Kinetics of the Continuous Alcoholic Fermentation of Blackstrap Molasses

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The very important problems concerning fermentation kinetics have only recently received some attention (Calam *et al.*, 1951; Gaden, 1955a, b, 1956; Borzani, 1957; Kosin, 1957). In the particular case of continuous alcoholic fermentation, a great number of papers have been published (Alzola, 1940, 1945; Bilford *et al.*, 1942; Owen, 1948; Asai *et al.*, 1952, 1953; Mariller *et al.*, 1952; Borzani, 1953, 1957; Ueda *et al.*, 1954; Ueda, 1955a, b) but only one (Borzani, 1957) gives some attention to the kinetics of the process. In that article (Borzani, 1957) the author postulates an empirical equation relating the fermentation cycle time (T, hr) of a complete continuous fermentation and the concentration of total reducing sugars (C, g per L) of the feeding mash:

$$T = 2.08 \times 10^{-3}C^2$$

and says that this equation can be justified theoretically with the hypothesis that the consumption of sugar is a reaction of kinetic order -1 ($dc/dt = -k/c$). The author further states that "another experimental approach is being tried to confirm these results."

That theoretical justification, however, is not correct. In fact, the rate equation mentioned

$$\frac{dc}{dt} = -\frac{k}{c} \tag{1}$$

admits that the fermentation rate (dc/dt) is influenced only by the temperature (since k is a function of the temperature) and by the sugar concentration of the feeding mash; the equation (1) does not consider the influence of the microorganism concentration, which depends on several factors, for example, sugar concentration, feed rate, and agitator speed. We believe that only a coincidence can explain the agreement between the theoretical and the empirical equations presented in the mentioned paper.

The correct rate equation of a fermentation of kinetic order n can be written:

$$\frac{dc}{dt} = -kMc^n \tag{2}$$

where c and M are, respectively, the sugar concentration and the microorganism concentration at the time t, and k is the velocity constant.

If we assume that a fermentor is being continuously fed fresh mash at a constant rate, ϕ, and that fermenting material is being continuously withdrawn from the vessel at the same rate (thus, a constant volume, V, of liquid is maintained in the fermentor), and if we call C_i the sugar concentration of the feeding mash, C_f and M, respectively, the sugar concentration and the microorganism concentration of the withdrawal medium at steady state, equation (2) will give:

$$n \neq 1: \frac{1}{1-n}(C_i^{1-n} - C_f^{1-n}) = kM \cdot \frac{V}{\phi} \tag{3}$$

$$n = 1: \lg \frac{C_i}{C_f} = kM \cdot \frac{V}{\phi} \tag{4}$$

Equations (3) and (4) can be rearranged:

$$n \neq 1: \frac{C_i^{1-n} - C_f^{1-n}}{M} = k' \cdot \frac{1}{\phi} \tag{5}$$

$$n = 1: \frac{1}{M} \cdot \lg \frac{C_i}{C_f} = k'' \cdot \frac{1}{\phi} \tag{6}$$

showing that a linear relation exists between $1/\phi$ and a function of C_i, C_f and M that depends on n.

This report presents the results obtained on laboratory-scale continuous alcoholic fermentations, carried out to study the kinetic order of the process, varying the feed rate (then varying the fermentation cycle time, V/ϕ), the sugar concentration of the feeding mash, and the agitator speed.

The equations presented in this paper were derived by the application of the least squares method to the experimental values.

Materials and Methods

The fermentation equipment is shown in figure 1. The mash feeder used, described by Borzani and Vairo (1959), assures practically constant feeding rates.

Blackstrap molasses with about 50 per cent of total reducing sugars, calculated as glucose, was used.

Ten L of concentrated mash (about twice as concentrated as the desired mash) were prepared by diluting the blackstrap molasses with tap water; $NH_4H_2PO_4$ (0.4 per cent of the molasses weight) was added and the solution was heated for 15 min at 100 C and for 15 min at 110 to 120 C to clarify the medium. After 24 to 48 hr of rest, the clarified mash was siphoned, diluted

with tap water to attain a desired sugar concentration, and distributed in the fermentor (2.8 L) and in the storage flasks. The vessels containing the diluted mash, the connection tubes, and the pumps were then sterilized by autoclaving at 120 C for 15 to 20 min. After sterilization the equipment was mounted with care to avoid contamination. Ammonium phosphate, monobasic (2.0 g per L) and $MgSO_4 \cdot 7H_2O$ (0.25 g per L) were then added to the mash, as sterilized solutions, to correct the deficiencies of N, P, and Mg normally observed in molasses. The fermentor was then inoculated with a suspension of 30 g of pressed yeast[1] in 100 to 180 ml of sterilized water; this quantity of yeast assures an initial yeast concentration of 0.9×10^{11} to 1.3×10^{11} cells per L (Borzani, 1955). After the fermentation was completed (24 to 28 hr), the continuous feeding with fresh mash was started. Temperature was controlled between 29 and 31 C. Feeding rate was periodically calculated from the measurement of the volume of fermenting medium obtained in a given time. The steady state, attained 12 to 15 hr later and maintained for at least 4 hr, was controlled by measuring periodically the specific gravity of the fermenting medium. Samples of the fermenting medium were collected for analytical determinations. For measurement of the alcohol content of the completely fermented mash, a sample of

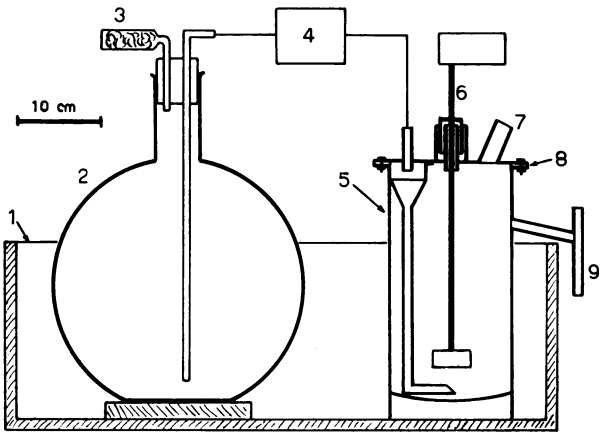

Figure 1. Schematic representation of the fermentation equipment. *1.* Water bath; *2,* fresh mash storage flask; *3,* air filter; *4,* mash feeder; *5,* Inox fermentor; *6,* agitator; *7,* sample tube; *8,* asbestos gasket; and *9,* draw off.

TABLE 1

Effect of initial sugar concentration, feeding rate, and agitation speed on continuous fermentation

Agitator Speed	Mash Sugar Conc*	Feeding Rate	Withdrawal Medium (at Steady State)		Alcohol Content of the Completely Fermented Mash	Fermentation Efficiency
			Sugar conc*	Yeast conc		
rpm	*g/L*	*L/hr*	*g/L*	*10^{10} cells/L*	*% vol at 15C*	*ml alcohol/kg glucose*
0	128.7	0.916	121.5	1.3	6.3	490
	132.3	0.979	115.0	3.4	6.4	480
	132.6	0.648	116.7	2.4	—	—
	133.9	0.689	118.0	3.2	6.7	500
	162.8	0.909	154.9	1.4	9.0	550
	166.5	0.483	138.2	3.9	8.9	530
	176.2	0.640	150.9	3.3	9.0	510
	188.2	0.880	180.4	1.0	9.9	530
500–530	102.0	1.011	89.5	2.8	4.6	450
	103.5	0.707	77.2	5.5	5.3	510
	105.3	0.469	40.2	8.5	5.1	480
	122.2	0.398	46.8	7.6	6.3	520
	130.0	0.666	89.7	7.8	6.6	510
	130.7	0.981	107.9	4.6	6.5	500
	132.0	0.466	58.5	10.0	7.2	550
	132.9	0.676	91.3	6.0	7.0	530
	150.6	0.648	115.4	6.2	8.0	530
	158.1	0.920	130.7	5.2	8.4	530
	159.4	0.441	80.8	9.8	8.5	530
	160.0	0.985	145.1	5.0	8.5	530
	175.0	0.608	145.8	4.6	9.2	530
	178.4	0.426	97.7	7.0	9.2	520
	201.2	0.903	186.2	2.8	9.3	460

* Calculated as glucose.

[1] Prepared by Standard Brands of Brazil, Inc., São Paulo, Brazil.

TABLE 2

Determination of the kinetic order of the continuous alcoholic fermentation

Agitator Speed	C_i*	$1/\phi$*	$\frac{C_i^{1-n} - C_f^{1-n}}{M} \times 10^{9}$*					$\frac{1}{M} \cdot \lg \frac{C_i}{C_f} \times 10^{12}$*
			$n = -1$	$n = -0.5$	$n = 0$	$n = 0.3$	$n = 0.5$	
rpm								
0	128.7	1.09	141	9.5	0.56	0.092	0.0258	1.95
	132.3	1.02	128	8.5	0.51	0.084	0.0233	1.79
	132.6	1.54	163	10.9	0.65	0.107	0.0288	2.27
	133.9	1.45	170	8.4	0.50	0.081	0.0225	1.72
	162.8	1.10	185	11.0	0.58	0.089	0.0228	1.59
	166.5	2.07	220	13.3	0.72	0.112	0.0288	2.07
	176.2	1.56	252	14.8	0.77	0.118	0.0300	2.05
	188.2	1.14	277	15.0	0.75	0.110	0.0260	1.77
500–530	102.0	0.99	87	6.7	0.45	0.081	0.0235	2.06
	103.5	1.41	86	6.8	0.48	0.169	0.0250	4.13
	105.3	2.13	112	9.7	0.77	0.151	0.0463	4.93
	122.2	2.51	167	13.5	0.99	0.185	0.0550	5.45
	130.0	1.50	114	8.2	0.52	0.089	0.0250	2.08
	130.7	1.02	118	8.1	0.50	0.083	0.0225	1.81
	132.0	2.15	141	10.8	0.74	0.133	0.0388	3.57
	132.9	1.48	156	11.1	0.69	0.118	0.0338	2.73
	150.6	1.54	151	9.9	0.57	0.092	0.0253	1.87
	158.1	1.09	153	9.7	0.53	0.089	0.0228	1.60
	159.4	2.27	192	12.4	0.80	0.134	0.0328	3.00
	160.0	1.02	92	5.6	0.30	0.047	0.0125	0.86
	175.0	1.64	202	11.9	0.63	0.096	0.0245	1.71
	178.4	2.35	317	20.2	1.15	0.184	0.0498	3.73
	201.2	1.11	205	11.2	0.53	0.076	0.0198	1.19

* C_i = initial sugar concentration as glucose, g per L; C_f = sugar concentration as glucose at steady state, g per L; M = yeast concentration at steady state, cells per L; and ϕ = feeding rate, L per hr. n = kinetic order.

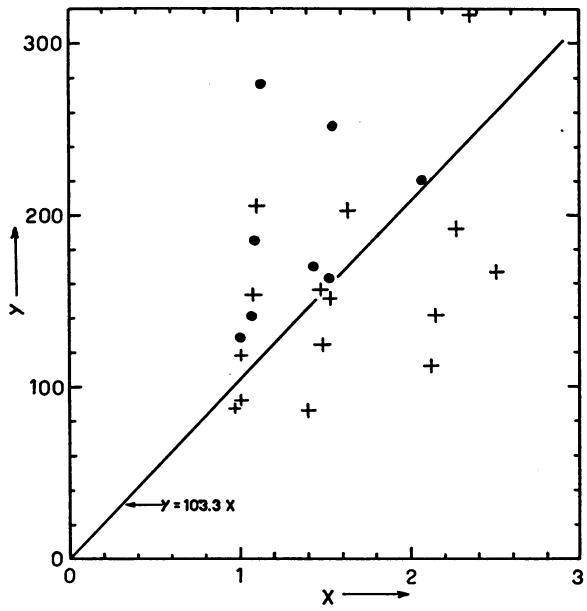

Figure 2. Graphical representation of the fermentation kinetics supposing a kinetic order −1.

$$Y = \frac{C_i^2 - C_f^2}{M} \times 10^9$$

$$X = \frac{1}{\phi}$$

C_i = initial sugar concentration as glucose, g per L.
C_f = sugar concentration as glucose at steady state, g per L.
M = yeast concentration at steady state, cells per L.
ϕ = feeding rate, L per hr.
● = experiments without agitation.
+ = experiments with agitation.

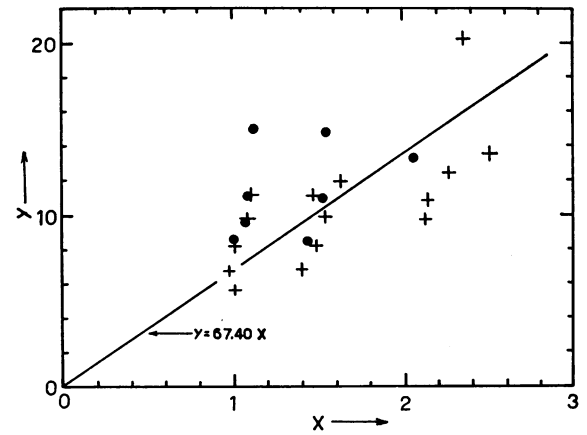

Figure 3. Graphical representation of the fermentation kinetics supposing a kinetic order −0.5.

$$Y = \frac{C_i^{1.5} - C_f^{1.5}}{M} \times 10^9$$

$$X = \frac{1}{\phi}$$

C_i = initial sugar concentration as glucose, g per L.
C_f = sugar concentration as glucose at steady state, g per L.
M = yeast concentration at steady state, cells per L.
ϕ = feeding rate, L per hr.
● = experiments without agitation.
+ = experiments with agitation.

about 0.5 L was incubated at 30 to 32 C until the fermentation was complete. In the experiments with agitation, the agitator was started just before the inoculation.

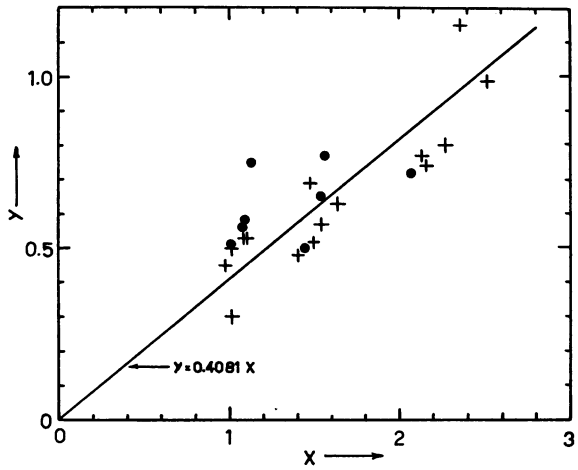

Figure 4. Graphical representation of the fermentation kinetics supposing a kinetic order 0.

$$Y = \frac{C_i - C_f}{M} \times 10^9$$

$$X = \frac{1}{\phi}$$

C_i = initial sugar concentration as glucose, g per L.
C_f = sugar concentration as glucose at steady state, g per L.
M = yeast concentration at steady state, cells per L.
ϕ = feeding rate, L per hr.
● = experiments without agitation.
+ = experiments with agitation.

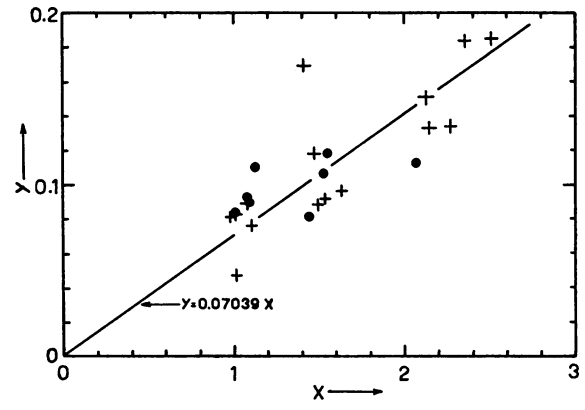

Figure 5. Graphical representation of the fermentation kinetics supposing a kinetic order 0.3.

$$Y = \frac{C_i^{0.7} - C_f^{0.7}}{M} \times 10^9$$

$$X = \frac{1}{\phi}$$

C_i = initial sugar concentration as glucose, g per L.
C_f = sugar concentration as glucose at steady state, g per L.
M = yeast concentration at steady state, cells per L.
ϕ = feeding rate, L per hr.
● = experiments without agitation.
+ = experiments with agitation.

The specific gravity was determined as follows: about 5 ml of the fermenting liquid were rapidly boiled to kill the cells, and then were rapidly cooled; 2.0 ml of the cooled liquid were weighed and the specific gravity was then calculated.

Sugar concentrations (Falcone *et al.*, 1959) of the fresh mash and of the fermenting liquid, alcohol content (Borzani and Falcone, 1952) of the completely fermented medium, and yeast counts of the fermenting liquid by the hemocytometer chamber (White, 1954), were determined.

Results

The experiments were run with two different values of the agitator speed (0 and 500 to 530 rpm), varying the mash sugar concentration (102.0 to 201.2 g per L), and the feeding rate (0.398 to 1.011 L per hr). The results are presented in table 1. The alcohol content of the fermented mash, as well as the fermentation efficiency, were not used in the calculations; these values were determined only to control the goodness of the fermentation.

Discussion

Based on the experimental values presented (table 1) and on equations (5) and (6), the determination of the kinetic order of the process was tried in the following way.

Assume, for instance, based on the known fact that many enzymatic reactions are of zero-order, that the kinetic order of the continuous alcoholic fermentation is *zero*. In this case, equation (5) can be written:

$$\frac{C_i - C_f}{M} = k' \cdot \frac{1}{\phi}$$

The experimental values of C_i, C_f, M, and ϕ (table 1) permit to calculate $(C_i - C_f)/M$ and $1/\phi$ for each continuous fermentation test; plotting $(C_i - C_f)/M$ versus $1/\phi$, a linear function must be obtained if the kinetic order is zero.

Table 2 shows the results obtained applying equations (5) and (6) to the values of table 1, with six different values of n: -1; -0.5; 0; 0.3; 0.5; and 1.

Figures 2 to 7, obtained from table 2, show that the most probable value of the kinetic order of the continuous fermentation lies between 0 and 0.5. Figure 2 shows also that a kinetic order -1, proposed in a previous paper (Borzani, 1957), can not be admitted.

Acknowledgment

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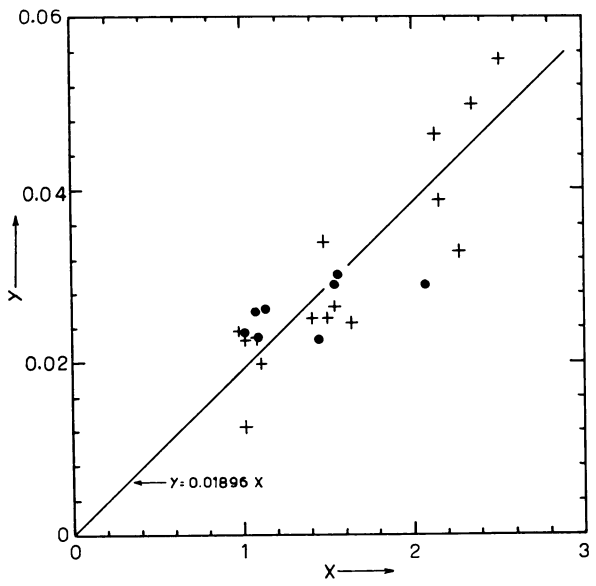

Figure 6. Graphical representation of the fermentation kinetics supposing a kinetic order 0.5.

$$Y = \frac{C_i^{0.5} - C_f^{0.5}}{M} \times 10^9$$

$$X = \frac{1}{\phi}$$

C_i = initial sugar concentration as glucose, g per L.
C_f = sugar concentration as glucose at steady state, g per L.
M = yeast concentration at steady state, cells per L.
ϕ = feeding rate, L per hr.
● = experiments without agitation.
\+ = experiments with agitation.

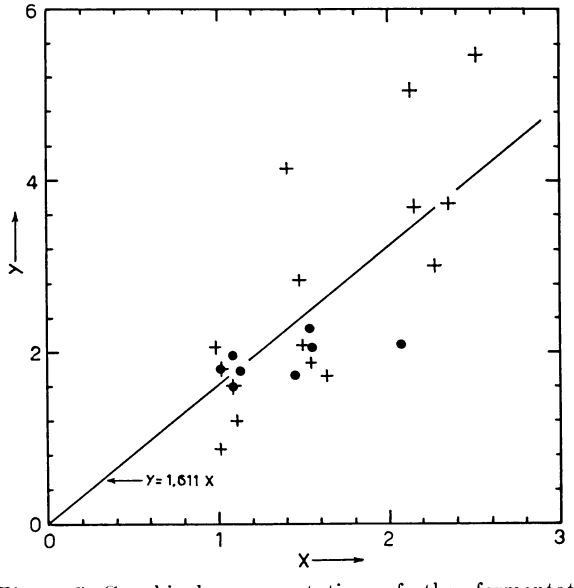

Figure 7. Graphical representation of the fermentation kinetics supposing a kinetic order 1.

$$Y = \frac{1}{M} \times \lg \frac{C_i}{C_f} \times 10^{12}$$

$$X = \frac{1}{\phi}$$

C_i = initial sugar concentration as glucose, g per L.
C_f = sugar concentration as glucose at steady state, g per L.
M = yeast concentration at steady state, cells per L.
ϕ = feeding rate, L per hr.
● = experiments without agitation.
\+ = experiments with agitation.

SUMMARY

Experiments were carried out in an attempt to determine the kinetic order of the continuous alcoholic fermentation of blackstrap molasses, varying the initial sugar concentration, the feeding rate, and the agitator speed. The results obtained lead to the conclusion that the kinetic order of the process is probably 0 to 0.5, and that the value $n = -1$, presented previously (Borzani, 1957), can not be admitted.

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