## SUMMARY

Many microorganisms are able to use the energy derived from the oxidation of $C_1$- compounds to synthesize cell material from these compounds via special metabolic pathways. In the past ten years the physiology and biochemistry of microorganisms able to grow on $C_1$- compounds has been investigated extensively. However, so far little attention has been paid to the regulation of the synthesis of enzymes specifically involved in the $C_1$- assimilation pathways. A few years ago, studies on this regulatory problem were initiated in our laboratory. As a first approach, the regulation of the enzymes of the Calvin (RuBP) cycle was investigated. This pathway is present in photosynthetic organisms and in bacteria which are able to synthesize cell material via assimilation of $CO_2$, energized by the oxidation of inorganic compounds, like thiosulphate and hydrogen gas. This so-called autotrophic growth is also possible in a relatively small group of bacteria which energize this process by the oxidation of simple organic compounds, like methanol and formate. The bacterium *Pseudomonas oxalaticus* OX1 belongs to this group because the organism grows autotrophically on formate. When the organism is grown on a "heterotrophic" substrate (e.g. oxalate, glyoxylate and acetate) the enzymes of the Calvin cycle are absent and cell material is synthesized by the assimilation of these organic compounds. This bacterium is therefore a facultative autotroph and because, as indicated above, it presumably regulates the synthesis of Calvin cycle enzymes in an efficient manner, it was selected as a model organism for the present study (chapter 1). Since formate is an intermediate in the dissimilation of oxalate ($C_2$- compound), metabolic regulation during growth on oxalate and on mixtures of oxalate and formate was studied as well.

Oxalate and formate are both toxic for the organism and cause problems when used in concentrations required for growth in batch culture. In order to optimize the conditions of growth, a kinetic study of the inhibitory effects of these compounds was performed (chapter 2). Optimum growth was obtained at relatively low and constant concentrations of oxalate or formate in the culture when the rapid increase in the pH of the culture during growth on these substrates was counteracted by the controlled addition of oxalic acid and formic acid, respectively (extended culture technique). In this way maximum specific growth rates and high cell yields were obtained at concentrations of approximately 15 mM oxalate or 20 mM formate in the culture.

In addition to the toxic nature of oxalate and formate, growth on these substrates causes also special problems because of their highly oxidized state. Complete oxidation of oxalate and formate to $CO_2$ results in the production of only two electrons. To study this in more detail the energetics of growth of *Pseudomonas oxalaticus* on these substrates was investigated (chapter 3 and 4). Whereas the energy yield from the oxidation of oxalate and formate is the same, there is a large difference in the energy- and reducing power requirement for the synthesis of cell material during growth on these substrates. Energetically, autotrophic $CO_2$ fixation during growth on formate is a far more expensive process than heterotrophic carbon assimilation from oxalate. Therefore it was

very surprising to find that the molar growth yields of the organism on oxalate and formate were rather close. Further investigations showed that, when comparing the metabolism of oxalate and formate, the energy requirement for the translocation of these compounds from the medium into the cell is rather different. For oxalate transport, via an inducible active transport system, about twice as much energy is required as for formate transport, which occurs via passive diffusion. The results indicated that during growth on oxalate about 50% of the energy potentially available in the substrate is required for its transport. This difference in energy requirement for the transport of the substrates explains why the molar growth yields on the two substrates are similar.

The results of studies on the regulation of the synthesis of key enzymes in the specific carbon assimilation pathways during growth on mixtures of the "autotrophic" substrate formate and a "heterotrophic" substrate in batch- and in continuous culture, are presented in chapter 5, 6 and 7. In batch culture the synthesis of enzymes of the Calvin cycle was strongly repressed in the presence of the "heterotrophic" substrates acetate, glycollate and glyoxylate. Formate only functioned as an ancillary energy source in the metabolism of these substrates.

Although repression of autotrophic enzymes was also evident in carbon- and energy-limited continuous cultures, the degree of this repression was less strong and depended on the dilution rate and the ratio of the concentrations of the "heterotrophic" substrate and formate in the medium reservoir. Following the observation that the presence of formate does not always result in synthesis of Calvin cycle enzymes, whereas synthesis does occur at low dilution rates in an oxalate-limited continuous culture and in a mutant with a block in the carbon assimilation pathway from oxalate, it was concluded that synthesis of Calvin cycle enzymes is not induced by formate but regulated by a derepression/repression mechanism. The seemingly inductive effect exerted by formate on the synthesis of these autotrophic enzymes is therefore caused by its function as an energy source, presumably reducing the intracellular concentration of intermediates for biosynthesis of cell material, including the repressor molecule(s), which results in derepression.

The available evidence indicates that the enzymes specifically involved in the metabolism of oxalate are indeed induced by oxalate or a closely related metabolite. In the metabolism of oxalate oxalyl-CoA is situated at the branch point of the dissimilatory- and the assimilatory pathway. To regulate the flow of oxalate carbon over these two pathways in a balanced way the organism must possess some additional control mechanism. Evidence for the presence of such a control mechanism was obtained in experiments in which a culture of the organism was transferred from oxalate to formate. This transfer resulted in a 3-fold increase in the intracellular ratio of NADH/total NAD and a 1.5-fold increase in NADPH/total NADP ratio. Another indication for control over the flow of oxalate carbon was obtained in continuous culture experiments. When formate was added to the reservoir of an oxalate-limited continuous culture, the flow of oxalate carbon over the assimilatory pathway increased with the increasing concentration of formate in the feed. These results indicate that the distribu-

tion of oxalyl-CoA over dissimilation and assimilation is presumably regulated by the cell in a simple way, namely by the relative levels of reduced and oxidized pyridine nucleotides.

When *Pseudomonas oxalaticus* was grown in batch culture on mixtures of acetate and oxalate or formate and oxalate diauxic growth was observed with acetate and formate utilized first. The available evidence indicates that this is caused by effects of formate and acetate on the oxalyl-CoA cycle in the metabolism of oxalate (chapter 8). Simultaneous utilization of both substrates from these mixtures was observed in carbon- and energy source-limited continuous cultures over a range of dilution rates. Since under the latter conditions of growth the concentration of these substrates is low, compared to growth in batch culture, this indicates that in Nature, with low and limiting concentrations of carbon- and energy sources, simultaneous utilization of homologous substrates will be the predominant condition.

The present study established the involvement of a cytoplasmic membrane-bound formate dehydrogenase in the metabolism of both oxalate and formate. In studies with cytoplasmic membrane vesicles it was shown (chapter 9) that this enzyme feeds the electrons derived from formate oxidation into the respiratory chain at the level of cytochrome b. Since the affinity constant ($K_m$) for formate of the cytoplasmic NAD-dependent formate dehydrogenase and the membrane-bound enzyme are of the same order of magnitude, it has to be expected that both enzymes compete for formate inside the cell. Physiologically, the two important processes of generation of reducing power for biosynthesis and membrane energization for ATP synthesis and other energy requiring purposes may thus be accomplished by the two separate enzymes.