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Methane production from acetamide in an upflow anaerobic sludge-blanket reactor based on a synergistic association between an aerobic rod and methanogens

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Abstract Acetamide degradation was investigated in a bench-scale upflow anaerobic sludge-blanket (UASB) reactor, successively fed with acetamide, acetate and acetamide, over a period of 343 days, at different hydraulic retention times (t_{HR}). The reactor was seeded with the sludge previously described [Guyot et al. (1994) Appl Microbiol Biotechnol, 42:452–456], in which methanogenesis from acetamide was performed through a synergistic relationship between an acetamide-degrading, aerobic rod and methanogens. When the reactor was fed acetamide, the chemical oxygen demand (COD) removal efficiency was 86% at volumetric loads less than 1.18 kg COD m^{-3} day^{-1}. At higher volumetric loads, the efficiency decreased markedly, e.g. 50.9% at a volumetric organic load of 3.39 kg COD m^{-3} day^{-1} (1 day t_{HR}) with an accumulation of both acetamide and acetate. The same reactor, when fed with acetate at t_{HR} 1 day, reached a high COD removal (99%). Evidence of the inhibition of acetate degradation by acetamide is presented. After a long period (135 days) without feeding the reactor with acetamide, the sludge reactor was still capable of degrading acetamide when this substrate was supplied again. It seems that the synergistic degradation of acetamide by aerobes and methanogens present in the UASB reactor sludge is stable over a long period (343 days), in spite of limiting concentrations of dissolved oxygen in the feed.

Introduction

Industrial wastewaters (e.g. pharmaceutical, chemical, petrochemical) are often an aqueous mixture of chemicals, which may be toxic, recalcitrant or inhibitory or can produce degradation products with these adverse effects.

The systematic study of the microbial anaerobic biodegration of chemicals generated by the industry (Battersby and Wilson 1989; Shelton and Tiedje 1984), can provide information to allow the potential for the treatment of industrial wastewaters to be evaluated. This can lead sometimes to unexpected results, which might have some industrial consequences (Guyot et al. 1994; Macarie and Guyot 1992).

Few data exist on the anaerobic biodegradability of acetamide. It is widely employed in the lacquer, cosmetics, explosives, textiles and pharmaceutical industries (Moretti 1978) and it is also produced by acetonitrile biodegradation (DiGeronimo and Antoine 1976).

Up to now, the importance of aerobic and facultative bacteria within anaerobic sludge has not been extensively evaluated, especially their contribution during the biodegradation of some recalcitrant molecules in environments supposed to be anoxic, such as anaerobic digestors. Recently some work has focused on the possibility of strict or facultative aerobes and strict anaerobes co-existing in an unique environment (Gerritse and Gottschal 1993; Guyot and Fajardo 1993; Guyot et al. 1993; Kato et al. 1993; Wu et al. 1987). A previous study, showed that methane production from acetamide in batch culture, by the sludge sampled from the acetamide-fed UASB reactor used in the present work, was a two-step reaction. This reaction involved a synergestic coupling between a sporulating gram-positive, strictly aerobic rod, which transformed acetamide to acetate and ammonia, and methanogens (Guyot et al. 1994). Because of its simple chemical structure and degradation pathway, acetamide is a convenient molecule for the study of aerobic/anaerobic microbial interactions. In order to know the stability and the efficiency of this natural synergistic association between aerobes and anaerobes, acetamide degradation and methanation by this particular sludge were

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investigated over 343 days, in a bench-scale upflow anaerobic sludge blanket (UASB) reactor successively fed with acetamide, acetate and acetamide.

Materials and methods

Reactor set-up and inoculation

During this study, a 1.25-l bench-scale glass UASB reactor was operated at 35°C, 0.77 kPa (mean local pressure). It was seeded with 500 ml sludge from a 40-m^3 pilot UASB reactor treating baker's yeast factory wastewaters. This sludge had 26.2 g/l total suspended solids and 12 g/l of volatile suspended solids. First it was adapted for 15 days in batch culture within the UASB reactor with a synthetic medium containing 5 g/l acetamide, then for a 2.5-month period in continuous flow at a volumetric loading rate of 1.13 kg chemical oxygen demand (COD) m^{-3} day^{-1}. Later, the reactor was operated in continuous flow at different hydraulic retention times (t_{HR}), with the same sterilized synthetic medium. The reactor was fed either acetamide, acetate or acetamide. The acetamide or acetate concentrations are reported in Tables 1–3. The Balch et al. (1979) medium 1 without yeast extract and biotrypcase was used throughout this study. The feed medium was maintained under aerobiosis and stirred with a magnetic stirrer. Under these conditions, the dissolved oxygen concentration in the feed was 5 mg/l (0.16 mM). The oxygen concentration was measured with an oxymeter (Hach Chemical Company).

Analysis

Acetamide and acetate were determined by gas/liquid chromatography (flame ionisation detector), using a non-packed capillary column (0.54 mm × 10 m) (AT-1000, Alltech).

Methane was analysed as previously described (Guyot et al. 1990).

Ammonia was determined colorimetrically by the Nessler method as described by Rodier (1978).

Chemical oxygen demand (COD) and total and volatile suspended solids (VSS) were determined according to APHA (1985).

Results

First feeding with acetamide (days 1–176)

During the first 176 days, the UASB reactor was operated with acetamide as substrate at $t_{HR} = 4$, 3, 2 or 1 days (Fig. 1), corresponding to the following volumetric organic loads: 0.865, 1.180, 1.850, 3.390 kg COD m^{-3} day^{-1}, respectively. The corresponding acetamide loads (mmol acetamide applied g VSS^{-1} day^{-1}) (Table 1) did not exceed the initial specific sludge activity (5.52 mmol acetamide degraded g VSS^{-1} day^{-1}) calculated from previously published data (Guyot et al. 1994), except at 1 day t_{HR}. Under these conditions, sludge saturation was not expected in the range of $t_{HR} = 4$–2 days. The COD removal efficiencies at organic loading rates of 1.180 kg and 0.865 kg COD m^{-3} day^{-1} were indeed relatively high, 85.8% and 86.1%, respectively. However, at higher but still relatively low organic loads, 1.85 COD m^{-3} day^{-1} and 3.39 kg COD m^{-3} day^{-1}, acetamide and acetate started to accumulate (Fig. 2), resulting in a decrease of the COD removal efficiencies to 64.6% and 50.9% respectively (Fig. 1). At $t_{HR} = 1$ day volumetric organic load = 3.39 kg COD m^{-3} day^{-1}), acetate, acetamide and ammonia concentrations in the effluent were 13.5 mM (SD = 1.9), 13.9 mM (SD = 5.9) and 54.0 mM (SD = 8.2) respectively.

pH variations were not responsible for the decrease of acetamide and acetate removal efficiency, since the sludge pH was near neutrality at all t_{HR} tested (Table 1). The average influent pH was 6.4 (SD = 0.2) and the effluent pH was above 8 (Table 1), reflecting the acid/base equilibrium resulting from ammonia and bicarbonate production.

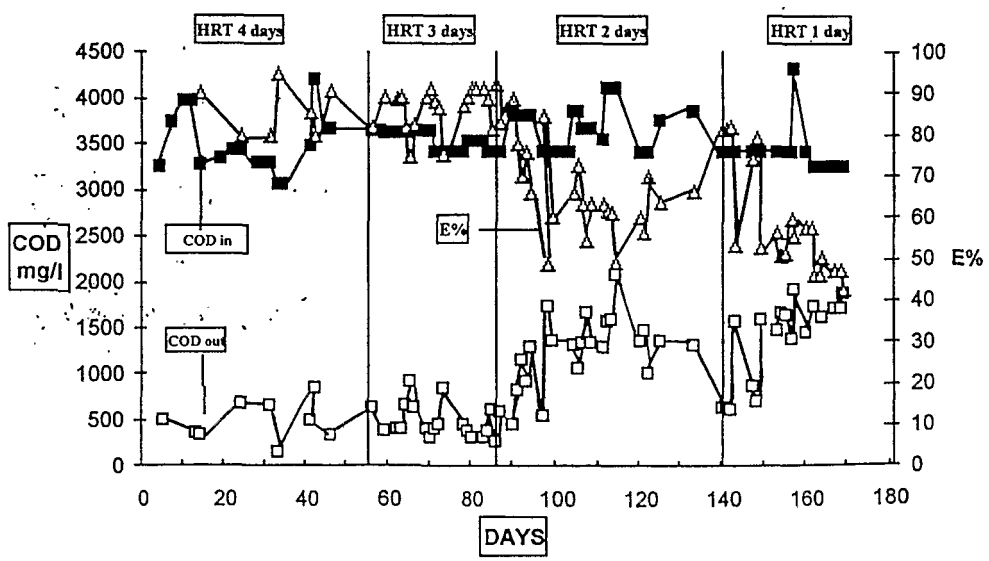

Fig. 1 Chemical oxygen demand (*COD*) removal efficiencies (*E*%) of the acetamide-fed reactor at different hydraulic retention times (*HRT*). *COD in*: COD in the feed, *COD out*: COD in the reactor effluent

Table 1 Methanogenesis from acetamide during days 1–176 at different hydraulic retention times (t_{HR}). *VSS* volatile suspended solids

Parameter	$t_{HR} = 4$	$t_{HR} = 3$	$t_{HR} = 2$	$t_{HR} = 1$
Acetamide concentration in the feed medium (mM)	55.5	48	50.6	55.2
Loading rates of acetamide (mmol fed/g VSS^{-1} day^{-1})	2.31	2.64	4.22	9.2
Average sludge pH	7.47 (SD = 0.04)	7.73 (SD = 0.31)	7.38 (SD = 0.06)	7.36 (SD = 0.08)
Average effluent pH	8.08 (SD = 0.21)	8.62 (SD = 0.23)	8.59 (SD = 0.21)	8.47 (SD = 0.22)
$\%CH_4$ in the biogas (%)	58.9	37.7	41.9	56.5
Measured production rate of CH_4(ml l $reactor^{-1}$ 35°C, 7 kPa) day^{-1}) at	167	178	460	548
Theoretical production rate of CH_4 (ml l $reactor^{-1}$ day^{-1}) at 35°C 7 kPa	386	525	620	896
Differences between the theoretical rates and the measured rates of CH_4 production (%)	57	66	26	39

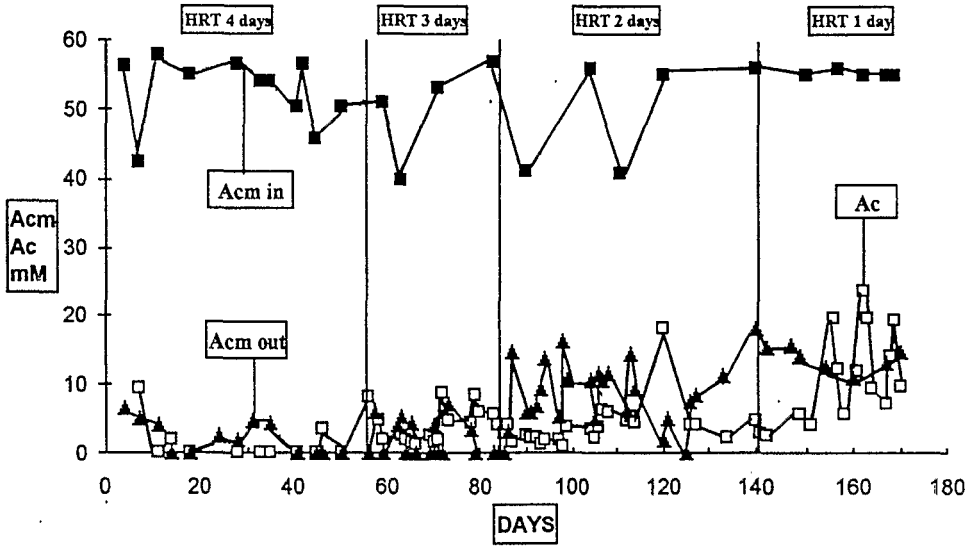

Fig. 2 Acetamide and acetate accumulation in the bench-scale upflow anaerobic sludge-blanket reactor at different hydraulic retention times (*HRT*). *Acm in*: acetamide concentration in the feed, *Acm out*: acetamide concentration in the reactor effluent, *Ac* acetate in the reactor effluent

At low volumetric organic loads, discrepancies between the measured and theoretical rates of methane production were observed (Table 1). When the reactor wall was struck, large bubbles of gas entrapped within the sludge were released. At these loads, the low methane production rates, together with the absence of mechanical mixing, might have favoured gas accumulation. At higher volumetric loads (1.85 and 3.39 kg COD m^{-3} day^{-1}) the differences decreased, probably because of better mixing in relation with a higher gas production.

Study of the acetate inhibition with the UASB sludge (days 177–312)

Ammonia was reported to inhibit methanogenesis from acetate by the aceticlastic methanogen *Methanosaeta* (*Methanothrix*) *concilii* at concentrations between 40 mM and 120 mM (Sprott and Patel 1986). This compound is suspected to be responsible for the acetate accumulation in the reactor. In order to examine this hypothesis, the reactor was fed acetate at $t_{HR} = 1$ and 2 days to determine the ability of the sludge to degrade acetate. The reactor was subsequently fed again with acetate at $t_{HR} = 1$day, before the feed was started at the same t_{HR} with a mixture of acetate and two different ammonia concentrations: 21.3 mM (SD = 2.4) and 54.1 mM (SD = 4.8) (Table 2).

With the acetate-fed reactor, at $t_{HR} = 1$ and 2 days the COD removal efficiencies were very high (Table 2), which demonstrates that the sludge was capable of degrading acetate efficiently. The results were better than those obtained when the reactor was fed acetamide, at similar volumetric organic loads. When ammonia was fed together with acetate, the COD removal

Table 2 Effect of ammonia on acetate degradation by the sludge of the bench-scale UASB reactor (days 177–312) *COD* chemical oxygen demand

Parameter	Duration of the experiment (days) 40	28	16[a]	20	15	12
t_{HR} (days)	1	2	1	1	1	1
Acetate concentration in the influent (mM)	54.7 (SD = 4.5)	56.0 (SD = 4.4)	52.0 (SD = 2.6)	56.1 (SD = 4.3)	55.4 (SD = 5.6)	56.6 (SD = 0.6)
Ammonia concentration in the influent (mM)	0	0	0	21.1 SD = 2.5	54.4 SD = 5.4	0
pH of the influent medium	5.20 (SD = 0.20)	5.60 (SD = 0.50)	5.98 (SD = 0.02)	5.94 (SD = 0.05)	5.99 (SD = 0.02)	6.33 (SD = 0.58)
pH of the sludge	7.60 (SD = 0.20)	7.44 (SD = 0.09)	7.40 (SD = 0.00)	7.57 (SD = 0.06)	7.30 (SD = 0.10)	7.55 (SD = 0.07)
pH of the effluent	8.84 (SD = 0.02)	8.69 (SD = 0.13)	8.71 (SD = 0.15)	8.64 (SD = 0.11)	8.35 (SD = 0.09)	8.64 (SD = 0.09)
COD removal (%)	98.85 (SD = 0.07)	99.39 (SD = 0.25)	99.09 (SD = 0.06)	99.13 (SD = 0.32)	99.08 (SD = 0.26)	98.62 (SD = 0.55)
Biogas at 35°C, (ml/l reactor^{-1} day^{-1}) 77 kPa	1923 (SD = 162)	1388 (SD = 203)	1023 (SD = 159)	1867 (SD = 587)	1938 (SD = 503)	2080 (SD = 463)
CH_4 in the biogas (%)	81 (SD = 5)	71 (SD = 5)	76 (SD = 5)	72 (SD = 4.3)	76 (SD = 2)	81 (SD = 7)

[a] An adaption period at t_{HR} = 1 day

Table 3 Persistence of acetamide biodegradation in the bench-scale UASB reactor. The acetamide concentration in the influent was adjusted to 56 mM

Parameter	Days 313–322	Days 323–343
t_{HR} (days)	1	4
pH of the sludge	6.64 (SD = 0.07)	7.00 (SD = 0.25)
COD removal efficiency	53.3 (SD = 21.2)	96.2 (SD = 1.2)
Biogas (ml/l reactor^{-1} day^{-1})	264 (SD = 83)	326 (SD = 49)
CH_4 in the biogas (%)	21.7 (SD = 8.6)	40.0 (SD = 11.8)
Acetamide concentration in the effluent (mM)	17.5 (SD = 3.1)	0.6 (SD = 0.2)
Acetate concentration in the effuent (mM)	11.2 (SD = 2.8)	0.3 (SD = 0.2)

efficiency did not change significantly (Table 2). Therefore, ammonia was not responsible for the inhibition of acetate degradation.

After these experiments, at day 300, the reactor was fed with acetate alone at t_{HR} = 1 day (Table 2) for 12 days, as an intermediary stabilization period before the last stage of the study was begun.

Persistence of acetamide degradation and reproducibility of the inhibition of acetate degradation (days 313–343)

During the previous 135 days, the reactor had not been fed acetamide. In order to know whether the sludge was still capable of degrading acetamide and whether the inhibition of acetate degradation was reproducible, acetamide was fed again at t_{HR} = 1 and 4 days. At t_{HR} = 1 day (days 313–322) (Table 3), the COD removal efficiency was similar to the values obtained during the first experiment at the same t_{HR} (days 140–176) (Table 1) and acetate and acetamide accumulation was observed once more (Table 3). At t_{HR} = 4 days (days 323–343), the COD removal efficiency was high (Table 3). The sludge reactor retained the ability to degrade acetamide efficiently at very low volumetric organic loads.

Discussion

Acetamide can be converted to methane in a bench-scale UASB reactor only at very low volumetric organic loads. Nevertheless, the COD removal efficiency decreased and acetamide and acetate started to accumulate when the volumetric organic load was increased. This was unexpected at such volumetric loads. For instance, in the case of a comparable substrate: acrylic acid (a low-molecular-mass compound found in wastewaters of the acrylate industry), the efficiency of COD removal was 97.1% at a volumetric load of 3.8 kg COD m^{-3} day^{-1} (Dohányos 1988). The acetate accumulation was surprising. Experimental data showed that neither the inefficiency of the sludge in acetate degradation nor ammonia was responsible for the increase of acetate concentration in the reactor effluent. On the basis of these observations, there is strong evidence that acetamide inhibits the aceticlastic reaction. In high-rate digestors, such an inhibition

might limit the anaerobic treatment efficiency of effluent containing acetamide or nitrile compounds, such as acetonitrile, since acetamide is an intermediary product of acetonitrile bioconversion into acetate (DiGeronimo and Antoine 1976, Maestracci et al. 1984). The observed acetamide accumulation could be explained by product inhibition (acetate); Maestracci et al. (1984) reported a slight competitive inhibition by acetate of an acrylamidase produced by *Brevibacterium* sp.

Discrepancies between theoretical and measured rates of methane production were explained mainly by gas accumulation in the sludge at low volumetric loads. Nevertheless, a small part of the COD was probably used by the aerobic bacterium. The acetamide-degrading microorganism might compete to some extent with aceticlastic methanogens for the use of acetate, since it is also a substrate of this aerobic bacterium (Guyot et al. 1994). Two moles of oxygen are needed to oxidize completely one mole of acetamide or acetate to CO_2 and H_2O. The dissolved oxygen concentration in the feed (0.16 mM) in comparison to the acetamide concentration (near 50 mM) was very low. Therefore, the aerobic degradation of acetamide was limited by the oxygen availability for the growth of the acetamide-degrading aerobic rod, and the contribution of this reaction to the COD removal cannot be significant. In spite of oxygen limitations, the acetamide-degrading aerobic rod would produce enough amidase to convert acetamide to acetate; this reaction does not require oxygen. This hypothesis is congruent with previous experiments in batch culture: the sludge maintained an amidase activity at decreasing rates when it was successively transferred under strictly anaerobic conditions (Guyot et al 1994). The fact that oxygen availability was limited, might in this case be a favourable factor allowing enough amidase production without significant aerobic acetate consumption.

Two reasons might explain the persistence of acetamide degradation, the acetamide-degrading microorganism isolated from this sludge is sporulated, and it can use acetate as substrate. The ability to degrade acetamide over a long period (343 days) may indicate that the synergy between aerobes and anaerobes was stable. Different studies have shown that (a) strict aerobes can be isolated from anaerobic digestor sludges (Toerien 1967; Guyot et al. 1994), (b) strict anaerobes can be present in oxygenated environments such as activated sludge (Guyot and Fajardo 1993; Wu et al. 1987) or granular sludge exposed to oxygen (Kato et al. 1993) and (c) strict aerobes and anaerobes can form stable associations to degrade organic compounds (Gerritse and Gottschal 1993; Guyot et al. 1994). These results strengthen the previously expressed idea (Guyot et al. 1994) that a new concept of wastewater treatment could be evolved. This would involve the association of aerobic and strict anaerobic microorganisms in a single reactor, taking advantage of their respective metabolic properties under both conditions.

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