Methanogenic and Nonmethanogenic Activity of Granulated Sludge in Anaerobic Baffled Reactor

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The methanogenic and nonmethanogenic activities of sludge from anaerobic baffled reactor (ABR) were investigated. The lab-scale ABR treated synthetic wastewater containing starch and peptone. The activities were measured at two values of organic loading rate (OLR) – 3.5 kg m^{-3} d^{-1} and 10 kg m^{-3} d^{-1} (as COD – Chemical Oxygen Demand). The performance of ABR was compared with the performance of a UASB (Upflow Anaerobic Sludge Bed) reactor operated under the same conditions. For the kinetic tests corresponding substrates were used: the starch for hydrolytic activity, the glucose for acidogenic activity, and the sodium acetate for methanogenic activity. $NaHCO_3$ was added to each test with the aim to keep pH in a normal range of operation. For ABR the maximal measured specific hydrolytic activity was 36.8 kg kg^{-1} d^{-1}, maximal specific acidogenic activity 38.1 kg kg^{-1} d^{-1}, and maximal specific methanogenic activity 1.51 kg kg^{-1} d^{-1} (at OLR 10 kg m^{-3} d^{-1}). The activities of the anaerobic sludge from UASB reactor at OLR 10 kg m^{-3} d^{-1} were as follows: hydrolysis 3.52 kg kg^{-1} d^{-1}, acidogenesis 1.12 kg kg^{-1} d^{-1}, and methanogenesis 0.66 kg kg^{-1} d^{-1} The measurements have shown that the rate-limiting step of anaerobic degradation for the used system substrate—reactor was the methanogenic phase.

The biochemistry and microbiology of anaerobic digestion is a complex biogenic process involving a number of microbial populations, often linked by their individual substrate and product specificities. As shown in Fig. 1, the conversion of complex substrate ingredients proceeds *via* the formation of numerous intermediate products. The first group of organisms which take place in anaerobic digestion are the hydrolytic fermentative (acidogenic) bacteria. These bacteria hydrolyze the complex polymer substrate to organic acids, alcohols, sugars, hydrogen, and carbon dioxide. The second group are hydrogen-producing and acetogenic organisms, which convert the fermentation products of the previous step (hydrolysis and acidogenesis) into acetate and carbon dioxide. The third group are the methanogens, which convert simple compounds as acetic acid, methanol, and carbon dioxide + hydrogen into methane. In examining the anaerobic degradation process of complex organic substrates (as proteins, carbohydrates, lipids) six distinct steps can be identified (*Gujer* and *Zehnder* [1]):

1. Hydrolysis of organic polymers.

2. Fermentation of amino acids and sugars to hydrogen, acetate and short-chain VFA (volatile fatty acids) and alcohols.

3. Anaerobic oxidation of long-chain fatty acids and alcohols.

4. Anaerobic oxidation of intermediary products such as volatile acids (except acetate).

5. Conversion of acetate into methane by acetotrophic organisms.

6. Conversion of hydrogen into methane by hydrogenotrophic organisms (carbon dioxide reduction).

Some authors present anaerobic degradation process by more than six steps. *Harper* and *Pohland* [2] have identified nine recognizable steps. These authors, for example, have separated anaerobic oxidation by obligate hydrogen-producing acetogens and by nitrate- and sulfate-reducing bacteria. Generally we can say, anaerobic process is determined by four main steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

Considering easy biodegradable materials (containing short-chain VFA, monomeric saccharides, *etc.*) the limiting step of anaerobic degradation is the methanogenic step (step *5.* and *6.* mentioned above). On the other hand, during the anaerobic digestion of complex materials (*e.g.* agricultural wastes, which are mainly composed of cellulosics, lipids, and proteins or wastewaters from food industry), the limiting step of the process is often the hydrolytic step.

The selection of the most suitable equipment to be employed in the anaerobic treatment of individual waste or wastewater depends on the substrate nature and so on the limiting step of the process. Several analyses, such as the Chemical Oxygen Demand (COD), the Total Organic Carbon (TOC), VSS *vs.* SS (Volatile

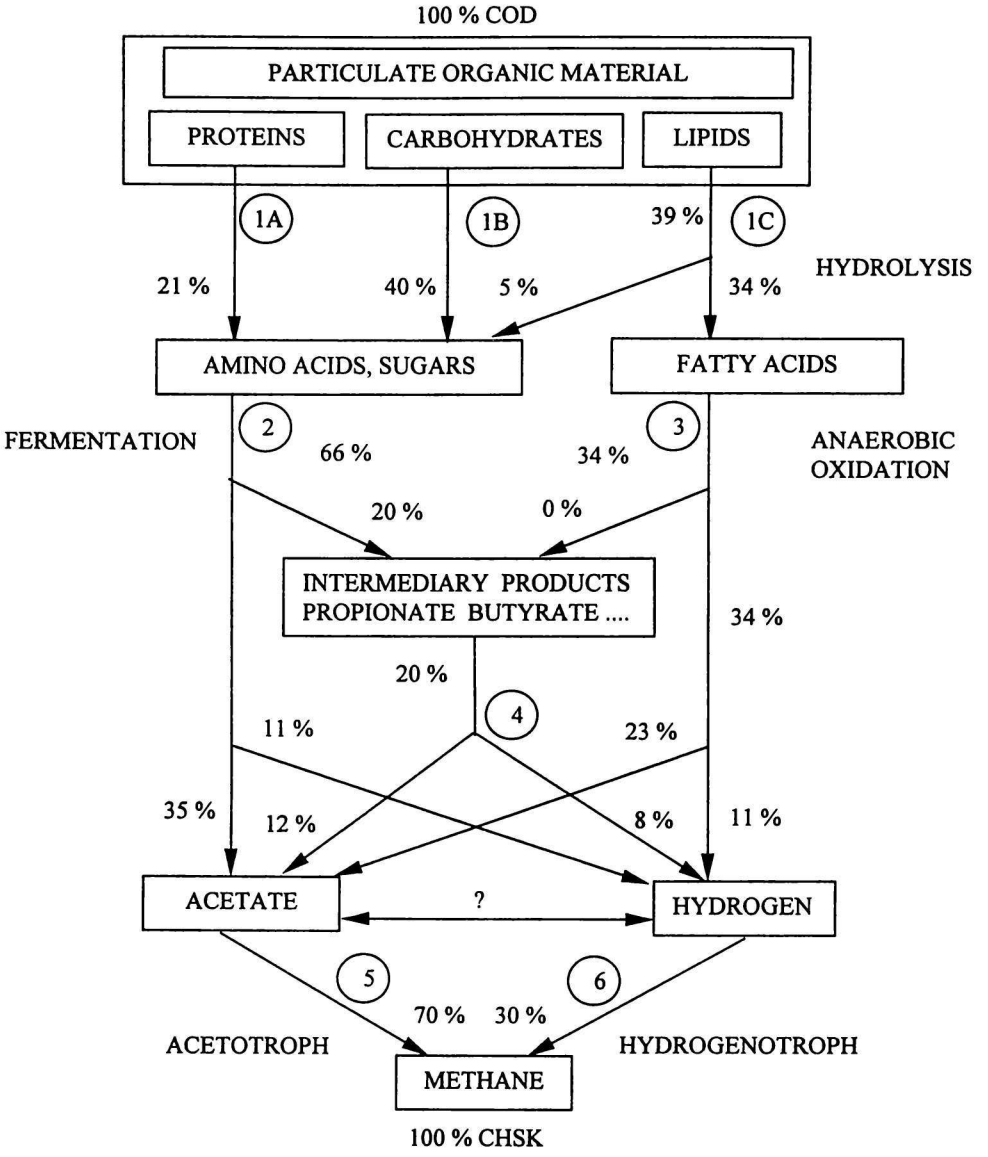

Fig. 1. Schematic diagram showing the conversion reaction taking place in the anaerobic digestion of complex substrates [1].

Suspended Solids/Suspended Solids), proteins, fats, and carbohydrates content give a good information about nature of complex substrates. However, from these data it is difficult to determine exactly which of the anaerobic digestion steps is the limiting one. It is evident that the experimental method for determining the microbial activity in the different step of the degradation of complex substrate is necessary.

Anaerobic sludge activity measurement can be considered in two different ways: an overall measurement which gives information about the whole degradative activity and an activity measurement of each basic stage of the process. A further interesting application of activity tests is to determine the toxic or inhibition effect of a substrate on an anaerobic sludge.

In our previous works [3, 4] we have studied the behaviour of an anaerobic baffled reactor (ABR) in wastewater treatment. From these works it followed that a cascade arrangement in ABR can cause the segregation of the individual anaerobic process phases (hydrolysis, acidogenesis, acetogenesis, and methanogenesis). From this point of view the performance of the ABR was compared with the performance of the UASB reactor operated under the same conditions. As main compounds of the synthetic nonacidified wastewater starch and peptone were used. In this work methanogenic and nonmethanogenic activity of anaerobic sludge in each ABR compartment is measured and discussed. Also, these activities were compared with the activity of sludge from UASB reactor.

EXPERIMENTAL

The experimental equipments described in works

Table 1. Synthetic Wastewater Composition (COD of 2000 mg dm^{-3})

Component	Concentration / mg dm^{-3}
Starch	1500
Peptone	333
$NaHCO_3$	max. 1000
NH_4—N	26.7
PO_4—P	6.7
$CaCl_2$	27.7
$MgSO_4 \cdot 7H_2O$	50.7

[3, 4] were used. The laboratory model of ABR consisted of four compartments and its useful volume was 13.05 dm^3. The UASB reactor was made from a tube with an inner diameter of 7 cm and its volume was 3.33 dm^3. The synthetic wastewater composition is shown in Table 1. The hydrolytic kinetic test, acidogenic test, and the test of specific methanogenic activity were used to determine the kinetic parameters of the anaerobic process. Sludge from all four compartments of the ABR was used as inoculum in the determination of the kinetic parameters. Inoculum concentration was 0.25—4.8 g dm^{-3} VSS (Volatile Suspended Solids). For the kinetic tests corresponding substrates were used: 1.5 g dm^{-3} of starch for hydrolytic step, 1.5 g dm^{-3} of glucose for acidogenic step, and 2.0 g dm^{-3} of sodium acetate for methanogenic step. 1.0 g dm^{-3} of $NaHCO_3$ was added to each test with the aim to keep pH in a normal range of operation.

Calculations and activity expressions are presented in the work by *Soto et al.* [5]. The activity (Ac) is usually expressed as g COD per g VSS per day and calculated from the substrate consumption rate (*e.g.* hydrolysis, acidogenesis) or from product formation rate (*e.g.* acetogenesis, methanogenesis).

From substrate consumption rate

$$\mathrm{Ac_s} = -\frac{1}{\rho(\mathrm{VSS})} \frac{\mathrm{d}\rho(\mathrm{COD})}{\mathrm{d}t} \quad (\mathrm{g\ g^{-1}\ d^{-1}}) \qquad (1)$$

From methane production

$$\mathrm{Ac_{CH_4}} = -\frac{1}{\rho(\mathrm{VSS}) V_\mathrm{R} f_1} \frac{\mathrm{d}V(\mathrm{CH_4})}{\mathrm{d}t} \quad (\mathrm{g\ g^{-1}\ d^{-1}}) \qquad (2)$$

where $V(CH_4)$ is the cumulative methane production, V_R the useful volume of the reactor, and f_1 a conversion factor which represents the COD value of the unit of methane volume.

The hydrolytic activity was determined as maximum consumption rate of starch, expressed as grams of COD of starch per gram sludge VSS per day (according to eqn (*1*)). The acidogenic activity was determined as maximum consumption rate of glucose, expressed as grams of COD of glucose per gram sludge VSS per day (eqn (*1*)). Finally, the maximum specific methanogenic activity was determined as maximum rate of methane production, expressed as grams of COD of methane per gram sludge VSS per day (eqn (*2*)).

Determination of VSS was carried out as proposed by *Standard Methods* [6]. Glucose concentration was evaluated by determining the amount of reducing sugars in sample using the dinitrosalicylic acid (DNS) reactive. Starch content was estimated as the difference between the total sugars (by using the phenolsulfuric reagent) and the reducing sugar amount in the sample. Methane production was measured according to work [5].

RESULTS AND DISCUSSION

A lab-scale mesophilic ABR and a UASB reactor treating synthetic nonacidified wastewater (see Experimental) were operated at gradually increased OLR (organic loading rate). The possibility of segregation of the anaerobic process phases along the ABR (hydrolysis and acidogenesis are determining processes in first compartments, methanogenesis is determining process in the last compartment) caused biomass stratification. This fact is evident from the values of pH, too. The pH in the ABR rose from the first to the last compartment. The average pH in the compartments were 6.3; 6.6; 6.7; resp. 6.8. In each compartment different biomass was cultivated. It was clear also from visual observation. In the first compartment granular sludge of a light-grey colour with large portion of suspended biomass was cultivated. The sludge bed of the last compartment was formed by fully granulated aggregates of microorganisms. Characterization of the biomass by its methanogenic and nonmethanogenic activity was made at two values of OLR: 3.5 kg m^{-3} d^{-1} and 10 kg m^{-3} d^{-1} (as COD).

Hydrolytic, acidogenic, and methanogenic activities of the sludge from all four compartments of ABR were determined. Fig. 2 shows the activity values for starch removal, Fig. 3 for glucose removal, and Fig. 4 for methane production from acetate. As it can be observed, specific hydrolytic and acidogenic activities were the highest in the first compartment, while these activities remained almost constant in other compartments. If we compare the hydrolytic and acidogenic activity in the first compartment at OLR 3.5 kg m^{-3} d^{-1} and 10 kg m^{-3} d^{-1} we can note that during the operating of the ABR biomass with high hydrolytic and acidogenic activity was cultivated in this compartment. The maximum specific methanogenic activity in ABR was obtained in the second compartment and it decreased towards the last compartment. We assume that methanogenic activity in the first compartment was strongly influenced by the acidifying environment. A considerable increase of methanogenic activity in the second and third compartments was observed at

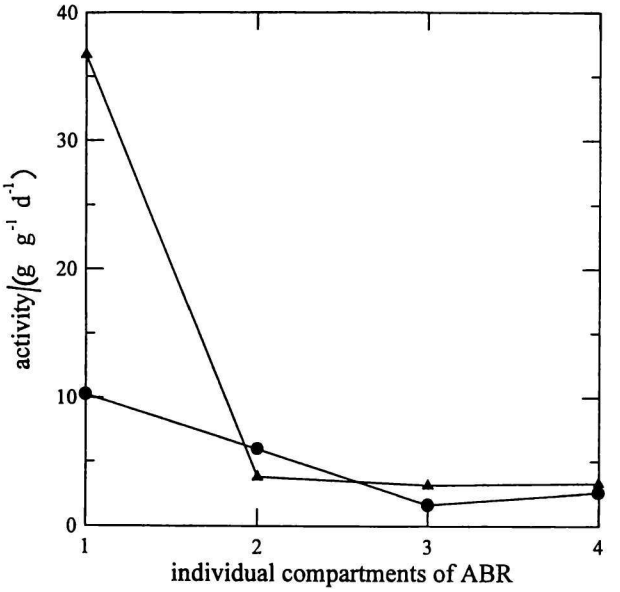

Fig. 2. Hydrolytic activity of the sludge from the ABR. ● OLR 3.5 kg m^{-3} d^{-1}, ▲ OLR 10 kg m^{-3} d^{-1}.

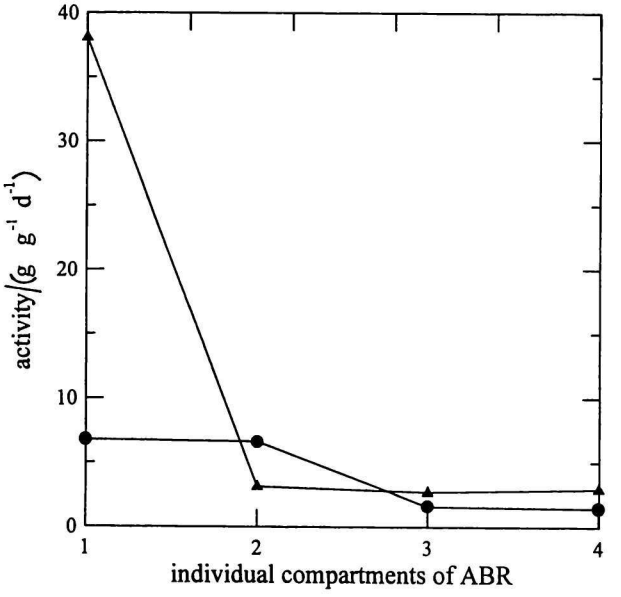

Fig. 3. Acidogenic activity of the sludge from the ABR. ● OLR 3.5 kg m^{-3} d^{-1}, ▲ OLR 10 kg m^{-3} d^{-1}.

OLR 10 kg m^{-3} d^{-1}. The maximal measured specific hydrolytic activity was 36.8 kg kg^{-1} d^{-1}, maximal specific acidogenic activity 38.1 kg kg^{-1} d^{-1}, and maximal specific methanogenic activity 1.51 kg kg^{-1} d^{-1}. These values correspond with the values mentioned in literature [5, 7, 8].

The activity of the sludge from UASB reactor at OLR 10 kg m^{-3} d^{-1} is shown in Table 2. This activity is comparable with activity of the sludge from ABR at the same OLR.

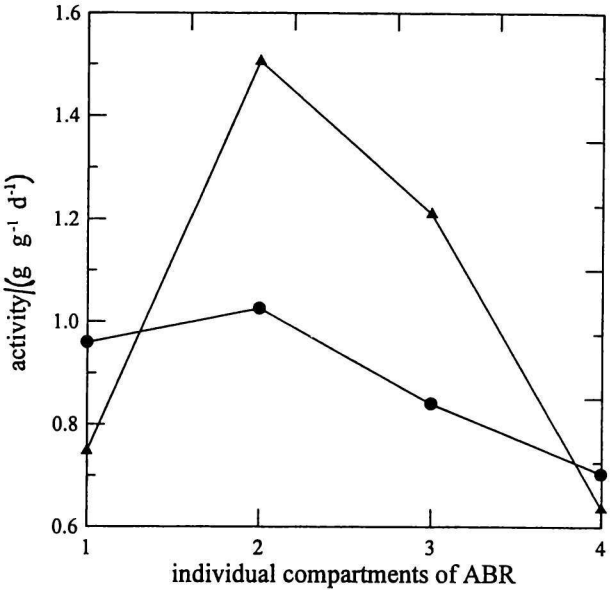

Fig. 4. Methanogenic activity of the sludge from the ABR. ● OLR 3.5 kg m^{-3} d^{-1}, ▲ OLR 10 kg m^{-3} d^{-1}.

Table 2. Methanogenic and Nonmethanogenic Activity of the Sludge from the UASB Reactor at OLR 10 kg m^{-3} d^{-1}

Activity	Ac/(kg kg^{-1} d^{-1})
Hydrolytic	3.52
Acidogenic	1.12
Methanogenic	0.66

From our work [4] it resulted that a wide application of the ABR can mostly be seen in treating nonacidified wastewater, where a two-stage anaerobic treatment is recommendable. As shown by the measurements of the methanogenic and nonmethanogenic activity done in this work, starch is not a convenient substrate for supporting of this idea. The slowest, or rate-limiting step in anaerobic degradation of used substrates was the methanogenic phase. Therefore we did not observe any significant difference between ABR and UASB reactor performance.

CONCLUSION

The tests of methanogenic and nonmethanogenic activity were used for the characterization of the sludge from a lab-scale anaerobic baffled reactor. Results from this study can be summarized as follows:

– During the operation of ABR hydrolytic, acidogenic, and methanogenic activity of the anaerobic sludge increased considerably.

– The maximal specific hydrolytic activity was 36.8 kg kg^{-1} d^{-1}, maximal specific acidogenic activity 38.1

kg kg^{-1} d^{-1}, and maximal specific methanogenic activity 1.51 kg kg^{-1} d^{-1}.

– In a complex multistep process such as anaerobic digestion, the kinetic characteristics of the slowest step govern the overall rate of anaerobic degradation. Our measurements showed that this rate-limiting step for the used system substrate—reactor was the methanogenic phase.

– Therefore, it is concluded that refractory organic compounds such as cellulosic materials, where the hydrolysis rate of complex materials limits the overall digestion rate can be a more convenient substrate for confirmation of ABR advantages.

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