IAE ACADEMY INSTITUTE FOR ENGINEERS

GATE BT

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Plant, Animal and Microbial Biotechnology Formula Sheet

INSIDE

- 28 formulas in 5 topics
- When to use each one
- Taken from the free topic notes on iaeacademy.in

Online, one page per topic: **iaeacademy.in/study/plant-animal-microbial-biotechnology/formula-sheet**

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Plant tissue culture, regeneration and secondary metabolites

STOCK DILUTION

$$C_1 V_1 = C_2 V_2$$

V_1 is the volume of stock at concentration C_1 needed to make volume V_2 of medium at concentration C_2. Put both concentrations in one unit and both volumes in one unit before substituting (1 mg/mL = 1000 mg/L).

EXPONENTIAL GROWTH

$$X = X_0 \, e^{\mu t}$$

Biomass concentration X at time t during the exponential phase; X_0 is the biomass at the start of that phase and μ the specific growth rate.

SPECIFIC GROWTH RATE

$$\mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1}$$

Calculated from two points within the exponential phase; the unit is per time (for example per day).

DOUBLING TIME

$$t_d = \frac{\ln 2}{\mu} \approx \frac{0.693}{\mu}$$

Time for biomass to double in the exponential phase; t_d is in the same time unit as $1/\mu$.

NUMBER OF DOUBLINGS

$$n = \frac{\ln(X / X_0)}{\ln 2}$$

Number of doublings n needed to grow from X_0 to X in the exponential phase; they take time $t = n \, t_d$.

GROWTH INDEX

$$\text{Growth index} = \frac{X_f - X_i}{X_i}$$

X_i is the initial and X_f the final fresh weight, dry weight or cell concentration of a culture cycle; it is a ratio with no unit. Some define growth index as the fold increase X_f / X_i instead, so check which definition a question uses.

BIOMASS YIELD ON SUBSTRATE

$$Y_{X/S} = \frac{X_f - X_i}{S_i - S_f}$$

Biomass formed per unit of substrate (for example sucrose) consumed over the same interval; in g biomass per g substrate when X and S are both in g/L.

PACKED CELL VOLUME

$$\text{PCV}\ (\%) = \frac{V_{\text{cells}}}{V_{\text{culture}}} \times 100$$

Volume of the settled or centrifuged cell pellet as a percentage of the total culture volume sampled.

Protoplast fusion and transgenic plants

CHROMOSOME NUMBER OF A SYMMETRIC SOMATIC HYBRID

$$2n_{\text{hybrid}} = 2n_A + 2n_B$$

Applies when one protoplast of each parent fuses, the nuclei fuse and no chromosomes are lost. The hybrid of two diploid parents is an amphidiploid (allotetraploid). Chromosome loss or elimination makes the number lower, and a hybrid made with irradiated donor protoplasts keeps fewer chromosomes.

FRACTION OF SELFED PROGENY CARRYING AT LEAST ONE TRANSGENE LOCUS

$$P = 1 - \left(\frac{1}{4}\right)^{n}$$

For a primary transformant that is hemizygous at n independent, unlinked single-copy loci, with each locus carrying a dominant, fully expressed marker and no loss in transmission or viability, selfed. At one locus the selfed progeny are 1/4 homozygous, 1/2 hemizygous and 1/4 without the transgene, so 3 with the transgene to 1 without and P = 3/4. Two loci give 15:1. Does not apply to linked loci or to plastid genes, which are maternally inherited.

FRACTION OF PROGENY CARRYING AT LEAST ONE TRANSGENE LOCUS WHEN CROSSED TO A NON-TRANSGENIC PLANT

$$P = 1 - \left(\frac{1}{2}\right)^{n}$$

For the same hemizygous transformant (n independent, unlinked single-copy loci) crossed with a non-transgenic plant. Each locus is passed to half the progeny, so one locus gives 1:1.

Animal cell culture: media, preservation and growth

EXPONENTIAL GROWTH

$$\frac{dX}{dt} = \mu X \qquad X = X_0\, e^{\mu t}$$

X is the viable cell concentration (cells/mL), X_0 its value at the start of the exponential phase, and μ the specific growth rate (per hour); valid only in the exponential phase.

SPECIFIC GROWTH RATE IN LOG PHASE

$$\mu = \frac{\ln(X_2/X_1)}{t_2 - t_1}$$

X in cells/mL and t in hours, so μ is in per hour; both points must lie in the exponential phase.

DOUBLING TIME

$$t_d = \frac{\ln 2}{\mu} \approx \frac{0.693}{\mu}$$

Convention used here: t_d = ln 2/μ, in the same time unit as μ; exponential phase only.

NUMBER OF DOUBLINGS

$$n = \log_2 \frac{X}{X_0} \approx 3.32 \log_{10} \frac{X}{X_0} \qquad X = X_0\, 2^{\,t/t_d}$$

For exponential growth from X_0 with no lag, n doublings take n × t_d. For a cell line, the population doubling level adds n at each subculture (cells harvested over cells seeded); the passage number counts subcultures only.

MONOD MODEL (SUBSTRATE-LIMITED GROWTH)

$$\mu = \frac{\mu_{\max} S}{K_s + S}$$

S is the limiting substrate concentration (for animal cells often glucose or glutamine), μ_max the maximum specific growth rate and K_s the substrate concentration at which μ = μ_max/2. When S is much larger than K_s, μ ≈ μ_max.

HAEMOCYTOMETER COUNT

$$\text{cells/mL} = \text{mean count per large square} \times \text{dilution factor} \times 10^{4}$$

In the standard chamber one large square is 1 mm × 1 mm with depth 0.1 mm, so its volume is 0.1 mm³ = 10^-4 mL. Cells on two agreed sides of a square are counted, those on the other two are not. The dilution factor includes the stain: a 1:1 mix with trypan blue gives 2.

VIABILITY BY TRYPAN BLUE EXCLUSION

$$\text{Viability}(\%) = \frac{\text{unstained (live) cells}}{\text{total cells counted}} \times 100$$

Live cells with intact membranes exclude the dye; dead cells with damaged membranes take it up and look blue.

Hybridomas, stem cells, cloning and transgenic animals

OFFSPRING OF A HETEROZYGOUS INTERCROSS (A = NULL ALLELE)

$$\mathrm{Aa} \times \mathrm{Aa} \;\rightarrow\; \tfrac{1}{4}\,\mathrm{AA} : \tfrac{1}{2}\,\mathrm{Aa} : \tfrac{1}{4}\,\mathrm{aa}$$

For one autosomal locus with Mendelian segregation and equal viability of all genotypes. The fraction of homozygous null (knock-out) pups is 1/4. To be homozygous null at two unlinked loci the chance is 1/4 x 1/4 = 1/16. If the null homozygote dies as an embryo, live pups are +/+ : +/- = 1 : 2, so 2/3 of them are heterozygous.

Microbial products, recombinant protein production and screening

SPECIFIC GROWTH RATE

$$\mu = \frac{1}{X}\frac{dX}{dt}$$

X is biomass concentration (g/L). In exponential growth with constant μ, $X = X_0 e^{\mu t}$.

DOUBLING TIME

$$t_d = \frac{\ln 2}{\mu}$$

Valid only in the exponential phase, where μ is constant. μ is in h^{-1}, so t_d is in hours.

MONOD EQUATION

$$\mu = \frac{\mu_{\max} S}{K_s + S}$$

S is the concentration of the growth-limiting substrate (g/L). K_s, the saturation constant, is the value of S at which $\mu = \mu_{\max}/2$. When $S \gg K_s$, $\mu \approx \mu_{\max}$; when $S \ll K_s$, $\mu \approx (\mu_{\max}/K_s)S$. Linearised (Lineweaver-Burk form): $1/\mu = 1/\mu_{\max} + (K_s/\mu_{\max})(1/S)$. Applies when one substrate limits growth.

BIOMASS YIELD COEFFICIENT

$$Y_{X/S} = \frac{\Delta X}{-\Delta S}$$

Grams of biomass formed per gram of substrate consumed, taken as constant over the culture. Similarly $Y_{P/S} = \Delta P/(-\Delta S)$ for product and $Y_{P/X} = \Delta P/\Delta X$.

LUEDEKING-PIRET MODEL

$$\frac{dP}{dt} = \alpha\frac{dX}{dt} + \beta X$$

α (g product per g biomass) is the growth-associated coefficient and β (g product per g biomass per h) the non-growth-associated coefficient. A purely growth-associated product has $\beta = 0$; a purely non-growth-associated product, such as a typical secondary metabolite, has $\alpha \approx 0$. Mixed products such as lactic acid need both terms.

CHEMOSTAT AT STEADY STATE

$$\mu = D = \frac{F}{V}, \quad S = \frac{K_s D}{\mu_{\max} - D}, \quad X = Y_{X/S}(S_0 - S)$$

F is the feed flow rate (L/h), V the culture volume (L), D the dilution rate (h^{-1}) and S_0 the limiting-substrate concentration in the feed. Assumes a sterile feed, Monod kinetics, constant yield, and no death or maintenance. Washout occurs when D reaches $\mu_{\max} S_0/(K_s + S_0)$, the highest growth rate the feed can support.

ALCOHOLIC FERMENTATION OF GLUCOSE

$$\mathrm{C_6H_{12}O_6} \rightarrow 2\,\mathrm{C_2H_5OH} + 2\,\mathrm{CO_2}$$

Theoretical ethanol yield is $2 \times 46/180 = 0.511$ g ethanol per g glucose, and CO_2 is $2 \times 44/180 = 0.489$ g per g glucose. Real yields are lower because some sugar goes to cells and by-products.

BIODIESEL TRANSESTERIFICATION

$$\mathrm{Triglyceride} + 3\,\mathrm{CH_3OH} \rightarrow 3\,\mathrm{FAME} + \mathrm{glycerol}$$

Three moles of methanol per mole of triglyceride, usually with excess methanol to push the equilibrium forward and an alkali catalyst; lipases can also catalyse it.

THERMAL DEATH KINETICS AND D VALUE

$$\ln \frac{N_0}{N} = k_d t, \qquad D = \frac{\ln 10}{k_d} = \frac{2.303}{k_d}$$

Assumes first-order death at a constant temperature. N is the number of viable cells or spores, k_d the specific death rate (min^{-1}), and D the time at that temperature that cuts the viable count tenfold (one log). The z value is the temperature rise that cuts D tenfold.

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