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Notes

Reassessment of the Product Inhibition in Alcohol Fermentation

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Empirical equations

The product inhibition pattern which was extracted from the experiments of alcohol fermentation with a respiration-deficient mutant of *Saccharomyces cerevisiae* is as follows:

$$\mu=\mu_0 e^{-k_1 p}\frac{S}{K_s+S} \quad \cdots\cdots(1)$$

$$\nu=\nu_0 e^{-k_2 p}\frac{S}{K'_s+S} \quad \cdots\cdots(2)$$

where

μ = specific growth rate, hr^{-1}
μ_0 = specific growth rate at $p=0$, hr^{-1}
ν = specific rate of ethanol production, hr^{-1}
ν_0 = specific rate of ethanol production at $p=0$ hr^{-1}
p = product (ethanol) concentration, g/l
S = substrate (glucose) concentration, g/l
K_s, K'_s = saturation constants, g/l
k_1, k_2 = empirical exponents, l/g

A chemostatic cultivation of the yeast cells in single vessels (limiting substrate = glucose), adding ethanol artificially, if needed, into an influent of fresh medium was required to elaborate on the kinetic pattern as formulated in Equns. (1) and (2).

The Lineweaver-Burk plot, either $1/\mu$ vs. $1/S$ or $1/\nu$ vs. $1/S$, parameter in each plot being the concentration of ethanol, p constructed a bundle of straight lines. These lines converged onto the abscissa. Clearly, the reading on the abcsissa gave the value of $-1/K_s$ or $-1/K'_s$ in Equation (1) or (2). The above-mentioned correlation, $1/\mu$ (or $1/\nu$) vs. $1/S$ justified the non-competitive pattern of the product (ethanol) inhibition in this alcohol fermentation.

The reading of intersection of each straight line on the ordinate in the Lineweaver-Burk plot gave the value of μ (or ν) as affected by the ethanol concentration, p, assuming that neither the cellular growth nor the fermentation was limited by the substrate concentration of glucose, S.

Incidentally, if the values of μ (or ν) are plotted against the values of p on a semi-logarithmic paper, a straight line could be assumed without difficulty through the data points in each case as are demonstrated in Figs. 4 and 7 in the previous work[2].

Experimental data taken from the batch culture of the cells[1] in shaken flasks for the growth and in the Warburg manometer for the fermentation activity followed also the same pattern on the semi-logarithmic paper as included in the same figures referred to above. Needless to say, the experimental data in batch where no substrate was limiting the cellular activity were taken carefully.

The point worthy of reassessment

Although the previous study revealed clearly the non-competitive inhibition of ethanol in the anaerobic cultivation of the Baker's yeast cells, the point which requires due reassessment here lies in the effect of p as formulated in the exponential term.

As far as the exponential term is appreciated, the empirical exponents, k_1 and k_2 (see Equns. (1) and (2)) which depended apparently on the procedure of cultivation, batch and continuous, are lacking in any particular significance from the viewpoint of enzyme kinetics. However, Eq. (2) was applied successfully to a prediction of the accumulation rate of ethanol in the later phase of the "sake" brewing. The effect of p on ν in Eq. (2) was extrapolated to the case of $p \fallingdotseq 20$ (vol %), far exceeding the experimental range from which the equation was derived. The successful application to the practice was beyond any stretch of the theoretical background.

Despite a congenital lack of the theoretical significance, the previous equations are deemed valid in connection with the application to the "sake" brewing, because the enzymatic reappraisal due to be made here could not be extrapolated to the actual situation in the brewing. This sort of controversial inconsistency points out an aspect of the very complicated nature of ethanol inhibition in practice, to which the monolithic approach seems still prohibitive.

Reassessment

Since the non-competitive inhibition was apparent from the previous Lineweaver-Burk plot, the intersection of each straight line with the ordinate of the plot should indicate the following relationships between $1/\mu$ and p or $1/\nu$ and p.

$$\frac{1}{\mu}=\left(1+\frac{p}{K_p}\right)\frac{1}{\mu_0} \quad \cdots\cdots(3)$$

$$\frac{1}{\nu}=\left(1+\frac{p}{K'_p}\right)\frac{1}{\nu_0} \quad \cdots\cdots(4)$$

provided: K_p, K'_p=empirical constants.

If one replots the values of $1/\mu$ (or $1/\nu$) against p with reference to the data shown in Figs. 4 and 7 in the previous study[2], a straight line can be expected,

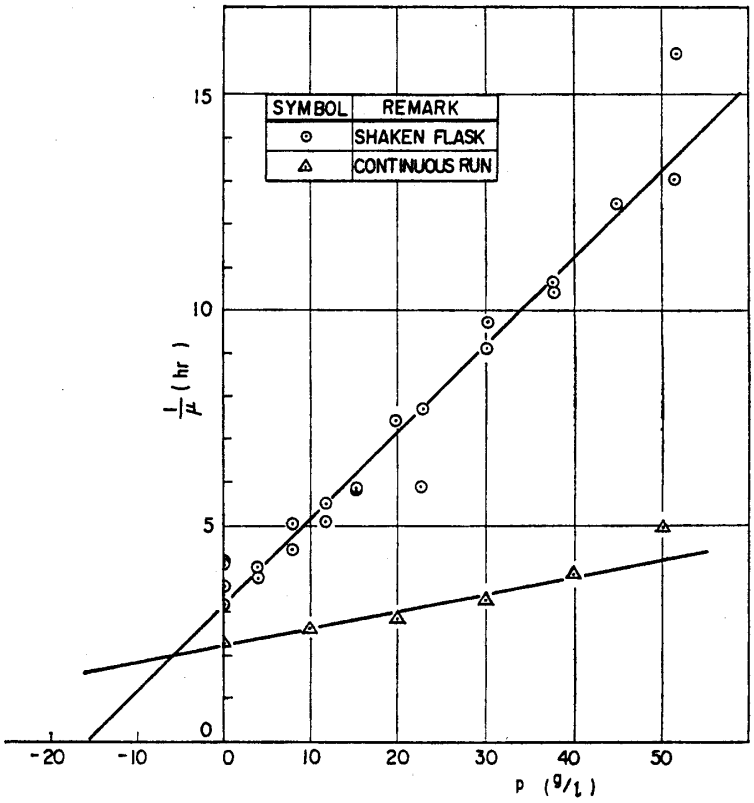

Fig. 1. $\frac{1}{\mu}$ vs. p.

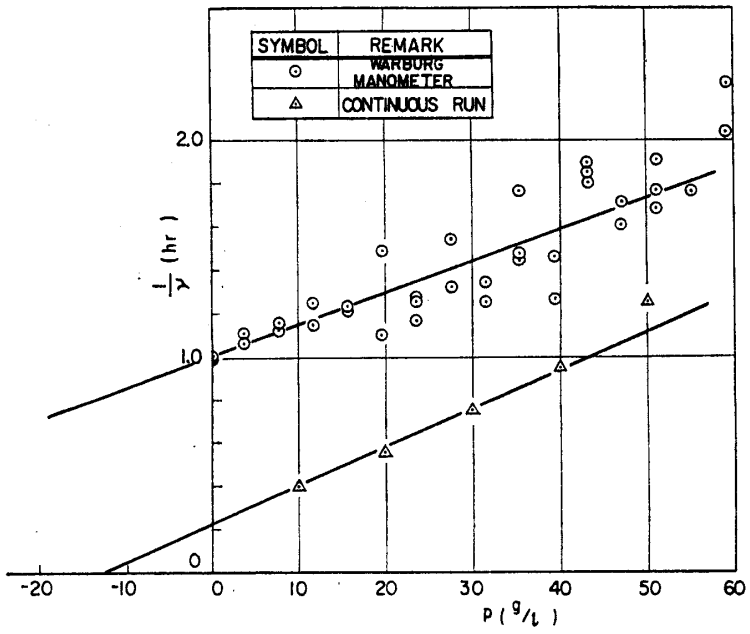

Fig. 2. $\frac{1}{\nu}$ vs. p.

respectively so far as the non-competitive inhibition is acceptable. The rearrangement of the data points of the previous figures is shown in Figs. 1 and 2 in this work. It is not prohibitive to draw each straight line as shown in the figures to determine from the slope of the line the values of K_p and K'_p (see Table 1).

Table 1. Values of K_p and K'_p.

Culture		K_p(g/l)	K'_p (g/l)
Batch	Shaken flask for K_p Warburg manometer for K'_p	16.0	71.5
Continuous run		55.0	12.5

This replotting to yield the straight lines as shown in Figs. 1 and 2 sounds peculiar in view of the previous plot of the same data on the semi-logarithmic paper. However, this dual arrangement of the data points stems apparently from a narrow range of ethanol concentration p($p=0$ to $p=60$ g/l) ever observed in each experimentation.

As far as the fitness of the data points with the straight line is solely concerned, any preference between the former semi-logarithmic plot and the current plot as exemplified in Figs. 1 and 2 is difficult, indeed.

To recapitulate, the previous equations are reassessed as follows:

$$\mu=\frac{\mu_0}{1+\frac{p}{K_p}}\cdot\frac{S}{K_s+S} \quad \cdots\cdots(5)$$

$$\nu=\frac{\nu_0}{1+\frac{p}{K'_p}}\cdot\frac{S}{K'_s+S} \quad \cdots\cdots(6)$$

It is interesting to find out a striking similitude between the product inhibition *in vivo* and the typical pattern of the non-competitive inhibition of a pure enzyme *in vitro*. If and only if a *de facto* simulation between the living system of this alcohol fermentation and the purely enzymatic environment is possible, the physiological significance of K_p and K'_p becomes more lucid.

The fact that the K_p value in the shaken flasks is smaller than in the continuous run as evident from Table 1 suggests the possibility that the chemical affinity of ethanol with a key enzyme, if any, participating in the cellular growth becomes manifested in the shaken flask experiment.

On the other hand, it is also suggested from the table in comparing the K'_p values between the Warburg manometer and the continuous cultivation that the inhibition of ethanol against another key enzyme responsible for the fermentation activity becomes more revealed in the continuous run.

The intriguing observation that the association of ethanol with the key enzymes both for the cellular growth and the fermentation activity depends presumably, as shown from the table, on the procedure of cultivation is suggestive of another aspect of the multi-lateral features of the viable cells. Doubtlessly this interpretation of the living materials presents one of the basic information relevant to the

scale-up in the fermentation industry.

Finally, it seems worthwhile to mention briefly that Equns. (5) and (6) reassessed here are more suited in formulation for further analysis of "stability" of this fermentation, if required.

References

1) Nagatani, M., Shoda, M., Aiba, S.: *J. Ferment. Technol.*, **46**, 241 (1968).

2) Aiba, S., Shoda, M., Nagatani, M.: *Biotechnol. and Bioeng.*, **10**, 845 (1968).

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