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## **Assessment of susceptibility of substrates on biochemical degradation using AT<sub>4</sub> and BMP tests**

### **Summary**

The physico-chemical composition of substrate influences their aerobic or anaerobic biodegradability. Based on the results of biodegradability of selected substrates (chicken manure, corn silage, ground mushrooms, grass, haulm tomatoes) using oxygen test (AT<sub>4</sub>) and anaerobic (BMP) was determined correlations between these parameters. It has been shown that long-term test BMP can be replaced by test AT<sub>4</sub> and methane production studied substrates can be determined from the relationship:  $BMP = 1.2 + 44.3 AT_4$  with  $R^2 = 0.9$ .

Keywords: AT<sub>4</sub>, BMP, biodegradability, waste

### **1. Introduction**

Proper waste management in the context of sustainable development includes the prevention and minimization of waste generation, the recovery of materials and energy, as well as reducing their hazardous properties. Removal of organic matter from waste can be achieved using of mechanical-biological or thermal processing methods [9, 14]. In accordance with the legal requirements, waste disposal by deposition them in landfills must be replaced by other techniques [5, 21]. Among these techniques, particular attention is paid to the use of aerobic or anaerobic decomposition of organic substrate.

The growing interest of anaerobic processes results from the strategy of energy development, which is associated with the use of non-conventional energy sources, in this case the gas

fermentation. [15]. Exemplary amounts of the biogas obtained from a variety of organic waste is shown in Fig. 1.

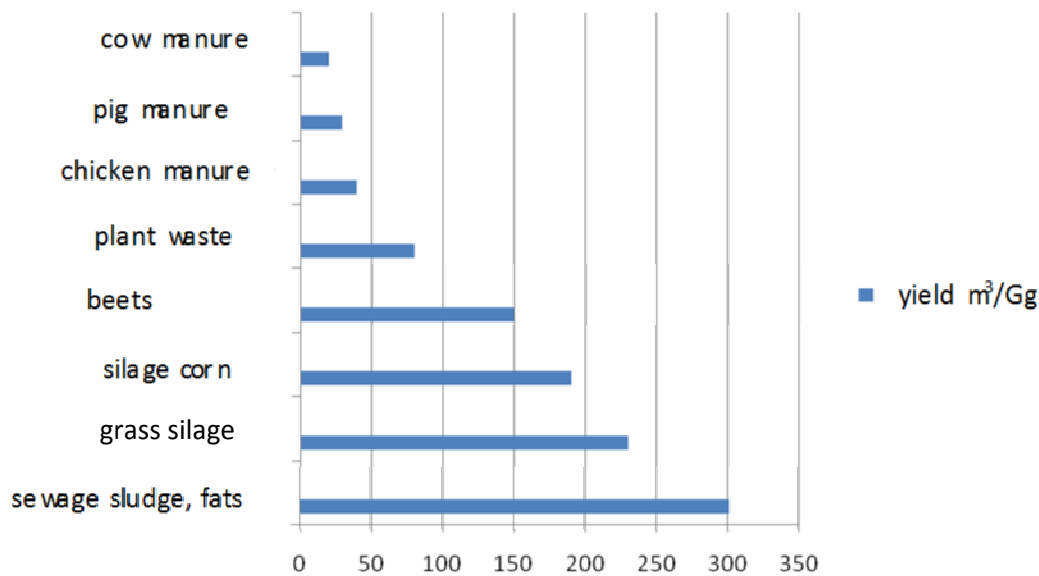


Fig. 1. The average yield of biogas produced with tons of animal and plants waste [6]

The data (Fig. 1) show that the greatest efficiency of biogas production are characterized by sewage sludge and fat. The development of fermentation technology is focused on increasing its efficiency and on the extension of the types utilized of organic substrates. Fermentation of two or more ingredients joined in homogeneous mixture is called co-fermentation process [9, 15]. The main components used in the process are, for example, sewage sludge, manure, waste from the production of poultry manure, bio-fraction of municipal waste, waste paper, waste from the food industry, biomass and silage. Co-fermentation is the preferred method because it allows better use of the available capacity of the digesters, and well prepared, balanced chemically substrate allows for a higher degree of degradation of components from the same time a higher biogas production, better quality of digested sludge, better properties of fertilizer in comparison with the fermentation of the separate components. The diverse composition of the substrate allows selection of optimal parameters what has a beneficial effect on the efficiency of the fermentation process. The course of the fermentation process depends on biodegradability of the substrate, suitable groups of microorganisms and

environmental parameters, e.g.: pH, temperature, oxidation-reduction potential, alkalinity, the volatile organic acids content, the concentration of nutrients (C/N) content in the substrate, toxic compounds content and the level of fragmentation of the substrate [9, 16].

It can be concluded that the process of co-digestion efficiency of biogas production and its quality depends largely on the type of co-substrates and a method for carrying out fermentation.

## **2. Methods of assess susceptibility of waste to biodegradation**

Biodegradation studies are conducted to determine the susceptibility to degradation of the single or mixtures compounds. The speed and effectiveness of the biological degradation of the substrate depends on the following parameters [10,19]:

- the concentration of substrates,
- the masses and degree of adaptation of microorganisms,
- the presence of nutrients (nitrogen and phosphorus, trace elements),
- the presence of an activating or inhibitory substances,
- environmental conditions (temperature, pH).

The susceptibility of organic compounds biodegradability also depends on their physical and chemical properties e.g.: volatility or water solubility. Susceptibility of waste to biodegradation can be determined using both tests: anaerobic (respiratory static test ( $AT_4$ ) and dynamic test respiratory ( $DT_4$ ) or anaerobic: biochemical methane potential (BMP) [1-3, 10, 11, 20].

Evaluation of biodegradability using  $AT_4$  test is a practical tool to quickly assess the susceptibility of substrates for degradation after just 4 days, while the BMP test requires 21 days (partial production of biogas) or 100 days (total biogas production) [17]. Assessment of susceptibility of the substrate to biodegradation using spirometry test is now routinely used to

assess the potential of biogas waste [17]. In the literature can be find information about the correlation between the AT<sub>4</sub> and BMP [4, 13, 18, 20].

### 2.1 Static respiratory test (AT<sub>4</sub>)

Static respiration test [2, 8, , 12, 17, 20] is a parameter determining the oxygen demand required for the decomposition of an organic substrate within four days. The AT<sub>4</sub> test measured decrease of oxygen concentration inside the reactor. Research can be carried out both on a substrate of solid and liquid.

The AT<sub>4</sub> test belongs to closed semiconductor techniques. It requires monitoring of oxygen consumption over time, and releases carbon dioxide is bound by the alkaline absorbent.

The value of AT<sub>4</sub> for the selected components of the waste are shown in Table 1.

Table 1. Values of the indicator AT<sub>4</sub> for the selected waste [6]

Substrate	AT <sub>4</sub> [g/kg DM]
Cellulose	84,9
Newspapers	76,6
Corrugated paper	12,5
Grass	119,0
Branches	57,1
Plants	137,0
Meat	150,0
Cotton	12,9
Wool	17,0
Diapers	86,1
Compost	26,3

The guidelines of the Department of Waste Management in the Ministry of the Environment [21] concerning the requirements for the processes of composting, fermentation and mechanical - biological treatment of waste dictated in the years 2009-2010 to implement measurements of AT<sub>4</sub> as a complementary parameter for assessing the degree of stabilization of waste.

## 2.1 Biochemical methane potential

Biochemical methane potential (BMP) [3, 6, 7, 11] is a test to rank biodegradability of organic substrate under anaerobic conditions. It is a tool to assess the fermentation process and the its kinetics used not only for waste, but also for waste water and sewage sludge.

Production and composition of biogas measured in a test BMP depend closely on the type of substrates. Table 2 shows the methane production from the selected waste.

Table 2. Methane production from selected waste [6]

Substrate	BMP [dm <sup>3</sup> /kg VS]
Cellulose	136
Newspapers	76
Corrugated paper	320
Grass	225
Branches	93
Plants	312
Meat	633
Cotton	26
Wool	21

Diapers	278
Compost	24

The data presented in Table 2 show that the for individual substrates in the methane fermentation process are obtained different values of methane production dependent on the biodegradability of the substrate. The substrates characterized by the highest methane production are meat and vegetable.

### 3. Experimental

In the studies used a waste's substrate from the agri-food sector as: silage corn, ground mushrooms, chicken manure, grass and haulm tomatoes. The susceptibility of these substrates on the aerobic and anaerobic biodegradation evaluated based on tests AT<sub>4</sub> and BMP, respectively. Substrates shredding to the size <20 mm. The physico-chemical composition of substrate was evaluated based on the following parameters: dry matter, loss on ignition, COD, pH, Kjeldahl nitrogen, ammonia and phosphorus. Analyses were performed according to the methodology in force in Poland.

#### 3.1. Methodology for determining parameter AT<sub>4</sub>

Determination of AT<sub>4</sub> was performed a static method using Oxitop apparatus. A view of the elements the test bench are shown in Fig. 2. Time measurement of microbial activity was 4 days. The total duration of the study also takes into account the period of adaptation of microorganisms to new environmental conditions. It was assumed that the adaptation of microorganisms time ends when the average of three-hour measuring the oxygen concentration reaches 25% value of the maximum oxygen demand. The study was conducted at a constant temperature of 20 ° C in a thermostatic cabinet.



Fig. 2. The test bench - AT<sub>4</sub>

The oxygen consumption by the microorganisms calculated from the equation:

$$AT_4 = \frac{M_R(O_2)}{R \cdot T} \cdot \frac{V_{fr}}{m_{Bt}} \cdot \Delta p \quad (1)$$

where:

AT<sub>4</sub> - biodegradability, mgO<sub>2</sub>/g DM,

M<sub>R</sub> (O<sub>2</sub>) - the molar mass of oxygen (32000 mg/mol)

V<sub>fr</sub> - volume of free gas, dm<sup>3</sup>,

R -General gas constant (83,14 hPa mol<sup>-1</sup> K<sup>-1</sup>)

T - measured value of temperature (293 K)

m<sub>Bt</sub> - dry mass of waste in a sample including mineral fraction g DM,

Δp - pressure drop, hPa.

The volume of free gas calculated by the formula:

$$V_{fr} = V_S - V_{AG} - V_{AM} - V_{Bf} \quad (2)$$

where:

$V_{fr}$  - volume of free gas,  $dm^3$ ,

$V_S$  - the total volume of vessel,  $dm^3$ ,

$V_{AG}$  - the volume of vessels on absorbent,  $dm^3$ ,

$V_{AM}$  - absorbent volume,  $dm^3$ ,

$V_{BF}$  - sample volume,  $dm^3$ .

### 3.2. Test BMP

Studies of the biochemical methane potential of substrates was carried out in anaerobic reactors with a capacity of  $2.5 dm^3$ . The process was conducted for a period of 21-30 days, at a temperature of  $37^\circ C$  in a thermostatic cabinet. Biogas from reactors was collected using 300 ml syringe. In biogas was measured content:  $CH_4$ ,  $CO_2$ ,  $O_2$ ,  $NH_2$ ,  $H_2S$ . View of the test bench is shown in Fig. 3.

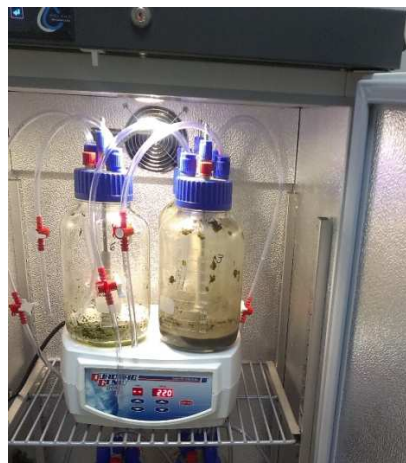


Fig. 3. The test bench - BMP

The amount of the substrate and the inoculum was determined on the basis of a expected production of biogas. It biogas production in each bioreactor was assumed on the level 3000 ml with concentration of methane - 60%. It was assumed that from the 1 g COD is obtained 395 ml of  $CH_4$ .



The efficiency of decomposition of an organic substrate (COD) to methane will be defined by the equation:

$$K_{CH_4} = \frac{(V_{bp} - V_{bk})}{COD_s \cdot (395)} \quad (3)$$

where:

$K_{CH_4}$  – COD conversion into methane, %

$V_{bp}$  – the total volume of the methane produced in the reactor, ml

$V_{bk}$  – the total volume of the methane produced in the reactor control, ml

$COD_s$  – COD value of the substrate, g

395 – Theoretical methane production from 1 kg of COD determined for standardized conditions:  $T=35^\circ\text{C}$ ,  $p=1$  atm.

#### 4. Results and discussion

The test results physico-chemical parameters of the substrates shown in Table 3. In the group of analyzed substrates highest content of organic dry matter in the range of 760.0 - 945.5 g/kg DM characterized by corn silage and grass and haulm tomatoes. Chicken manure was characterized by a dry matter content of organic matter at the level of 700.3 g/kg DM and compared to other substrates highest nitrogen content of 60.0 g/ kg DM. Designated for substrates quotient C/N was in the range from 11 to 57. The highest value of the ratio C/N was obtained for corn silage.

**Table 3.** Composition of the physico-chemical substrates

<b>Paramter</b>	<b>Unit</b>	<b>Surface of mushrooms</b>	<b>Chicken manure</b>	<b>Maize silage</b>	<b>Grass</b>	<b>Haulm tomatoes</b>
<b>pH</b>		6,2	7,7	5,8	6,5	5,5
<b>Dry matter</b>	g/kg	414,5	212,3	264,2	220,6	133,1
<b>VS</b>	g/kg DM	590,2	700,3	945,5	770,3	760,0
<b>C/N</b>		16	15	57	11	31
<b>Kjeldahl nitrogen</b>	g/kg DM	25,4	59,6	16,5	48,8	24,2
<b>Ammonium</b>	g/kg DM	0,7	10,8	1,7	6,6	0,6
<b>Phosphorus</b>	g/kg DM	576	1072	352	688	728
<b>COD</b>	g/kg DM	740,1	1156,1	1884,2	1345,3	1184,1

The test results AT4 and BMP efficiency of decomposition of organic compounds (COD) methane (KCH<sub>4</sub>) shown in Table 4.

**Table 4.** Assessment of biodegradability of substrates

<b>Paramter</b>	<b>Unit</b>	<b>Surface of mushrooms</b>	<b>Chicken manure</b>	<b>Maize silage</b>	<b>Grass</b>	<b>Haulm tomatoes</b>
<b>AT4</b>	g O <sub>2</sub> /kg DM	53	109	200	162	148
<b>BMP</b>	dm <sup>3</sup> /kg DM	122	185	320	237	185
<b>K<sub>CH4</sub></b>	%	41,7	40,5	42,9	44,5	39,5

Studies have shown that the highest value of AT4 on the level of 200 gO<sub>2</sub>/kg DM obtained for corn silage, and the lowest (53 g O<sub>2</sub>/kg DM) for ground mushrooms. The highest methane

production ( $320 \text{ dm}^3/\text{kg DM}$ ) was obtained from anaerobic digestion of corn silage.

For all of substrates tested (Table 4) the efficiency of decomposition of organic compounds was  $> 40\%$ . Statistical analysis of the results of studies made it possible to demonstrate the relationship between the organic content in the substrate and the value of  $AT_4$  (Fig.4.). The correlation between these parameters can be described by the equation:

$$AT_4 = 0,4VS - 178,7 \quad (4)$$

with a correlation coefficient  $R^2 = 0,9$ .

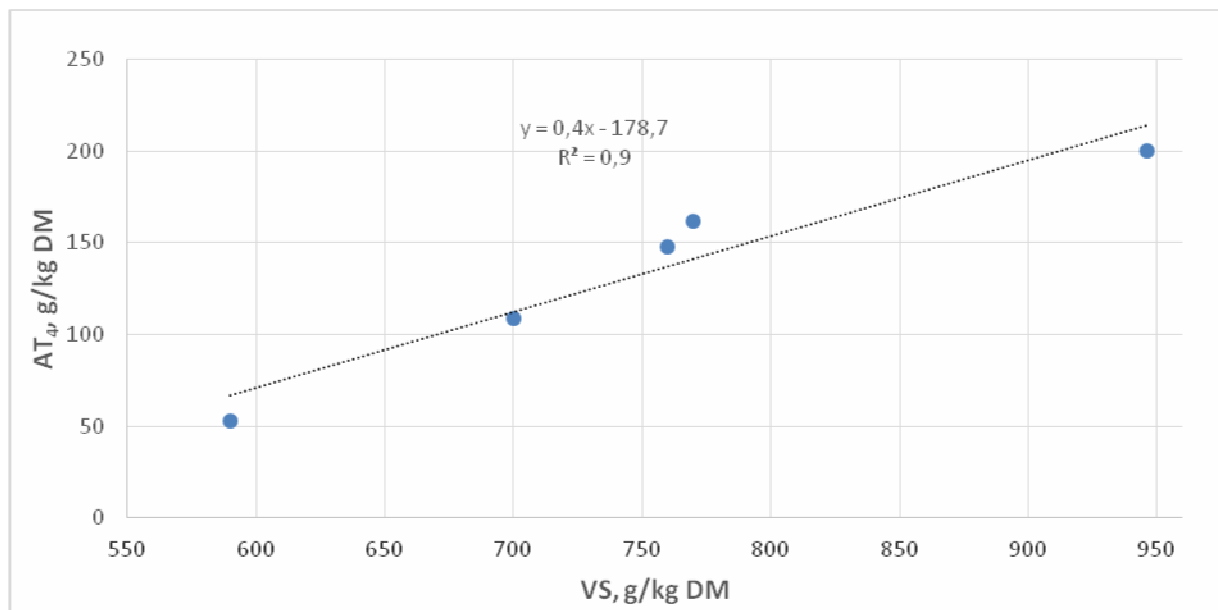


Fig. 4. The relationship between the organic content (VS) in the substrate and the value of  $AT_4$

With the increase in the organic content of a substrate increases the value of  $AT_4$ . The relationship between the organic content in the substrate, and the production of methane is shown in Fig. 5.

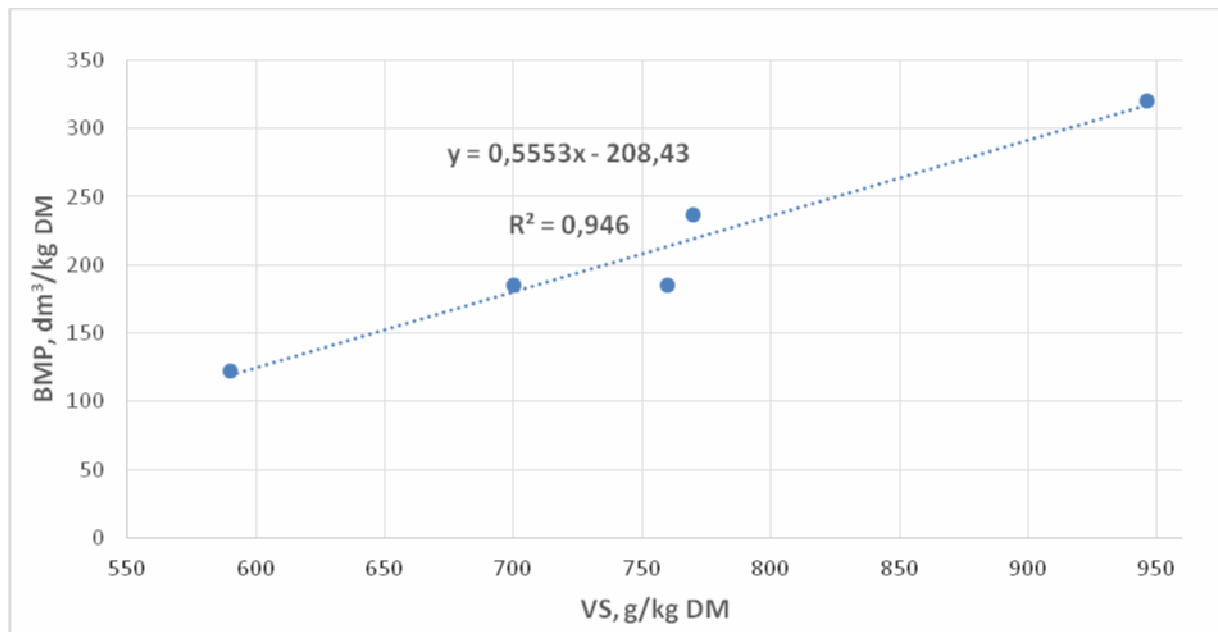


Fig. 5. The relationship between the organic content in the substrate (VS) and the value of the BMP

In the study was received a good mathematical correlation between the content of organic compounds, and the production of methane, with a coefficient of determination  $R^2 = 0,946$ . This relationship for the tested of substrates can be described as a mathematical equation:

$$\text{BMP} = 0,55\text{VS} - 208,4 \quad (5)$$

Also in this case the increase of the organic content of the substrate causes an increase in methane production.

The results confirm that there is a relationship between the AT<sub>4</sub> and BMP (fig. 6). In the study was o obtained a linear relationship as:

$$\text{BMP} = 1,2 \text{ AT}_4 + 44,3 \text{ przy } R^2 = 0,9 \quad (6)$$

With the increase of participation in substrates bioavailable organic matter the productivity of methane was increasing.

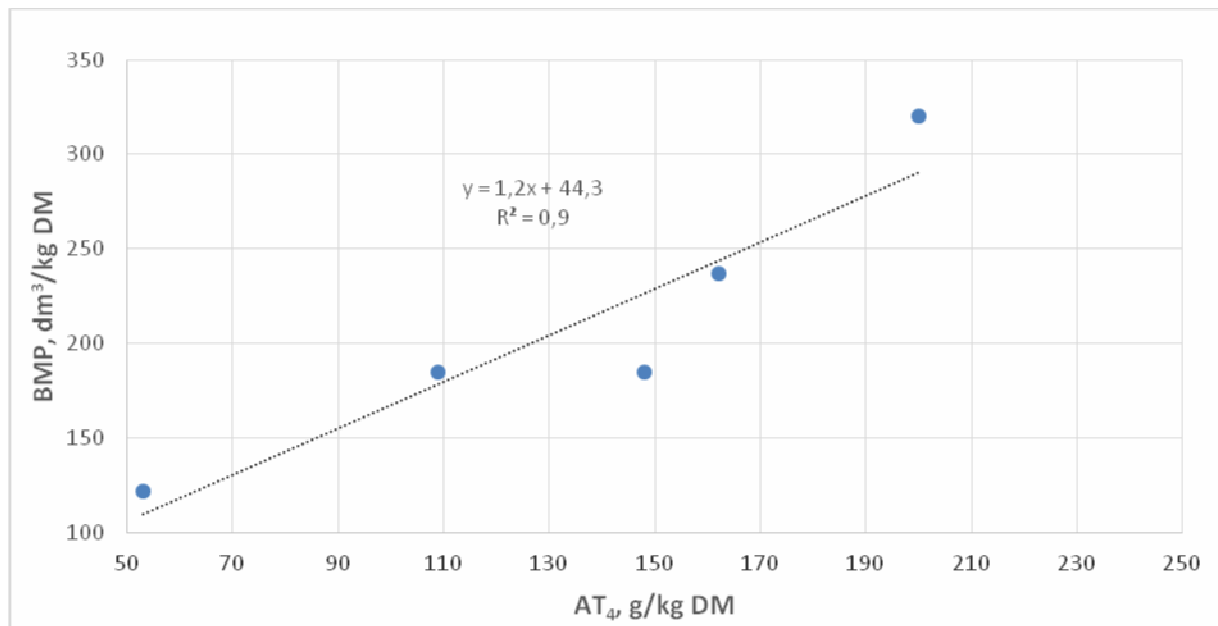


Fig. 6. The relationship between AT<sub>4</sub> and BMP value

Test results and their mathematical interpretation shown that based on AT<sub>4</sub> of substrates can be estimated methane production. Estimation of the biogas potential of substrates using test AT<sub>4</sub> is in practice more justified than the BMP test which is expensive and time-consuming, requiring 21 (partial biogas production) to 100 days (total biogas production).

## 5. Conclusion

The results of these tests show that:

1. The highest content of organic matter are characterized by maize silage, grass and haulm tomatoes.
2. The highest quotient C/N determined for silage and haulm tomatoes.
3. AT<sub>4</sub> of 200 gO<sub>2</sub> / kg DM and the highest production of methane (320 dm<sup>3</sup>/kg DM) was obtained for corn silage.

4. For all tested substrates obtained the efficiency of anaerobic digestion of > 40%.
5. Statistical analysis showed a high correlation between the content of organic compounds in the substrates, and the value of AT<sub>4</sub> and BMP and between BMP and AT<sub>4</sub>.
6. The BMP test can be replaced by test AT<sub>4</sub>. For tested substrates were determined the relationship as:  $BMP = 1.2 + 44.3 AT_4$  with  $R^2 = 0.9$ .

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## Figure legends

Fig. 1. The average yield of biogas produced with tons of animal and plants waste

Fig. 2. The test bench - AT4

Fig. 3. The test bench – BMP

Fig. 4. The relationship between the organic content (VS) in the substrate and the value of AT4

Fig. 5. The relationship between the organic content in the substrate (VS) and the value of the BMP

Fig. 6. The relationship between AT4 and BMP value

