Summary

Assemblase ® is an industrially applied immobilized penicillin-G acylase. It catalyzes the synthesis of the semi-syntheticb-lactam antibiotic cephalexin (cex) from 7-amino deacetoxy cephalosporinic acid (7-adca) and (d-)(-)-phenylglycine amide (pga). In addition to the coupling reaction, also two unwanted side-reactions are catalyzed: the hydrolysis ofpgato the inactive by-product phenylglycine (pg) and the hydrolysis ofcexto 7-adcaandpg. Compared to a free-enzyme reaction,cexsynthesis with Assemblase ® is sub-optimal, both in terms of selectivity forcexsynthesis overpghydrolysis (decreased synthesis/hydrolysis ratio,s/h) and ofcexyield on both substrates.

In literature, an extensive body of data was available on Assemblase ® -catalyzed cephalexin synthesis. However, quantitative information on the Assemblase ® performance on the basis of the underlying physical and chemical mechanisms was scarce at best. Because the phenomena involved take place at various scales, it was decided to study them at an increasing level of detail (resolution), until an adequate description of the system would be obtained. First, an inventory was made of available techniques for visual inspection of the Assemblase ® interior, both with respect to intra-particle enzyme distribution and support matrix characteristics (Chapter 2).

Subsequently, the kinetic behaviour of Assemblase ® was analyzed by external, macroscopic means, i.e. without any intra-particle observations (Chapter 3). The size-dependent quantitative enzyme loading of Assemblase ® was determined. From macroscopic observations on mass-transfer limitedcex-synthesis experiments, a heterogeneous intra-particle enzyme distribution with most biocatalyst present in the outer 100mm of the particle was estimated.However, this could not provide a sufficientdescription of the particle behaviour in various reaction conditions.

Being an essential characteristic of Assemblase ® , the size-dependent quantitative intra-particle enzyme distribution was measured (Chapter 4). To this end, multiple particles of different sizes were each cut in multiple sections, which were immunochemically labelled for penicillin-G acylase presence. After enzyme detection with light microscopy (lm), image analysis showed that the intra-particle distributions resembled unsteady-state enzyme-diffusion profiles. These profiles were successfully expressed by a size-dependent Fourier number for (enzyme) mass transport.

The relation between heterogeneity in enzyme concentration and in the structure of the support material was studied at high resolution (Chapter 5). Globally, transmission electron microscopy (tem) confirmed the size-dependent enzyme gradient observed with light microscopy. Locally, however, abrupt deviations in enzyme concentration were observed at the particle surface (1.4-fold) and in areas (designated halos) that surrounded internal voids (7.7-fold). Cryogenic field-emission scanning electron microscopy (cryo-fesem) on cryo-planed surfaces related these local heterogeneities to local deviations in the matrix structure. Cryo-fesemadditionally indicated that the matrix was locally enriched in chitosan, which is one of the two matrix polymers of Assemblase ® . This was supported by a basic thermodynamic line of reasoning on polymer demixing during particle formation.

Although the resolution oftemand cryo-fesemanalysis is exemplary, these methods each study a single phenomenon and each requires its own sample preparation method. This inevitably complicates the interpretation of the results. Therefore, a technical feasibility study was set

up, to evaluate if ambient-temperaturefesemcould serve as an integral alternative to study the above-mentioned phenomena in a single sample with a single piece of equipment (Chapter 6). The results were benchmarked against previous results obtained withtemand cryo-fesem. Although integration of different measurements withfeseminvolved some concessions with respect to resolution, for the Assemblase ® system this integrated approach proved to be an attractive alternative to the two previously used methods.

From data gathered hitherto, a single, representative Assemblase ® particle was inferred, with a typical particle size and enzyme distribution. Combined with additional experiments on intra-particle reactant diffusivities, an integrated mechanistic model for Assemblase ® -catalyzedcexsynthesis was developed (Chapter 7). The model features, among other things, reactions in a heterogeneous enzyme system, electrostatically coupled diffusive mass transfer, and pH-dependent dissociation of reactants.

The model was successfully validated against synthesis experiments for conditions ranging from heavily diffusion-limited to hardly diffusion-limited, including substrate concentrations from 50 to 600 mM, temperatures between 273 and 303 K and pH's between 6 and 9. The pH gradients inside Assemblase ® duringcexsynthesis as reported by others were predicted correctly. The model provides physical insight in the complex interplay between the individual processes leading to the sub-optimal cephalexin synthesis with Assemblase ® , and may therefore give important clues for future biocatalyst design.

Although the integrated mechanistic model identifies the bottlenecks of the biocatalytic system, it does not determine the scientific area into which future improvements should be made. This depends on the outcome of an economic evaluation, which lies outside of the scope of this work. Therefore, in Chapter 8 an overview is given of promising developments in enzyme biochemistry, material science, reactor engineering and micro-technology that all can become relevant in future biocatalyst design.