation of the organic matter, especially of the available cellulose, is observed.

Wasted fruits and vegetables – wheat straw

Table 7. The rate of biodegradation and biogas production from anaerobic co-digestion of WFV/WS mixtures.

Substrate ratio WFV / WS [% w/w]	Biodegradation ratio, [%]	Biogas production, [cm^3/g]	Yield of methane, [cm^3/g]
100 / 0	78.9	0.910	0.52
80 / 20	76.5	0.852	0.49
75 / 25	78.7	0.860	0.51
70 / 30	82.1	0.855	0.47
65 / 35	79.5	0.826	0.45
50 / 50	58.5	0.708	0.38

The maximal biomethane yield was achieved using mixture WFV/WS = 75/25 but the maximal biodegradation of organic matter is observed when the WFV/WS ratio was 70/30.

IV. AcoD OF A MIXTURES OF THREE SUBSTRATES

More complicated was the design of the experiments when all three substrates must be changed simultaneously, which means studying the influence of a combination of three factors. This required a special kind of coordinate system shown in Fig. 4, which reflect the change in substrate volumes ratios. In this part of the research, a total of over 240 experiments were conducted in 9 series, covering a period of about 9 months of continuous work.

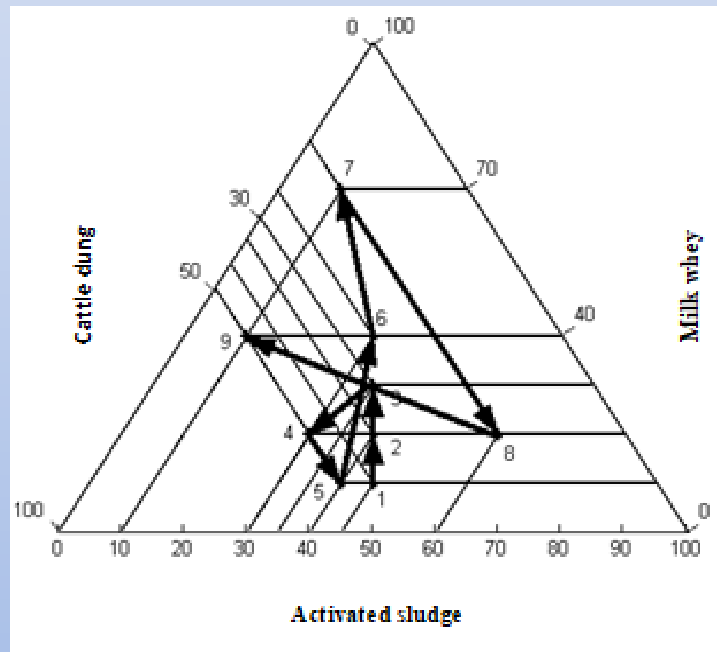

Fig. 4. A coordinate system to reflect the change in the ratios of the substrates in mixtures of three substrates and the route of the conducted series of experiments. Point 1 – starting series (AS:CM:W = 45:45:10)

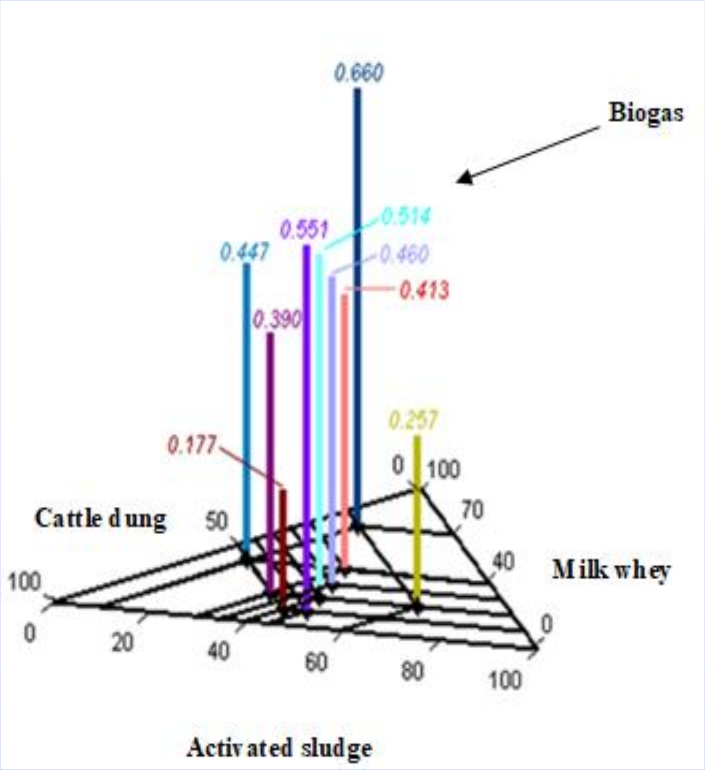

Fig. 5. Biogas production for mixture of three substrates (D = 0.0025 day^{-1})

The obtained results give reason to consider that when using mixtures of three substrates, in general, the trends for the influences of individual substrates are preserved, but the sharpness of the effects observed in the case of double mixtures is reduced. Furthermore, masking effects were observed when using mixtures of three substrates. They are expressed in the fact that the mixtures of three substrates do not allow obtaining such high values of Q as in the double mixtures. **The highest value of Q for ternary mixtures is 60% lower than that obtained for binary mixtures.** Perhaps this is due to the high content of hard-to-digest fats in the milk whey or deteriorated ratio of C:N.

Referring to these results, an logical extend of our research was the biodegradation of ternary mixtures in a **cascade of two serially connected laboratory bioreactors**, both with only methane production. The ratio of the working volumes of the first to the second bioreactors is 1:10. Experiments were carried out with **mixtures of wasted activated sludge, milk whey and waste from biodiesel production.** Chemical (triton) and biological (ramnolipid) surfactants are added into bioreactors in order to stimulate the activity of microorganisms. The total reduction of COD and BOD at the effluent compared to the influent reaches 13 times (4.5 times in the first and 2.9 times in the second bioreactors) at $D = 0.025$ day^{-1}. The total methane yield increases by 50-60%. The addition of surfactants leads to the timely increase of biogas yields, but also helps to restart the processes after their failures due to various factors.

As technology advances, the **two-stages AcoD** should persist in enhancing biogas production.

V. CONCLUSIONS

Original scientific results were obtained in the study of AcoD of **double and triple mixtures** of organic waste in laboratory conditions.

It has been proven that under the conditions of the performed experiments – AcoD of double and triple mixtures of complex substrates - waste from agriculture, the food industry and wastewater treatment plants (cattle manure, milk whey and activated sludge) **the steady state of the bioreactors is reached for about 10-12 days.** This result can be significantly useful for optimizing studies of such processes where long-term experiments have to be carried out.

High process intensity can be observed in some of the dual mixtures. In this aspect**, the double mixture "cattle manure – milk whey" in the ratio 70:30 vol.%, in which the highest values of the flow rate of the released biogas were obtained, seems promising.**

Our results discover opportunities for their application in industrial scale. However, an essential requirement for commercializing the AcoD of livestock manures is **to situate livestock farms and co-substrate sources as close as possible to the digestion facility, ensuring the overall process is economically viable.**

The increasing trends of the feasibility and applicability of biogas production indicate the potential for using different kinds of feedstock waste in the production of biogas through the AcoD process. **However, some challenges need to be confronted to convert laboratory-scale production to the industrial level.** Many aspects must be maintained, such as buffering capacity, the production rate of biogas, nutritional balance, and microbiological stability.

Further research is necessary to develop a systematic framework that ensures the effective implementation of different wastes management, considering technical, environmental, agronomic, economic, and social perspectives.

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Thank you!