Sulfate reduction and fermentation of amino acids in industrial waste water.
Nanninga, Hendrik Jan

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SUMMARY

Waste water from the industrial production of potato-starch contains a large quantity of qualitatively heterogeneous organic and inorganic compounds. Therefore, the anaerobic treatment of this type of waste water involves a large number of microbial processes such as the fermentation of carbohydrates and amino acids, the acetogenic degradation of fatty acids, methane production and sulfate reduction. The bacterial reduction of sulfate, resulting in the formation of hydrogen sulfide ($H_2S$), is a process of great importance in several industrial purification plants. A major aim of the present study is to gain more insight in this particular process thus enabling a better process control.

In the introduction of this thesis (Chapter I) a survey is given of compounds which may interfere with the anaerobic purification process of potato waste water. The most significant compounds are hydrogen, ammonium, sulfite and $H_2S$. Besides a brief discussion of the kind of problems these compounds may cause, some possible solutions to these problems are suggested.

During the anaerobic treatment the waste water of the potato-starch factory in De Krim (AVEBE, The Netherlands) passes successively a sedimentation pond, an upflow reactor and a methane reactor. The degradation of the quantitatively most important compounds is described in the chapters II and III. Carbohydrates and citrate were already fermented before they reached the upflow reactor. The degradation of free amino acids and small peptides took place mainly in the sedimentation pond and in the upflow reactor whereas the degradation of protein and longer peptides took place in the upflow reactor and methane reactor. Bacterial species involved in the anaerobic degradation of glutamate, aspartate and lactate were isolated and classified. Acetate, propionate and butyrate were the main organic fermentation products formed by these bacteria. This agrees well with the relatively high concentrations of these fatty acids found in the purification plant. With respect to the reduction of oxidized
sulfurous compounds it is important that this process is completed before the waste water stream reaches the methane reactor. This allows the removal of $H_2S$ prior to this reactor, thus ensuring that the biogas produced and effluent of the methane reactor contain low amounts of (corrosive) $H_2S$. However, in practice sulfate reduction was demonstrated both in the upflow and the methane reactor. Several factors may be responsible for this incomplete reduction of sulfate in the upflow reactor such as the hydraulic retention time, $pH$, temperature, availability of growth substrates and the type of interaction between sulfate-reducing bacteria and other bacterial species. In the following chapters these factors have been investigated.

In the chapters IV, V and VI the occurrence of interactions between sulfate-reducing and amino acid-fermenting species has been described. In potato waste water glutamate and aspartate (along with their amides) are by far the most abundant amino acids. Bacterial species involved in the degradation of these amino acids in the purification plant were isolated via direct dilutions (see also Chapter II) and via chemostat enrichments. In all cases the predominant aspartate-fermenting species appeared to belong to the genus *Campylobacter*. With glutamate as a substrate clostridia became dominant in chemostat enrichments carried out at a high dilution rate ($0.25 \text{ h}^{-1}$) and appeared most numerous in direct dilutions of the reactor fluid. One strain has been characterized in detail and was classified as *Clostridium cochlearium*. However, in chemostat enrichments carried out at low dilution rates ($0.025-0.12 \text{ h}^{-1}$) other species predominated. Again one of these strains was characterized in detail and classified as a new species, *Selenomonas acidaminophila*. These differences in the outcome of chemostat enrichments at different dilution rates could be explained by differences in the affinity for the growth-limiting substrate glutamate. The amino acid-fermenting species mentioned so far may affect the metabolism of sulfate-reducing bacteria in several ways, especially when hydrogen ($H_2$) is involved. Hydrogen can be utilized by several sulfate-reducing species as an energy source. Thus $H_2$ produced by *C. cochlearium* may stimulate sulfate reduction whereas *Campylobacter* spec. and
S. acidaminophila are potential competitors for H₂. In addition to the degradation of aspartate and glutamate, the degradation of other amino acids such as valine and leucine has also been studied. The fermentation of valine and leucine usually yields H₂. Accumulation of H₂ leads to the inhibition of the fermentation of these amino acids. It is therefore not surprising that the presence of H₂-scavenging species, such as several sulfate-reducing species, strongly accelerated the degradation of these amino acids. It was also found that some valine- and leucine-fermenting organisms were able to prevent the accumulation of H₂ through the reductive conversion of acetate into butyrate.

In the chapters VII and VIII five sulfate-reducing species are described which were isolated from the purification plant in De Krim (see Chapter III). One of these species turned out to be capable of growth with methanol plus sulfate, to ferment glycerol to 1,3-propanediol plus 3-hydroxypropionate and to possess a considerable quantity of intracellular membrane structures. This new species has been named Desulfovibrio carbinolicus. The isolated species were able to metabolize overlapping ranges of compounds. In the presence of sulfate good growth by one or more species was always possible with H₂, lactate, propionate or ethanol (doubling time 3.0-6.3 h). To explain the incomplete reduction of sulfate in the upflow reactor (see also Chapter III) the values of these doubling times are quite important. In this reactor the hydraulic retention time is 9.5 h. This implies that the doubling time of a species required to prevent washout of cells from the reactor should be less than 6.6 h. Of the substrates which permit such a short doubling time propionate is abundantly present in the upflow reactor. However, depression of the rate of growth of the propionate-metabolizing bacteria by the relatively low pH in the upflow reactor may be responsible, at least partly, for the incomplete reduction of sulfate. A solution to this problem might be the application of support material in the upflow reactor in order to increase the biomass of sulfate-reducing bacteria. The first results of a laboratory experiment with a small upflow reactor filled with porous support material appeared very promising.