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Biodegradation of Hexamethylenetetramine in Anaerobic Baffled Reactor

M. Hutnan*, M. Drtil, J. Derco, L. Mrafkova

Department of Environmental Science, Faculty of Chemical Technology, Slovak University of Technology,
Radlinskeho 9, 812 37 Bratislava, Slovak Republic

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Abstract

Due to its structure, hexamethylenetetramine (HMT) is a slowly biodegradable substance, hydrolysing in acid environment to ammonium and formaldehyde. Formaldehyde is toxic under normal conditions for biological treatment processes. Following an adaptation, a formaldehyde concentration of 1000 mg·l^{-1} may become biodegradable both in aerobic and anaerobic conditions. Acidic conditions for hydrolysis may be created chemically or by using natural biological treatment processes. The process of nitrification may be used in aerobic treatment, when H^+ ions are created and pH drops below 5. For anaerobic treatment, the process of acidification may be used, when volatile fatty acids are formed and pH of the environment is decreasing. This work examines HMT degradation in an anaerobic baffled reactor (ABR). Spatial segregation of individual anaerobic degradation phases was used in the ABR, when hydrolysis and acidification in the first reactor compartments are accompanied by low pH values in these compartments. ABR performance was compared with UASB (upflow anaerobic sludge bed) reactor performance working under the same conditions. The removal efficiency of 95.8% HMT in ABR reactor and of 85.4% in UASB reactor, respectively, was measured at the organic loading 4 kg·m^{-3}·d^{-1} and retention time 84 hours. 98% overall COD (chemical oxygen demand) removal was measured in ABR reactor and 95.6% in UASB reactor. The ABR proved to be the appropriate reactor for HMT removal. Better results of HMT removal in the ABR reactor in comparison with the UASB reactor were proved.

Keywords: anaerobic wastewater treatment, biodegradation of hexamethylenetetramine, anaerobic baffled reactor, UASB reactor

Introduction

Hexamethylenetetramine (HMT) is a colourless, crystallic, water-soluble compound (813 g·l^{-1} at 12°C). It is used for the production of plastic materials, explosives, phenolic resins, pharmaceutics, disinfecting materials, in the rubber industry as an additive, etc. HMT may also be formed in wastewater with high concentrations of formaldehyde and ammonia. HMT therefore must be considered a real wastewater pollutant. Its molecule has a cyclic symmetric structure. It is very stable, thus creating significant problems in the treatment of wastewater containing HMT.

One of alternatives to remove HMT is its degradation in acidic condition to formaldehyde and ammonia:

$$(CH_2)_6N_4 + 4H^+ + 6H_2O \rightarrow 4NH_4^+ 6HCOH \qquad (1)$$

Formaldehyde is a biodegradable compound. There are several references in the literature for the biological removal of HMT without previous chemical hydrolysis, i. e. pH ≥ 7. Such biodegradation has been described by authors Gomolka and Gomolka [1]. However, a sufficient

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*Corresponding author

adaptation of microorganisms is a required condition. In addition, authors did not take into account the fact that nitrification of NH_4^+ reduced the pH value to 6.0, and it is very likely that HMT removal had started only after this pH decrease. Smith and Colquhoun [2] showed that 73% HMT was removed at pH=6.9, when no free formaldehyde formed in the hydrolysis was detected. Hydrolysis following the equation (1) may also be slowly performed under neutral conditions, creating a balance with a very low concentration of free formaldehyde. Microorganisms continuously remove these hydrolysis products, shifting the balance of the equation (1) to the right, which may result in measurable HMT degradability after longer retention times. The primary condition for decay is to maintain sufficiently high sludge age, when very slowly growing microorganisms remove HMT.

Nitrification is one of the options to create and maintain acidic conditions. HMT is very often accompanied with NH_4^+ ions present in wastewater and ammonia is also formed in hydrolysis – as outlined in equation (1). Nitrification has two phases, which are described by the following two equations:

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O \qquad (2)$$

$$2NO_2^- + O_2 \rightarrow 2\ NO_3^- \qquad (3)$$

The stoichiometry of equations 1, 2, and 3 shows that 72 mmol of NH_4 is formed from 1 mmol HMT (140 mg), and 4 mmol H^+ after their nitrification.

Bodík *et al*. [3] used lower pH in the nitrification to remove HMT, employing semicontinuous and continuous activated sludge process with the starch-peptone basic substrate. HMT was added to this basic substrate so that the ratio of substrate to HMT (in COD units) was 1:10 (total substrate COD was 4200 $mg \cdot l^{-1}$). HMT removal in semicontinuous system was measured with the sludge age ranging from 5 to 50 days. The higher sludge age meant a higher nitrification effectiveness and a lower pH in the laboratory model. Decreasing pH increased HMT removal efficiency. HMT removal efficiency of 52.5% was reported with 50 day sludge age (pH in the reactor was 5.2), while the HMT removal efficiency of 70% was reported in the continuous reactor with the sludge age of 50 days and pH 5.6. Drtil *et al*. [4] also used semi-continuous and continuous model for HMT removal, though combining suspended and biofilm biomass. Polyurethane foam cubes (approximately 1 cm^3) were used as a carrier material for the biofilm biomass. In the semicontinuous model in the work [4], the HMT degradation was slow, arising from relatively high pH at the beginning of the semicontinuous cycle. Average HMT removal efficiency reached 43.7%. Average HMT removal efficiency in the continuous model reached 68.7%, with pH values ranging from 3.8 to 4.0 in the continuous model. However, simultaneous denitrification in the carrier was demonstrated. OH^- ions formed in the micro-environment of the polyurethane cubes resulted in pH higher than that in the bulk liquid. Painter and King [5] showed 100% effectiveness of HMT biodegradability using an activated sludge and ISO Die-Away test. Laboratory activated sludge models exhibited less than 25% HMT removal. Authors explained this fact by HMT hydrolysis during the long-term test using the above methodology (28 days), when biodegradable formaldehyde and ammonium were formed. Smith and Colquhoun [2] measured 87% effectiveness of HMT biodegradability, using the above test.

A pathway for the aerobic biodegradation of HMT was proposed in the work of Colquhoun [6].

Even though more information is available on anaerobic formaldehyde treatment, there are also few references in the literature on HMT removal in anaerobic conditions. Similar to the aerobic formaldehyde treatment, most of the works were focused on formaldehyde toxicity during anaerobic treatment [7, 8, 9]. In these works the formaldehyde concentration of 100 – 400 $mg \cdot l^{-1}$ (giving 50% inhibition in the single kinetic tests) depends on the co-substrate used. Formaldehyde with concentration 2 $g \cdot l^{-1}$ may be treated in continuous anaerobic reactor after a sufficient adaptation phase [8]. Dohanyos *et. al*. [10] studied anaerobic HMT removal. From their work it resulted that up-flow biofilm reactor was appropriate for the anaerobic treatment of the HMT-containing substrate. Methanol was used as the main substrate, with formaldehyde as an additive in the early phases of the process, and HMT was added afterwards. Total substrate COD was 15,000 $mg \cdot l^{-1}$, maximum formaldehyde concentration was 1.5 $g \cdot l^{-1}$, and maximum HMT concentration was 1 $g \cdot l^{-1}$. HMT was added to the substrate following the successful adaptation of an anaerobic filter to formaldehyde. Maximum HMT removal efficiency was 95.6%, the overall COD removal efficiency was 98%, with the reactor organic loading rate 4-4.3 $kg \cdot m^{-3} \cdot d^{-1}$ and retention time 3-3.5 days. Lower pH in the bottom part of the anaerobic filter was observed during operation (about 5.95). Measurements also proved higher acidification in this part of the reactor. Maximum HMT removal efficiency was achieved when the substrate was injected to three openings alongside the reactor. The lowest pH (4.3) was measured at a height of 10 cm. Hydrolysis, followed by formaldehyde decay, were the major mechanisms for anaerobic HMT removal [10].

Our work investigates possibilities of treating HMT in anaerobic baffled reactor (ABR). ABR belongs to the high-rate anaerobic reactors with a sludge bed, where the granulated biomass may be cultivated. As results from our previous works [11, 12, 13] this reactor is appropriate for the treatment of substrates with the lower acidification potential. Spatial segregation of individual anaerobic degradation phases (hydrolysis, acidogenesis, acetogenesis and methanogenesis) caused biomass stratification and pH gradient alongside the reactor. These characteristics show that ABR could be used for the treatment of wastewater containing HMT. In the work presented we compared an ABR operation with the UASB reactor operating under the same conditions and with the same substrate.

Materials and Methods

The construction of used laboratory models is shown in Fig. 1. Both the ABR and UASB reactor models were made from plexiglass. The effective reactor volume was 13.05 l for the ABR and 3.7 l for the UASB reactors. Reactors were filled with the granulated sludge of the same concentration in both reactors (20 g·l^{-1}, volatile suspended solids – VSS 14.5 g·l^{-1}). The ABR and UASB reactor were operated under the same conditions. The reactor operation was split to three periods.

Synthetic wastewater composition is given in Table1. During the first period (0-120 days) the synthetic substrate contained glucose, sodium acetate and HMT. Substrate COD was 6000 mg·l^{-1}. Sodium acetate was removed from the substrate in the second period (120-180 days) and glucose was the major substrate component, with the substrate COD 6000 mg·l^{-1}. Organic loading of the reactor in these periods ranged from 1 to 7 kg·m^{-3}·d^{-1}. In the third period glucose was used in the concentration necessary to reach the substrate COD 14,000 mg·l^{-1}. Phosphorus was added to the substrate in the ratio of COD: P = 500:1. No ammonium nitrogen was added to the substrate. We assumed that the amounth of ammonium nitrogen formed from HMT was sufficient. Micronutrients were added to the COD ratio as given in [12]. Reactors were operated at 37°C.

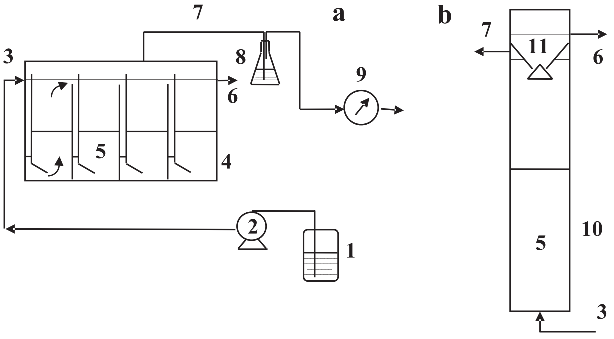

Fig. 1. Laboratory models of the ABR (a) and the UASB reactor (b).

1 - synthetic wastewater, 2 - peristaltic pump, 3 - influent, 4 - ABR, 5 - sludge bed, 6 - effluent, 7 - biogas outlet, 8 - 4 M solution of NaOH, 9 - methane production measurement, 10 - UASB reactor, 11-g-l-s separator

All analyses were performed using standard methods [14]. HMT was measured after the hydrolysis with strong acid at 60°C as free formaldehyde, using the hydroxylamine amperometric titrimetric analysis [15]. Free formaldehyde was measured in the reactors (difference between formaldehyde fixed in HMT and total formaldehyde). All measurements showed only minimal amounts of free formaldehyde. Thus, all concentrations of total formaldehyde listed below represent formaldehyde fixed in HMT.

Results and Discussion

The initial reactor organic load of 1 kg·m^{-3}·d^{-1} was gradually increased by wastewater flow. Fig. 2 shows reactor loads in the first period (0-120 days) and in the second period (120-180 days). Reactor organic loading in the third period was 4 kg·m^{-3}·d^{-1}, retention time 84 hours. COD ratio to total formaldehyde are given in Table 2. Fig. 3 shows the effluent concentration of nonfiltered COD from the ABR and UASB reactor during the I-st and II-nd periods.

The effluent concentrations of COD from the ABR were lower and more stable during the first and second period than from the UASB reactor. No significant biomass washout was observed. This was also confirmed by small differences between the filtered and nonfiltered COD effluent values. Smaller differences between effluent filtered and nonfiltered COD were observed in the ABR, when compared to the UASB reactor, suggesting lower washout of anaerobic biomass from the ABR. In the second period in particular, when glucose was used as substrate, the ABR operation was more stable than that of the UASB reactor. The effluent COD concentrations from the UASB reactor even exceeded 1000 mg·l^{-1} (see Fig. 3). However, a relatively low efficiency of the HMT removal was reached during the first and second periods (up to 50%). It is obvious that the HMT and total COD removal efficiency were higher in the ABR (Table 2). Fig. 4 shows the average influent and effluent concentrations of total formaldehyde for both reactors at organic loading values during periods I, II and III.

Total formaldehyde removal efficiency was low in the first period. Alongside the ABR, the pH gradient was observed. This is in agreement with our previous reports [11, 12] when the substrate with the high acidi-

Table 1. Synthetic wastewater composition.

Component	I. period	II. period	III. period
	Concentration (g·l^{-1})	Concentration (g·l^{-1})	Concentration (g·l^{-1})
Total COD	6.0	6.0	14.0
Sodium acetate	3.19	-	-
D-Glucose	4.21	5.7	13.1
HMT	0.23-0.69	0.23-0.69	0.92

Table 2. Average values of the ABR and UASB reactor parameters.

Period	B_V [kg·m^{-3}·d^{-1}]	Days	HRT [h]	Influent HMT concentration [mg·l^{-1}]	COD:HMT ratio	ABR		UASB	
						Average efficiency of HMT removal [%]	Average efficiency of COD removal [%]	Average efficiency of HMT removal [%]	Average efficiency of COD removal [%]
I.	1	8-19	144	300	20:1	77.3	94.6	46.3	90.8
	1.5	20-75	96	300	20:1	74.7		37.7	
	3	76-93	48	600	10:1	37.3		30	
	4	94-110	36	700	8.5:1	38.7		31.6	
	6	111-119	24	700	8.5:1	41.6		33.2	
II.	6	120-172	24	700	8.5:1	46.2	91.8	36.7	89.1
	7	173-180	20.5	900	6.7:1	44		25.3	
III.	4	180-210	84	1200	11.6:1	95.8	98	85.4	95.6

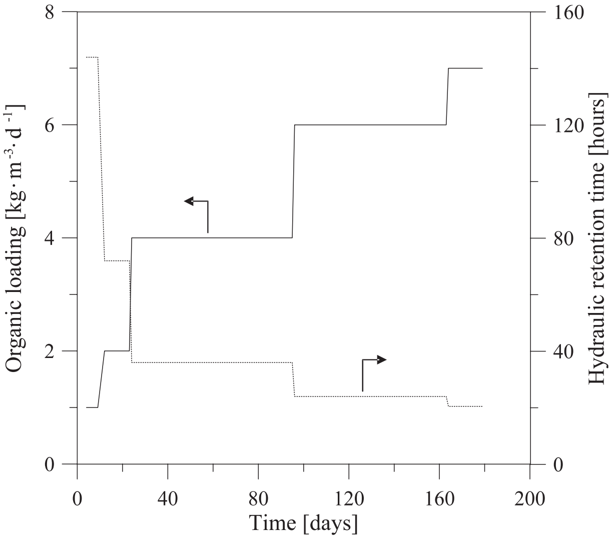

Fig. 2. Course of the organic loading and hydraulic retention time in the ABR and UASB reactor (in I. and II. period).

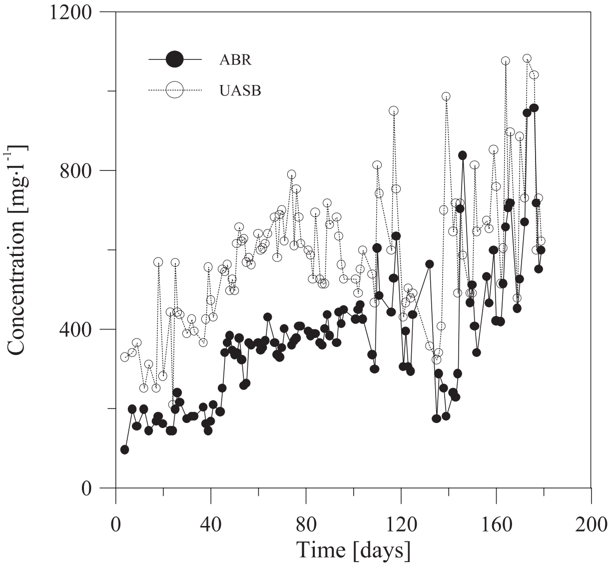

Fig. 3. Concentration of nonfiltered COD in the effluent from the ABR and the UASB reactor (in I. and II. period).

fication potential was used. When the HMT removal was studied, no $NaHCO_3$ was used to maintain pH. In the first compartment of the ABR, the pH was around 6.5. These pH values are much higher than those measured in our earlier studies [11, 12], as pH in the absence of $NaHCO_3$ dropped below 5. We suggest that relatively high pH values in the first compartment of the ABR were caused by the HMT buffering ability and basic reaction of sodium acetate as a salt of strong base and weak acid. Therefore, in the second period we decided to remove sodium acetate from the substrate. It was assumed that higher concentrations of glucose would support acidification, the pH in the first compartment of the ABR would drop and hydrolysis of HMT should increase. Indeed, after removal of sodium acetate, pH in the first compartment of ABR dropped to 5.6. Removal efficiency of HMT increased slightly in both the ABR and the UASB reactor, even though only a small increase was observed. The data are shown in Table 2. It is important to note however, that pH 5.6 was thus still too high to observe more effective HMT hydrolysis.

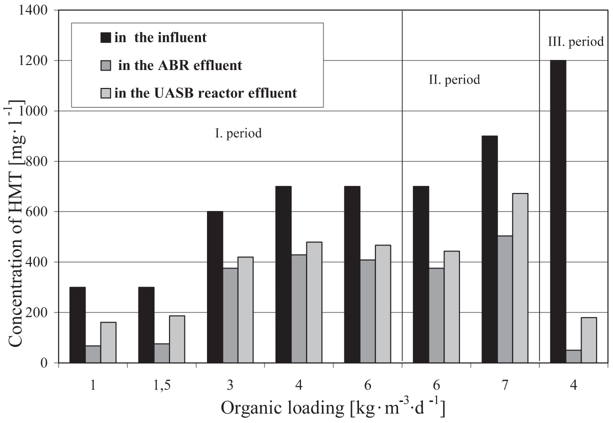

Fig. 4. Average HMT concentration during individual operational periods of the reactors.

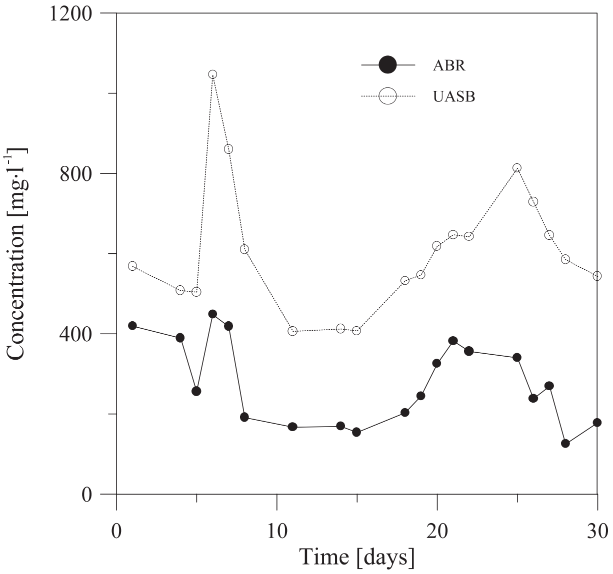

Fig. 5. Concentration of nonfiltered COD in the effluent from the ABR and the UASB reactor in third period.

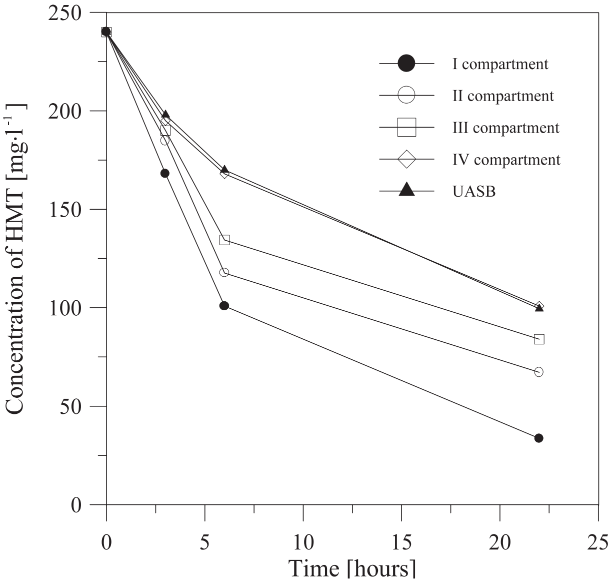

Fig. 6. Course of the hydrolytic tests in the ABR and in theUASB reactor.

Synthetic wastewater COD was increased significantly in the third period, maintaining lower pH at the beginning of the ABR by acidification. Results of studies carried out by Dohanyos *et al.* [10] who measured HMT removal in anaerobic filter, were used for the choice of the synthetic wastewater concentration. Composition of wastewater is shown in Table 1. pH dropped to 4.8 in the first compartment of the ABR, the average pH value in the second compartment was 5.6. The average pH values in the last compartment were 6.3-6.7. Organic loading was 4 kg·m^{-3}·d^{-1}, with HRT 84 hours. Maximum reached HMT removal efficiency, as well as maximum removal of the total formaldehyde were 95.8% in the ABR and 85.4% in the UASB reactor after 30 days of operation (210 days of operation in total) (see Table 2), respectively. Total COD removal efficiency was 98% in the ABR and 95.6% in the UASB reactor. Fig. 5 shows nonfiltered COD in the effluent from both reactors. Whereas COD value did not exceed 500 mg·l^{-1} in the ABR, COD values in the UASB reactor were even higher than 1000 mg·l^{-1}. COD removal efficiency did not drop below 96%. Therefore, it can be concluded that the ABR reactor reached the HMT removal parameters similar to those described in Ref. [10], yet with a much shorter adaptation period. Dohanyos *et al.* [10] reached these parameters after 500 days of an anaerobic filter operation. The ABR proved better HMT removal efficiency than the UASB reactor.

Biomass with the higher portion of the acidification microorganisms grew in the first two compartments of the reactor, decreasing COD removal. An amperometric titrimetric method with hydroxylamine was used during the operation to measure the concentration of free formaldehyde. Free formaldehyde formed in the HMT hydrolysis was quickly removed. Only a small amount of free formaldehyde (16 – 30 mg·l^{-1}) was detected in the first compartment of the ABR. No free formaldehyde was observed in any other compartment or at the effluent. Total volatile fatty acid (VFA) concentration was measured during the operation in the ABR and in the effluent from both reactors. Kapp titrimetric method [16] was used for VFA measurements. VFA concentration was given as mg·l^{-1} of acetic acid. The effluent VFA concentrations increased in both reactors on the 120th day of the operation, when the period of the operation changed (removing sodium acetate from the substrate). VFA concentration became stable in the ABR reactor during the first and the second period, reaching values below 200 mg·l^{-1} of acetic acid; VFA concentrations in the UASB reactor were higher (upto 600 mg·l^{-1}) and oscillating. The VFA concentration in ABR decreased from the first to the last compartment during the reactor performance (1st compartment – 906 mg·l^{-1}, 2nd compartment – 661 mg·l^{-1}, 3rd compartment – 448 mg·l^{-1}, and 4th compartment – 200 mg·l^{-1}). This indicates that no VFA accumulated in the reactor and that methanogenesis was not inhibited.

Hydrolysis and methanogenesis tests with the granulated sludge from the ABR compartments were performed after the third period was finished. The substrate

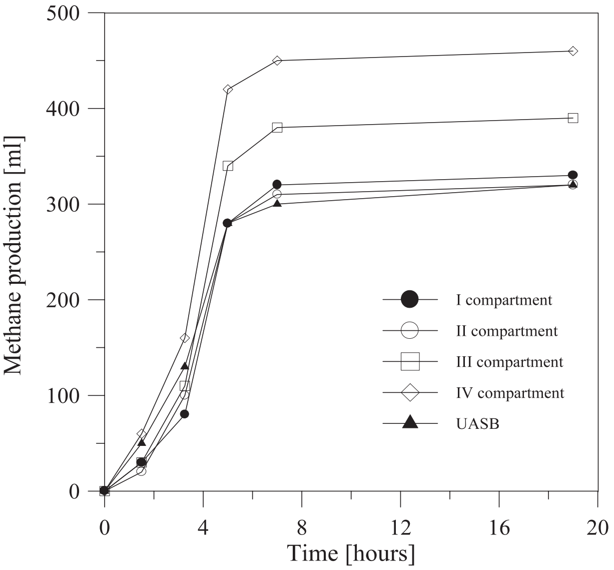

Fig. 7. Course of the methanogenic tests in the ABR and in the UASB reactor.

Table 3. Activity of the anaerobic sludge from the ABR and from the UASB reactor.

Activity $[g\cdot g^{-1}\cdot d^{-1}]$	Individual compartments of ABR				UASB reactor
	1.	2.	3.	4.	
Hydrolytic	0.055	0.046	0.021	0.023	0.021
Methanogenic	0.704	0.704	0.852	0.910	0.529

containing glucose and HMT was used for all hydrolysis tests. COD to total formaldehyde concentration was the same as in third period, i. e. 11.6:1. Volume of the total and free formaldehyde was measured by the amperometric titrimetric analysis. VSS values were the same in all tests – 12 $g\cdot l^{-1}$. The paper by Hutnan *et al.*, [17] gives detailed methodology for methanogenic and non-methanogenic activity tests. Values of maximum hydrolytic activity in the individual ABR compartments and the UASB reactor were calculated from the HMT concentration decrease rate. Values of the maximum specific methanogenesis rate were calculated from the methane production rate. Figs. 6 and 7 show results of these tests. Table 3 gives values of the maximal hydrolytic and methanogenic activities.

The maximum hydrolytic activity is given in grams of removed fixed formaldehyde to g VSS per day. The highest hydrolytic activity was observed in the first section of the ABR reactor. The sludge activity value in the UASB reactor was comparable with the activity of the anaerobic biomass in the third section of the ABR. The amount of free formaldehyde was measured during our tests, but it was negligible. Free formaldehyde was quickly removed, similar to the operation of the reactors.

The values of the methanogenic biomass activity given in g COD of methane to g VSS per day were the same in the first and second sections, and higher in sections three and four. Despite the inhibition of methanisation in the first section arising from the low pH, tests proved high methanogenic activity also in this section. Methanogenic rate was lower in the UASB reactor than in the ABR. Tests also indicated that the construction of the ABR creates better conditions for HMT hydrolysis.

Conclusions

The following conclusions have resulted from the operation of the reactors and tests of methanogenic and non-methanogenic anaerobic sludge activity:

- spatial segregation of individual anaerobic degradation phases (hydrolysis, acidogenesis, acetogenesis and methanogenesis) caused biomass stratification and pH gradient alongside the ABR. Therefore, the ABR is suitable for the treatment of slowly biodegradable HMT. Acidification conditions in the first ABR compartment allow its hydrolysis to ammonium ions and free formaldehyde, which is removed immediately.
- COD and HMT removal efficiencies were higher in the ABR, compared to those reached in the UASB reactor.
- kinetic tests proved higher (doubled) hydrolytic activity of an anaerobic biomass in the first ABR compartment, compared to the hydrolytic activity in the UASB reactor.
- these processes may be used for the treatment of industrial HMT-containing wastewater (e. g. Chemko Strazske in Slovakia), or water with the higher content of ammonium ions and formaldehyde, or in the wastewater where formaldehyde can be formed as a by-product.
- the ABR reactor reached the HMT removal parameters similar to those described in the study by Dohanyos *et al.* [10], who measured HMT removal in anaerobic filter. However, in the ABR, shorter adaptation period was needed (two or three times).

Acknowledgements

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