Elemental Balance -- Yeast growing on ethanol -- Given RQ=CER/OUR and rate of acid or base addition, find biomass yield from substrate Y_x.

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$$C_2H_6O + a\,O_2 + b\,NH_3 \longrightarrow c\,C_6H_{10}O_3N + d\,H_2O + e\,CO_2 + f\,C_2H_4O_2$$

(ethanol) (yeast) (acetic acid)

Both the uptake of NH_3 and the production of acetic acid make the fermentation broth acidic. Hence, we need to add a base solution to maintain the pH at a preset level.

$(NH_4)_2SO_4 \longrightarrow 2\,NH_3 + 2\,H^+ + SO_4^{2-}$ (H_2SO_4 is a strong acid; it is completely dissociated.)

$CH_3COOH \longrightarrow H^+ + CH_3COO^-$ (Acetic acid is a weak acid.)

(HAc) (Ac)

The reason acetic acid is considered a weak acid is that, in an aqueous solution containing only acetic acid, most of the acetic acid is present in the undissociated form HAc. We will demonstrate this numerically here.

Given

1. Dissociation constant for acetic acid: $\frac{H\cdot Ac}{HAc} = K_a$

2. Conservation of acetate species: $HAc_0 = HAc + Ac$

3. Contribution of H^+ from dissociation of HAc to Ac^- and H^+: $H = H_0 + Ac$
   where pH_0 is the pH of the original solution.

$$\mathrm{Find}(H, Ac, HAc) \rightarrow \begin{bmatrix} \frac{1}{2}\cdot H_0 - \frac{1}{2}\cdot K_a + \frac{1}{2}\cdot\sqrt{H_0^2 + 2\cdot H_0\cdot K_a + K_a^2 + 4\cdot K_a\cdot HAc_0} & \frac{1}{2}\cdot H_0 - \frac{1}{2}\cdot K_a - \frac{1}{2}\cdot\sqrt{H \ldots} \\ \frac{-1}{2}\cdot H_0 - \frac{1}{2}\cdot K_a + \frac{1}{2}\cdot\sqrt{H_0^2 + 2\cdot H_0\cdot K_a + K_a^2 + 4\cdot K_a\cdot HAc_0} & \frac{-1}{2}\cdot H_0 - \frac{1}{2}\cdot K_a - \frac{1}{2}\cdot\sqrt{H \ldots} \\ HAc_0 + \frac{1}{2}\cdot H_0 + \frac{1}{2}\cdot K_a - \frac{1}{2}\cdot\sqrt{H_0^2 + 2\cdot H_0\cdot K_a + K_a^2 + 4\cdot K_a\cdot HAc_0} & HAc_0 + \frac{1}{2}\cdot H_0 + \frac{1}{2}\cdot K_a + \frac{1}{2} \ldots \end{bmatrix}$$

For acetic acid: $K_a := 1.85\cdot 10^{-5}$

Conc. of Ac^-: $Ac(H_0, HAc_0) := \frac{-1}{2}\cdot H_0 - \frac{1}{2}\cdot K_a + \frac{1}{2}\cdot\sqrt{H_0^2 + 2\cdot H_0\cdot K_a + K_a^2 + 4\cdot K_a\cdot HAc_0}$

Conc. of HAc: $HAc(H_0, HAc_0) := HAc_0 - Ac(H_0, HAc_0)$

pH of an acetic acid solution: $H(H_0, HAc_0) := H_0 + Ac(H_0, HAc_0)$

Example: For a 1M acetic acid solution, the pH is: $-\log\left(H\left(10^{-7}, 1\right)\right) = 2.367$

On the other hand, when pH is controlled or known, we can find the fraction that is dissociated at a given pH with the following equation.

Fraction that is dissociated: $$f_{Ac} = \frac{Ac}{HAc_0} = \frac{Ac}{HAc + Ac} = \frac{\frac{Ac}{HAc}}{1 + \frac{Ac}{HAc}} = \frac{\frac{K_a}{H}}{1 + \frac{K_a}{H}} = \frac{K_a}{H + K_a}$$

$$f_{Ac}(pH) := \frac{K_a}{10^{-pH} + K_a}$$

Examples: $pH := 7$ $f_{Ac}(pH) = 0.995$ ← Almost all of acetic acid is in the dissociated acetate form, though acetic acid is generally considered a weak acid.

$pH := 5$ $f_{Ac}(pH) = 0.649$ ← Some of acetic acid is in the acetate form.

$pH := -\log(K_a)$ $f_{Ac}(pH) = 0.5$ ← At pH=pK_a, half of acetic acid is in the acetate form.

Thus, to maintain the pH at a given constant value, we need to add OH^- at the same rate H^+ is generated from ammonia uptake and dissociation of acetic acid that is produced by the yeast cells.

$Q = (b + f \cdot f_{Ac}) \cdot rate$ ... rate of base addition ("+" for base addition, "-" for acid addition.)

We divide Q by OUR to turn the rate of base addition, which is an extensive variable, into an intensive variable suitable for calculating the stoichiometric coefficients.

$$q = \frac{Q}{OUR} = \frac{b + f \cdot f_{Ac}}{a}$$

Elemental Balance Given

C: $2 = 6 \cdot c + e + 2 \cdot f$

H: $6 + 3 \cdot b = 10 \cdot c + 2 \cdot d + 4 \cdot f$

N: $b = c$

O: $1 + 2 \cdot a = 3 \cdot c + d + 2 \cdot e + 2 \cdot f$

Note: "e" means exponential in Mathcad. "ε" works in Mathcad v5, but is strictly treated as an antisymmetric tensor function and does not work in Mathcad v7. Here we add a period (which is invisible) to fool Mathcad.

Measurements: $RQ = \frac{e}{a}$ ... respiratory quotient

$$q = \frac{b + f \cdot f_{Ac}}{a}$$

We have six equations and we can solve for six stoichiometric coefficients (a, b, c, d, e, f). Find the analytical solution symbolically via |Math|SmartMath|.

$$\text{Find}(a,b,c,d,e,f) \rightarrow \begin{bmatrix} -2\cdot\dfrac{(11\cdot f_{Ac}-4)}{(2\cdot q+8-8\cdot RQ-24\cdot f_{Ac}+25\cdot f_{Ac}\cdot RQ)} \\ 4\cdot\dfrac{(2\cdot q-2\cdot f_{Ac}+3\cdot f_{Ac}\cdot RQ)}{(2\cdot q+8-8\cdot RQ-24\cdot f_{Ac}+25\cdot f_{Ac}\cdot RQ)} \\ 4\cdot\dfrac{(2\cdot q-2\cdot f_{Ac}+3\cdot f_{Ac}\cdot RQ)}{(2\cdot q+8-8\cdot RQ-24\cdot f_{Ac}+25\cdot f_{Ac}\cdot RQ)} \\ \dfrac{(22\cdot q+8-44\cdot f_{Ac}+33\cdot f_{Ac}\cdot RQ)}{(2\cdot q+8-8\cdot RQ-24\cdot f_{Ac}+25\cdot f_{Ac}\cdot RQ)} \\ -2\cdot RQ\cdot\dfrac{(11\cdot f_{Ac}-4)}{(2\cdot q+8-8\cdot RQ-24\cdot f_{Ac}+25\cdot f_{Ac}\cdot RQ)} \\ -2\cdot\dfrac{(11\cdot q-4+6\cdot RQ)}{(2\cdot q+8-8\cdot RQ-24\cdot f_{Ac}+25\cdot f_{Ac}\cdot RQ)} \end{bmatrix}$$

Coefficients at: $pH := 7$ $\quad f_{Ac} := f_{Ac}(pH)$ $\quad f_{Ac} = 0.995$

$$\text{coeff}(RQ,q) := \begin{bmatrix} -2\cdot\dfrac{(11\cdot f_{Ac}-4)}{(2\cdot q+8-8\cdot RQ-24\cdot f_{Ac}+25\cdot f_{Ac}\cdot RQ)} \\ 4\cdot\dfrac{(2\cdot q-2\cdot f_{Ac}+3\cdot f_{Ac}\cdot RQ)}{(2\cdot q+8-8\cdot RQ-24\cdot f_{Ac}+25\cdot f_{Ac}\cdot RQ)} \\ 4\cdot\dfrac{(2\cdot q-2\cdot f_{Ac}+3\cdot f_{Ac}\cdot RQ)}{(2\cdot q+8-8\cdot RQ-24\cdot f_{Ac}+25\cdot f_{Ac}\cdot RQ)} \\ \dfrac{(22\cdot q+8-44\cdot f_{Ac}+33\cdot f_{Ac}\cdot RQ)}{(2\cdot q+8-8\cdot RQ-24\cdot f_{Ac}+25\cdot f_{Ac}\cdot RQ)} \\ -2\cdot RQ\cdot\dfrac{(11\cdot f_{Ac}-4)}{(2\cdot q+8-8\cdot RQ-24\cdot f_{Ac}+25\cdot f_{Ac}\cdot RQ)} \\ -2\cdot\dfrac{(11\cdot q-4+6\cdot RQ)}{(2\cdot q+8-8\cdot RQ-24\cdot f_{Ac}+25\cdot f_{Ac}\cdot RQ)} \end{bmatrix}$$

Example:

$$\text{coeff}(0.55, 0) = \begin{bmatrix} 2.105 \\ 0.211 \\ 0.211 \\ 2.686 \\ 1.158 \\ -0.212 \end{bmatrix}$$

... both ethanol and acetic acid are consumed as substrate for growth.

$$\text{coeff}(0.5, 0.1) = \begin{bmatrix} 1.918 \\ 0.164 \\ 0.164 \\ 2.37 \\ 0.959 \\ 0.028 \end{bmatrix}$$

... ethanol is consumed, and acetic acid is produced.

Molecular Weight

$MW_{ethanol} := 46 \qquad MW_{yeast} := 144$

Each coefficient is now described in terms of RQ, Q, and OUR.

$$c(RQ, q) := \frac{4 \cdot (2 \cdot q - 2 \cdot f_{Ac} + 3 \cdot f_{Ac} \cdot RQ)}{2 \cdot q + 8 - 8 \cdot RQ - 24 \cdot f_{Ac} + 25 \cdot f_{Ac} \cdot RQ}$$

$$Y_x(RQ, q) := \frac{c(RQ, q) \cdot MW_{yeast}}{MW_{ethanol}}$$

The denominator of c cannot be zero. $2 \cdot q + 8 - 8 \cdot RQ - 24 \cdot f_{Ac} + 25 \cdot f_{Ac} \cdot RQ < 0$

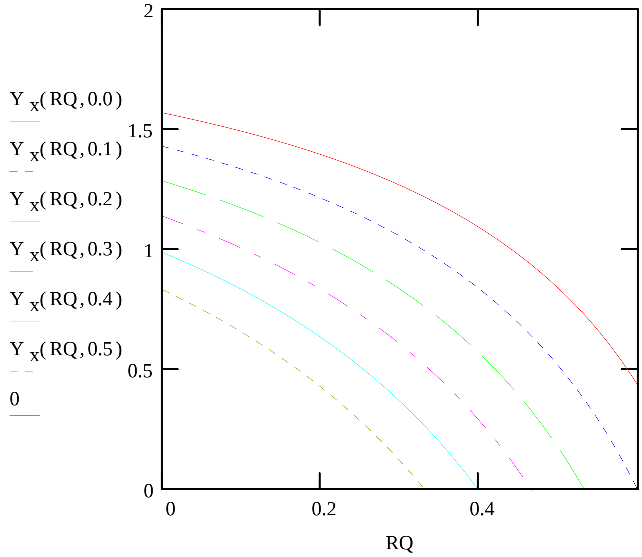
which leads to the restriction: $q < -\left(\frac{25}{2} \cdot f_{Ac} - 4\right) \cdot RQ + 12 \cdot f_{Ac} - 4$ or $RQ < 2 \cdot \frac{-q - 4 + 12 \cdot f_{Ac}}{-8 + 25 \cdot f_{Ac}}$

The coefficient c should be positive. $2 \cdot q - 2 \cdot f_{Ac} + 3 \cdot f_{Ac} \cdot RQ < 0$

which leads to the more restrictive restriction: $q < f_{Ac} - \frac{3}{2} \cdot f_{Ac} \cdot RQ$ or $RQ < \frac{2 \cdot f_{Ac} - 2 \cdot q}{3 \cdot f_{Ac}}$

Example: when $q := 0.1$ The upper limit of RQ is $\frac{2 \cdot f_{Ac} - 2 \cdot q}{3 \cdot f_{Ac}} = 0.6$

Y_x as a function of RQ for $RQ := 0, 0.01 .. 0.6$

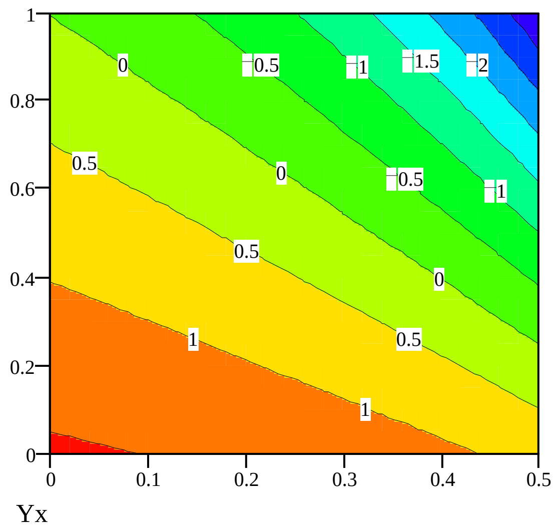


In general, for a given value of q, a high value of RQ means less cell yield; this is because more C in the substrate (ethanol) is diverted to CO_2. Likewise, for a given value of RQ, a high value of q means less cell yield as more C is diverted to acetic acid.

Contour Plot

$i := 0 .. 25 \qquad RQ_i := 0.02 \cdot i$

$j := 0 .. 20 \qquad q_j := 0.05 \cdot j \qquad Yx_{i,j} := Y_x(RQ_i, q_j)$



⇑ Increasing q; ⇒ increasing RQ

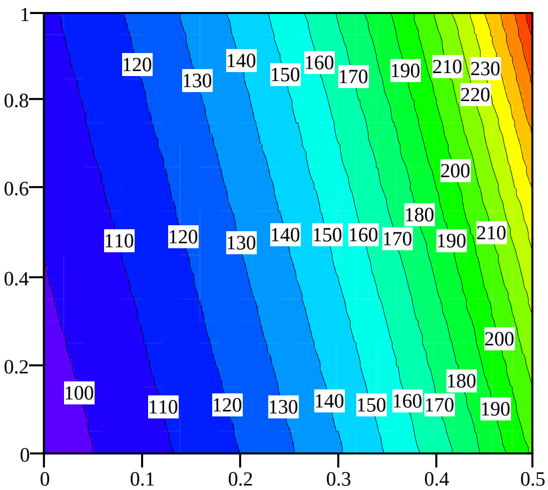
Oxygen utilization rate (mole O_2 per mole of ethanol consumed) at pH=7.

$$a(RQ,q) := \frac{-2\cdot\left(11\cdot f_{Ac} - 4\right)}{2\cdot q + 8 - 8\cdot RQ - 24\cdot f_{Ac} + 25\cdot f_{Ac}\cdot RQ}$$

Assume the heat of reaction to be $Q_{oxygen} := 27$ kcal/mole oxygen-released electron.

Heat evolution rate (kcal per mole of ethanol consumed).

$$Q_{heat}(RQ,q) := 4\cdot a(RQ,q)\cdot Q_{oxygen} \qquad Q_{Heat_{i,j}} := Q_{heat}\left(RQ_i, q_j\right)$$



Q_{Heat}

⇑ Increasing q; ⇒ increasing RQ

$$\sqrt{H_0^2 + 2\cdot H_0\cdot K_a + K_a^2 + 4\cdot K_a\cdot HAc_0}$$

$$\sqrt{H_0^2 + 2\cdot H_0\cdot K_a + K_a^2 + 4\cdot K_a\cdot HAc_0}$$

$$\cdot\sqrt{H_0^2 + 2\cdot H_0\cdot K_a + K_a^2 + 4\cdot K_a\cdot HAc_0}$$