SECTION II: KINETICS AND BIOREACTOR DESIGN:

LESSON 9.- Examples and exercises

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Example #1:

Experimental data allowed us to know that a certain microorganism is able to convert two thirds (w/w) of the carbon present in the substrate (hexadecane or glucose) to biomass. Calculate:

a) The stoichiometric coefficients for the bioprocesses:

$$C_{16}H_{34} + aO_2 + bNH_3 \xrightarrow{cells} cC_{4,4}H_{7,3}N_{0,86}O_{1,2} + dCO_2 + eH_2O$$

$$C_6H_{12}O_6 + aO_2 + bNH_3 \xrightarrow{cells} cC_{4,4}H_{7,3}N_{0,86}O_{1,2} + dCO_2 + eH_2O$$

b) Yields $Y_{X/S}$, $Y_{X/O2}$ for both reactions.

USEFUL INFORMATION:

Mw(glucose) = 180,1 g/mole; Mw(hexadecane) = 226 g/mole

Am(H) = 1 amu; Am(C) = 12 amu; Am(O) = 16 amu; Am(N) = 14 amu;

Example #1:

a) Stoichiometric coefficients obtaining using hexadecane.

$$C_{16}H_{34} + aO_2 + bNH_3 \xrightarrow{cells} cC_{4,4}H_{7,3}N_{0,86}O_{1,2} + dCO_2 + eH_2O$$

Carbon balance: 16 = 4,4·c + d

Oxygen balance: 2·a = 1,2·c + 2·d+e

Hydrogen balance: 34+3·b = 7,3·c + 2·e

Nitrogen Balance: b = 0,86·c

2/3 C in hexadecane are transformed into biomass:

(2/3)·16=4,4·c ➔ 10,67=4,4·c ➔ **c=2,42**

Example #1:

a) Stoichiometric coefficients obtaining using hexadecane.

Carbon balance: $16 = 4{,}4 \cdot c + d$

Oxygen balance: $2 \cdot a = 1{,}2 \cdot c + 2 \cdot d + e$

Hydrogen balance : $34 + 3 \cdot b = 7{,}3 \cdot c + 2 \cdot e$

Nitrogen Balance : $b = 0{,}86 \cdot c$

$$\left\{\begin{array}{rcl} 4{,}4 \cdot c + d & = & 16 \\ 2 \cdot a - 1{,}2 \cdot c - 2 \cdot d - e & = & 0 \\ 3b - 7{,}3 \cdot c - 2 \cdot e & = & -34 \\ b - 0{,}86 \cdot c & = & 0 \\ c & = & 2{,}42 \end{array}\right.$$

2/3 C in hexadecane are transformed into biomass:

$(2/3) \cdot 16 = 4{,}4 \cdot c$ ➔ $10{,}67 = 4{,}4 \cdot c$ ➔ **$c = 2{,}42$**

Example #1:

a) Stoichiometric coefficients obtaining using hexadecane.

$$\begin{cases} 4{,}4\cdot c + d = 16 \\ 2\cdot a - 1{,}2\cdot c - 2\cdot d - e = 0 \\ 3b - 7{,}3\cdot c - 2\cdot e = -34 \\ b - 0{,}86\cdot c = 0 \\ c = 2{,}424 \end{cases}$$

d = 5,333

a = 12,428

e = 11,279

b = 2,085

$$C_{16}H_{34} + 12{,}43\cdot O_2 + 2{,}085\cdot NH_3 \xrightarrow{\text{células}} 2{,}424\cdot C_{4,4}H_{7,3}N_{0,86}O_{1,2} + 5{,}33\cdot CO_2 + 11{,}279\cdot H_2O$$

Example #1:

a) Stoichiometric coefficients obtaining using glucose.

$$C_6H_{12}O_6 + aO_2 + bNH_3 \xrightarrow{cells} cC_{4,4}H_{7,3}N_{0,86}O_{1,2} + dCO_2 + eH_2O$$

Carbon balance: 6 = 4,4·c + d

Oxygen balance: 6 + 2·a = 1,2·c + 2·d+e

Hydrogen balance : 12+3·b = 7,3·c + 2·e

Nitrogen balance: b = 0,86·c

2/3 C in glucose are transformed into biomass:

(2/3)·6=4,4·c ➔ 4=4,4·c ➔ **c=0,909**

Example #1:

a) Stoichiometric coefficients obtaining using glucose.

Carbon balance: $6 = 4{,}4 \cdot c + d$

Oxygen balance: $6 + 2 \cdot a = 1{,}2 \cdot c + 2 \cdot d + e$

Hydrogen balance: $12 + 3 \cdot b = 7{,}3 \cdot c + 2 \cdot e$

Nitrogen balance: $b = 0{,}86 \cdot c$

$$\left\{\begin{array}{lll} & 4{,}4 \cdot c + d & = 6 \\ 2 \cdot a & -1{,}2 \cdot c - 2 \cdot d - e & = -6 \\ & 3 \cdot b - 7{,}3 \cdot c - 2 \cdot e & = -12 \\ & b - 0{,}86 \cdot c & = 0 \\ & c & = 0{,}909 \end{array}\right.$$

2/3 C in glucose are transformed into biomass:

$(2/3) \cdot 6 = 4{,}4 \cdot c \rightarrow 4 = 4{,}4 \cdot c \rightarrow$ **$c = 0{,}909$**

Example #1:

a) Stoichiometric coefficients obtaining using glucose.

$$\begin{cases} 4{,}4\cdot c + d = 6 \\ 2\cdot a - 1{,}2\cdot c - 2\cdot d - e = -6 \\ 3\cdot b - 7{,}3\cdot c - 2\cdot e = -12 \\ b - 0{,}86\cdot c = 0 \\ c = 0{,}909 \end{cases}$$

d = 2,000

a = 1.473

e = 3,855

b = 0,782

$$C_6H_{12}O_6 + 1{,}473\cdot O_2 + 0{,}782\cdot NH_3 \xrightarrow{cells} 0{,}909\cdot C_{4,4}H_{7,3}N_{0,86}O_{1,2} + 2\cdot CO_2 + 3{,}855\cdot H_2O$$

Example #1:

b) Yields $Y_{X/S}$, $Y_{X/O2}$ using hexadecane.

$$C_{16}H_{34} + 12{,}43 \cdot O_2 + 2{,}085 \cdot NH_3 \xrightarrow{cells} 2{,}424 \cdot C_{4,4}H_{7,3}N_{0,86}O_{1,2} + 5{,}33 \cdot CO_2 + 11{,}279 \cdot H_2O$$

$$Y_{X/S} = \frac{c \cdot Mm_{biomass}}{Mm_{hexadecane}} = \frac{(2{,}424) \cdot (91{,}34\ g/mole)}{(226\ g/mole)} = 0{,}98 \frac{g\ cells}{g\ substrate}$$

$$Y_{X/O2} = \frac{c \cdot Mm_{biomass}}{a \cdot Mm_{oxygen}} = \frac{(2{,}424) \cdot (91{,}34\ g/mole)}{(12{,}43) \cdot (32\ g/mole)} = 0{,}557 \frac{g\ cells}{g\ oxygen}$$

Example #1:

b) Yields $Y_{X/S}$, $Y_{X/O2}$ using glucose.

$$C_6H_{12}O_6 + 1{,}473 \cdot O_2 + 0{,}782 \cdot NH_3 \xrightarrow{cells} 0{,}909 \cdot C_{4,4}H_{7,3}N_{0,86}O_{1,2} + 2 \cdot CO_2 + 3{,}855 \cdot H_2O$$

$$Y_{X/S} = \frac{c \cdot Mm_{biomass}}{Mm_{glucose}} = \frac{(0{,}909) \cdot (91{,}34\ g/mole)}{(180 g/mole)} = 0{,}462 \frac{g\ cells}{g\ substrate}$$

$$Y_{X/O2} = \frac{c \cdot Mm_{biomass}}{a \cdot Mm_{oxygen}} = \frac{(0{,}909) \cdot (91{,}34\ g/mole)}{(1{,}473) \cdot (32\ g/mole)} = 1{,}761 \frac{g\ cells}{g\ oxygen}$$

Example #2:

Calulate stoichiometric coefficients for the expression describing aerobic growth of *Saccharomyces cerevisiae* using glucose as limiting nutrient.

USEFUL INFORMATION:

Respiratory Exchange Ratio RER=1,033 mole CO_2/mole O_2.

Average molecular formula $CH_{1,703}N_{0,171}O_{0,459}$

Example #2:

$$C_6H_{12}O_6 + aO_2 + bNH_3 \xrightarrow{cells} cCH_{1,703}N_{0,171}O_{0,459} + dCO_2 + eH_2O$$

Balance de C: 6 = c + d ➔ $c + d = 6$

Balance de O: 6 + 2·a = 0,459·c + 2·d+e ➔ $2\cdot a - 0{,}459\cdot c - 2\cdot d - e = -6$

Balance de H: 12+3·b = 1,703·c + 2·e ➔ $3\cdot b - 1{,}703\cdot c - 2\cdot e = -12$

Balance de N: b = 0,171·c ➔ $b - 0{,}171\cdot c = 0$

RER = 1,033: 1,033 = d/a ➔ $1{,}033\cdot a - d = 0$

Example #2:

$$C_6H_{12}O_6 + aO_2 + bNH_3 \xrightarrow{cells} cCH_{1,703}N_{0,171}O_{0,459} + dCO_2 + eH_2O$$

$$\left.\begin{array}{l} c + d = 6 \\ 2\cdot a - 0{,}459\cdot c - 2\cdot d - e = -6 \\ 3\cdot b - 1{,}703\cdot c - 2\cdot e = -12 \\ b - 0{,}171\cdot c = 0 \\ 1{,}033\cdot a - d = 0 \end{array}\right\} \Rightarrow \left\{\begin{array}{l} a = 3{,}951 \\ b = 0{,}328 \\ c = 1{,}918 \\ d = 4{,}082 \\ e = 4{,}859 \end{array}\right.$$

$$C_6H_{12}O_6 + 3{,}95\cdot O_2 + 0{,}328\cdot NH_3 \xrightarrow{cells} 1{,}92\cdot CH_{1,703}N_{0,171}O_{0,459} + 4{,}08\cdot CO_2 + 4{,}86\cdot H_2O$$

Example #3:

The growth rate in the presence of excess carbon substrate of a *Stepinpooni* bacterium can be described by the following equation:

$$R_X(g/L.h) = 0{,}5 \cdot C_X \cdot \left(1 - \frac{C_X}{20}\right)$$

Plot the growth rate and cell concentration as a function of time if 0.4 g of cells are inoculated into a 2 L work volume reactor.

Identifie the values of the parameters of the previous equation.

Example #3:

We are considering **logistic equation in its differential form:**

$$C_X = \frac{C_{X0} \cdot \exp(\mu.t)}{1 - \frac{C_{X0}}{C_{Xm}} \cdot (1 - \exp(\mu.t))}$$

In order to identify each parameter:

$$R_X(g/L.h) = \mu \cdot C_X \cdot \left(1 - \frac{C_X}{C_{Xm}}\right)$$

$\mu = 0{,}5 \quad h^{-1}$ $\qquad$ $C_{Xm} = 20 \quad g/L$

According to data within the wording:

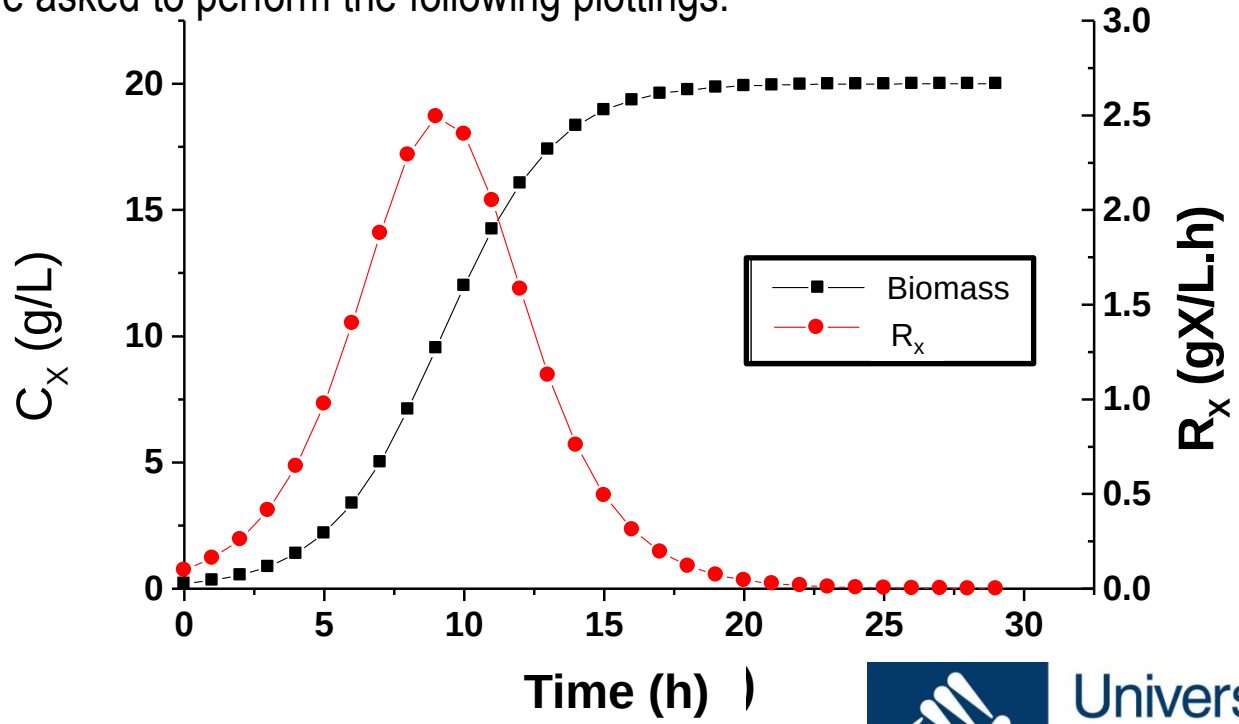
$$C_{X0} = \frac{0{,}4g}{2L} = 0{,}2 \quad g/L$$

Example #3:

Thus:

$$C_X = \frac{0{,}2 \cdot \exp(0{,}5.t)}{1 - \frac{0{,}2}{20} \cdot (1 - \exp(0{,}5.t))}$$

We are asked to perform the following plottings:



Example #4:

A battery of seven experiments has been carried out to study the influence of a limiting substrate, **S**, on the growth of a given microorganism.

Using the following information, obtained at short reaction times, work out the equation and the parameters relating the specific growth rate (**μ**) to the average substrate concentration (**S**) .

t(h)	0,54	0,36	0,33	0,35	0,37	0,38	0,37
[S] (g/L)	137,0	114,0	90,0	43,0	29,0	9,0	2,0
[X]o (g/L)	15,5	23,0	30,0	38,5	48,5	58,3	61,3
[X]t (g/L)	23,0	30,0	38,5	48,5	58,3	61,3	62,5

Example #4:

$$R_X = \frac{\mu_m \cdot [S]}{k_S + [S]} \cdot [X] = \mu \cdot [X]$$

$$\mu = \frac{\mu_m \cdot [S]}{k_S + [S]}; \mu = \frac{R_X}{[X]} = \frac{1}{[X]} \cdot \frac{d[X]}{dt}$$

Different linearization can be considered:

$\Delta[X] = [X]_t - [X]_0$; $[X]_{average} = (X_t + X_0)/2$; $[S]_{average}$ = experimental piece of data

$$\mu \approx \frac{1}{[X]} \cdot \frac{\Delta[X]}{\Delta t} = \frac{1}{[X]} \cdot \frac{\Delta[X]}{t}$$

Example #4:

t(h)	[S] (g/l)	[X]o (g/l)	[X]t (g/l)	Δ[X] (g/L)	$[X]_{aver.}$ (g/L)	$[S]_{aver.}$ (g/L)	r (g/(L·h))	$\mu(h^{-1})$
0,54	137	15,5	23	7,50	19,25	137	13,89	0,72
0,36	114	23	30	7,00	26,50	114	19,44	0,73
0,33	90	30	38,5	8,50	34,25	90	25,76	0,75
0,35	43	38,5	48,5	10,00	43,50	43	28,57	0,66
0,37	29	48,5	58,3	9,80	53,40	29	26,49	0,50
0,38	9	58,3	61,3	3,00	59,80	9	7,89	0,13
0,37	2	61,3	62,5	1,20	61,90	2	3,24	0,05

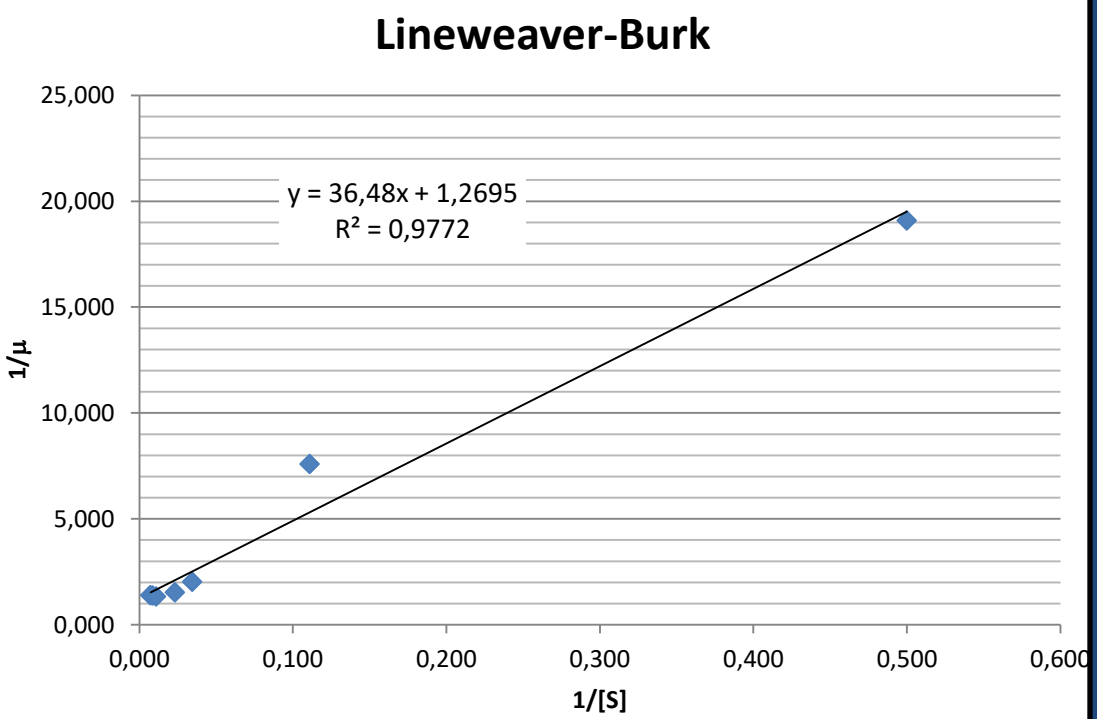
1. Lineweaver-Burk

$$\frac{1}{\mu} = \frac{k_S}{\mu_m} \cdot \frac{1}{[S]} + \frac{1}{\mu_m}$$

$$\mu_m = 0{,}876\ h^{-1}$$

$$K_S = 32{,}17\ g/L$$

$[S]_{aver}$ (g/L)	$\mu(h^{-1})$	$1/[S]_{aver}$ (L/g)	$1/\mu$ (h)
137	0,72	0,007	1,386
114	0,73	0,009	1,363
90	0,75	0,011	1,330
43	0,66	0,023	1,523
29	0,50	0,034	2,016
9	0,13	0,111	7,575
2	0,05	0,500	19,086



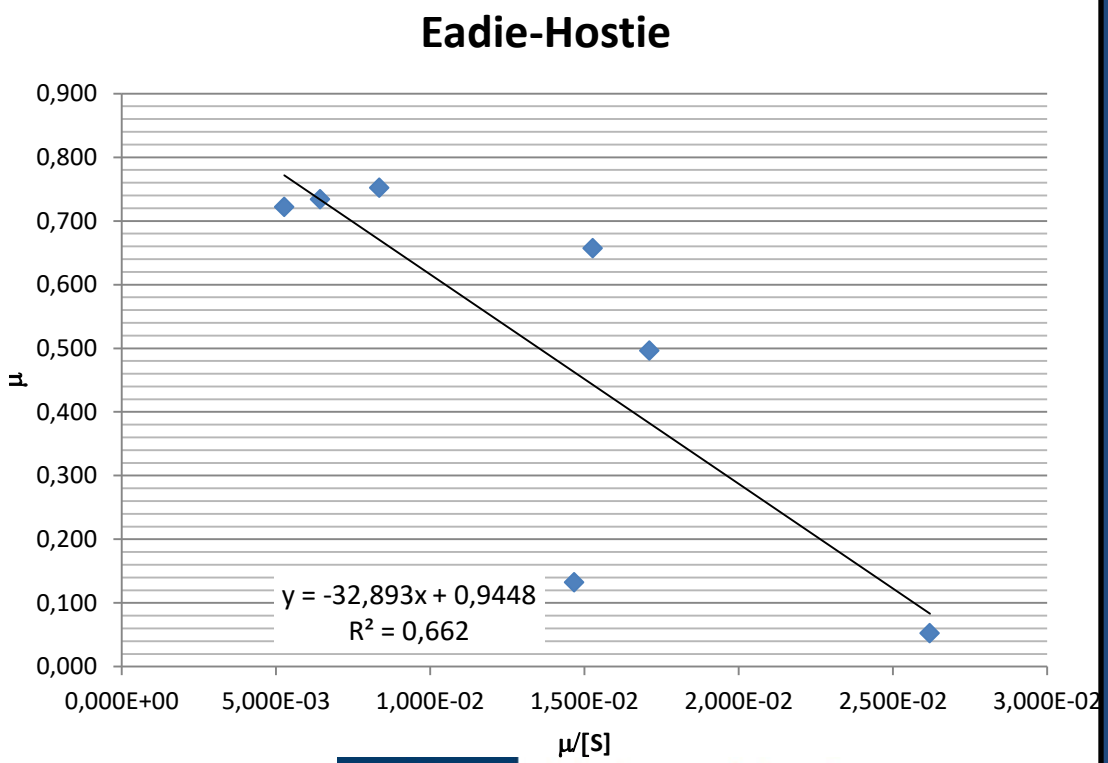
2. Eadie-Hostee

$$\mu = \mu_m - k_S \cdot \frac{\mu_m}{[S]}$$

$$\mu_m = 0{,}876 h^{-1}$$

$$K_S = 27{,}55 g/L$$

$[S]_{aver}$ (g/L)	$\mu(h^{-1})$	$\mu/[S]_{aver}$ (L/(g·h))	$\mu(h^{-1})$
137	0,72	5,266E-03	0,72
114	0,73	6,436E-03	0,73
90	0,75	8,356E-03	0,75
43	0,66	1,527E-02	0,66
29	0,50	1,710E-02	0,50
9	0,13	1,467E-02	0,13
2	0,05	2,620E-02	0,05



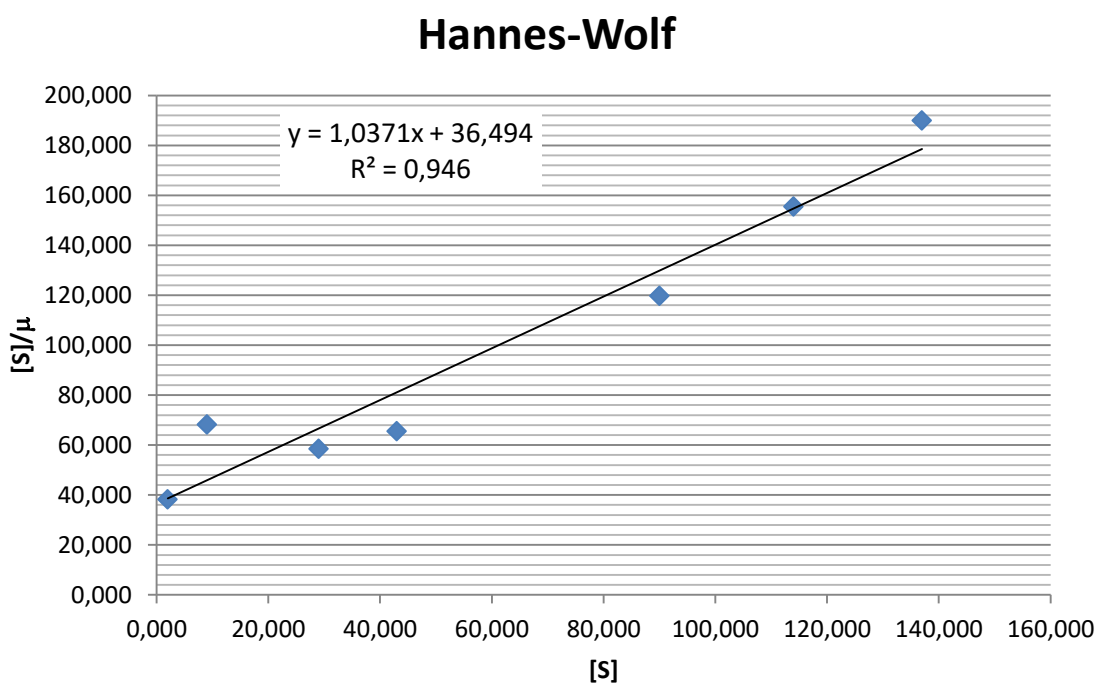
3. Hannes-Wolf

$$\frac{[S]}{\mu} = \frac{k_S}{\mu_m} + \frac{1}{\mu_m} \cdot [S]$$

$[S]_{aver}$ (g/L)	$\mu(h^{-1})$	$[S]_{aver}$ (g/L)	$[S]/\mu$ (g·h/(L))
137	0,72	137	189,882
114	0,73	114	155,366
90	0,75	90	119,674
43	0,66	43	65,468
29	0,50	29	58,468
9	0,13	9	68,172
2	0,05	2	38,172

$$\mu_m = 0{,}884\, h^{-1}$$

$$K_S = 28{,}87\, g/L$$



Example #5:

Using the following information, obtain the values for the kinetic parameters describing growth under limiting nutrient conditions.

t (h)	[X] (g/L)	[S] (g/L)
0	0,100	40,0
1	0,134	39,9
2	0,180	39,8
3	0,241	39,7
4	0,323	39,5
5	0,433	39,3
6	0,580	39,0

t (h)	[X] (g/L)	[S] (g/L)
7	0,777	38,5
8	1,041	38,0
9	1,393	37,2
10	1,863	36,2
11	2,488	34,8
12	3,315	32,9
13	4,402	30,5

t (h)	[X] (g/L)	[S] (g/L)
14	5,804	27,2
15	7,546	22,8
16	9,486	17,1
17	10,454	9,6
18	12,500	1,1
19	12,520	1,1
20	12,555	1,1

Example #5:

$$R_X = \frac{\mu_m \cdot [S]}{k_S + [S]} \cdot [X] = \mu \cdot [X]$$

$$\mu = \frac{\mu_m \cdot [S]}{k_S + [S]} = \frac{1}{[X]} \cdot \frac{d[X]}{dt}$$

Example #5:

$$\left.\begin{aligned}\Delta[X] &= [X]_n - [X]_{n-1} \\ \Delta t &= t_n - t_{n-1}\end{aligned}\right\} \quad \mu \approx \frac{1}{[X]} \cdot \frac{\Delta[X]}{\Delta t}$$

$$[X]_{average} = \frac{[X]_n + [X]_{n-1}}{2}$$

$$[S]_{average} = \frac{[S]_n + [S]_{n-1}}{2}$$

Example #5:

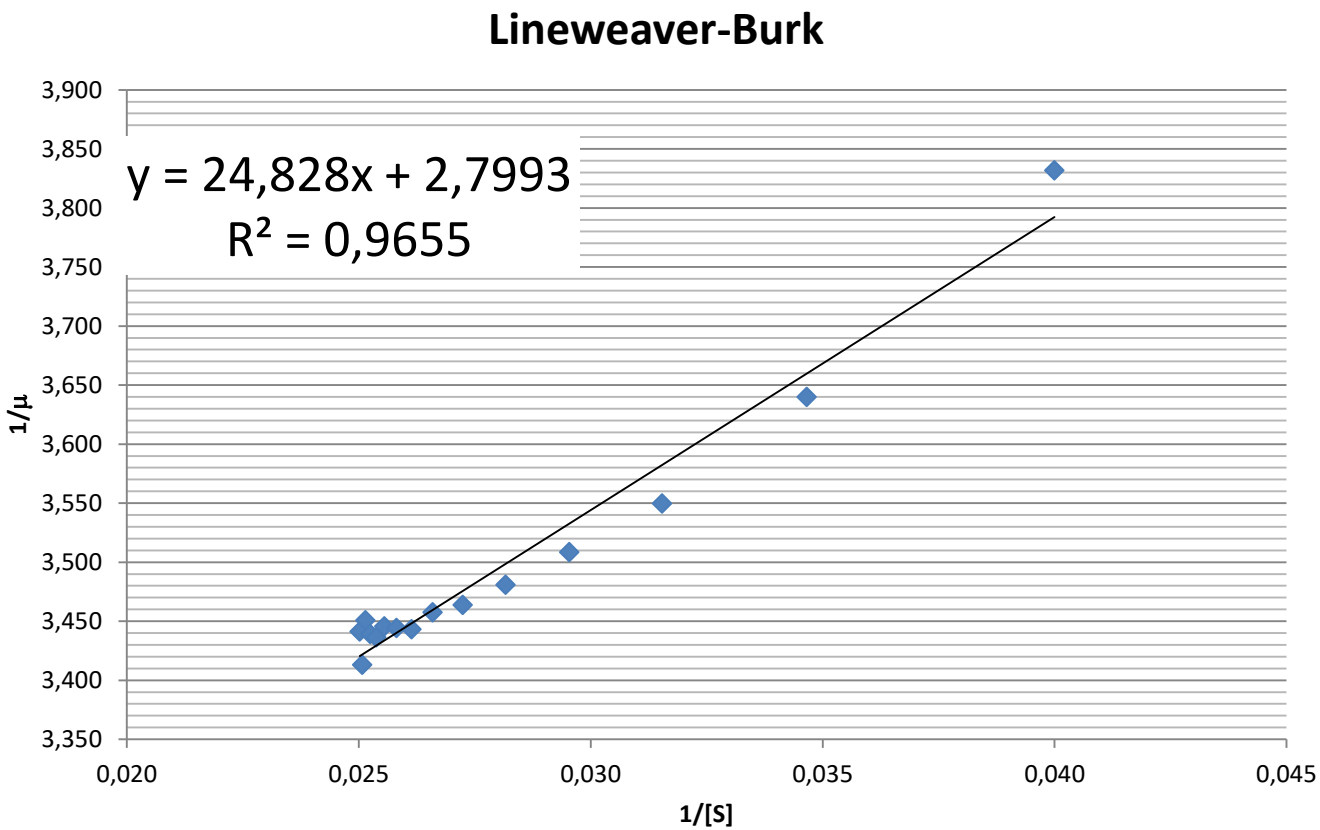
Experimental data were transformed using Lineweaver-Burk linerization:

$[S]_{aver}$ (g/L)	$\mu(h^{-1})$	$1/[S]_{aver}$ (L/g)	$1/\mu$ (h)	$[S]_{aver}$ (g/L)	$\mu(h^{-1})$	$1/[S]_{aver}$ (L/g)	$1/\mu$ (h)	$[S]_{aver}$ (g/L)	$\mu(h^{-1})$	$1/[S]_{aver}$ (L/g)	$1/\mu$ (h)
-	-	-	-	38,735	0,290	0,026	3,444	28,850	0,275	0,035	3,640
39,965	0,291	0,025	3,441	38,250	0,290	0,026	3,443	25,000	0,261	0,040	3,832
39,880	0,293	0,025	3,413	37,600	0,289	0,027	3,457	19,950	0,228	0,050	4,390
39,765	0,290	0,025	3,451	36,700	0,289	0,027	3,464	13,350	0,097	0,075	10,300
39,600	0,291	0,025	3,439	35,500	0,287	0,028	3,481	5,355	0,178	0,187	5,609
39,400	0,291	0,025	3,436	33,850	0,285	0,030	3,508	1,110	0,002	0,901	625,500
39,135	0,290	0,026	3,446	31,700	0,282	0,032	3,550	1,110	0,003	0,901	358,214

Example #5:

Ks = 8.87 g/L

μ^{max} = 0.357 h^{-1}



Example #6:

The growth of baker's yeast (*S. cerevisiae*) using glucose as a carbon source can be described according to the following equation:

$$C_6H_{12}O_6 + 3O_2 + 0.48NH_3 \xrightarrow{\text{células}} 0.48C_6H_{10}NO_3 + 4.32CO_2 + 3.12H_2O$$

In order to employ a batch reactor of 100,000 L so that a final biomass concentration of 50 dwg/L can be reached, answer the following questions:

Example #6:

$$C_6H_{12}O_6 + 3O_2 + 0.48NH_3 \xrightarrow{cells} 0.48C_6H_{10}NO_3 + 4.32CO_2 + 3.12H_2O$$

a) Calculate concentration and total amount for glucose and ammonium sulphate in the medium.

b) Calculate Y_{XS} and Y_{XO2}

c) Calculate the amount of oxygen required.

d) If r_X = 0.7 dwg /(L·h), calculate OUR (g O_2 /(L · h)

Example #6:

$$C_6H_{12}O_6 + 3O_2 + 0.48NH_3 \xrightarrow{cells} 0.48C_6H_{10}NO_3 + 4.32CO_2 + 3.12H_2O$$

a) Calculate concentration and total amount for glucose and ammonium sulphate in the medium.

$$V \cdot [X] = 10^5 L \cdot 50\ dwg / L = 5 \cdot 10^6\ dwg\ X$$

$$Mm(X) = 144\ g/mole$$

$$Mm(glu) = 180\ g/mole$$

$$Mm((NH_4)_2SO_4) = 132{,}1\ g/mole$$

Example #6:

$$C_6H_{12}O_6 + 3O_2 + 0.48NH_3 \xrightarrow{cells} 0.48C_6H_{10}NO_3 + 4.32CO_2 + 3.12H_2O$$

a) Calculate concentration and total amount for glucose and ammonium sulphate in the medium.

$$5 \cdot 10^6\ g\ X \cdot \frac{1\ mole\ X}{144\ g\ X} \cdot \frac{1\ mole\ Glu}{0.48\ mol\ X} \cdot 180\ g\ Glu = 13020833\ g\ Glu$$

$$[Glu] = \frac{13020833\ g\ Glu}{100000\ L} = 130.2\ g/L\ Glu$$

Example #6:

$$C_6H_{12}O_6 + 3O_2 + 0.48NH_3 \xrightarrow{cells} 0.48C_6H_{10}NO_3 + 4.32CO_2 + 3.12H_2O$$

a) Calculate concentration and total amount for glucose and ammonium sulphate in the medium.

$$5{\cdot}10^6\ g\ X \cdot \frac{1\,mol\ X}{144\,g\ X} \cdot \frac{1\,mole\ NH_3}{1\,mole\ X} \cdot \frac{1\,mole\ (NH_4)_2SO_4}{2\ mole\ NH_3} = 17361\,mole\ (NH_4)_2SO_4$$

$$17361\,mole\ (NH_4)_2SO_4 \cdot \frac{132{,}1\ g\ (NH_4)_2SO_4}{mole\ (NH_4)_2SO_4} = 2293402{,}8\ g\ (NH_4)_2SO_4$$

$$[(NH_4)_2SO_4] = \frac{2293402{,}8\ g\ (NH_4)_2SO_4}{100000\ L} = 22{,}9\ g/L\ (NH_4)_2SO_4$$

Example #6:

$$C_6H_{12}O_6 + 3O_2 + 0.48NH_3 \xrightarrow{cells} 0.48C_6H_{10}NO_3 + 4.32CO_2 + 3.12H_2O$$

b) Calculate Y_{XS} and Y_{XO2}

$$Y_{X/S} = \frac{c \cdot Mm_{biomass}}{Mm_{glucose}} = \frac{0.48 \cdot (144\ g/mole)}{(180\ g/mole)} = 0{,}384 \frac{g\ cells}{g\ substrate}$$

$$Y_{X/O2} = \frac{c \cdot Mm_{biomass}}{a \cdot Mm_{oxygen}} = \frac{(0.48) \cdot (144\ g/mole)}{(3) \cdot (32\ g/mole)} = 0{,}72 \frac{g\ cells}{g\ oxygen}$$

Example #6:

$$C_6H_{12}O_6 + 3O_2 + 0.48NH_3 \xrightarrow{cells} 0.48C_6H_{10}NO_3 + 4.32CO_2 + 3.12H_2O$$

c) Calculate the amount of oxygen required.

$$Y_{X/O2} = 0{,}72 \frac{g\ cells}{g\ oxygen}$$

$$O_2 = Biomass \cdot \frac{1}{Y_{X/O2}} = 5 \cdot 10^6\ gX \frac{1}{0{,}72 \frac{g\ cells}{g\ oxygen}} =$$

$$= 6944444\ g\ oxygen$$

Example #6:

$$C_6H_{12}O_6 + 3O_2 + 0.48NH_3 \xrightarrow{cells} 0.48C_6H_{10}NO_3 + 4.32CO_2 + 3.12H_2O$$

d) If r_X = 0.7 dwg /(L·h), calculate OUR (g O_2 /(L · h)

$$OUR = q_{O2} \cdot [X] = \frac{\mu_g}{Y_{X/O2}} \cdot [X] = \frac{r_X}{Y_{X/O2}}.$$

$$OUR = \frac{0.7 \dfrac{g\ X}{L \cdot h}}{0{,}72 \dfrac{g\ X}{g\ oxygen}} = 0.972 \frac{g\ O_2}{L \cdot h}$$

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