6. OTHER EFFECTS ON ENZYME ACTIVITY

- Ionic strength
- pH
- TEMPERATURE
- Shear
- Pressure (hydrostatic)
- Surface tension
- Chemicals (alcohol, urea, H_2O_2...)
- Light, sonication, ionising radiations

Reverzible
changes
Irreverzible

Active side chains

Changes in activity of proteins are caused by changes of amino acid side chains.

Acidic: –COOH: Asp, Glu Basic: $-NH_2$: Lys, Arg
(and terminal –COOH and $-NH_2$)
amide: $-CO-NH_2$: Asn, Gln

Polar: –OH: Ser, Thr -SH: Cys, $-S-CH_3$: Met

Imidazole: His Guanidin: Arg

H-bonds: C=O …… H-O- C=O …… H-NH-

Effect of pH

Proteins: + and – charged side chains ← their charge depends on dissociation ← determined by pH → it effects the active centre.

Recharge of enzyme:

$$E \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} E^- \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} E^{2-}$$

Only E^- is active!

Ratio of active enzymes: $Y^- = E^- / E_0$

$$E \rightleftharpoons E^- + H^+$$
$$E^- \rightleftharpoons E^{2-} + H^+$$

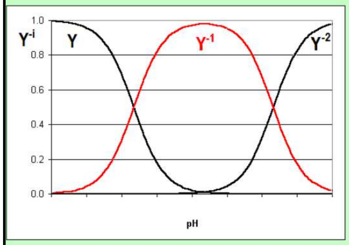
$$K_1 = \frac{H^+ \cdot E^-}{E}$$

$$K_2 = \frac{H^+ \cdot E^{2-}}{E^-}$$

$$E_0 = E + E^- + E^{2-}$$

Michaelis-féle pH függvények: $Y^- = \dfrac{1}{1 + H^+/K_1 + K_2/H^+}$

Effect of pH



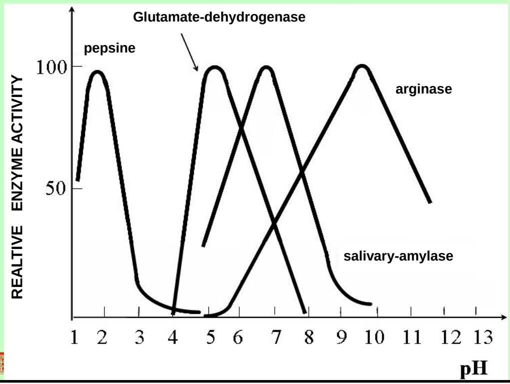
$$Y^{-} = \frac{1}{1 + H^{+}/K_1 + K_2/H^{+}}$$

$$H^{+}_{optimum} = \sqrt{K_1 K_2}$$

$$(pH)_{optimum} = \frac{1}{2}(pK_1 + pK_2)$$

$$V_{max} = k_2 E_0 Y^{-} = k_2 E_0 \frac{1}{1 + H^{+}/K_1 + K_2/H^{+}}$$

Effect of pH



Effect of pH temperature

Double effect
- Increasing reaction rate
- decrease: denaturation
  - irreversible
  - reversible

depends on treatment time, too!

$$\frac{dE_a}{dt} = -kE_a \longrightarrow E_a(t) = E_{a0} e^{-kt}$$

Effect of pH temperature

$$E_a \underset{}{\overset{K_d}{\rightleftarrows}} E_i \qquad \frac{E_i}{E_a} = K_d = \exp\left(\frac{-\Delta G_d}{RT}\right) = \exp\left(\frac{-\Delta H_d}{RT}\right)\exp\left(\frac{\Delta S_d}{R}\right)$$

S_d = ~900 KJ/mol.K

H_d = 280-310 KJ/mol

Large: sensitively reacts on small change (one H-bond: 12,5-29,3 kJ/mol)

If: $V_{max} = k_2(T) E_a$

$E_0 = E_a + E_i \rightarrow E_a = \frac{E_0}{1+K_d}$

and $k_2(T) = \beta\left(\frac{k_B T}{h}\right) e^{\Delta S^*/R} \cdot e^{-E/RT}$

$$V_{max} = \frac{\alpha T e^{-E/RT}}{1 + e^{\Delta S^*/R} \cdot e^{-\Delta H_d/RT}}$$

α = combination of ($\beta, k_B, h, E_0, \Delta S^*$)

K_m also depends on T!

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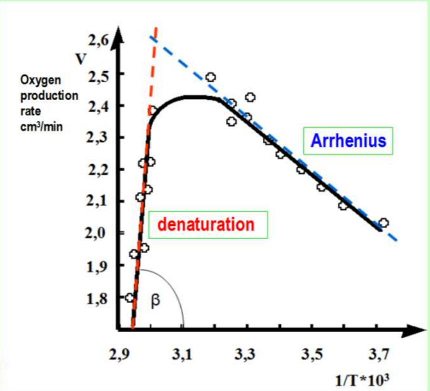
BME Alkalmazott Biotechnológia és Élelmiszertudomány Tanszék

Effect of pH temperature



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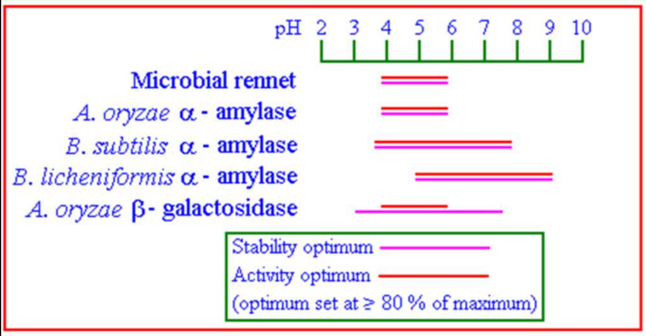
pH optimum and stability



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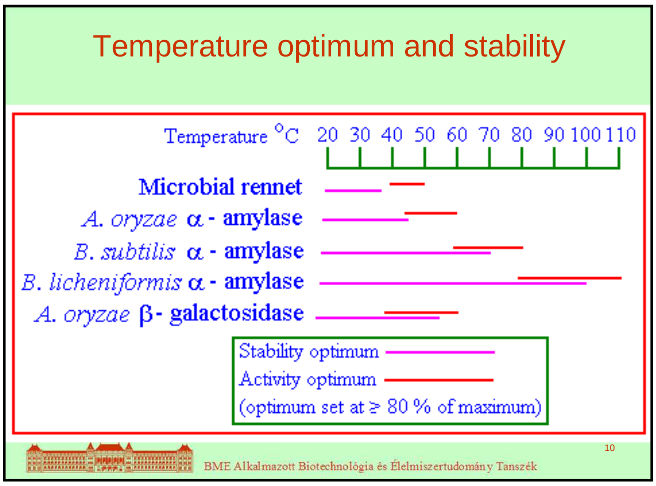
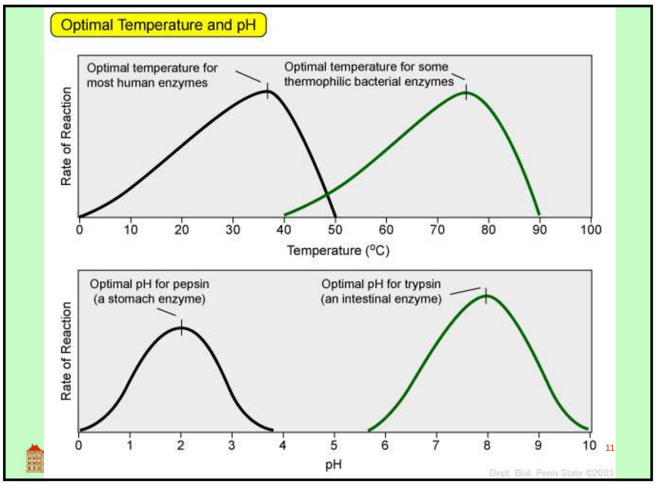
BME Alkalmazott Biotechnológia és Élelmiszertudomány Tanszék


Temperature optimum and stability
Temperature °C
20 30 40 50 60 70 80 90 100 110
Microbial rennet
A. oryzae α - amylase
B. subtilis α - amylase
B. licheniformis α - amylase
A. oryzae β- galactosidase
Stability optimum
Activity optimum
(optimum set at ≥ 80 % of maximum)
10
BME Alkalmazott Biotechnológia és Élelmiszertudomány Tanszék


Optimal Temperature and pH
Optimal temperature for most human enzymes
Optimal temperature for some thermophilic bacterial enzymes
Rate of Reaction
0 10 20 30 40 50 60 70 80 90 100
Temperature (°C)
Optimal pH for pepsin (a stomach enzyme)
Optimal pH for trypsin (an intestinal enzyme)
Rate of Reaction
0 1 2 3 4 5 6 7 8 9 10
pH
11