

Kinetics (rates) of fungal growth

$$R = \frac{\Delta \text{ in some measure of growth}}{\Delta t}$$

Focus on 5* of 6 situations that concern practical measurements of fungal biomass

- 1.* Growth of populations of unicells
(reproductive growth)



- 2.* Hyphal growth by apical extension & branching (nonreproductive growth)



- 3.* Unrestricted growth
4. Restricted growth (some factor limiting)
- 5.* Surface growth
- 6.* Submerged growth

114

Types of growth as related to rates (kinetics)

1. Unicellular growth - usually involves doubling rates (exponential rates)
2. Hyphal growth - may or may not involve doubling rates
3. Unrestricted growth - maximum rates &/or no limiting factors
4. Restricted growth - less than maximum rates
5. Surface growth - air-substrate interface growth (somewhat restricted because substrate not equally available to all parts of fungus)
6. Submerged growth - may or may not be at max rates

115

Question? What to measure for index of fungal growth?

Key - parameter measured must be known to be proportional to criterion of growth being considered

Measured parameter must directly correlate with increase in biomass (protoplasm)

Could measure* -

1. Δ in linear dimensions (colonies, hyphae)
2. Δ in mass or weight
3. Δ in cell #
4. Δ in volume
5. Δ in metabolic activity
6. Δ in quantity of cell constituent
7. Δ in absorbance

* all have been used for fungi

116

Practical considerations of measuring fungal growth (which is best?)

1. Linear measurements - e.g.
 - a. Increase in colony diameter*
 - B. Increase in colony margin*
in "race tube"
 - c. Increase in hyphal length using microscopic techniques **

Advantages:

1. Simplicity
2. Nondestructive

Disadvantages:

1. No necessary correlation between increase in colony diameter and increase in total biomass

* Use - good for evaluating growth rates of strains of same species

** Excellent, but difficult

117

2. Dry weights - most widely used particularly for molds - or as correlates* of other measures for yeasts (e.g. absorbance $x = y$ mg dry wt)

* Should correlate with balanced growth.

Advantages:

- a. Probably one of best measures for mold growth except when linear measurements appropriate
- b. Excellent for yeasts, but time consuming

Disadvantages:

- a. Destructive
- b. Requires culture sampling which must be precise
- c. Near impossible to uniformly sample mold culture*

* Why

- Hard to pipette mold mycelium
- Mycelium not always uniformly distributed in media
- Culture homogeneity difficult to insure

121

3. Estimates of cell #s

- a. Viable counts (plate counts)
- b. Particle counts (haemocytometer, coulter counters, etc.)

Good for unicells - yeasts & spore counts

Poor for molds - because:

- 1) Hypha of any length would yield only one (1) viable or Particle count
- 2) Difficult to sample
- 3) Correlations inaccurate

Most often used for yeast measures, and estimates of spore, germling or blended hyphal inoculum levels.

124

4. Cell volume - (packed cells)
 - o.k. for unicells & spores, sometimes germlings
 - poor in general for molds because packing is dependent on:
 - a. Length of hyphae
 - b. Degree of branching
 - c. Endogenous substrate reserves
5. Turbidity or absorbance
 - a. Extremely useful for spores, unicells, germlings or germination activity
 - b. Poor for molds
 - 1) Hyphae do not always form homogeneously
 - 2) Filaments do not uniformly scatter light

Can get good data with yeasts.

127

6. Metabolic activity
 - Good in well-defined situations e.g., O_2 | CO_2 | during "balanced growth"
 - Problems: data may be misleading -e.g., because of generally high levels of endogenous reserves, fungi often show high rates of O_2 uptake and CO_2 evolution in the absence of growth
 - Same for product formation
 - Substrate utilization
 - Enzymatic activity
7. Compositional changes same
 - Good for certain balanced growth situations, e.g. > dna

131

Review

Kinetics (rates) of fungal vegetative growth

$$\text{Rate} = \frac{\Delta \text{ in some measure of growth}}{\Delta t}$$

Comparisons of reproductive growth (yeast growth) and nonreproductive growth (mold growth) involve different considerations

Practical considerations:

What to measure? parameter must directly correlate with increase in biomass.

Measures:

1. Linear dimensions
2. Mass or weight
3. Cell # or viable #
4. Volume
5. Metabolic activity
6. Cell constituent (e.g. protein)
7. Absorbance or transmittance

133

Questions to ask related to the kinetics of fungal growth

1. What conclusions with fungi, particularly molds, have resulted from the collection of data by techniques reviewed?
2. How fast do fungi grow?
 - a. Do fungi grow as fast as bacteria?
 - b. Do molds grow exponentially?*

* Can/do they ever double all their cell constituents proportionally with time?

134

How fast do molds grow?
What controls rates of mold growth?

Conclusions* of Henderson Smith ~ 1920:

1. Rate of growth of a mold during any one period of time is not a function necessarily of total fungal biomass -although this may contribute
2. Rate controlled by # of hyphal tips
3. Rates at which tips are supplied nutrients either by absorption or translocation
4. Rates of branching

* Not readily apparent because mold growth most easily demonstrated to show constant growth rates (arithmetic increases)

136

Conclusions

1. Both yeasts and molds can be demonstrated to grow logarithmically
2. Usually mold exponential growth occurs for only relatively short period while colony establishes itself into circle or sphere shape*, **
3. Mold exponential growth is correlated with rapid branching to --> 2 or 3D colonies
4. Rapid growth rates must involve protoplasm contributions and syntheses from areas not actually involved in apical extension (tips)

* then by definition is restricted

** use of strains that fragment at high rates extends periods of demonstrable exponential growth (strain selection in industry)

144

Zolakar's calculations (1950) w/ *N. crassa*

1. *N. crassa* extends its tip at maximum rates of 100 $\mu\text{m}/\text{min}$ (6 mm/hr).
- he did this by measuring microscopically & in race tubes.
2. Hyphae needed 2 hours to double protein content (..) took 2 hrs to double protein,
but only an hr to double length
3. Means protein synthesized behind tip contributes to elongation rate (translocation)
4. Need a minimum of 12 mm of hyphal length to sustain an elongation rate of 100 $\mu\text{m}/\text{min}$

$$100 \mu\text{m} = 12 \text{ mm length}$$

$$100 \mu\text{m} (\text{per min extension rate}) \times 120 \text{ min (time to double protein)} = 1200 \mu\text{m} = 12 \text{ mm}$$