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ANAEROBIC TREATMENT OF 'ACID WATER' (METHANE PRODUCTION IN A SULFATE-RICH ENVIRONMENT)

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ABSTRACT

The anaerobic purification of 'acid water', *i.e.* wastewater of the industrial production of fatty acids, was studied. A particular problem results from the presence of sulfate in this wastewater as this can be converted to sulfide. Nevertheless the results of the anaerobic treatment of this wastewater were promising. A total organic carbon reduction of 80-90% was attained. The sulfide production was less than expected relative to the theoretical maximum. Hypotheses to explain the successful competition of methane-producing bacteria with sulfate-reducing bacteria were formulated.

INTRODUCTION

Anaerobic wastewater treatment is applied to a variety of wastewaters. Each type of wastewater requires a specific approach, since under anaerobic conditions highly specialized groups of microorganisms are involved in the breakdown of different compounds. Variations in environmental conditions and wastewater composition may have a striking effect on the composition of the microbial population and thus on the applicability of the anaerobic waste treatment. A particular problem results from the presence of sulfate in the wastewater, as this is often converted quantitatively to sulfide by the sulfate-reducing bacteria. These bacteria compete with methane-producing bacteria for available substrates, such as acetate and hydrogen. The presence of sulfide in the effluent and biogas is highly undesirable because of its toxicity and corrosive properties. In a study on the anaerobic purification of so-called 'acid water', *i.e.* the wastewater from industrial production of fatty acids, it was found that sulfide production was only a part of the theoretical maximum on the basis of the available reduction capacity in the influent. This paper describes and analyses the results obtained and presents a number of hypotheses, which may explain the relatively successful competition of methanogenic bacteria with sulfate-reducing bacteria for the available energy sources.

MATERIALS AND METHODS

Wastewater composition

glycerol	.4	-	4	g/l
fatty material	.01	-	3.7	g/l
sulfate	10	-	40	g/l
phosphorus	.2	-	.8	g/l
nitrogen	1×10^{-2}	-	6×10^{-2}	g/l
iron	1×10^{-2}	-	4×10^{-2}	g/l
nickel	$.1 \times 10^{-3}$	-	127×10^{-3}	g/l
pH	.8	-	2.3	
COD	2	-	6	g O_2/l

Wastewater was enriched with .5 g/l ammonium chloride before anaerobic treatment.

Artificial wastewater composition

glycerol	1.2	g/l
sulfate, as sodiumsulfate	10	g/l
Na_3PO_4 . 12 aq.	3	g/l
nitrogen, as ammonium chloride	5×10^{-2}	g/l

Inoculum

As an inoculum for the anaerobic reactor, sludge was used from the methane-forming reactor of a two-stage anaerobic wastewater treatment installation fed with a sucrose solution.

Experimental set-up

The experimental set-up is given in figure 1. pH-control was performed in a vessel before the reactor, because pH-control in the reactor would cause undesirable pH gradients. Gasrecirculation was applied to enhance mixing of sludge and wastewater in the reactor.

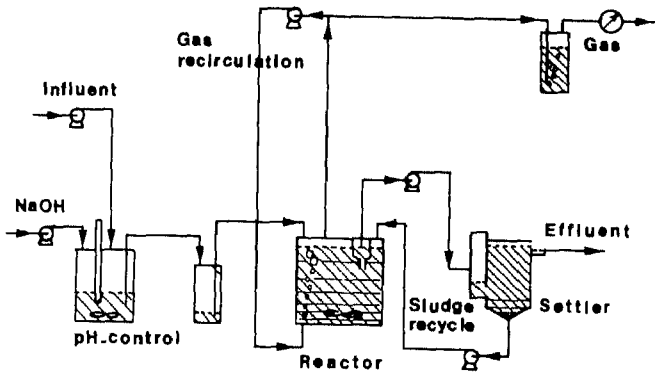
Gas production

Gas production was estimated by a precision wet gasmeter (Meterfabriek Dordrecht, The Netherlands).

Gas composition

The gas composition was analyzed gaschromatographically. Hydrogen, nitrogen, oxygen, methane and carbon dioxide were determined quantitatively. Sulfide, both in gaseous and in liquid phase, was determined iodometrically. To determine dissolved inorganic carbon, a liquid sample was acidified with concentrated sulfur-

Fig.1. Experimental set-up.



ic acid in a closed bottle provided with a septum. Hydrogen was added as an internal standard. Then the gas composition was analyzed.

It appeared that diffusion of methane, oxygen and hydrogen sulfide across tubing and sealing material could not be neglected in these small-scale experiments. From the data of experiment 4, a methane loss of about 8 mmole per day and a hydrogen sulfide loss (by diffusion and oxidation) of about 1 mmole per day could be calculated. This could be verified by diminishing the surface of non-gastight material in the experimental set-up; a decrease in gas-losses by about 50% was observed. Data on gas composition were corrected for diffusion or oxidation losses. After correction, balance equations over experiments 1, 2 and 3 closed to within 4%.

Total organic carbon

Total organic carbon was estimated on a Tocsin aqueous carbon analyzer (Phase Separations, U.K.).

Glycerol

Glycerol was determined enzymatically (Boehringer, cat.no. 148270).

Fatty acid

Fatty acid was determined on a Becker Packard gaschromatograph Type 437, provided with a Porapak QS column.

RESULTS

The efficiency of removal of total organic carbon (TOC) from the 'acid water' was measured in a series of experiments in the experimental set-up depicted in figure 1. At the same time the formation of sulfide was followed. Table 1 shows that a TOC reduction of 80-90% was accomplished indicating that the anaerobic treatment is a promising option in the treatment of this wastewater.

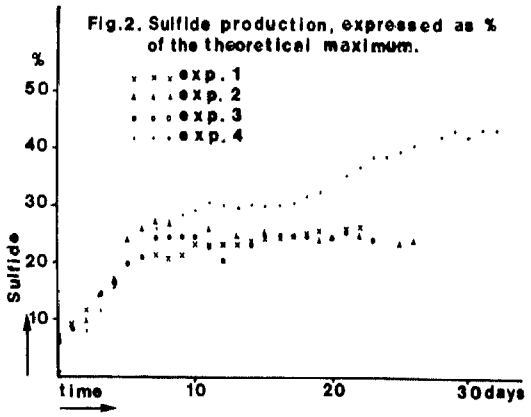
TABLE 1

Total organic carbon (TOC) reduction and sulfide production in 'acid water' during anaerobic treatment.

Experiment		Load	Influent			Effluent		
			glycerol	sulfate	TOC	sulfide production		TOC
no.	type 1)	g.COD / g DM.d	mM C	mM	mM C	theor. max. mM	observed % of theor. max.	mM C
1	W	.05	56	120	80	39.7	23	10.5
2	W	.06	24	100	62	28.1	26	11.0
3	W	.16	56	120	80	39.7	25	11.0
4	A	.16	39	104	39	22.8	30	-

1) W = wastewater, A = artificial medium.

Figure 2 shows how the sulfide production, expressed as % of the theoretical maximum increased during the first phase of the experiments. After about 10 days, the sulfide production stabilized at 20-30% of the theoretical maximum value. A pseudo steady state was reached.



The data on feed and product composition were used to set up elemental and reduction equivalent balances [1]. The possible sulfide production originating from H_2 and the possible methane production derived from acetate can be calculated (Table 2). For that purpose, the following assumptions were made:

1. The maximal production of hydrogen gas is realized when glycerol is converted to acetate and hydrogen. (Depending on the pathway of conversion the production of hydrogen gas may decrease, but one mole of glycerol will always give at least one mole of acetate.)
2. Methane may be formed either from acetate or from CO_2 and H_2. In our system sulfide is produced by sulfate-reducing bacteria from H_2 only and not from acetate. This assumption is based on studies on microbial sulfate reduction, showing that acetate-oxidizing sulfate-reducing bacteria are generally not present in fresh-water environments [2].
3. Organic compounds in 'acid water' are glycerol and carbohydrates. These substrates are metabolized via the EMP-pathway.
4. Biomass carbon of hydrogen-utilizing sulfate reducers is derived for 70% from acetate and for 30% from CO_2 [3,4]. In the calculations these figures can be used for the acidogenetic population too. For the production of 1 C-mole biomass 0.7 mole H_2 is used.

TABLE 2

Theoretical and experimental data concerning methane and sulfide production (concentrations expressed in mM).

Experiment		Available substrates by acidogenesis[1]		Methane		Sulfide	
no.	time period days	H_2	acetate	Theor. on acetate	Exp.[2]	Theor. on H_2	Exp.[2]
2	15-26	39.0	15.2	15.2	17.2	9.8	7.0
3	7-23	59.0	19.7	19.7	-	14.8	9.7
4	14-19	39.0	12.5	12.5	15.9	9.8	6.8
5[3]	-	34.5	11.7	11.7	13.4	8.6	7.3

[1] Corrected for biomass production.
[2] Corrected for diffusion losses.
[3] This experiment was not included in figure 1.

Comparison of the theoretical and experimental production values showed that sulfide production appeared to be only 25-30% of the theoretical maximum (Table 1). Moreover, the production was less than expected if hydrogen was the only energy source (Table 2). Methane production, if from acetate only, appeared to be higher than expected. Therefore, the methanogenic population produced methane from acetate as well as from H_2/CO_2.

DISCUSSION

The population of methane producers can grow on acetate and H_2 only. Thus competition between sulfate reducers and methanogens focus on these two substrates. As mentioned before, we assume that of each mole of glycerol a maximum of one mole of acetate will be formed, irrespective of the route followed in the mixed culture. This assumption was supported by the observation that acetic acid was the only detectable fatty acid in the effluents during steady states. However, other compounds such as propionic acid and butyric acid which were present during the first phase of the experiments, could have been below the detectable level during steady states because of their high turn-over. When these fatty acids are incompletely oxidized by sulfate reducers no hydrogen is produced. Nevertheless, the overall data on sulfide and methane production show that the methane-producing population must have utilized H_2 as additional substrate. This is an unexpected result, because:

1. growth rates of sulfate-reducing bacteria on hydrogen are higher than growth rates of methanogenic bacteria,
2. the affinity for hydrogen of sulfate reducers is larger,
3. thermodynamically, microbial sulfate reduction appears to be slightly favoured above methane production.

At present no clear explanation is available. Hypotheses that might explain the phenomenon of the successful competition of the methanogenic population for the available substrates are:

1. Iron-limitation could inhibit growth of the sulfate-reducing bacteria. Although the iron concentration in the influent was rather high, the free-iron concentration in the reactor might be extremely low, due to the formation of either iron(III)hydroxide or iron(II)sulfide. Although iron determinations in influent and effluent gave relatively high values (influent 300 µM iron, effluent 2 µM iron), it is questionable if iron is in an acceptable form for the bacteria.
2. The results might be explained by a model for microbial competition, which is based on the observation that some bacteria are able to grow mixotrophically; they can utilize two substrates simultaneously. Such organisms may outcompete a seemingly better-equipped organism specialized to one substrate [5]. In the present case mixotrophic growth of methane-producing bacteria, such as *Methanosarcina*, on the substrates acetate and hydrogen is considered. The methanogens would be able to attain a certain population size on the available acetate and then be able to consume a part of the available H_2 in spite of its lower affinity for hydrogen relative to sulfate reducers.
3. A third group of bacteria, the acetogens, could affect the competition. When they convert H_2 and CO_2 into acetate, there would be a drain of available H_2 from the sulfate reducers to methanogens. This is not very likely, however,

because studied on mixed cultures of acetogenic bacteria and methanogens generally show that the acetogens prefer in mixed cultures an organic carbon source, which they convert to acetate, and leave the available H_2 and CO_2 for the methanogens [6].

Certainly other explanations cannot be excluded at the present time; for instance the effect of pH or the presence of an unknown inhibitor in the influent. Research is in prospect to evaluate the described hypotheses.

CONCLUSION

Anaerobic treatment of decanted 'acid water' results in a purification of 80-90%.

Production of toxic hydrogen sulfide is much less than expected on the basis of available reduction capacity and sulfate. This can be explained either by iron limitation of the growth of sulfate-reducing bacteria or by the phenomenon that the sulfate-reducing bacteria in the system are specialized on hydrogen as energy source and methane-producing bacteria have the opportunity to grow mixotrophically on acetate and hydrogen.

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