Product Inhibition in Sludge Digestion

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Abstract. Hydrogen and acetate inhibit the microbial degradation of propionate and ethanol in digesting sludge. Propionate oxidation is more sensitive than ethanol oxidation to product activities. This difference can be explained on thermodynamic grounds. It is concluded that the limiting factors for complete anaerobic mineralization of biodegradable organic matter in digesting sludge are to be sought in the boundary conditions for the exergonic oxidation of propionate. The different ecological significances of hydrogen and acetate as inhibitory agents of fatty acid degradation in sludge digestion are discussed. Hydrogen partial pressure and alkalinity are recommended as parameters in monitoring sludge digestion.

Introduction

Hydrogen was shown to reversibly inhibit the growth of several anaerobic bacteria which produce this gas during fermentation [2, 5, 8]. Monocultures of hydrogen-forming anaerobes usually excrete higher amounts of fermentation products such as ethanol and less hydrogen than mixed cultures containing hydrogen-utilizing species [1, 3, 9, 13, 14]. The influence of hydrogen on growth yield and product formation depends on both species and substrates, or in other words, on the pathways of hydrogen formation [3]. The action of hydrogen on product formation in facultative and anaerobic chemotrophs was explained by the thermodynamics of hydrogen-yielding processes [15]. Consequently, the influence of hydrogen on fermentations which potentially include hydrogen formation may be interpreted as a thermodynamic control mechanism acting by product inhibition.

We show in this work the product inhibition caused by hydrogen as well as by acetate of some important catabolic reactions in digesting sludge.

Materials and Methods

Sludge

Samples of digested sludge were anaerobically taken from the digester of the municipal waste treatment plant in Opfikon near Zürich. The sludge was well stabilized (≈ 40 days solids residence time at 33°C), and the content of dry matter was 2.9%, with 37% organic material. Volatile fatty acids were very low (less than $50\ \mu\text{moles/liter}$ propionic and butyric acid and around $0.5\ \text{mmoles/liter}$ acetic acid), and the pH was 7.4. The temperature and pH conditions prevailing in the digester were also maintained in the laboratory experiments.

Chemicals

All chemicals (p.a.) were obtained from commercial sources. Traces of oxygen in the gases were removed by passing them over heated copper (340°C) and through columns of Oxisorb (Messer Griesheim, Düsseldorf; [16]).

Apparatus

Figure 1 shows the experimental arrangement. Defined mixtures of carbon dioxide, hydrogen, and helium were continuously produced and moistened by a gas-mixing device. Carbon dioxide pressure was used to control the pH at 7.4. The lab-scale digester equipped with two flat blade stirrers (1000 rpm) and four baffles had a volume of 11 liters. Batches of 7 liters of sludge were used. The pressure in the reactor was kept between 1.03 and 1.06 atm. Sludge samples were taken every hour through a valve preventing the access of oxygen to the reactor. Water vapor and hydrogen sulfide were removed from the digester gas by a silica gel filter. All tubes and connections were of stainless steel (Hoke Inc., Cresskill, N.J.).

Fig. 1. Apparatus used for the study of product inhibition by hydrogen and acetate in digesting sludge.

Gas Analysis

The amount of gas leaving the digester was measured by a wet test gas meter (Elster, Mainz-Kastel). At intervals of 1 hour, the composition of the gas mixture was determined by a two-column gas chromatograph (molecular sieve 5Å and Porapak Q) connected to a thermoconductivity detector (Gow Mac Instruments Co. Ltd., Shannon).

Volatile Fatty Acids

Sludge samples were centrifuged, and the clear supernatant was acidified with conc. HCl. The volatile fatty acids were determined by a Pye Unicam Series 104 two-column chromatograph (Porapak QS) and flame-ionization detectors.

Results

Influence of Hydrogen on Propionate Degradation

As already mentioned, the sludge from the waste treatment plant had a very low content of volatile fatty acids. The propionate metabolizing enzyme systems, e.g., were saturated only to 20% [6]. Therefore, the concentration of propionate at the beginning of the experiments was made up to 2 to 3 mmol/liter by adding calcium propionate to the sludge. The decrease of the propionate concentration was then strictly linear. Addition of hydrogen gas immediately stopped the propionate consumption, as shown in Fig. 2A.

Fig. 2. Influence of hydrogen on the propionate degradation in sludge digestion. (A): Inhibition of the propionate concentration decrease by hydrogen. (B): Enhanced decrease of the acetate concentration and linear accumulation of propionate resulting from a continuous addition of hydrogen. (C): No detectable effect of a low hydrogen concentration on the acetate and propionate turnover.

In analogy to propionate, the acetate concentration in the original sludge could only half-saturate the acetate-consuming systems [6]. Calcium acetate additions to the sludge to initial concentrations of 2 to 3 mmol/liter acetate resulted in a linear decrease of the acetate. The rate of the acetate concentration decrease could be accelerated instantly by raising the partial pressure of hydrogen above 0.005 atm. Simultaneously, propionate began to accumulate at a constant rate (Fig. 2B). Smaller pressures of hydrogen gas had no measurable effect on the acetate and propionate turnover (Fig. 2C).

Influence of Acetate on Propionate Degradation

At the beginning of these experiments, calcium propionate was added to the sludge in order to obtain initial propionate concentrations of 2 to 3 mmol/liter. After 3 hours, calcium acetate was added up to a final concentration of 8 mmol/liter acetate. There was no effect on the rate of decrease of the propionate concentration. Additions up to 80 mmol/liter acetate (40 mmol/liter calcium acetate), however, stopped the oxidation of propionate. A control experiment with 40 mmol/liter calcium chloride showed that an effect of the calcium ion concentration was not involved (Fig. 3).

Influence of Hydrogen and Acetate on Ethanol Degradation

In a further series of experiments, ethanol was added to the sludge to a concentration of 2 to 3 mmol/liter. In contrast to the general assumption of ethanol being an unimportant intermediate product in natural anaerobic environments [4], our sludge showed an immediate and rapid ethanol consumption at a rate of about 0.25 mmol/liter/hour. The addition of hydrogen up to a partial pressure of

Fig. 3. Influence of acetate on the propionate degradation in digesting sludge. (A): Addition of 4 mmol/liter calcium acetate: no effect on the propionate oxidation. (B): Addition of 40 mmol/liter calcium acetate: stop of the propionate degradation. (C): 40 mmol/liter calcium chloride: no effect on the decrease of the propionate concentration.

Fig. 4. Influence of hydrogen and acetate on ethanol degradation in digesting sludge. (A): No detectable effect of 0.025 atm hydrogen on ethanol degradation. (B): Inhibition of ethanol oxidation by pressures of hydrogen > 0.05 atm. (C): No effect of 40 mmoles/liter calcium acetate on ethanol degradation; inhibition of ethanol degradation by 40 mmoles/liter calcium acetate and at hydrogen pressures of > 0.01 atm.

0.02 atm did not influence the ethanol degradation rate (Fig. 4A). Increased hydrogen pressures of 0.07 atm stopped the ethanol conversion (Fig. 4B). A concentration of 80 mmoles/liter acetate (40 mmoles/liter calcium acetate) had no effect on ethanol degradation. In conjunction with a hydrogen pressure of only 0.01 atm, however, the decrease of the ethanol concentration was stopped instantaneously (Fig. 4C).

Discussion

Our results indicate that hydrogen as well as acetate may stop the oxidation of ethanol and propionate in digesting sludge. The necessary concentrations for inhibition are at variance for different substrate-product relationships. Propionate degradation was inhibited by either 0.005 atm hydrogen gas or 80 mmoles/liter acetate, whereas the inhibition of ethanol oxidation required a pressure of 0.07 atm hydrogen or 80 mmoles/liter acetate plus 0.01 atm hydrogen. This difference in sensitivity to product inhibition can be explained by the thermodynamics of the metabolic reactions of fatty acids and alcohols at anaerobic conditions. According to Bryant et al. [2], ethanol is degraded as follows:

Our previous observations [6], which are in agreement with earlier results [7, 10, 11], indicate that propionate is decomposed according to the equation

¹ Thermodynamic data from Thauer et al. [12].

Preliminary experiments in our laboratory with enrichment cultures and digested sludge indicate an anaerobic butyrate oxidation according to

The three reactions are endergonic under standard conditions ($\Delta G^{0'} > 0$). $\Delta G^{0'}$ of the propionate oxidation is more positive than $\Delta G^{0'}$ of the ethanol oxidation, which already points to a greater sensitivity of the propionate oxidation to product inhibition. If one calculates the Gibbs free energy $\Delta G'$ for "natural conditions," i.e., the activities of educts and products as measured in the *milieu extérieur* of the cells, the thermodynamic relationships become much more informative. The Gibbs free energy under natural conditions is

$$\Delta G' = \Delta G^{0'} + RT \ln \frac{[\text{products}]}{[\text{educts}]}$$

Since the anaerobic degradation of alcohols and fatty acids, according to equations 1 through 3, is essentially controlled by the acetate concentration and the partial pressure of hydrogen, we may estimate the limit of negative $\Delta G'$ values as a function of the activities of the two products (Fig. 5). With due reservations toward such arithmetics (in fact, we ignore educt and product activities at the site of reaction), the result gives a clear indication of the boundary conditions for the functioning of sludge digestion in a well-equilibrated system. As might be expected, the oxidation of propionate is thermodynamically less favorable than the oxidation of ethanol and butyrate, since 1 mole of propionate yields 3 moles of hydrogen gas compared to only 2 moles of hydrogen produced from 1 mole of ethanol or butyrate. The experimental findings on the higher sensitivity of propionate oxidation to hydrogen and acetate activities in comparison to ethanol and butyrate oxidation (Fig. 2 versus Fig. 4) confirm the theoretical considerations. Therefore, the boundary conditions for the exergonic oxidation of propionate are found to be decisive for the complete mineralization of biodegradable organic matter in digesting sludge.

Fig. 5. Boundary lines for $\Delta G' \geq 0$ for the oxidation of propionate, butyrate, and ethanol in function of the acetate concentration and the partial pressure of hydrogen at conditions similar to those in well-digested sludge. 1, 2, 3: reactions mentioned in the discussion. $[\text{HCO}_3^-] = 0.1$ mole/liter; $\text{pH} = 7.0$; all other concentrations = 0.1 mmole/liter; 25°C .

Although the activities of acetate and hydrogen resulting in negative values of $\Delta G'$ are in the same order of magnitude (Fig. 5), the partial pressure of hydrogen is ecologically the dominating factor. The reason is the low solubility of hydrogen in water. An interruption of the hydrogen consumption by lithotrophic methanogens will therefore immediately increase the hydrogen pressure to such an extent that the degradation of higher fatty acids is inhibited. The acetate concentration, however, will change only slightly. If this situation prevails for a longer period, fatty acids accumulate and decrease the alkalinity which is responsible for the buffering of the pH around 7.5 [16]. As the bicarbonate buffer becomes weaker than the fatty acid buffer, the resulting drop of the pH will cause the digestion to fail. It is obvious, therefore, that the partial pressure of hydrogen and the alkalinity are the essential parameters to be monitored in order to detect an undesirable development of a sludge digestion as early as possible. If a stable digestion is to be achieved, the partial pressure of hydrogen in the digester gas should not be allowed to exceed 10^{-4} atm, and the molar bicarbonate concentration should be at least one order of magnitude higher than the total molarity of volatile fatty acids.

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