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INHIBITION OF THE ANAEROBIC ACETATE DEGRADATION BY FORMATE.

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SUMMARY: Granular sludge from an UASB reactor fed with VFA showed a very low affinity for formate which provide little support to the theory of interspecies formate transfer. It is shown that formate can inhibit acetate degradation by anaerobic sludge.

INTRODUCTION:

Formate has an important role in the anaerobic degradation of organic matter, as one of the major products of fermentative reactions (Guyot and Brauman, 1986), and as one of the main substrates for methanogenic bacteria (Balch et al, 1979). The majority of the hydrogenophilic methanogens which cannot use acetate as energy source, can use formate as the other substrate for methane production (Guyot and Brauman, 1986). Hydrogen is known to inhibit methanogenesis from acetate by some acetoclastic methanogens of the Methanosarcina type (Ferguson and Mah, 1983) but not with the Methanothrix type bacteria (Zehnder et al, 1980). Furthermore Guyot (1986) shown that formate can inhibit methanogenesis from acetate in pure cultures of Methanosarcina barkeri 227 and Methanosarcina thermophila. However the possible inhibitory effect of formate on the acetoclastic reaction by sludge from anaerobic reactors remained to be investigated.

case our reactor would also have selected a microbial population able to use formate efficiently.

The effect of different formate concentrations on the acetoclastic reaction was determined and the maximal rate of acetate consumption for each curve (Table 1) was calculated by double reciprocal plot from each point of the curves. $1/V = f(1/S)$:

formate	Vm	standard deviation
mM	mmol acetate/l.h	
0	3.02	0.25
5	2.50	0.10
50	2.31	0.07
100	2.05	0.13

Table 1. Effect of different formate concentrations on the rate of acetate degradation, for the same initial acetate concentration (8.5 mM) (triplicate experiments).

Clearly table 1 demonstrates the inhibitory effect of various formate concentrations on acetate degradation; at 100 mM the maximal rate (Vm) is decreased by one third. This confirms with anaerobic sludge the experiment performed with pure cultures of Methanosarcina (Guyot, 1986) and adds new perspectives in the field of the inhibition of anaerobic digestion. Since formate, like hydrogen, is a major product of the first step of anaerobic degradation of organic matter, and nevertheless the sludge capacity to use formate is low, as we described, we suspect that formate accumulation in such a digester may cause either a decrease of reactor performances or a digester failure. Another interesting observation made by Belay et al (1986), is the inhibition between pH 5.8 to 6.2 of both growth and methanogenesis of Methanococcus thermolithotrophicus grown on H_2-CO_2 in presence of formate; it would be valuable to define the extent of such an inhibition with other hydrogenophilic methanogens. We note that formate might not be inhibitor of the Methanothrix type of bacteria, since they have a formate dehydrogenase and the hydrogen evolved by formate breakdown does not inhibit them (Zehnder et al, 1980). Thus the effect of formate or hydrogen on the acetoclastic reaction in anaerobic reactors might greatly depend on the relative proportion of Methanosarcina and Methanothrix. Therefore, there is a double interest to look for the enrichment of a digester sludge with Methanothrix, because of its high affinity for low acetate concentrations and its potential resistance to inhibition by either hydrogen or formate. In the future the definition of an index which would characterize the ratio Methanosarcina/Methanothrix for a sludge, might help to forecast the ability of an anaerobic reactor inoculum to be inhibited by either formate or hydrogen at the level of the acetoclastic reaction.

MATERIALS AND METHOD:

UASB reactor: a 4.5 litre UASB reactor was continuously fed during one year with a mixture of acetic (3.5 g/l) and propionic (1 g/l) acids as carbon and energy sources, in the following salt medium (mg/l):
NH_4HCO_3 (1000), $NaHCO_3$ (600), $(NH_4)_2SO_4$ (250), K_2HPO_4 (130), $CaCl_2.2H_2O$ (200), $MgCl_2.6H_2O$ (10), $FeSO_4.7H_2O$ (14), $(NH_4)_6Mo_7O_{24}.4H_2O$ (10), $MnCl_4.H_2O$ (1), $CuCl.2H_2O$ (0.1), $NiCl.6H_2O$ (0.2), $CoCl_2.6H_2O$ (0.05).

Experimental techniques: anaerobic techniques as described by Hungate (1969) and Balch et al (1976) were used throughout.

Experiments: kinetic experiments were run in 60 ml serum bottles which contained each 10 ml of granular sludge and 10 ml of the same mineral solution used for the reactor. The granular sludge (1 mm to 2 mm) was sampled 24 hours before the beginning of the experiments, and allowed to stay under vacuum in the pre-chamber of an anaerobic hood (Mc Coy), to allow the consumption of residual substrates from the reactor and accelerate the gas removal. Effectively, 24 hours later, controls run without substrate indicated no detectable acetate or propionate. At the beginning of the experiment (time 0), concentrated stock solutions of acetate and (or) formate were injected into the serum bottles. For the inhibition experiments, for a constant acetate concentration, different formate concentrations were added at the same time. For the determination of the apparent saturation constant (K'm) of formate, different formate concentrations were tested as substrate. From the time-course of formate methanogenesis at different concentrations, a doble reciprocal plot was used to calculate K'm. Experiments were run in triplicate.

Analytical techniques: acetate was analyzed by gas chromatography using a flame ionization detector and a stainless steel column packed with Porapack Q (80-100 mesh). Volatile Suspended Solids (VSS) were analyzed according to the standard methods (1980).

RESULTS AND DISCUSSION.

Determination of the kinetic parameters for formate methanogenesis by the granular sludge, gave the specific rate of formate degradation (Asp) as 3 mmol/g VSS.h and a K'm of 11 mM. These values compared to those found by Schauer et al (1982) for Methanobacterium formicicum (Asp: 37 mmol/g VSS.h, K'm: 0.58 mM) show that the UASB reactor had selected a very poor formate using microbial population, probably because the reactor was exclusively fed with acetate and propionate. Thus the sludge might be enriched with hydrogenophilic methanogens, acetoclastic methanogens, and obligate hydrogen producing acetogens. We must conclude, in view of the kinetic data, that the selected hydrogenophilic methanogens present little affinity for formate and are mainly unable to use formate. This observation and others published elsewhere (Guyot and Brauman, 1986) do not support the theory of interspecies formate transfer described by Thiele and Zeikus (1988), since in that

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