



**A SURFACE MODEL FOR THE KINETIC STUDY OF HYDROCARBON
UTILIZATION BY YEASTS**

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In spite of the relative limited amount of experimental data regarding the kinetics of microbial growth on hydrocarbons, several mathematical models were recently proposed and successfully tested (Aiba, Haung, Moritz & Someya, 1969; Erickson, Humphrey & Prokop, 1969; Erickson & Humphrey, 1969 a; Erickson & Humphrey, 1969 b). Dunn (1968) suggested a model based on the applicability of the Langmuir's law to the adsorption of cells by the hydrocarbon drops but, as far as we know, such a model was not compared with experimental results.

The purpose of this paper is to present a new surface model in order to interpret the kinetics of pure hydrocarbon consumption by yeast cells.

Basic hypothesis

The model presented here is based on the following fundamental assumptions:

1. The microbial cells are attached at the hydrocarbon drop surface. Free cells will exist only when the drop surface becomes saturated with cells.
2. The specific growth rate of cells attached to hydrocarbon drops is maximum and constant, whatever will be the cell and hydrocarbon concentrations in the fermenting system. The value of that maximum specific growth rate is governed by the other experimental conditions.
3. The specific growth rate of non-attached cells is negligible when compared with the specific growth rate of the cells attached to hydrocarbon drops.
4. The end of the exponential growth phase

is attained when the hydrocarbon drops become saturated with cells.

There are some experimental evidences supporting the above hypothesis number 1 and 3.

In fact, the microscopic examination of several hydrocarbon fermentations during the exponential growth phase show a great number of yeast cells attached at the droplets surface, and a negligible number of free cells. In certain cases it is necessary to add a surfactant (Aiba, Moritz, Someya & Haung, 1969) in order to separate the cells from the hydrocarbon drops. Such experimental observations can be appointed as a reasonable support to the proposed hypothesis number 1.

In a recent paper, Aiba, Moritz, Someya & Haung (1969) presented the results of tests carried out in order to compare the specific growth rates of adsorbed and of non-adsorbed yeast cells. Hydrocarbons fermentations by *Candida guilliermondii* Y-8 were realized in the presence and in the absence of biologically inert surfactants leading to the following values:

a) in the absence of surfactant:
 $\mu \cong 0.14 \text{ h}^{-1}$;

b) in the presence of surfactant:
 $\mu \cong 0.06 \text{ h}^{-1}$.

That is, when the yeast adsorption was not inhibited, the specific growth rate was about 2.5 larger.

As a matter of fact, even in the presence of a surfactant it is impossible to avoid completely the contacts between the yeast cells and the hydrocarbon droplets. The real value of the specific growth rate of non-attach-

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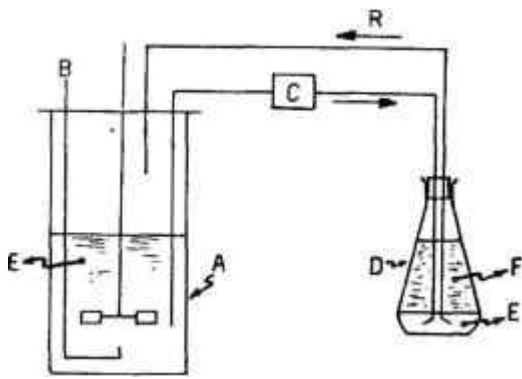


Figure 1. Equipment used for the measurement of the specific growth rate of non-adsorbed cells.

A — 5 liters fermentor containing 2.5 liters of aqueous medium.

B — Air inlet.

C — Pump.

D — 500 ml erlenmeyer flask containing 200 ml of aqueous medium and 200 ml of oil.

E — Aqueous medium.

F — Oil.

R = Aqueous medium rate = 2.5 to 3.0 l/h.

cd cells, then, must be smaller than the above reported figure.

We tried to measure the real specific growth rate of non-adsorbed cells using the equipment represented in Figure 1. Experiments were also carried out in batch fermentation vessels to measure the specific growth rate in the aqueous medium, and in the presence of hydrocarbon drops. The experimental conditions described by Aiba, Moritz, Somaya & Haung (1969), using Diesel oil as

hydrocarbons source, were adopted in all tests. Figure 2 shows the results obtained. It is then possible to state that the specific growth rate of non-attached cells is at least ten times smaller than the specific growth rate of the adsorbed cells. Our hypothesis number 3 then can be accepted.

Otherwise, if the hydrocarbon is the sole growth limiting substrate, the above fundamental assumptions number 2 and 4 seem logical and its consequences will be presented in this paper.

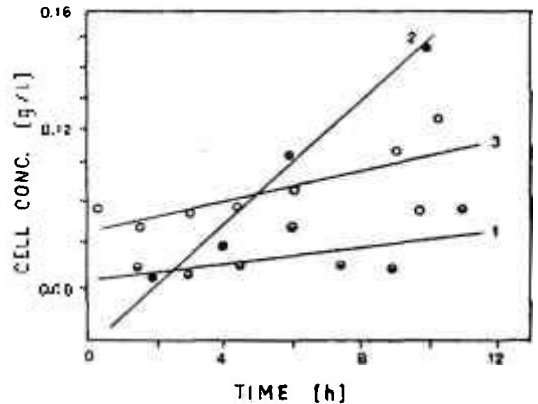


Figure 2. Propagation of yeast cells with bench-scale fermentor.

Curve 1 — Aqueous medium ($\mu = 0.012 \text{ h}^{-1}$).

Curve 2 — Aqueous medium and oil drops ($\mu = 0.081 \text{ h}^{-1}$).

Curve 3 — Aqueous medium and oil, using the equipment represented in Figure 1 ($\mu = 0.018 \text{ h}^{-1}$).

The surface kinetic model

The following nomenclature is adopted:

- a = Drop surface area covered by cells per unit volume, L^{-1} .
- a_0 = Initial a value, L^{-1} .
- A = Interfacial area per unit volume, L^{-1} .
- d = Volume-surface mean diameter of drop, L .
- K = Sec equation (2), dimensionless.
- K_{av} = Average K value, dimensionless.
- m = Average mass (dry matter) of a single cell, M .
- n = Number of cells per unit volume, L^{-3} .
- n_0 = Initial n value, L^{-3} .
- n_s = Number of cells per unit volume when drop surface becomes saturated with cells, L^{-3} .
- n' = Number of adsorbed cells per unit volume when $t > t_s$, L^{-3} .
- n'' = Number of cells per unit volume produced from time t_s to time t , L^{-3} .
- N = Number of hydrocarbon drops per unit volume, L^{-3} .
- r = Radius of spherical cell, L .
- R = Radius of drop, L .
- R_0 = Initial R value, L .
- S = Substrate concentration, ML^{-3} .
- S_0 = Initial S value, ML^{-3} .
- S_s = Substrate concentration when drop surface becomes saturated with cells, ML^{-3} .
- t = Time, T .

- t_s = Time at which drop surface becomes saturated with cells = Exponential growth phase duration, T.
 t_h = Time for total hydrocarbon consumption, T.
 X = Cell concentration (dry matter), ML^{-3} .
 X_0 = Initial X value, ML^{-3} .
 X_s = Cell concentration when drop surface becomes saturated with cells, ML^{-3} .
 X' = Concentration of adsorbed cells when $t > t_s$, ML^{-3} .
 Y = Yield in cell mass (dry matter) per unit mass of substrate, dimensionless.
 Δ = R/r , dimensionless.
 λ = See equation (18), $L^{-3/2} T^{-1}$.
 μ = Specific growth rate, T^{-1} .
 ρ = Substrate density, ML^{-3} .
 ω = Area of drop surface covered by a single spherical cell, L^2 .
 ω_{av} = Average ω value, L^2 .

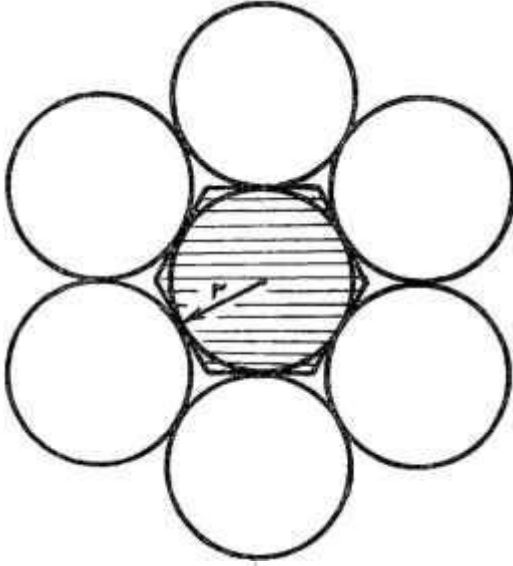


Fig. 3. Spherical cells packed on a plane surface.

In the following considerations $t=0$ is the moment at which the exponential growth phase begins.

If the yeast cells are packed on a plane surface, the area "covered" by each cell will be $2\sqrt{3}r^2$ (Figure 3). When the cell is attached to a drop surface, the area "covered" by the cell can be calculated by:

$$\omega = 12 \Delta^2 r^2 \left[\frac{\pi}{6} - \text{arc sin} \frac{\sqrt{1 - \left(\frac{1}{\Delta - 1}\right)^2}}{2} \right] = K r^2 \quad (1)$$

where

$$K = 12 \Delta^2 \left[\frac{\pi}{6} - \text{arc sin} \frac{\sqrt{1 - \left(\frac{1}{\Delta - 1}\right)^2}}{2} \right] \quad (2)$$

Figure 4 shows that equation (2) can be substituted by a more simple one:

$$K = 2\sqrt{3} \frac{\Delta}{2.5 + \Delta} \quad (3)$$

When the drop radius varies from R_0 to zero, equation (3) permits to calculate the average value of K :

$$K_{av} = \frac{2\sqrt{3}}{R_0} \left[R_0 - 2.5r \ln \left(1 + \frac{R_0}{2.5r} \right) \right] \quad (4)$$

and then we can calculate the average value of the area "covered" by a cell when the drop radius varies from R_0 to zero:

$$\omega_{av} = K_{av} \cdot r^2 \quad (5)$$

Let us consider the system at a time $t < t_s$. We can write:

$$X = X_0 \cdot e^{\mu t} \text{ or } n = n_0 \cdot e^{\mu t} \quad (6)$$

and

$$S = S_0 - \frac{X_0}{Y} (e^{\mu t} - 1) \quad (7)$$

But the interfacial area A (per unit volume) is given by:

$$A = \left(\frac{36 \pi N}{\rho^2} \right)^{1/3} S^{2/3} \quad (8)$$

then:

$$A = \left(\frac{36 \pi N}{\rho^2} \right)^{1/3} \left[S_0 - \frac{X_0}{Y} (e^{\mu t} - 1) \right]^{2/3} \quad (9)$$

It is convenient to emphasize, at this point, that the drop radius calculated by

$$R = \left(\frac{3}{4 \pi \rho N} \right)^{1/3} S^{1/3}$$

is equal to $d/2$.

Otherwise, the area of drop surface (per unit volume) "covered" by the cells at time t , is:

$$a = K_{av} \cdot r^2 n_0 e^{\mu t} \quad (10)$$

When the drop surface becomes saturated with cells at time t_s , equations (9) and (10) permit us to write:

$$\left(\frac{36 \pi N}{\rho^2} \right)^{1/3} \left[S_0 - \frac{X_0}{Y} (e^{\mu t_s} - 1) \right]^{2/3} = K_{av} \cdot r^2 n_0 e^{\mu t_s} \quad (11)$$

It is then possible to calculate the time t_s at which drop oil surface becomes saturated with cells. According to our hypothesis number 3, the value of t_s must be equal to the exponential growth phase duration.

When $t = t_s$, the values of X_s , n_s and S_s are:

$$X_s = X_0 e^{\mu t_s}$$

$$n_s = n_0 e^{\mu t_s}$$

$$S_s = S_0 - \frac{X_0}{Y} (e^{\mu t_s} - 1)$$

Let us consider now the system at a time $t > t_s$:

$$\frac{dX'}{dt} = \mu X' \therefore dX' = \mu X' \cdot dt$$

where X' is the adsorbed cell concentration, corresponding to a number of cells per unit volume equal to n' . The cell production dX' is a consequence of a substrate consumption dS :

$$dS = -\frac{dX'}{Y} = -\frac{\mu X'}{Y} dt = -\frac{\mu m n'}{Y} dt \quad (12)$$

this oil consumption leads to a decrease of interfacial area calculated from equations (8) and (12):

$$dA = -\left(\frac{32 \pi N}{3 \rho^2}\right)^{1/3} \frac{\mu m}{Y} S^{-1/3} n' \cdot dt \quad (13)$$

But the drop surface is always saturated with cells when $t > t_s$. It is then possible to state:

$$K_{av} \cdot r^2 n' = \left(\frac{36 \pi N}{\rho^2}\right)^{1/3} S^{2/3} \therefore$$

$$\therefore S^{-1/3} = \left[\frac{K_{av} \cdot r^2}{\left(\frac{36 \pi N}{\rho^2}\right)^{1/3}} \right]^{-1/2} (n')^{-1/2} \quad (14)$$

Equations (13) and (14) give:

$$dA = -\left(\frac{32 \pi N}{3 \rho^2}\right)^{1/3} \frac{\mu m}{Y} \left[\frac{K_{av} \cdot r^2}{\left(\frac{36 \pi N}{\rho^2}\right)^{1/3}} \right]^{-1/2} (n')^{1/2} \cdot dt \quad (15)$$

Due to the interfacial area decrease dA , a certain number of cells, dn' , will be expelled from the drop surface:

$$K_{av} \cdot r^2 \cdot dn' = dA \quad (16)$$

From equations (15) and (16) we can establish:

$$dn' = -\lambda (n')^{1/2} \cdot dt \quad (17)$$

where

$$\lambda = \frac{1}{K_{av} \cdot r^2} \left(\frac{32 \pi N}{3 \rho^2}\right)^{1/3} \frac{\mu m}{Y} \left[\frac{K_{av} \cdot r^2}{\left(\frac{36 \pi N}{\rho^2}\right)^{1/3}} \right]^{-1/2} \quad (18)$$

Equation (17) permits to calculate the number of cells (per unit volume) attached to the drop surface at a time $t > t_s$. In fact:

$$\int_{n_s}^{n'} \frac{dn'}{(n')^{1/2}} = -\lambda \int_{t_s}^t dt \therefore$$

$$\therefore \sqrt{n'} = \sqrt{n_s} - \frac{\lambda}{2} (t - t_s) \quad (19)$$

It is possible, from equation (19), to evaluate the time for the total hydrocarbon consumption, that is, the time when $n' = \text{zero}$:

$$t_k = t_s + \frac{2 \sqrt{n_s}}{\lambda} \quad (20)$$

Let us calculate now the total number of cells (per unit volume) at time $t > t_s$. Such a number of cells is equal to the sum of the number of cells at the saturation moment (n_s) plus the number of cells produced from time t_s to time t .

According to our basic assumption, the following equation can be stated:

$$dn = \mu n' \cdot dt = \mu \left[\sqrt{n_s} - \frac{\lambda}{2} (t - t_s) \right]^2 \cdot dt \quad (21)$$

relating n and t , when $t > t_s$. Calling n' the number of cells (per unit volume) produced from time t_s to time t , equation (21) gives:

$$n'' = \mu \left[n_s (t - t_s) + \frac{\lambda^2}{12} (t - t_s)^3 - \frac{\lambda \sqrt{n_s}}{2} (t - t_s)^2 \right] \quad (22)$$

Then, the total number of cells per unit volume at time $t > t_s$ will be:

$$n = n_s + \mu \left[n_s (t - t_s) + \frac{\lambda^2}{12} (t - t_s)^3 - \frac{\lambda \sqrt{n_s}}{2} (t - t_s)^2 \right] \quad (23)$$

In a similar manner, it is possible to establish the correlation between the substrate concentration S and time t , when $t > t_s$:

$$S = S_s - \frac{\mu m}{Y} \left[n_s (t - t_s) + \frac{\lambda^2}{12} (t - t_s)^3 - \frac{\lambda \sqrt{n_s}}{2} (t - t_s)^2 \right] \quad (24)$$

Results and discussion

Figure 5 shows the curves relating the exponential phase duration (t_s) with the initial substrate concentration (S_0) for different values of the initial drop radius (R_0), calculated from equation (11) assuming $X_0 = 0.1$ g/l, $\rho = 0.76$ g/ml, $Y = 0.75$, and $\mu = 0.15$ h⁻¹ (experimental conditions described by Aiba, Moritz, Someya & Haung, 1969). The values of r (1.5×10^{-3} mm) and n/X (2.0×10^{11} cells/gram of dry matter) were measured in our laboratory from a culture of *Candida guilliermondii* Y-8. Figure 5 shows also that the model presented here is in good agreement

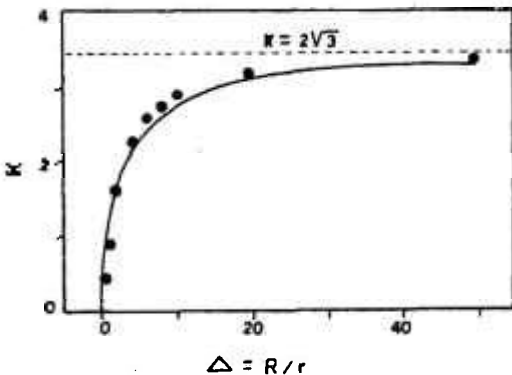


Figure 4. Comparison between the exact values of K calculated by equation (2) (points ●) and the approximate curve represented by equation (3).

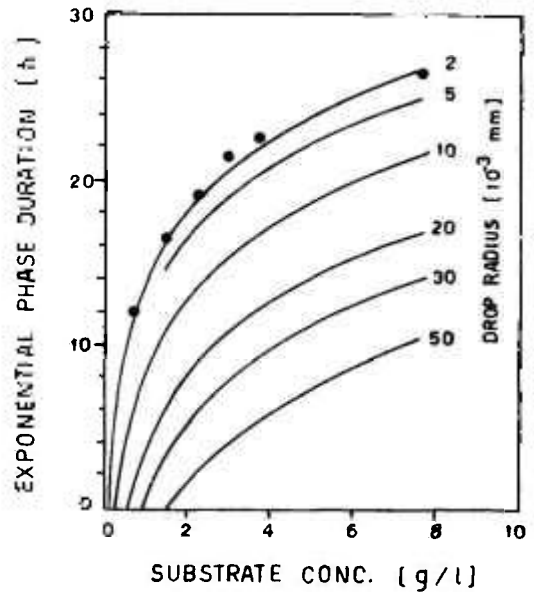


Figure 5. Comparison of data of Aiba, Moritz, Someya & Haung (1969) with the proposed model.

with the results presented by Aiba, Moritz, Someya & Haung (1969) for bench-scale fermentor tests, assuming $R_0 = 2.0 \times 10^{-3}$ mm. It must be emphasized, at this point, that according the results presented by Aiba, Haung, Moritz & Someya (1969), R_0 values varying from 2.5×10^{-3} mm to 5×10^{-2} mm were found; an average value of $2.0 \times$

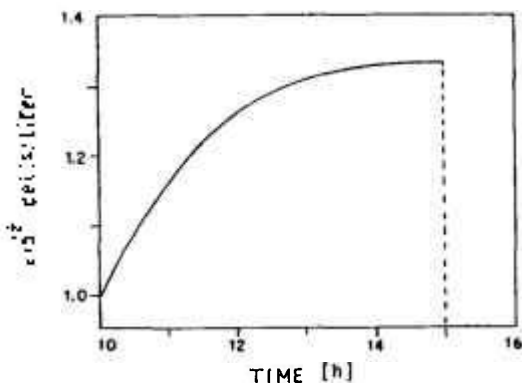


Figure 6. Batch growth curve after the exponential phase, assuming:
 $n_s = 1.0 \times 10^{12}$ cells/liter;
 $\mu = 0.20 \text{ h}^{-1}$;
 $\lambda = 0.4 \times 10^6 \text{ l}^{-1/2} \text{ h}^{-1}$;
 $t_s = 10 \text{ h}$;
 $t_h = 15 \text{ h}$ (calculated from the above values).

$\times 10^{-3}$ mm was never obtained. As a matter of fact, the droplets diameter measurements carried out by Aiba, Haung, Moritz & Someya (1969) did not avoid the drops coalescence, leading very probably to average values higher than the real average drop diameter. This fact can explain the agreement of the experimental points represented in Figure 5 with the theoretical curve obtained assuming $R_o = 2.0 \times 10^{-3}$ mm.

Otherwise, the growth curves obtained by Aiba, Moritz, Someya & Haung (1969) in bench-scale fermentor experiments, show that at the end of the exponential phase the hydrocarbon concentrations were practically

zero, that is, the difference $t_h - t_s$ must be negligible when compared with t_s . The differences $t_h - t_s$ were calculated from equation (20) in two cases, leading to the following results, in very good agreement with the experimental data:

1st case ($S_o = 0.76 \text{ g/l}$ or 0.10 vol. \% ;
 $t_s \cong 12 \text{ h}$):

$$t_h - t_s = 0.00008 \text{ h}$$

2nd case ($S_o = 7.6 \text{ g/l}$ or 1.0 vol. \% ;
 $t_s \cong 36.5 \text{ h}$):

$$t_h - t_s = 0.0001 \text{ h}$$

In order to show the aspect of the growth curve after the exponential phase, equation (23) is represented in Figure 6 assuming $n_s = 1.0 \times 10^{12}$ cells/liter, $\mu = 0.20 \text{ h}^{-1}$, $t_s = 10 \text{ h}$, and $\lambda = 0.4 \times 10^6 \text{ l}^{-1/2} \text{ h}^{-1}$.

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ABSTRACT

A mathematical model for the kinetic study of pure liquid hydrocarbon utilization by yeasts is proposed based on the following assumptions:

1. The microbial cells are adsorbed by the hydrocarbon drops.
2. The specific growth rate of adsorbed cells is maximum and constant.
3. The specific growth rate of non-adsorbed cells is negligible.
4. The end of the exponential growth phase is attained when the hydrocarbon drops become saturated with cells.

and assuming also that the hydrocarbon drops have the same diameter and their number is constant during the fermentation. A good agreement of the proposed model with experimental results presented by Aiba et al. (J.Ferm.Technol., 47, 203, 1969) was observed.

RESUMO

Apresenta-se um modelo matemático para o estudo da cinética de utilização de hidrocarbonetos líquidos puros por leveduras, baseado nas seguintes hipóteses:

1. As células são adsorvidas pelas gotas de hidrocarboneto.
2. A velocidade específica de reprodução das células adsorvidas é máxima e constante.
3. A velocidade específica de reprodução das células não adsorvidas é desprezível.
4. A fase exponencial de reprodução termina no momento em que as gotas de hidrocarboneto se saturam com células.

e admitindo ainda que as gotas têm mesmo diâmetro e que seu número é constante durante a fermentação. A aplicabilidade do modelo foi verificada com valores experimentais obtidos por Aiba e colaboradores (J.Ferm.Technol., 47, 203, 1969).

RÉSUMÉ

On présente dans cet article un modèle mathématique pour la cinétique de l'utilisation des hydrocarbures liquides pures par les levures, ayant comme point de départ les hypothèses suivantes:

1. Les cellules sont adsorbées par les gouttes d'hydrocarbure.
2. La vitesse spécifique de reproduction des cellules adsorbées est maximale et constante.
3. La vitesse spécifique de reproduction des cellules non adsorbées est négligeable.
4. La phase exponentielle de reproduction fini au moment où les gouttes d'hydrocarbure sont saturées avec les cellules.

dans les hypothèses considérées ci-dessus on admet encore que les gouttes ont le même diamètre et que leur nombre est constant pendant le procès de fermentation. La viabilité du modèle a été vérifiée avec les valeurs expérimentales obtenues par Aiba et collaborateurs (J.Ferm.Technol., 47, 203, 1969).