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The effect of low temperature (5–29 °C) and adaptation on the methanogenic activity of biomass

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Abstract The influence of low temperature (5–29 °C) on the methanogenic activity of non-adapted digested sewage sludge and on temperature/leachate-adapted biomass was assayed by using municipal landfill leachate, intermediates of anaerobic degradation (propionate) and methane precursors (acetate, H_2/CO_2) as substrates. The temperature dependence of methanogenic activity could be described by Arrhenius-derived models. However, both substrate and adaptation affected the temperature dependence. The adaptation of biomass in a leachate-fed upflow anaerobic sludge-blanket reactor at approximately 20 °C for 4 months resulted in a sevenfold and fivefold increase of methanogenic activity at 11 °C and 22 °C respectively. Both acetate and H_2/CO_2 were methanized even at 5 °C. At 22 °C, methanogenic activities (acetate 4.8–84 mM) were 1.6–5.2 times higher than those at 11 °C. The half-velocity constant (K_s) of acetate utilization at 11 °C was one-third of that at 22 °C while a similar K_i was obtained at both temperatures. With propionate (1.1–5.5 mM) as substrate, methanogenic activities at 11 °C were half those at 22 °C. Furthermore, the residual concentration of the substrates was not dependent on temperature. The results suggest that the adaptation of biomass enables the achievement of a high treatment capacity in the anaerobic process even under psychrophilic conditions.

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Introduction

High-rate anaerobic processes have become an option for cost-effective and sustainable treatment of concentrated wastewaters. Anaerobic processes have been traditionally operated at 35–37 °C although the temperature of certain wastewater fractions might be either considerably warmer (e.g. pulp and paper industry) or cooler (e.g. breweries, landfills) than the above. Treating these concentrated fractions separately at initial temperatures would often be beneficial because of reduced resources and costs (e.g. no heating or cooling required). Promising results have already been reported in a few studies at 10–15 °C (Kennedy and van den Berg 1982; Koster and Lettinga 1985; Lin 1986; Viraraghavan and Kikkeri 1990; Kettunen and Rintala 1997).

Methanogenic activity and methane production have been observed at temperatures as low as 3–6 °C (Huser et al. 1982; Westermann and Ahring 1987; Kotsyurbenko et al. 1993). However, reports of a decrease in methanogenic (acetate utilisation) activity caused by a drop in temperature vary from study to study (Lawrence and McCarty 1969; Lin et al. 1987; Dinopoulou et al. 1988; Westermann et al. 1989; Rebac et al. 1995). Furthermore, the half-velocity constants of substrate utilisation have been reported both to increase (K_s; Lawrence and McCarty 1969; Lin et al. 1987) and to decrease (K_m; Westermann et al. 1989) at low temperature. Apparently the type of substrate and microbial culture (e.g. pure or mixed culture) and the experimental set-up used (e.g. batch or continuous experiment, temperature range) affect the activities and the constants obtained. Moreover, few studies (Rebac et al. 1995; Yan and Tay 1996) have focused on the effect on methanogenic activity of adapted biomass at low temperature.

The applicability of the anaerobic process to treatment of municipal landfill leachate is highly dependent on the characteristics of the leachate. Medium- to high-strength leachates have been successfully methanized even at 18–25 °C (Boyle and Ham 1974; Henry et al.

1987; Mennerich 1987). However, none of the studies has involved high-rate anaerobic treatment below 20 °C although, in a cold climate, leachate may be considerably cooler than that. Our previous results on a pilot scale showed that landfill leachates could be methanized even at 13 °C (Kettunen and Rintala 1997).

The aim of this study was to evaluate the effect of low temperature (5–29 °C) on the methanogenic activity of non-adapted digested sewage sludge (37 °C) and on temperature/leachate-adapted biomass. The activity was assayed by using municipal landfill leachate, intermediates of anaerobic degradation (propionate) and methane precursors (acetate, H_2/CO_2) as substrates. The leachate also contained acetate and propionate up to several hundred milligrams per litre (Kettunen and Rintala 1997). Kinetic models were used to asses the temperature dependence of methanogenic activity and substrate utilisation.

Materials and methods

Media

Municipal landfill leachate and synthetic media (acetate, propionate and H_2/CO_2) were used in the batch assays. The leachate (chemical oxygen demand, COD, 1500–4000 mg l^{-1}) originated from an equalisation basin at the Ämmässuo landfill (Espoo, Finland; Kettunen and Rintala 1997). Acetic (4.2, 8.4, 16.8, 42, and 84 mM) and propionic acids (1.1, 2.8, and 5.5 mM) were supplied by J.T. Baker and Fluka Chemica (p.a. grade) respectively. H_2/CO_2 (80:20) was delivered by Oy AGA Ab, Finland. Inorganic macro- and micro-nutrients, listed elsewhere (Kettunen et al. 1996), were added to all the media and to the demineralized water used in the H_2/CO_2 assay. Further, a KH_2PO_4 (0.22 g l^{-1}) and $Na_2HPO_4 \cdot 2H_2O$ (0.104 g l^{-1}) solution was used as buffer in the H_2/CO_2 assay. The pH of the medium was adjusted to between 7.0 and 7.2 with 1 M NaOH or 0.1 M HCl.

Inocula

The inocula were digested sewage sludge from a 37 °C digester (The Viinikanlahti sewage treatment plant, Tampere, Finland) and the biomass from an upflow anaerobic sludge-blanket (UASB) reactor treating leachate at 13–28 °C (Kettunen and Rintala 1997). The inocula and temperatures used in the assays are summarised in Table 1. All the inocula were stored anaerobically at 4 °C for 0.4–2.5 months prior to assaying, which was considered to have negligible effect on the activity of the inocula (e.g. Wu et al. 1995).

Batch assays

Anaerobic batch assays were run in triplicate in 120-ml glass serum bottles. The total liquid volume was 48–60 ml, except that 25 ml was used in the H_2/CO_2 assay. In the H_2/CO_2 assay, demineralized water (plus nutrients) and inoculum were transferred into the bottles, the bottles were sealed with rubber stoppers and aluminium crimps, and the headspace was flushed with a H_2/CO_2 mixture. In all other assays, the media with or without the inoculum were transferred into the bottles, the contents and the headspace were flushed with nitrogen, and the bottles were sealed as described above. Finally, $Na_2S \cdot 9H_2O$ (0.25 g l^{-1}) was injected into the bottles to ensure anaerobic conditions during the assay. The bottles were incubated thermostatically (± 1 °C) in static cultures in the dark. Gas samples for methane analyses were taken periodically from the bottles with a pressurized-lock syringe. The bottles without added medium were incubated in all the assays, and the methane production observed was subtracted from that in the test samples.

Analyses

COD (dichromate method), volatile solids and volatile suspended solids (VSS) were determined according to the Finnish Standards (SFS standard 5504, 1988) and the standard methods (APHA 1992). Total organic carbon (TOC) was analysed with a TOC-5000 total organic carbon analyser (Shimadzu). Both the COD and TOC samples were filtered (Schleicher & Schüll ME25 membrane filter, 0.45 μm). pH was measured with an Orion research model SA250 pH meter. Methane was analysed by the gas chromatography /thermal conductivity detector technique (Perkin-Elmer Sigma, 2-m × 3-mm stainless-steel column packed with Porapak Q, 80–100 mesh; Supelco). The oven, injector and detector temperatures were 60 °C, 120 °C and 120 °C respectively. The carrier gas was helium at 20 ml min^{-1}.

Calculations

The specific methanogenic activity of the biomass (ml CH_4 g VSS^{-1} h^{-1}) was calculated from the linear part of a curve describing cumulative methane production (g VSS^{-1}) in time. The lag phase was determined as the time before methane was detected in an assay.

Several models have been suggested to describe the effect of either temperature or substrate on microbial activity and growth

Table 1 Media, inocula and temperatures used in the anaerobic batch assays. *VSS* volatile suspended solids, *SSD* sewage sludge digester, *UASB* upflow anaerobic sludge-blanket reactor (13–28 °C) treating municipal landfill leachate, *NA* not applicable

Medium	*T* in assay (°C)	Inoculum	Source of inoculum			Concentration of inoculum in vials (g VSS l^{-1})
			Reactor	Run time (days)	*T* (°C)	
Leachate	5–35	A	SSD	NA	37	1.9[a]
	11, 22	A	SSD	NA	37	1.1
	11, 22	B	UASB	114	24	1.0
Acetate	11	C	UASB	184	18	2.2
	22	D	UASB	135	23	1.9
	5–29	D	UASB	135	23	1.7
Propionate	11, 22	E	UASB	170	18	2.7–2.8
H_2/CO_2	5–29	F	UASB	226	14	8.3

[a] g volatile solids l^{-1}

(Heitzer et al. 1991; Pavlostathis and Giraldo-Gomez 1991). In the present study, the following equations (Eq. 1–4) were selected for calculations, on the basis of their wide use.

The temperature dependence of methanogenic activity was described with two models, the first one being the Arrhenius-type equation

$$k_{CH_4} = A \cdot e^{(-E_a/RT)} \quad (1)$$

where k_{CH_4} is the methanogenic activity (ml CH_4 g VSS^{-1} h^{-1}) at temperature T(K), A is the frequency factor (ml CH_4 g VSS^{-1} h^{-1}), E_a is the apparent activation energy (kJ mol^{-1}), R is the gas constant (0.008314 kJ mol^{-1} K^{-1}).

The second model was the Arrhenius-derived equation, which recognises biosynthesis and degradative processes (Pavlostathis and Giraldo-Gomez 1991)

$$k_{CH_4} = k_1 \cdot e^{a_1(T-T_X)} - k_2 \cdot e^{a_2(T-T_X)} \quad (2)$$

where k_{CH_4} is the methanogenic activity (ml CH_4 g VSS^{-1} h^{-1}) at the temperature T (°C). k_1 and a_1 are kinetic constants for temperatures below the optimum when degradation (the second term in Eq. 2) is negligible, k_2 and a_2 are kinetic constants for the second term and T_X is the optimum temperature (°C) up to which the activity increases exponentially. The first term is the most commonly used modification of the Arrhenius equation, and therefore it was alone fitted to the data (referred to as Eq. 2a) in addition to the whole equation (referred to as Eq. 2)

The dependence of methanogenic activity on the initial acetate concentration was described using the generalised Haldane equation

$$k_{CH_4} = \frac{k}{1 + \frac{K_s}{S} + \left(\frac{S}{K_i}\right)^n} \quad (3)$$

and the non-competitive inhibition equation

$$k_{CH_4} = \frac{kS}{(K_s + S)\left(1 + \frac{I}{K_i}\right)} \quad (4)$$

where k_{CH_4} is the methanogenic activity (ml CH_4 g VSS^{-1} h^{-1}), k is the maximum specific methanogenic activity, K_s is the half-velocity constant, K_i is the inhibition constant, S is the substrate concentration, I is the inhibitor concentration and n is a constant (order of inhibition). In parameter estimation, n was given values of 1 (referred to as Eq. 3a) and 2 (referred to as Eq. 3b).

The parameters in Eq. 1–4 were estimated using a non-linear least-square method; the minimum least-square value was iterated by the Newton method. Lack of fit was tested according to Berthouex and Brown (1994). The residual mean squares [RMS = RSS/(n–p) where RSS is the residual sum of squares and n–p degrees of freedom] of the fitted model were compared with the measurement error variance (s^2) by computing the F ratio (F = RMS/s^2) and comparing it with the tabulated F value (approximate 95% confidence level) having the appropriate degrees of freedom. The variance was assumed to be normally distributed.

Results

Methanogenic activity of non-adapted biomass at 5–35 °C with leachate as substrate

The influence of temperature (5–35 °C) on the methanogenic activity of non-adapted biomass (A) was studied in two consecutive feedings by using mesophilic-digested sewage sludge (37 °C) and the leachate (Table 2, Fig. 1a). In the first feeding (COD 3.5 g l^{-1}), methane production started within 10 days even at 5 °C, indicating the potential of the mesophilic biomass to initiate methanogenesis under psychrophilic conditions. The biomass showed good adaptation to low temperature, for in the second feeding (COD 3.9 g l^{-1}), methanogenic activities at 5–22 °C were two- to threefold higher than in the first feeding (Fig. 1a). Neither the adaptation nor the incubation temperature (13–35 °C) affected COD removal (84%–89%) and the final COD achieved (430–490 mg l^{-1}). At 5–9 °C, the assay was discontinued before the decline in methane production, and thus no comparison between the final COD values could be made. Without inoculum at 22 °C, the rate of methane production was low. However, 65% of the COD was removed by the micro-organisms in the leachate after 1.5 months (data not shown).

Equations 1 and 2a both described well the temperature dependence of the non-adapted biomass during the first feeding (Table 3). Equation 2 was rejected because of the negative k_2 value obtained. Contrary to the first feeding, Eq. 2 with the decay term best described temperature dependence in the second feeding, although neither equation fitted the data well. The change in the type of temperature-dependence model indicated alteration in the biomass as a result of low temperature.

Table 2 Lag-phases in methane production in the methanogenic activity assays with leachate, acetate, propionate or H_2/CO_2 as substrates. For inocula used see Table 1. Substrate concentration did not affect the lag phases. Values in parentheses were obtained in the assay with the adapted biomass (B) as inoculum. –Not determined

T (°C)	Lag phase (days)				
	Assays with temperature variation			Assays with variation of substrate concentration	
	Leachate inoculum A	Acetate inoculum D	H_2/CO_2 inoculum F	Acetate inoculum C, D	Propionate inoculum E
5	10	3	10	–	–
9–11	10 (0.4)	2	8	0.2	1
13–15	3	1	7	–	–
21–23	2 (0.2)	0.9	4	0.2	0.3
29	–	0.4	3	–	–
35	1	–	–	–	–

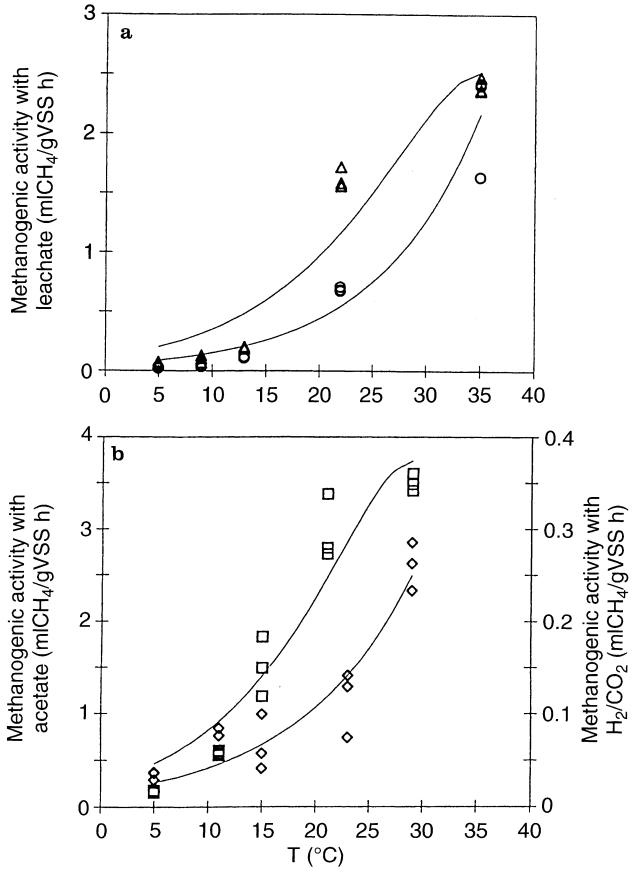

Fig. 1 The influence of temperature (5–35 °C) on methanogenic activity of (**a**) non-adapted biomass (A) and (**b**) adapted biomass (D and F). Substrates used: (**a**) leachate first feeding (○) and second feeding (Δ), (**b**) acetate (□) and H_2/CO_2 (◊). The coefficients of variation of triplicate (duplicate) samples were no more than 20%, 30%, 20% and 45% respectively. The lines are drawn according to the best-fit Arrhenius-derived model (see Table 3). *VS* volatile solids, (see Table 1) *VSS* volatile suspended solids

Methanogenic activity of adapted biomass at 11 °C and 22 °C with leachate as substrate

The effect of adapting biomass to low temperature and leachate was studied at 11 °C and 22 °C by assaying biomass (B) from a leachate-treating UASB reactor (inoculated with the non-adapted biomass A) after 4 months of operation at 20–28 °C (Kettunen and Rintala 1997). Adaptation increased significantly the methanogenic activity of the biomass under psychrophilic conditions. At 11 °C, the methanogenic activity of the adapted biomass (0.76 ± 0.04 ml CH_4 g VSS^{-1} h^{-1}, leachate COD 2.5 g l^{-1}) was sevenfold higher than that of the non-adapted mass while the respective increase at 22 °C was fivefold (3.12 ± 0.1 ml CH_4 g VSS^{-1} h^{-1}). At both 11 °C and 22 °C, methanogenic activities increased to a level previously obtained at around 10 °C higher temperatures with the non-adapted biomass. Methanogenic activities lower than the above were obtained with the diluted leachate (1:2) as substrate (data not shown), which suggested dependence of methanogenic activity on the substrate concentration. The adaptation of the biomass did not enhance COD removal (approx. 80% at both temperatures).

Methanogenic activity of biomass at 5–29 °C with acetate and H_2/CO_2 as substrates

The influence of temperature (5–29 °C) on the methanogenic activity of the adapted biomass (D and F) was also assayed with 16.8 mM acetate ($\approx K_s$ at 11 °C, see later) and H_2/CO_2 (80:20) as substrates (Fig. 1b). The start-up of methane production was retarded as the temperature decreased (Table 2) but, even at 5 °C, production started within 3 days with acetate and within 10 days with H_2/CO_2. With acetate as substrate, the influence of temperature on methanogenic activity was best described by the Arrhenius-derived Eq. 2 although none of the models fitted the data well (Table 3). A

Table 3 Kinetic constants for Eqs. 1 and 2 when leachate ($n = 15$), acetate (16.8 mM, $n = 15$), and H_2/CO_2 (20%:80%, $n = 14$) were used as substrates. $F_{0.05,13,13} = 2.58$, $F_{0.05,12,12} = 2.79$, $F_{0.05,11,11} = 2.83$, $F_{0.05,10,10} = 2.98$. The coefficient of variation of the final parameter estimates was less than 2% despite initial estimates 10^2 greater or smaller than the final ones. *NA* not applicable, T_X the optimum temperature, *RSS* the residual sum of squares

Substrate	Eq.	T_x (°C)	k_1 or A	a_1 or E_a	k_2	a_2	*RSS*	s^2	F
Leachate/ 1st feeding	1	NA	$4.15 \cdot 10^{13}$	78.42	NA	NA	0.512876	0.028825	1.369
	2a	37	2.679	0.106	NA	NA	0.537261	0.028825	1.434
Leachate/ 2nd feeding	1	NA	$2.91 \cdot 10^{9}$	53.51	NA	NA	1.819987	0.001749	80.05
	2a	37	2.912	0.073	NA	NA	1.965717	0.001749	86.46
	2[a]	20	10.0	0.136	9.03	0.141	1.129027	0.001749	58.68
Acetate	1	NA	$3.78 \cdot 10^{9}$	52.08	NA	NA	4.005567	0.034608	8.903
	2a	30	4.086	0.072	NA	NA	4.300524	0.034608	9.559
	2[a]	20	10.0	0.143	7.73	0.160	2.058643	0.034608	5.408
H_2/CO_2	1	NA	$3.79 \cdot 10^{9}$	59.03	NA	NA	0.012248	0.000444	2.299
	2a	30	0.274	0.094	NA	NA	0.010848	0.000444	2.036

[a] Pre-set boundaries were required ($k_1 \leq 10$, $a_1 \leq 1$) to obtain coefficients of variation less than 2%

similar residual concentration of acetate (5 mg TOC l^{-1} or less) was obtained at each temperature studied. With H_2/CO_2 as substrate, the Arrhenius-derived Eq. 2a without the decay term best described the methanogenic activity. Eq. 2 was rejected because of the negative k_2 value obtained. The methanogenic activity with H_2/CO_2 as substrate was less temperature-dependent than that with leachate or acetate, as indicated by the low temperature coefficient (a_1).

Influence of initial acetate and propionate concentrations on methanogenic activity of biomass at 11 °C and 22 °C

The influence of the initial substrate concentration on the methanogenic activity and on the kinetic parameters of the adapted biomass (C, D and E) was studied at 11 °C and 22 °C with acetate and propionate as substrates. With acetate as substrate (Fig. 2a), methanogenic activity surged up to a certain acetate concentration (approx. 15 mM at 11 °C and approx. 30 mM at 22 °C), after which a slight decline in the activity caused by substrate inhibition was observed. Methanogenic activities at 22 °C were 1.6–5.2 times higher than those at 11 °C (Fig. 2). At 11 °C, both the Haldane (Eq. 3a) and the non-competitive (Eq. 4) equations described methanogenic activity well (Table 4). The values for k, K_s, and K_i were 1.7–2.8 ml CH_4 g VSS^{-1} h^{-1}, 11–18 mM, and 30–49 mM respectively. At 22 °C, the best fit was obtained with the Haldane equation (Eq. 3a). However, two estimates of the parameters were found with and without a bounded range for K_s (Table 4). The K_s value, being higher than K_i, was not likely to be correct, on the basis of the findings at 11 °C and the values of K_s and K_i found in the literature (Yang and Okos 1987; Fukuzaki et al. 1990). Consequently, the following estimates for k, K_s and K_i at 22 °C were selected: 11 ml CH_4 g VSS^{-1} h^{-1}, 46 mM and 46 mM. Neither the initial acetate concentration nor temperature affected the residual concentration (10–35 mg TOC l^{-1}).

With propionate as substrate (Fig. 2b, Table 2), methanogenic activity seemed to increase with the increasing propionate concentration at both 11 °C and 22 °C, and no inhibition of methanogenesis was observed. Methanogenic activities at 11 °C were approximately half those at 22 °C. No kinetic model was fitted to the data as only three concentrations of propionate were assayed. Temperature did not affect the residual propionate concentration (5 mg TOC l^{-1} or less).

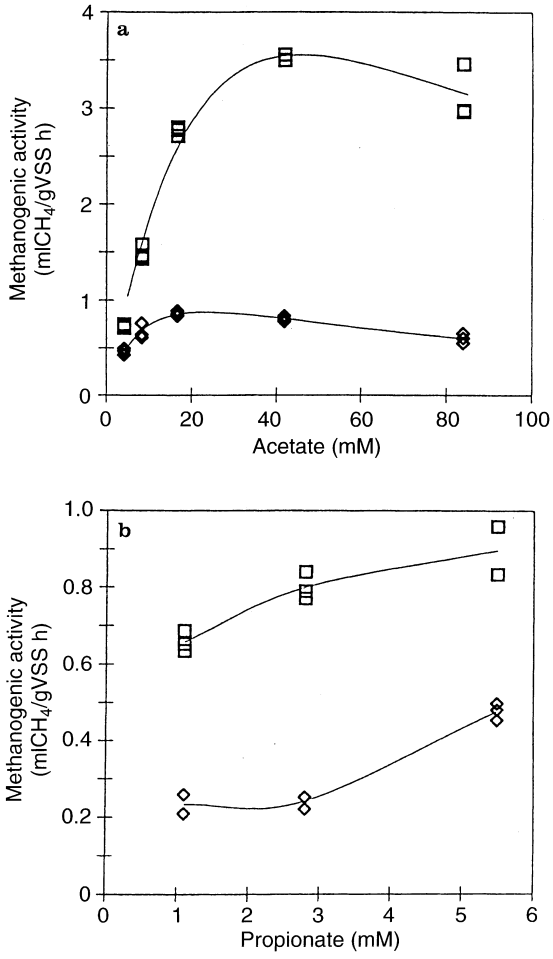

Fig. 2 The effect of initial (**a**) acetate (4.2–84 mM) and (**b**) propionate concentration (1.1–5.5 mM) on methanogenic activity of adapted biomass (C, D and E) at 11 °C (◊) and 22 °C (□). The coefficient of variation of triplicate (duplicate) samples was no more than 15%. In the acetate figure, the lines are drawn according to the best-fit substrate utilization model (Haldane, see Table 4) while, in the propionate figure, the lines represent averages of the samples

Table 4 Kinetic constants for the Eqs. 3 and 4 at 11 °C and 22 °C with acetate as substrate. $F_{0.05,12,12} = 2.69$. The coefficient of variation of the final parameter estimates was less than 2% despite initial estimates 10^2 greater or smaller than the final ones

T (°C)	Equation	k (ml CH_4 g VSS^{-1} h^{-1})	K_s (mmol)	K_i (mmol)	RSS	s^2	F
11	3a Haldane ($n = 1$)	1.725	11.38	48.62	0.024231	0.001671	1.208
	3b Haldane ($n = 2$)	1.139	5.805	90.11	0.032877	0.001671	1.640
	4 non-competitive	2.753	18.16	30.45	0.024231	0.001671	1.208
22	3a Haldane ($n = 1$)	16.86	79.60	23.98	0.318218	0.012744	2.081
	3a Haldane ($n = 1$)[a]	10.67	45.88	45.88	0.369260	0.012744	2.415
	3b Haldane ($n = 2$)	6.900	28.24	89.23	0.465558	0.012744	3.044
	4 non-competitive	13.83	51.53	51.53	0.638133	0.012744	4.173

[a] In the estimation, K was set to be equal to or less than K_i

Discussion

According to our knowledge, this was first time that the low-temperature kinetics of anaerobic reactor biomass has been assessed more extensively. The temperature dependence of methanogenic activity and the half-velocity constant of the biomass have to be considered thoroughly when designing and operating an anaerobic process at low temperature. Incorporation of temperature dependence equations with kinetic models for various treatment processes should allow process performance to be predicted at various temperatures (Novak 1974; Lin et al. 1987). In the present study, however, both the substrate (leachate, acetate or H_2/CO_2) and the adaptation of the biomass affected the type of best-fit temperature model and the kinetic parameters obtained. The most commonly used model (Arrhenius Eqs. 1 and 2a) was a poor predictor of the temperature dependence of the methanogenic activity of the adapted biomass. Consequently, the efficiency of an anaerobic process at low temperature could be significantly underestimated if the kinetic parameters are determined in batch experiments with non-adapted biomass.

The results showed that biomass from a mesophilic sewage sludge digester could be used to methanize medium-strength municipal landfill leachate at temperatures as low as 5 °C. Both acetate- and H_2/CO_2- utilising methanogens were able to function at such low temperatures. The adaptation of the biomass to low temperature/leachate enhanced its methanogenic activity significantly, a finding similar to those of Rebac et al. (1995) and Yan and Tay (1996). Rebac et al. (1995) reported a three- to fourfold increase in the acetate degrading activity of mesophilic granular sludge both at 10 °C and 20 °C, when an expanded granular-sludge-bed (EGSB) reactor fed with a volatile fatty acids solution was operated for 253 days at 10–12 °C. However, they observed no increased propionate-degrading activity. Yan and Tay (1996) found a higher than tenfold increase in methanogenic activity at 22 °C when a UASB reactor inoculated with digested sewage sludge and fed with brewery wastewater was run for 300 days at 18–26 °C. The increased activity may result from growth and enrichment of methanogens in addition to selection of methanogens with the highest activity under applied conditions, for example.

The methanogenic activities of the biomass with acetate or propionate as substrate were lower than those reported for EGSB reactor biomass at 10 °C (0.1–0.4 mg COD mg VSS^{-1} day^{-1}) and at 20 °C (0.3–1.0 mg COD mg VSS^{-1} day^{-1}) by Rebac et al. (1995). However, the propionate concentration used in the present study was significantly lower. Somewhat higher methanogenic activities with acetate as substrate have also been found in batch experiments at 15 °C and 20 °C (0.2 mg and 1 mg COD mg VSS^{-1} day^{-1} respectively; van den Berg 1977) with an acetate-acclimatised mixed culture. In the present study, methanogenic activities with H_2/CO_2 as substrate were significantly lower than with acetate at each temperature studied (5–29 °C), a finding similar to that of Kalyuzhnyi et al. (1996) at 35 °C. Higher activities with H_2/CO_2 might have been obtained as a result of enhanced gas transfer if mixing had been applied.

The methanogenic activities of the adapted biomass with leachate as substrate were comparable to those found with acetate in samples with the same initial COD (2.5–2.6 g l^{-1}). The highest methanogenic activity obtained at 22 °C was approximately half what Keenan et al. (1993) found at 35 °C for granular sludge from a leachate-treating UASB reactor. However, they did not report the COD of their leachate. According to the present findings, a temperature drop from 35 °C to 20 °C in a leachate treating process corresponds only to an approximately 30% decrease in methanogenic activity when the biomass has been adapted to low temperature.

The K_s of the biomass for acetate at 11 °C was approximately one-third that at 22 °C. Westermann et al. (1989) showed that the half-velocity constant (K_m) of *Methanosarcina barkeri* for both hydrogen and acetate decreased with decreasing temperature. In continuous-treatment studies, however, the half-velocity constant (K_s) was found to increase as the temperature decreased (15–35 °C; Lawrence and McCarty 1969; Lin et al. 1987). Other factors besides temperature, such as biomass retention time (Noike et al. 1985), might affect the K_s value obtained in continuous experiments. Unlike K_s, temperature seemed not to affect K_i in the present study. Further studies are needed to evaluate the dependence of K_s and K_i on temperature.

The K_s values (11–46 mM) in the present study were high compared to those found for acetate in continuous, mixed culture experiments at 10–35 °C (2.6–14 mM, Lawrence and McCarty 1969; 0.2–6.6 mM, Noike et al. 1985; 0.6 mM, Rebac et al. 1995). On the other hand, K_s values of 0.104–29.2 mM and 0.004–0.7 mM have been reported for *Methanosarcina* species (Yang and Okos 1987; Fukuzaki et al. 1990) and *Methanosaeta* (*Methanothrix*) species (Huser et al. 1982; Fukuzaki et al. 1990) at 35–37 °C. The respective K_i values were 8.3–811 mM and 4.6 mM. Apparently, high leachate COD (1.5–3.2 g l^{-1}) and relatively high loading rates (1.4–4 kg COD m^{-3} day^{-1} at 13–23 °C) applied in the UASB reactor (Kettunen and Rintala 1997) resulted in a high half-velocity and inhibition constants of the biomass.

In a continuous treatment process, high effluent quality should also be obtained at low temperature since the residual TOC/COD of acetate, propionate or leachate achieved in the batch experiments was not affected by temperature. Thus, for example, the higher effluent COD observed at 13 °C than at 18 °C in a leachate-treating UASB reactor (Kettunen and Rintala 1997) most probably resulted from an overloading and not from characteristics of the biomass.

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