Continuous Operation for Production of α-Galactosidase by a *Monascus* sp. molds

(Imanaka et al.)

α-Galactosidase

- Useful in the beet-sugar industry
- Decomposes raffinose, an inhibitor of sucrose crystallization

Major Findings from Batch Experiments

- Among 20 different carbon sources including glucose, fructose, mannitol, and starch, only four sugars were effective in inducing high α–galactosidase activity.
  - Strong inducers: galactose, melibiose, raffinose, stachyose
- Ammonium nitrate gave more enzyme production than other nitrogen sources.
  - Optimal NH_4NO_3 concentration: 0.3~0.5 wt %

Major Findings from Batch Experiments

- In a galactose medium, the cell mass is directly proportional to the α–galactosidase activity.
- When a mixture of glucose and galactose was used as the carbon source, diauxic growth was observed. (see Fig.)
- The α–galactosidase production does not start until the glucose is almost gone.
- Glucose concentrations greater than 0.05 wt % repress synthesis of the enzyme.

Batch Culture

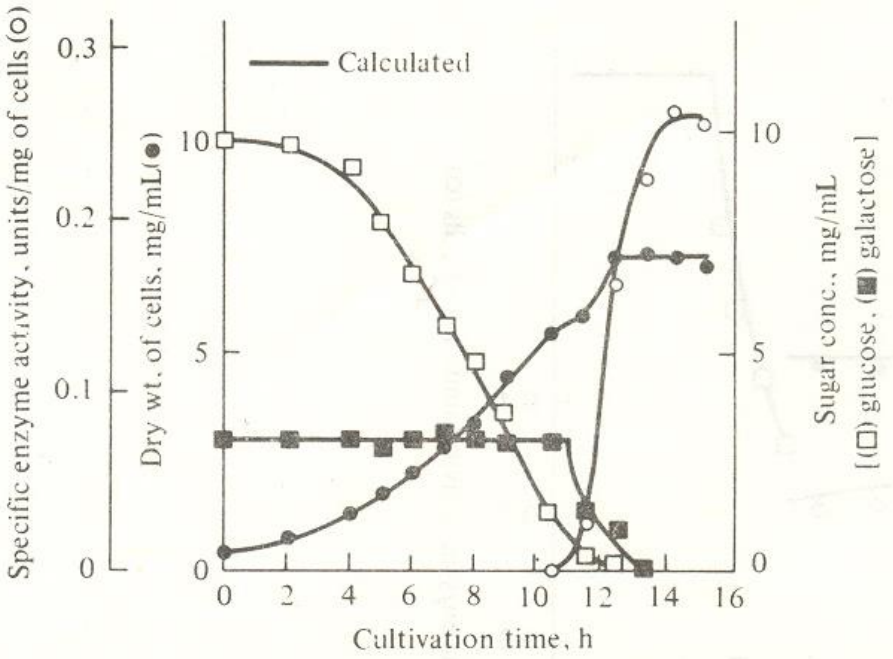

Figure 9E1.1 Results of batch cultivation of a *Monascus* sp. mold in a mixture of glucose and galactose [initial medium composition: glucose 1% (by weight), galactose 0.3%, NH_4NO_3 0.5%, KH_2PO_4 0.5%, $MgSO_4 \cdot 7H_2O$ 0.1%, yeast extract 0.01%]. The inoculum was grown in a glucose medium. The initial conditions used in the calculations were $x = 5 \times 10^{-4}$ g/mL, $s_1 = 1 \times 10^{-2}$ g/mL, $s_2 = 3 \times 10^{-3}$ gm/mL, $s_{2I} = 0$ μg/mg cell, $rs_{2I} = 0.910$ μg/mg cell, $e = 0$ units/mg cell. *[Reprinted from T. Imanaka et al., "Unsteady-state Analysis of a Kinetic Model for Cell Growth and α-Galactosidase Production in Mold," J. Ferment. Tech. (Japan), vol. 51, p. 423, 1973.]*

Chemostat Culture (shift-up)

- Discontinuity at D = 0.142 h^{-1}
  - At D<0.142
    - Both glucose and galactose are consumed.
    - α-galactosidase is produced.
  - At D>0.142
    - Only glucose is consumed.
    - α-galactosidase is not produced.
- When cultivated under relatively large specific growth rates (large D's), the mold preferentially feeds on glucose.

Chemostat Culture (shift-up)

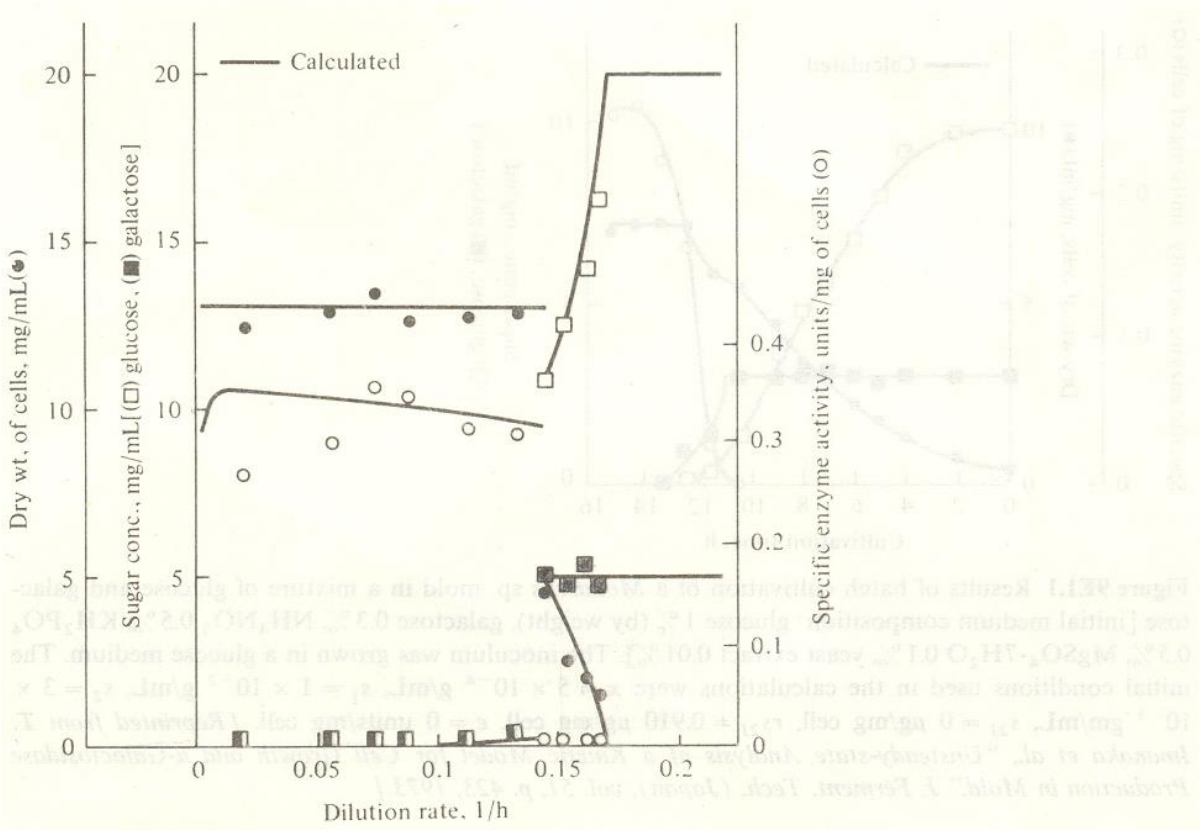

Figure 9E1.2 Steady-state cell and substrate concentrations and specific enzyme activity observed during gradual shift up of dilution rate for continuous culture (30°C). Initially the medium contains 2% glucose and 0.5% galactose. *[Reprinted from T. Imanaka et al., "Optimization of α-Galactosidase Production by Mold," J. Ferment. Tech. (Japan), vol. 50, p. 633, 1972.]*

Chemostat Culture (shift-down)

- Discontinuity at D = 0.008 h^{-1}
  - At D<0.008
    - Both glucose and galactose are consumed.
    - α-galactosidase is produced.
  - At D>0.008
    - Only glucose is consumed.
    - α-galactosidase is not produced.
- When cultivated under relatively large specific growth rates (large D's), the mold preferentially feeds on glucose.

Chemostat Culture (shift-down)

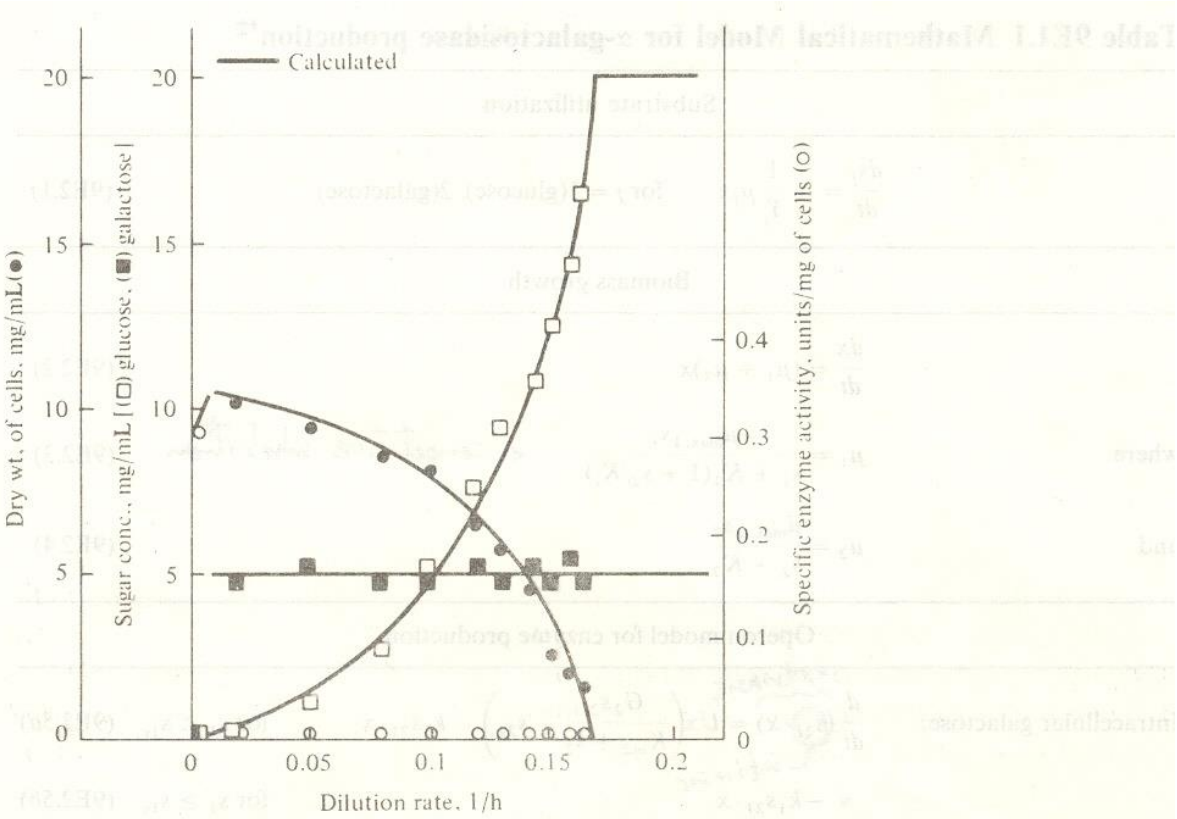

Figure 9E1.3 Steady-state cell and substrate concentrations and specific enzyme activity observed during gradual shift down of dilution rate for continuous culture (30°C). Initially the medium contains 2% glucose and 0.5% galactose. *[Reprinted from T. Imanaka et al., "Optimization of α-Galactosidase Production by Mold," J. Ferment. Tech. (Japan), vol. 50, p. 633, 1972.]*

Steady-state multiplicity and Hysteresis

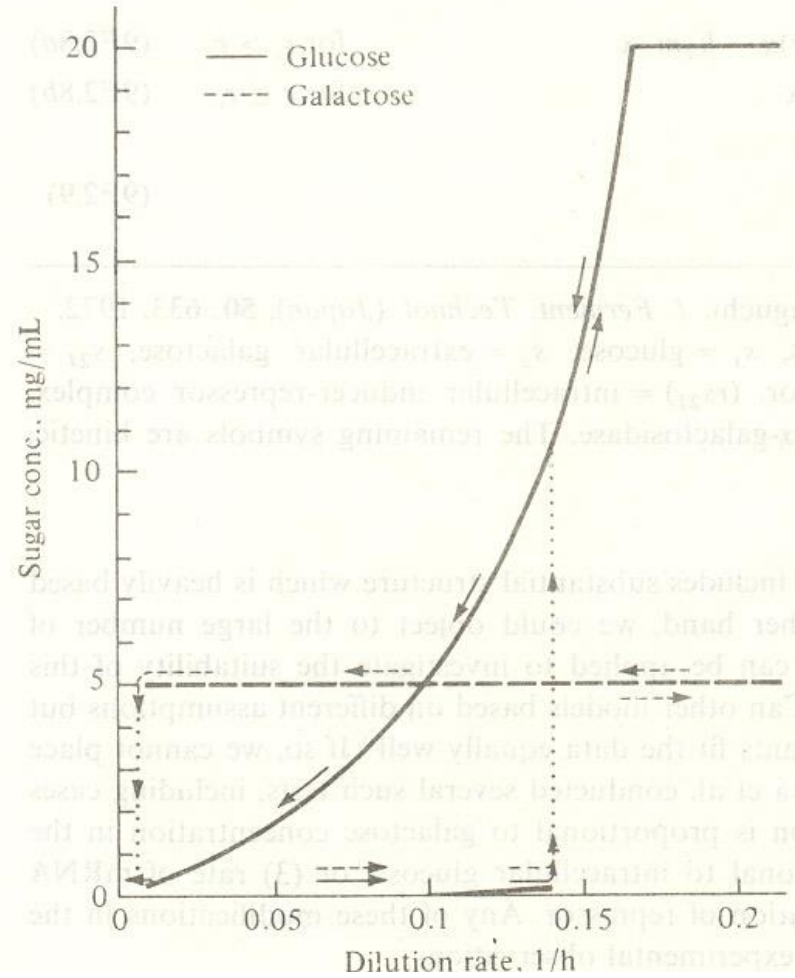

Figure 9E1.4 This replot of some of the curves from Figs. 9E1.2 and 9E1.3 displays steady-state multiplicity and hysteresis in continuous culture. Two stable steady states occur for dilution rates between 0.008 and 0.142 h^{-1}. Which is obtained depends on reactor start-up. *[Reprinted from T. Imanaka et al., "Optimization of α-Galactosidase Production by Mold," J. Ferment. Tech. (Japan), vol. 50, p. 633, 1972.]*

Table 9E1.1 are familiar from our earlier studies: the specific growth rate μ_2 based on galactose is of Monod form, while the specific growth rate of glucose μ_1 includes competitive inhibition by galactose. All the constants in these growth-rate functions were evaluated for two different media from continuous-culture experiments. Parameters labeled G in Table 9E1.2 correspond to a glucose medium (20 g glucose, 5 g NH_4NO_3, 5 g KH_2PO_4, 1 g $MgSO_4 \cdot 7H_2O$, 0.1 g yeast extract in 1000 mL tap water at pH 4.5) while the p subscripts refer to a galactose medium advantageous for enzyme production (5 g galactose, 5 g NH_4NO_3, 5 g KH_2PO_4, 1 g $MgSO_4 \cdot 7H_2O$ in 1000 mL tap water, pH 4.5).

The model for enzyme production is based upon the operon theory of induction, studied in Chap. 6. The specific rate of α-galactosidase synthesis is proportional to the intracellular concentration of mRNA which codes for that enzyme. This mRNA is assumed to decompose by a first-order reaction and is produced provided the intracellular concentration of repressor R is smaller than a threshold value r_c. Below this threshold value, lower r values cause increased specific rates of mRNA synthesis. The repressor is formed at constant specific rate k_2 and decomposes with first-order specific rate $k_2 r$. Repressor concentration is also reduced by complexing with the inducer, intracellular galactose.

The rate of galactose transport into the cell is given by the term in the intracellular galactose mass balance with coefficient U. To take into account the glucose effect this transport term is set equal to zero whenever the glucose concentration s_1 exceeds a critical value s_{1c}, which is taken to be 2.25×10^{-4} g/mL.

Little information is available for direct evaluation of the rate constants in the operon model. Values for k_3 and k_7 were assigned based on the assumption that the repressor and mRNA half-lives are 40 and 5 min, respectively. The other parameter values listed in Table 9E1.2 were estimated by trial and error to achieve a reasonable fit to the experimental data.

Mathematical Model

Table 9E1.1 Mathematical Model for α-galactosidase production[†‡]

Substrate utilization		
	$\frac{ds_j}{dt} = -\frac{1}{Y_j}\mu_j x$ for $j = 1$(glucose), 2(galactose)	(9E2
Biomass growth		
	$\frac{dx}{dt} = (\mu_1 + \mu_2)x$	(9E2
where	$\mu_1 = \frac{\mu_{\max,1} s_1}{s_1 + K_1(1 + s_2/K_i)}$ ← competitive inhibition	(9E2
and	$\mu_2 = \frac{\mu_{\max,2} s_2}{s_2 + K_2}$	(9E2

Mathematical Model

Operon model for enzyme production

Intracellular galactose:	$\frac{d}{dt}(s_{2I}\cdot x) = Ux\left(\frac{G_2 s_2}{K_{m2}+s_2} - s_{2I}\right) - k_1 s_{2I}\cdot x$	for $s_1 < s_{1c}$	(9E2
	$= -k_1 s_{2I}\cdot x$	for $s_1 \geq s_{1c}$	(9E2
Repressor:	$\frac{d}{dt}(r\cdot x) = k_2 s - k_3 r\cdot x - k_4 r\cdot s_{2I}\cdot x + k_5(rs_{2I})x$		(9W
Galactose-repressor complex:	$\frac{d}{dt}[(rs_{2I})x] = k_4 r\cdot s_{2I}\cdot x - k_5(rs_{2I})x$		(9E2
mRNA for galactosidase:	$\frac{d}{dt}(m\cdot x) = \begin{cases} k_6(r_c - r)x - k_7 m\cdot x \\ -k_7 m\cdot x \end{cases}$	for $r_c > r$ for $r \geq r_c$	(9E2 (9E2
Enzyme:	$\frac{d}{dt}(e\cdot x) = k_8 m\cdot x$		(9E2

† T. Imanaka, T. Kaieda, K. Sato, and H. Taguchi, *J. Ferment. Technol. (Japan)*, **50**: 633, 197

‡ Concentration variables are x = biomass, s_1 = glucose, s_2 = extracellular galactose, s_2 intracellular galactose, r = intracellular repressor, (rs_{2I}) = intracellular inducer-repressor comp m = intracellular mRNA, and e = intracellular α-galactosidase. The remaining symbols are kin yield, and transport parameters.

Parameter Values

Table 9E1.2 Parameter values for the mathematical model of α-galactosidase production[†]

Entries in the right column were evaluated experimentally; the remaining parameters were adjusted to fit the batch and continuous-culture results; G = glucose medium, 30°C, p = galactose medium, 35°C.

$k_1 = 40\ \text{h}^{-1}$	$\mu_{\max,1_G} = 0.215\ \text{h}^{-1}$
$k_2 = 1\ \text{mg/(mg cells} \cdot \text{h)}$	$\mu_{\max,2_G} = 0.208\ \text{h}^{-1}$
$k_3 = 1\ \text{h}^{-1}$	$K_{1_G} = 1.54 \times 10^{-4}\ \text{g/mL}$
$k_4 = 0.1\ \text{mg cells/(mg} \cdot \text{h)}$	$K_{2_G} = 2.58 \times 10^{-4}\ \text{g/mL}$
$k_5 = 1 \times 10^{-4}\ \text{h}^{-1}$	$\mu_{\max,1_p} = 0.190\ \text{h}^{-1}$
$k_6 = 1\ \text{h}^{-1}$	$\mu_{\max,2_p} = 0.162\ \text{h}^{-1}$
$k_7 = 8\ \text{h}^{-1}$	$K_{1_p} = 1.45 \times 10^{-4}\ \text{g/mL}$
$k_{8_G} = 3.2787\ \text{units/(mg mRNA} \cdot \text{h)}$	$K_{2_p} = 3.07 \times 10^{-4}\ \text{g/mL}$
$k_{8_p} = 5.0442\ \text{units/(mg mRNA} \cdot \text{h)}$	$K_i = 1.39 \times 10^{-4}\ \text{g/mL}$
$U = 100\ \text{h}^{-1}$	$Y_{1_G} = 0.530$
$G_2 = 1\ \text{mg/mg cells}$	$Y_{2_G} = 0.516$
$K_{m2} = 1 \times 10^{-8}\ \text{mg/mg cells}$	$Y_{1_p} = 0.377$
$s_{1c} = 2.25 \times 10^{-4}\ \text{g/mL}$	$Y_{2_p} = 0.361$
$r_c = 0.934\ \text{mg/mg cells}$	

[†] T. Imanaka, T. Kaieda, K. Sato, and H. Taguchi, *J. Ferment. Technol.*, **50**: 558, 1972.