SUMMARY

In recent years several types of obligately anaerobic bacteria have been discovered that can produce organic acids from methanol and CO_2. Vitamin B_{12} is a cofactor of the methyl-carrying enzyme of the C_1-pathway in these acidogens; they contain relatively high concentrations of corrinoids during growth on methanol. Most of these methylotrophic bacteria form acetate, but some species produce mainly butyrate. The production of these acids (e.g. butyrate) and vitamin B_{12} from low-price methanol are of industrial interest. However, industrial application requires substantial improvements in the growth and fermentation rates of the organisms. For this purpose, a better understanding of their metabolism is required.

In this thesis the results are reported of studies on growth and product formation of methylotrophic acidogenic bacteria in batch and continuous cultures. One of these acidogens, *Butyribacterium methylotrophicum*, was studied extensively. It is the first methylotrophic acidogenic bacterium whose potential for industrial application was recognized.

CO_2 is an essential cosubstrate during growth on methanol. Under these conditions part of the substrate is oxidized to CO_2 and the reducing equivalents generated are used to reduce the cosubstrate to the level of enzyme bound carbon monoxide, which is the precursor of the carboxyl group of the acid formed. Acid formation proceeds via acetyl CoA which is synthesized by the acetyl CoA synthase from the carbon monoxide moiety, coenzyme A and a methyl group (transferred from methanol by a methyltransferase). This metabolic route is called the acetyl CoA/CO dehydrogenase pathway (see chapter 1).

Growth of *Butyribacterium methylotrophicum* in batch culture was inhibited by the commonly employed sulfur source and reductant sulfide (chapter 2). Replacement of sulfide by dithionite or thiosulfate not only improved the maximal growth rate in batch and continuous culture considerably, but also the formation of cell aggregates and wall growth decreased. The use of the two alternative sulfur sources had no effect on the product ratio acetate to butyrate.

B. methylotrophicum was grown on methanol/CO_2 in a chemostat, but butyrate production was low (Chapter 3). The available literature reported changes in the product ratio of *B. methylotrophicum* towards more butyrate during growth in batch culture on methanol/CO_2, when the bicarbonate concentration was decreased and/or acetate was added to the medium. The results obtained after addition of acetate were confirmed by our experiments in batch culture, but the change in product ratio in the chemostat at different dilution rates was small.

In co-cultures of acidogens with non-methylotrophic hydrogenotrophs, part of the reducing equivalents generated during methanol oxidation by the acidogen were used as an energy donor by the hydrogenotrophs (chapter 4). Less acetate and/or butyrate were produced per mol methanol converted in favour of the production of sulfide or methane by (non-methylotrophic) sulfate-reducing bacteria or methanogens,

respectively. These results are explained in terms of interspecies hydrogen transfer between the acidogens and the sulfate-reducing or methanogenic bacteria. This process has ecological implications, because it shows that non-methylotrophic methanogenic and sulfate-reducing bacteria may be involved indirectly in the anaerobic conversion of methanol. The co-culture experiments were carried out with freshwater organisms and in order to get more information on the possible occurrence of this process in marine environments we had to set up co-cultures with marine bacteria. However, until then no methylotrophic acidogenic bacteria had been isolated from marine sediments. For the enrichment of marine acidogens specific substrates were used (addendum to chapter 4), because mainly methanogens were enriched in marine media containing methanol according to several reports. With vanillate we successfully enriched and isolated a strain of *E. limosum* from marine sediments and in coculture with a marine non-methylotrophic sulfate reducer we indeed observed sulfide production.

A specific substrate for the enrichment of acidogens from non-marine environments was betaine (*N,N,N*-trimethylglycine). *E. limosum* demethylates betaine to *N,N*-dimethylglycine and forms acetate and butyrate (see chapter 1). In our enrichments with marine mud and this substrate no acidogenic bacteria became dominant, but unexpectedly betaine was utilized by a non-methylotrophic *Desulfuromonas* strain. *Desulfuromonas* reductively cleaves betaine to trimethylamine and acetate, and part of the acetate is then oxidized to CO_2 to provide the reducing equivalents for the initial cleavage reaction (chapter 5). In the presence of sulfur, betaine was converted to trimethylamine and presumably CO_2 with the formation of sulfide; then, only traces of acetate were produced.

Acetate oxidation with sulfate as electron acceptor instead of sulfur has been described for several *Desulfobacter* species and therefore we expected the existence of *Desulfobacter* strains with the ability to oxidize betaine via an initial reductive cleavage. During a search for such betaine-metabolizing *Desulfobacter* strains we indeed isolated a sulfate-reducing betaine utilizer but instead of TMA the main product was *N,N*-dimethylglycine (chapter 6). The dominant bacterium was a strain of the genus *Desulfobacterium*. The species of this genus possess a variant of the acetyl CoA/CO dehydrogenase pathway. In this type of betaine metabolism one of the methyl groups is oxidized to CO_2 and the reducing equivalents generated are used for the reduction of sulfate.