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EFFECT OF SHORT-TERM TEMPERATURE INCREASE ON THE PERFORMANCE OF A MESOPHILIC UASB REACTOR

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ABSTRACT

A study was carried out to assess the effects of short-term temperature increments on the treatment efficiency and methane production of UASB reactors at a working temperature of 37-39°C. Two different substrates were used to determine the effects on the several bacterial groups involved in the digestion process. One reactor was fed with defined synthetic acidified wastewater the other with unacidified wastewater from a distillery process. Shocks of 5-24 hrs were applied at temperatures in the range of 45 to 61°C. Up to 45°C no detrimental effects were noticeable. Higher temperatures led to a sharp decrease of the activity of the different microbial populations as a result of elevated decay rates. Propionate oxidation turned out to be the most sensitive for temperature increments, whereas the acidogenic bacteria were least affected. Temperature shocks of 55 and 61°C led to a decrease of 50% of the overall efficiency after 10 and 3 hrs, respectively. By means of batch experiments decay rates of 0.44 and > 10 hr $^{-1}$ of the methanogenic bacteria were estimated at 55 and 65°C respectively. As temporary inactivation of the mesophilic bacteria during a temperature shock was found to be unlikely, reactor recovery is dependent on the bacterial growth and the biomass retention capacity of the reactor. When unacidified wastewater is treated, a pH decrease has to be considered during a temperature shock.

KEYWORDS

UASB; temperature; activity; decay rate; hydraulic retention time; mixing characteristics; recovery.

INTRODUCTION

Development of new reactor types with immobilized biomass has led to wide-spread possibilities for application of anaerobic wastewater treatment. The Upflow Anaerobic Sludge Bed (UASB) process receives the major interest for the treatment of high strength industrial waste waters at a temperature of 25-35°C (Lettinga et al., 1980, De Zeeuw, 1988). Industrial full-scale reactors tend to have a high process stability although sudden environmental changes, like temperature shocks, can have detrimental effects on the reactor performance. 45°C is mostly seen as the upper temperature limit for the mesophilic digestion process (Henze and Harremoes, 1983).

In full-scale reactors, temperature increments are experienced mainly due to problems in cooling systems in which hot wastewaters (60-90°C) are cooled down to moderate temperatures (30-40°C). Lescure et al. (1988) found that in a full-scale reactor operating at 36°C, exposure of the system to 50°C for 7 hours resulted in a decreased COD removal rate from about 85 % to 45 %. Sludge washout occurred as a consequence of the exposure.

The effects of a temperature shock depend on several factors like applied temperature, exposure time and bacterial composition. At temperatures above the maximum growth temperature, decay rates exceed the growth rate of bacteria which leads to a decrease of the sludge activity and reactor efficiency. This was described by Buhr and Andrews (1977) in a proposed kinetical model for the thermophilic digestion process, in correspondence with a model for the mesophilic digestion process (Lawrence and McCarty, 1969).

The aim of this study is to quantify the effect of a temperature increase on the performance of a mesophilic UASB reactor. As a result of bacterial decay, effluent COD concentrations will increase. Decrease of the overall efficiency during a temperature increase is, besides decay, dependent on the mixing characteristics and hydraulic retention time of the reactor. Under **not** completely mixed conditions the efficiency decrease will be lower than the actual sludge decay rate. The methane production rate reflects more the actual sludge activity, but gives only information about the methanogenic stage. If reactor conditions are kept the same, the rate of efficiency decrease correlates with sludge decay rates at different temperatures.

One goal of our research project is to assess the feasibility of mesophilic granular sludge for the start up of thermophilic reactors without a significant loss of the mesophilic activity. In this paper the effects of short term (5-24 hrs) temperature increments (up to 60-65°C) on the activity of mesophilic granular sludge in continuous flow reactors (UASB) are described. The effects on the degradation of defined synthetic acidified wastewater (VFA mixture), as well as unacidified wastewater from a distillery process (vinasse), were studied. All UASB-experiments were conducted under the same hydraulic and mixing characteristics. The reactors were **overloaded** to estimate the effects on the maximum activity of the sludge bed. In batch experiments, performed in serum bottles, different exposure times were applied to determine the decay rates of the methanogenic population.

MATERIALS AND METHODS

Reactors

Experiments were performed using upflow anaerobic sludge blanket (UASB) reactors with a working volume of 5.75 liters, equipped with a stirring blade which was used intermittently (2 seconds every 5 minutes at 100 rpm). Temperature was controlled by a thermostat -bathcirculator connected to the double wall of the reactor. Methane production was measured by a wet test gas meter at ± 20°C after passing the evolved biogas through a NaOH solution (1 M) and a column of soda lime pellets (Fig. 1).

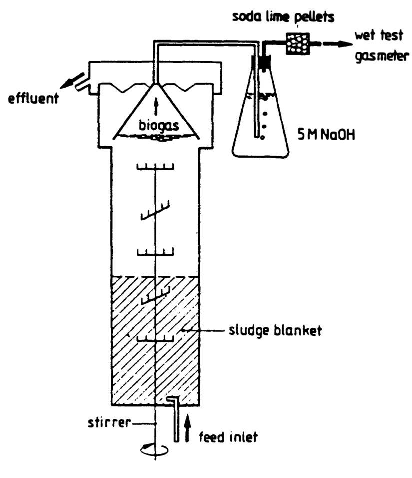

Figure 1. *Scheme of the 5.75 l UASB reactors.*

Seed sludge

The VFA fed Reactor (I) was seeded with mesophilic granular methanogenic sludge originating from the UASB reactor of the Aviko potato processing factory at Steenderen, The Netherlands. The vinasse fed Reactor (II) was seeded with a mixture of the above mentioned Aviko sludge and sludge from a mesophilic lab scale UASB reactor treating vinasse. The total amount of seed sludge for Reactor I was ± 13 g VSS/l and Reactor II ± 21 g VSS/l.

Medium

The reactors were fed with a concentrated stock solution which was diluted with hot tap water (containing ± 30 mg Ca^{++}/l) until the desired hydraulic retention time of ± 4 hrs was imposed to the system.
The substrate for Reactor I consisted of a partly neutralized (pH ± 6) VFA mixture: acetate, propionate, butyrate

$C_2:C_3:C_4$ = 3:1:1, (based on COD). Reactor II was supplied with sugarcane vinasse.
The final concentrations of basal nutrients in Reactor I were (mg/l): NH_4Cl, 170; $MgSO_4.7H_2O$, 111; KH_2PO_4 55, yeast extract 2. Per liter of influent 1 ml of a trace element solution was added containing (mg/l): $FeCl_2.4H_2O$ (2000), H_3BO_3 (50), $ZnCl_2$ (50), $CuCl_2.2H_2O$ (30), $MnCl_2.4H_2O$ (500), $(NH_4)_6Mo_7O_{24}.4H_2O$ (50), $AlCl_3.6H_2O$ (90), $CoCl_2.6H_2O$ (2000), $NiCl_2.6H_2O$ (92), $Na_2SeO.5H_2O$ (164), EDTA (1000), resazurin (200) and HCl 36% (1 ml/l). All chemicals were of analytical grade and were supplied by Merck, Darmstadt, FRG, except yeast extract which was provided by Gist Brocades, Delft, The Netherlands.
In the stock solution of Reactor II 0.8-1.0 g $NaHCO_3$/g COD was added, after which the pH was adjusted to 7-8.

Analyses

Volatile fatty acids (VFA) were determined by gas chromatography (glass column 2 m × 4 mm, packed with Supelcoport (100-120 mesh) coated with 10% Fluorad FC 431; temperatures (°C): column 130, injection port 200, flame ionization detector 280; carriergas (30 ml/min): N_2 saturated with formic acid). All other analyses were determined according to standard methods (APHA 1985).

Batch experiments

Batch-activity experiments were performed in 0.5 l serumbottles according to Field and Lettinga (1987) using Aviko sludge as seed (3.5 g VSS/l). Substrate was a 4 g COD/l VFA mixture ($C_2:C_3:C_4$ = 1:1:1 w/v, 24:34:41 based on COD). The effect of 6 different exposure times to high temperatures were measured (0-8 hrs.). Of every 3 bottles, 2 bottles were exposed to high temperatures, while 1 served as a control at 30°C. Before and after exposure, the maximum methanogenic activity of the granular sludge of each bottle was measured at 30 ± 1°C. During the exposures to high temperatures the sludge was unfed, which was also the case for the control bottles.

Calculations

Net growth rate per unit mass of methanogenic organisms becomes negative at high decay rates (Buhr and Andrews, 1977), resulting in a decreased activity of the methanogenic sludge. Decay rates were calculated from the slope of the logarithmic plot of the remaining mesophilic activity against exposure time, according to:

$$dA/dt = -k_d \times A_0 \quad (1),$$

in which A_0 = initial specific methanogenic activity (kg CH_4-COD/kg VSS.d) and k_d = decay rate (hr^{-1}).

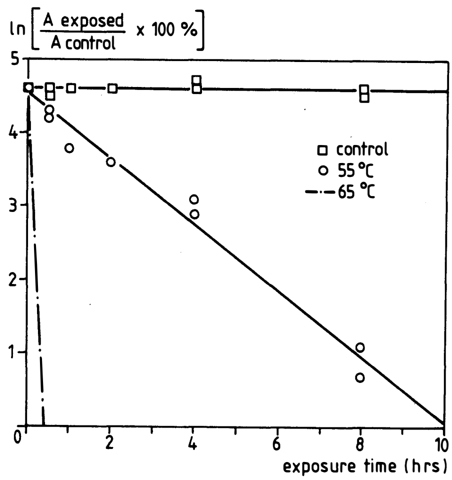

Figure 2. *Remaining mesophilic activity after exposure to 55°C (○) and 65°C (–·–).*

RESULTS

Batch experiments

The observed decrease of the mesophilic methanogenic activity after 0-8 hrs exposure to 55 and 65°C is shown in Fig. 2. Activity is expressed as the natural logarithm of the maximum activity after exposure, divided by the maximum activity of the control batches (x 100%).

A 30 minutes exposure to 65°C resulted in a complete loss of the methanogenic activity. According to equation (1) this means a decay rate of at least 10 hr^{-1} At 55°C a decay rate of ± 0.44 hr^{-1} was found.

Temperature shocks in UASB reactors

Reactor I, fed with a VFA mixture: After a start up period of 10 days at 39°C, organic loading rates of ± 15 kg COD/m^3.d were imposed (sludge loading rate: ± 1 kg COD/kg VSS.d), which resulted in increased effluent VFA's. During the whole course of the experiments the hydraulic retention time was ± 4 hrs.

Three temperature exposures were applied of 45, 55 and 61°C during 6-7 hrs. At the end of the last exposure, as no methane production was observed anymore, temperature increased accidentally up to 65°C. Temperature, loading rate and the resulting increased and decreased methane production are shown in Fig. 3a. During the temperature increase the methane production increased initially with 10-20%, followed by a sharp decline. Only at 45°C the methane production rate remained at an elevated level until the temperature was decreased to 39°C. No concomitant decreased VFA concentrations in the effluent were observed. After the desired temperature was reached a more or less linear increase of effluent VFA concentrations, or decrease of the overall efficiency, was measured, which is shown in Fig. 4. In this figure the efficiency is expressed as % of the initial efficiency at 39°C before every exposure. The rate at which the efficiency decreased was not the same for the individual VFA's. Propionate degradation decreased more rapidly than that of acetate and butyrate. Fig. 5 shows the rate of efficiency decrease for the individual VFA's during exposures to the different temperatures. The rate is estimated using the slope of the linear efficiency decrease of the individual fatty acids during an exposure to high temperatures and is expressed in %/hr. The effects of the 55°C exposure on the VFA-degradation capacity are tabulated in Table 1. In this table, besides recovery times for 100% recovery, also the doubling times of the involved groups of bacteria are given.

Propionate degradation did not recover within 3 weeks. No recovery at all was observed after the reactor was exposed to 60-65°C within 5 days.

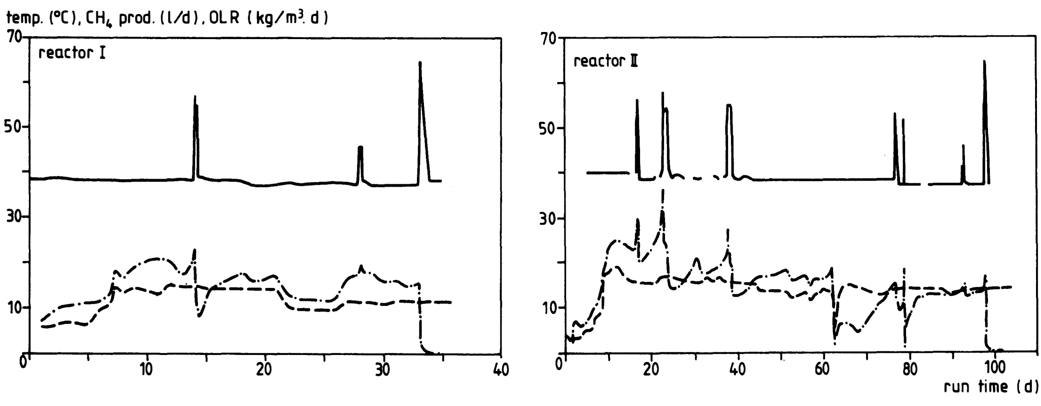

Figure 3a

Figure 3b

Figure 3a, 3b. *Experimental runs of Reactor I (left) and II (right). Mean reactor temperature (——) was ± 39°C; the average applied loading rate (– – –) was about 15 kg COD/m^3.d; methane production (–·–) is given in l/d.*

TABLE 1 Effect of 55°C Exposure on Mesophilic Sludge

VFA	efficiency decrease (%)	recovery time (d)	Td* (d)
C_2	40	6	7 [a]
C_3	75	-	5 [b]
C_4	10	2	2 [c]

* Doubling time (days) of specific enrichment cultures
[a] acetate: (Van den Berg et al., 1976; Van den Berg, 1977)
[b] propionate: (Koch et al., 1983; Gujer and Zehnder, 1983)
[c] butyrate: (Lawrence and McCarty, 1969)

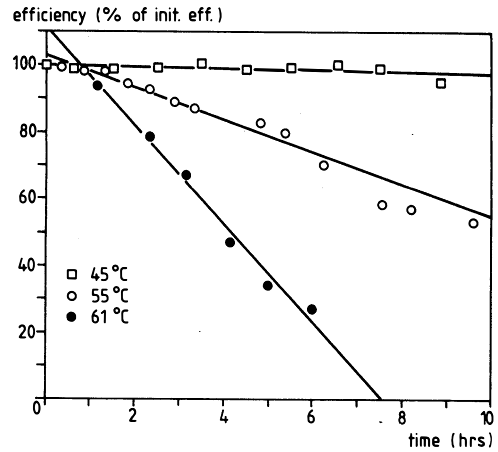

Figure 4. *Decrease of efficiency (based on COD removal) during exposure to 45°C (□), 55°C (○) and 61°C (●).*

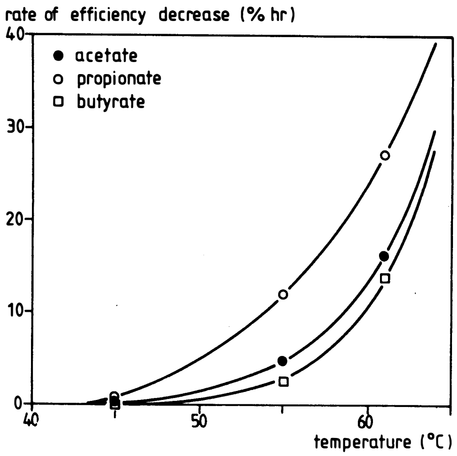

Figure 5. *Rate of efficiency decrease (%/hr) for acetate (●), propionate (○) and butyrate (□) at different temperatures in Reactor I.*

Reactor II, fed with vinasse: In Reactor II short term (± 5 hrs) temperature experiments were performed at 45, 55 and 61°C. Also twice, a 24 hours exposure was performed at 55°C. Fig. 3b shows the applied temperature, loading rate and methane production during the experimental periods. Temperature increments to 55°C on days 77 and 78 where accompanied with clogging problems, therefore results are not shown.

In all experiments increased methanogenic and acidogenic activities were observed during the initial stages of the temperature increase.

The rates of decreasing methanogenic and acidogenic activity at high temperatures are shown in Fig. 6. The rate of methanogenic activity decrease is estimated using the slope of the linear decrease of the methane production during an exposure to high temperatures. The rate of acidogenic activity decrease in Reactor II is calculated from the linear decrease of the amount of total COD which is converted to methane and VFA. Both rates are expressed in %/hr. Exposure to 45°C did not cause any clear decrease of activity. The values in Fig. 6 concerning the experiments conducted at 55°C are mean values of the period of decreasing activity. During the exposures acetate and propionate were the predominant acids accumulating. The observed recovery for acetate degradation was faster and more complete than for propionate degradation. However, this might be influenced by changes in the fermentation products due to temperature increase. After an exposure to 55°C, the time needed for 100 % recovery of the digestion process varied from 4 days to 15 days. Differences in recovery time can also be attributed to variations in the composition of the different vinasse stock solutions.

At 61°C methane production stopped rapidly during the exposure as is illustrated by the high rates of activity decrease in Fig. 6. No recovery of methane production occurred during the next 5 days after the exposure. The rate of activity decrease was lower for acidification than for methanogenesis. About 28 % of the influent COD was still acidified at the end of the exposure.

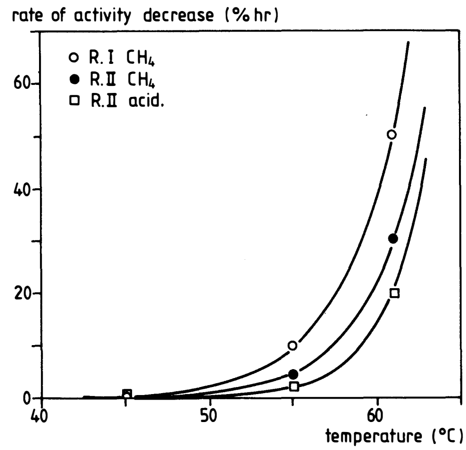

Figure 6. *Rate of methanogenic activity decrease in Reactor I (○) and Reactor II (●). Rate of acidogenic activity decrease in Reactor II (□).*

DISCUSSION

From the results it is clear that a temperature increase from 38°C to above 60°C leads to a rapid deterioration of the methanogenic, acetogenic and acidogenic activity. The exposures to temperatures exceeding 60°C resulted in a complete decay of the methanogenic and acetogenic bacteria, because no recovery was found during the following 5 days. In Reactor II, fed with vinasse, about 28 % of the influent COD was acidified after 5 hours exposure to 61-64°C and recovery of acidogenic bacteria was possible. But as the mesophilic acetogenic and methanogenic bacteria all decayed, no recovery of the process is possible without adding new seed material into the reactor.

According to equation (1), the actual sludge decay rate can be estimated from the decreasing methanogenic activity during a temperature increase in continuous systems. During the 55°C exposure in Reactor I, a decay rate of ± 0.25 hr^{-1} was estimated. However, by means of batch experiments a decay rate of 0.44 hr^{-1} at 55°C was found. This difference can be attributed to several factors.
1) Mesophilic activities during batch experiments were measured at ± 30°C, whereas the reactor temperature was 37-39°C. The applied temperature increment on the bacteria is different (25 and 17°C, respectively) although the same inoculum is used. 2) The temperature of the batches was increased within a few minutes, while the increase of the reactor temperature proceeded more gradually (1-3 hrs). A rapid increase of temperature might result in a more rapid and probably higher degree of deterioration of the bacterial membranes and enzymes. 3) It turned out as being quite difficult to obtain exactly the same temperature inside the sludge bed during an increase of the reactor temperature; this problem did not occur during batch experiments. 4) Temperature increments in continuous systems were performed under overloaded conditions, whereas the batches were unfed during the exposure. However, if the decay rates are much higher than the bacterial growth rates, then changes in the activity of the methanogenic sludge depend only on the decay rates.

Exposure of Reactor I to 55°C during a period of 7 hours, resulted in a decreased degradation of acetate, propionate and butyrate of 40%, 75% and 10% respectively (Table 1). The low decrease of the butyrate degrading

activity probably can be due to underloading of these bacteria. Recovery time needed for the degradation of acetate equals the doubling times for enrichment cultures on acetate, found by different authors (Table 1), while for butyrate it was longer than expected. The fact that within three weeks no recovery of the propionate degradation was observed can be due to lack of net growth, or to wash-out of propionate oxidizers. In all probability there was only decay and no temporary inactivation of the different bacteria.
The observed rates of decrease of acidogenic activity in Reactor II (Fig. 6) presumably are slightly lower than the actual rates, due to the fact that the gas production measured at time t cannot be related to the effluent concentration at the same time. Acidogenic activity is calculated from the amount of vinasse-COD which is converted to methane and VFA.

During the initial stages of the temperature increase there was an increase of 10-30% in methane production in all studies. As the temperature increase was gradual, (1-3 hours to desired temperature), the observed increased methane production occurred when the temperature was still 40-45°C. During the exposure to 45°C the methane production remained at 115% compared to 39°C. However, almost no lower effluent VFA values were observed.
Higher methane production rates can be due to the higher activity of methanogenic bacteria. Van den Berg et.al. (1976) found an optimum for the conversion rate of acetic acid at 40-45°C, but growth of the biomass was optimal at 35°C (Van den Berg, 1977). Speece and Kem (1970) reported a linear increase of the methane production of 70%, when the reactor temperature changed from 35 to 45°C. An irreversible deterioration of the mesophilic digestion process occurred only when shifting the temperature to 50°C; the inoculum was derived from a mesophilic sludge digester. Westermann et.al. (1989) described an increasing substrate consumption rate (Vmax) of Methanosarcina barkeri with increasing temperature (from 12 to 37°C), which was accompanied by a lower substrate affinity. A relatively higher increase of Vmax was observed at high substrate concentrations. However, a pure culture of Methanothrix soehngenii, common in reactors with low acetate concentrations like the ones in this study and the reactor from where the sludge originated, has its optimum temperature at 37°C. The methanogenic activity decreases by about 13 % at 45°C (Huser et al., 1982).
In the present study increased methane productions were accompanied by only a slight decrease of effluent VFA concentrations, and sometimes even no decrease was observed. Batch experiments conducted with mesophilic sludge, show a VFA build up in the medium during long term exposure of the sludge to high temperatures (55-65°C). This probably can be due to bacterial decay (data not shown). The released VFA, and higher turnover rates of cell components without VFA release, might be the cause of increased methane production at almost unchanged VFA concentrations in the effluent. Besides this, under **not** completely mixed conditions changes in effluent VFA concentrations due to a temperature increase are also dependent on the hydraulic and mixing characteristics of the used reactor, whereas the methane production is directly affected by temperature changes. This is illustrated by the observed higher rate of decreasing methanogenic activity compared to the rate of efficiency decrease for individual VFA's in Reactor I (Fig. 5, 6). Both rates increased exponentially with temperature, as they are correlated to the sludge decay rate.
Increase in methane production due to a lower solubility of methane at higher temperatures is negligible (max. ± 1%).

CONCLUSIONS

High temperatures (45-65°C) cause a serious drop in the activity of mesophilic granular sludge due to bacterial decay. Recovery of the mesophilic activity depends on bacterial growth and the fraction of new sludge that is retained in the reactor. This means that it is **not** possible to maintain the mesophilic activity of mesophilic granular sludge if this sludge is used for the start up of a thermophilic reactor.
During a temperature increase there is an increase of the methane production, caused by a higher methanogenic activity, followed by a sharp drop if the reactor temperature exceeds 45°C. Above 45°C decay rates exceed the growth rates of the bacteria present in mesophilic granular sludge. The decay rates increase exponentially with temperature and differ significantly between the different groups of bacteria. Propionate degradation was found to be the most sensitive to temperature increments whereas the acidogenic bacteria were least effected.
Exposure of a mesophilic process treating mainly unacidified wastewater to high temperatures results in unbalanced process conditions and probably changes in degradation pathways. Also the pH has to be followed in the event of temperature exposure, especially because the methanogens were found to be more sensitive than acidogenic bacteria. The most rapid way to get recovery is to add active sludge in the reactor.
Batch experiments performed as described in this paper do not predict accurately the effect of short term temperature increments on the performance of a mesophilic UASB reactor.

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REFERENCES

APHA, AWWA, WPCF (1985). Standard methods for the examination of water and wastewater; APHA Washington, D.C., USA.

Buhr, H.O. and J.F Andrews (1977). The thermophilic anaerobic digestion process; Wat. Res., Vol.11, pp. 129-143.

De Zeeuw, W. (1988). Granular sludge in UASB reactors; In: Proc. GASMAT Workshop, Lunteren, The Netherlands, 25-27 October 1987, Pudoc Wageningen, pp. 132-145.

Field, J.A. and G. Lettinga (1987). The methanogenic toxicity and anaerobic degradability of a hydrolyzable tannin; Wat. Res. Vol 21, No.3, pp 367-374.

Gujer, W. and A.J.B. Zehnder (1983). Conversion processes in anaerobic digestion; Wat. Sci. Techn. 15 (8/9), pp. 127-167.

Henze, M. and P. Harremoes (1983). Anaerobic treatment of wastewater in fixed film reactors - a literature review; Wat. Sci. Techn. 15, (8/9), pp. 1-101.

Huser B.A., K. Wuhrmann and A.J.B. Zehnder (1982). Methanothrix soehngenii gen. nov. sp. nov., a new acetotrophic non-hydrogen oxidizing methanobacterium; Arch. Microbiol. 132, pp. 1-9.

Koch, M., J. Dolfing, K. Wuhrmann and A.J.B. Zehnder (1983). Pathways of propionate degradation by enriched methanogenic cultures; Appl. Env. Microbiol. 45, pp. 1411-1414.

Lawrence, A.W., and P.L. McCarty (1969). Kinetics of methane fermentation in anaerobic treatment. J. Wat. Pol. Contr. Fed. 41, pp. R1-R17.

Lettinga, G., A.F.M. van Velsen, S.W. Hobma, W. de Zeeuw and A. Klapwijk (1980). Use of the Upflow Sludge Blanket Process (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment; Biotechn. Bioeng. 22, pp. 699-734.

Lescure, J.P., B. Delannoy, D. Verrier and G. Albagnac (1988). Consequence of a thermal accident on the microbial activity of an industrial anaerobic filter; In: Poster Papers Fifth Int. Symp. on Anaerobic Digestion, Bologna, Italy, 22-26 May 1988, pp. 211-214.

Speece, R.E. and J.A. Kem (1970). The effect of short-term temperature variations on methane production; J. Wat. Pol. Contr. Fed. 42, pp. 1990-1997.

Van den Berg, L., G.B. Patel, D.S. Clark and C.P. Lentz (1976). Factors affecting rate of methane formation from acetic acid by enriched methanogenic cultures; Can. J. Microbiol. 22, pp. 1312-1319.

Van den Berg, L. (1977). Effect of temperature on growth and activity of a methanogenic culture utilising acetate; Can. J. Microbiol. 23, pp. 898-902.

Westermann, P., B.K. Ahring and R.A. Mah (1989). Temperature compensation in Methanosarcina barkeri by modulation of hydrogen and acetate affinity; Appl. Env. Microbiol. 55, pp. 1262-1266.