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The estimation of changes in functional diversity of anaerobic community during co-substrates mesophilic digestion process.

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Anaerobic waste treatment technologies enable disposal of a wide variety of wastes, being substrates for this process. These include, inter alia, agricultural waste, or waste from the food industry, sewage sludge or municipal waste. In recent years there has beena significant increase in interest in the possibility of an aerobic organic waste degradation from various sources in order to obtain high-biogas production.

Transformation of complex organic compounds in organic wastes into CH_4 and CO_2, is possible due to the interaction of four different groups of microorganisms. These groups include the following: bacteria of primary fermentation, secondary fermentation bacteria (syntrophic andacetogenicbacteria) and two types of methanogens belonging to Archaea. Among the microorganisms, methanogens can be distinguished into psychro-, meso- and thermophilic. Mesophilic and thermophilic anaerobic bacteria show the highest activity in biogas production within the ranges of temperature, 28-42°C and 55-72°C respectively.

In our study we evaluated shifts in numbers of functional groups of anaerobic communities in mesophilic digestion process of co-substrates consisting of 25% fruit industry wastes, 25% dairy sewage sludge, 12% corn silage, 38% decoction grain, within 8 stages of this process (FM 1 - FM 8). Changes in anaerobes functional diversity were characterized by community level physiological profiling (CLPP) approach based on substrates utilization located on Biolog AN Plates, including: percentage of total carbon source utilization, significance of biodiversity indices, such as average well colour development (AWCD), Richness (R) and Shannon (H) indices; dendogram of carbon utilizations patterns, grouping particular stages community due to similarity according to the Sneath restrictive criterion (66%); and Cluster Analysis was performed to show which given substrates were utilized similarly.

Sample preparation procedure was conducted as follows: one g portions of soil were shaken in 99 mL of sterile peptone water for 20 minutes at 20°C and then were incubated at 4°C for 30 minutes. Next 100 µl of each sample were inoculated into each well of Biolog AN plates and then incubated in anaerobic atmosphere (85% N_2, 10% CO_2 and 5% H_2) at 27°C. The rate of utilization was indicated by the reduction of the tetrazolium, a redox indicator dye that changed from colourless into purple if substrates were utilised. Data were recorded with a plate reader at appropriate weave length AN plates and 750 nm every 24 h throughout 4 days. The data from 72 h were taken under consideration to evaluate CLPPs.

Proportion of carbon sources utilization (Fig.1.) differed according to the stage taken into consideration with no clear tendency. In stage FM 3 there were more amines and amides utilized and less substrates of polymer group. Dendogram (Fig. 2.) showed consistency to % of carbon

source utilization shifts. The most intensive utilized substrates are presented at Fig. 3. α-Methyl-D-Galactoside and α-D-Glucose in FM 2; Arbutin, Maltotriose in FM 3(carbohydrates) and L-Methionine and L-Alanine, L-Glutamic Acid (amino acids) in FM 2and FM 3, respectively.

Stage FM 3 differed significantly from others, also if biodiversity indices taken into consideration (Tab. 1).

This study highlighted changes in anaerobic community functional diversity during 8 stages of anaerobic digestion process. Particular stages were characterized by succession of different anaerobic populations involved in this process, which are important for effective run of the process.This culture-dependent approach revealed two stages with intensive degradation of large quantity of easily degradable substrates second and third stage of the process.

Scientific work was funded from the budget for science by National Centre of Research and Development in Poland. LIDER Programme 2011-2014

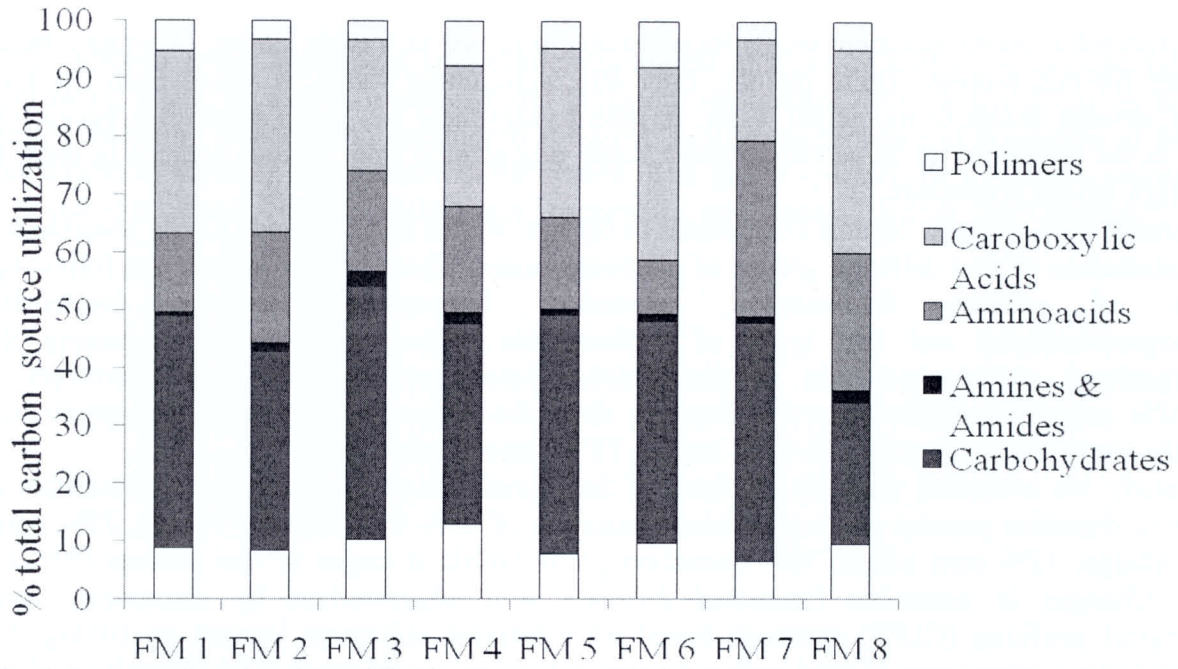


Fig. 1. Percent of total carbon utilization response tracked due to process stage

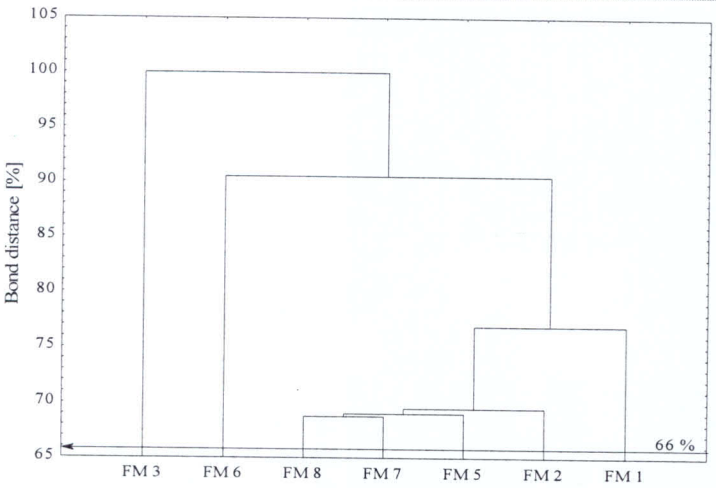
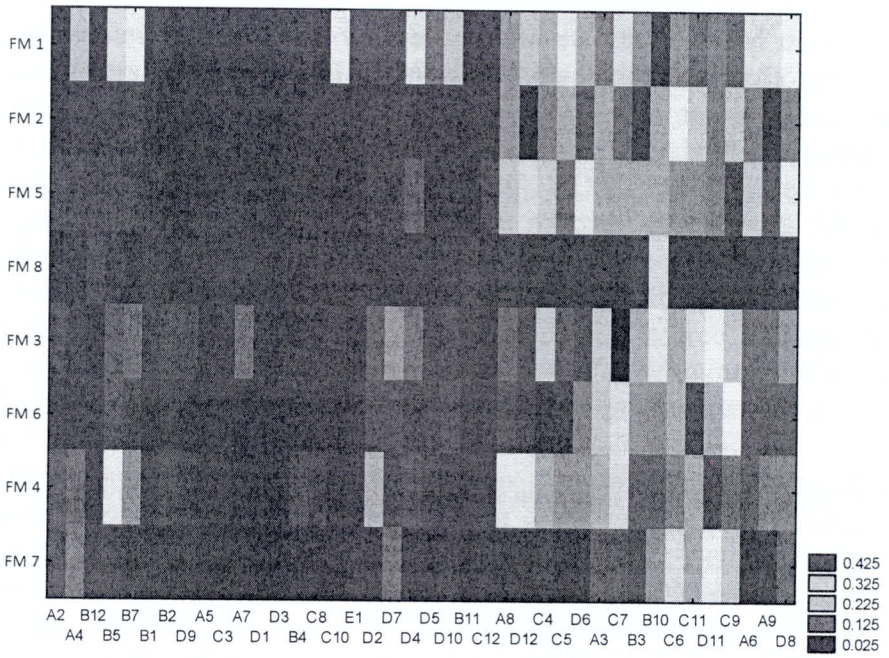


Fig. 2. Dendogram of carbon utilizations patterns of substrates located on Biolog AN Plates®

a)



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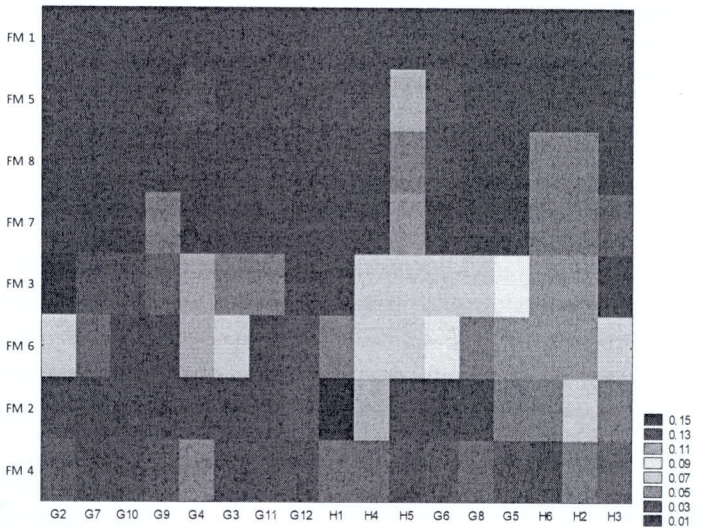

Fig. 3. Biolog AN Plate® carbon sources utilization intensity diagram of a) amino acids and b) carbohydrates

Tab. 1. Biodiversity indices in differed digestion stages calculated on Biolog AN Plate®

	R	H	AWCD
FM 1	6.00 ±1.00 b	3.46 ±0.01 c	0.06 ±0.00 b
FM 2	5.00 ±0.00 bc	3.23 ±0.01 d	0.05 ±0.00 cd
FM 3	12.00 ±0.58 a	4.00 ±0.07 a	0.10 ±0.01 a
FM 4	5.00 ±0.58 bc	3.85 ±0.10 b	0.06 ±0.01 bc
FM 5	4.00 ±0.00 c	3.47 ±0.03 c	0.05 ±0.01 d
FM 6	4.0 ±0.58 c	4.04 ±0.03 a	0.06 0.00 b
FM 7	2.00 ±0.00 d	3.43 ±0.06 c	0.03 ±0.01 e
FM 8	0.00 ±0.00 e	3.25 ±0.05 d	0.01 ±0.00 f