effect of immobilization on enzyme activity

Enzyme immobilization, while providing major advantages for industrial use, significantly alters an enzyme's catalytic activity compared to its free counterpart. The effect on enzyme activity is complex and influenced by the specific immobilization technique, the support material, and the enzyme's properties.

Effects on enzyme kinetics

Immobilization changes an enzyme's kinetic parameters, specifically the maximum reaction rate (V_{max}) and the Michaelis constant (K_m).

- **Reduced reaction rate (Vmax)** : Immobilization can lead to a decrease in the overall reaction rate due to mass transfer limitations, where the rate of substrate diffusion to the enzyme's active site is slower than the catalytic rate.
- **Altered affinity (Km):** The apparent K_m` (the substrate concentration at which the reaction rate is half of V_{max} is often altered.
  - **Increased apparent K_m**: Diffusional limitations can cause the substrate concentration around the enzyme to be lower than in the bulk solution, leading to a higher apparent K_m.
  - **Decreased apparent K_m**: If the support material has a charge that attracts the substrate, the local substrate concentration near the enzyme may be higher than in the bulk solution, resulting in a lower apparent K_m.

Physical and chemical effects

The new microenvironment created by the immobilization matrix introduces several factors that can affect an enzyme's catalytic efficiency.

- **Conformational changes and denaturation**: The binding process, particularly through strong covalent bonds, can cause the enzyme to lose its native three-dimensional structure. If the active site is distorted or blocked, catalytic activity will be significantly reduced.
- **Mass transfer limitations**: The diffusion of substrate molecules to the immobilized enzyme and the diffusion of product molecules away from it can become limiting factors. This is most prominent with porous supports or when working with large substrates.
- **Microenvironmental changes**: The support material's chemical nature can alter the local environment of the enzyme.
  - **pH shift**: If the support is charged, it can cause the local pH around the enzyme to be different from the bulk solution, shifting the enzyme's optimal pH.

° **Partitioning effects**: The support's properties can cause the substrate or product to accumulate (partition) near the enzyme surface, affecting local concentrations and reaction rates.

- **Steric hindrance**: The immobilization matrix can create physical barriers that block the access of large substrate molecules to the enzyme's active site, especially with techniques like entrapment.

Overall consequences on activity

While often leading to a reduction in initial activity, these same effects can improve an enzyme's performance over the long term.

• **Enhanced stability:** Immobilization often confers greater stability against temperature, pH, and denaturing agents by restricting the enzyme's conformational flexibility. For some multimeric enzymes, immobilization can prevent the dissociation of subunits, thereby preserving their activity.

• **Improved reusability**: The enhanced stability allows the enzyme to be reused repeatedly, which is a major benefit for industrial processes.

• **Reduced activity loss from autolysis**: For proteolytic enzymes, immobilization can reduce the autodigestion that would normally occur in the free, soluble state.