Microbial degradation kinetics of solid alkane dissolved in nondegradable oil phase

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Abstract

An *Acinetobacter* species was isolated and found to be able to grow on crude oil *n*-alkanes and solid alkanes at room temperature as the sole carbon source. The growth of the isolate on *n*-heneicosane dissolved in non-biodegradable pristane has been studied. A kinetic model of the growth of microorganism on the hydrophobic substrate dissolved in non-biodegradable oil droplet assuming direct contact of cell with oil droplet was developed and validated with a model system of crude oil biodegradation. The model was focused on the substrate transport to the cell being contact with the surface of droplet. The high value of saturation constant of *n*-heneicosane, $K_s = 0.086 \text{ kg m}^{-3}$, and the maximum specific growth rate, $\mu_{\text{max}} = 0.60 \text{ h}^{-1}$, were obtained. The transport limitation was considered and estimated. The high value of attached cell fraction was reasonable to explain the observed growth rate by the direct contact model and varied with time till it reached a plateau at the stationary growth phase. By considering the direct contact of the cells with the surface of pristane and the transport of *n*-heneicosane to the cell, the degradation of hydrophobic substrate in the oil phase could be elucidated. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Crude oil *n*-alkane; Biodegradation; Kinetic model; *Acinetobacter*

1. Introduction

Crude oil hydrocarbons are highly hydrophobic materials that can hardly be degraded or decomposed due to their poor availability for microorganisms [1]. Bioavailability of a chemical during bioremediation is determined by the rate of mass transfer relative to the intrinsic activity of microbial cells [2].

Different microbial activity to hydrophobic compounds can provoke the different degradation mechanisms, and vice versa. Three mechanisms by which hydrocarbons transported to microbial cells are generally considered [3]: (1) interaction of cells with hydrocarbons dissolved in the aqueous phase, (2) direct contact of cells with hydrocarbon drops (or particles) considerably larger than the cells, and (3) interaction of cells with solubilized, pseudosolubilized, or accommodated hydrocarbon in entities much smaller than the cells. The first case is generally thought to be incapable of supporting the observed rate of growth of microorganism in the degradation of long-chain alkanes due to low solubility. In the second case, microbial cells attach to the surface of oil drops and uptake the hydrocarbons presumably through diffusion or active transport. In this case, the available surface area of oil drops for cell attachment and the hydrophobicity of cell surface would be the critical factors since the only attached microorganisms are responsible for the degradation of hydrocarbons [4]. In the third case, hydrocarbon particles may attach to the whole surface of microorganism and can be taken up. Solubilization capacity of microorganism would have an important role in degradation.

However, when considering the bioremediation of crude oil contaminated area, neither the high solubility of crude oil components accounts for high aqueous concentration nor the abundant microbial population responsible for solubilization can be expected in environment, where the oil components are trapped in soil particles or floated in aqueous phase [5]. In such a condition, the solubilization effect of hydrocarbons may be restrained by their existence in oil matrix and may be also dependent on the attachment of microorganisms to oil surface. Thus the direct contact of cells with the surface of oil is thought to be important in the bioremediation of contaminated area with crude oil.
From such a point of view, a nonvolatile insoluble hydrocarbon was selected as a component of crude oil, and a nondegradable oil was introduced as a crude oil matrix to explicate microbial degradation of crude oil components. A kinetic model of the growth of microorganism on the hydrophobic substrate dissolved in the oil assuming direct contact of cells with oil phase was presented and experimental data were analyzed in terms of the model.

2. Materials and methods

2.1. Culture media

Mineral salts medium (MSM) [6] was used as a basal medium for the study of specified carbon source. For the screening of oil degraders, crude oil medium supplemented with nonionic surfactant was used. It contained 0.5% (v/v) Arabian light crude oil and 0.5% (v/v) Triton X-100 in MSM. Crude oil-agar was also prepared as solid medium which contained 1% (v/v) of crude oil, 0.24 mM of Triton X-100, and 1.5% (w/v) of agar in MSM. Luria–Bertani (LB) medium and LB agar were used for the preparation of inoculum and for the counting of colony forming unit (CFU), respectively.

2.2. Isolation and identification

After removing coarse particles from activated sludge, 100 μl of broth was inoculated in 100 ml of crude oil medium supplemented with nonionic surfactant. It was added 0.5% (v/v) Triton X-100 in MSM. Crude oil-agar was also prepared as solid medium which contained 1% (v/v) of crude oil, 0.24 mM of Triton X-100, and 1.5% (w/v) of agar in MSM. Luria–Bertani (LB) medium and LB agar were used for the preparation of inoculum and for the counting of colony forming unit (CFU), respectively.

2.3. Biodegradation of model oil

Biodegradation of model oil by the isolate was conducted using n-heneicosane as the sole source of carbon and energy and pristane as oil droplet phase. Each 1000 ml of MSM in the fermentor was supplemented with 10 ml of pristane carrying the desired amount of heneicosane, and autoclaved at 121°C for 15 min. For the preparation of inoculum, cells were harvested after a two-step precultivation in LB medium by centrifugation at 3000 x g for 10 min and washed twice using MSM. Each 10 ml of cell suspension in the MSM was used as inoculum. The rotation speed of dual impellers was kept constant at 600 rpm. Air was supplied to the culture through a 0.22 μm filter unit at 0.12 m³ h⁻¹. The temperature of the fermentor was kept at 27°C.

The cultivation was conducted for the additions of 25, 50, and 100 kg heneicosane per cubic meter of pristane in MSM. The cultivation for the pristane with no addition of heneicosane was also conducted as control.

2.4. Measurements

Microbial growth was measured as the number of viable cells by counting CFU. In case of model oil experiment, a 1 ml sample was added by Triton X-100 (final concentration of ca. 2%) to detach adhered cells from pristane. The number of free cells were measured from the precipitate after centrifugation at 5000 x g for 6 min, followed by the addition of MSM for suspension and of Triton X-100 for the same operation with total cells. The number of attached cells were calculated by subtracting the number of free cells from the number of total cells.

Hydrocarbons were analyzed by capillary column (0.25 mm in diameter and 50 m in length) gas chromatography (GC-17A, Shimadzu, Japan) equipped with flame ionization detector (FID). The column temperature was set at 150°C for 5 min and increased to 300°C by 10°C min⁻¹ and kept for 30 min. The temperatures of injector and FID were 320°C and 340°C, respectively. Helium was used as carrier gas at a flow rate of 1.5 ml min⁻¹.

3. Kinetic analysis

It is considered that the substrate dissolved in the oil phase is so hydrophobic that the substrate concentration in the aqueous phase is negligible. Microbial growth, therefore, can be expected only on the surface of the oil drop (Fig. 1). Since only attached microorganisms can uptake the substrate, microbial growth can be expressed in terms of the number of cells. Assuming that all the cells tend to adhere to the oil surface and there is no limit to the available surface area of oil drop, we can write the growth rate and the substrate uptake rate over an oil drop as follows:

\[
\frac{dn}{dt} = \mu n \\
\frac{ds}{dt} = \nu n
\]

(1)

(2)

If \( n \) reaches its limit value, where no more drop surface for attachment is available, it cannot increase any more, thus \( n \) in Eq. (1) is constant and \( \frac{dn}{dt} \) equals zero. Once the homogeneous reaction volume is expected with the assump-
tion of uniform distribution of oil drops by the complete mixing, any substrate consumption over the whole reactor volume can be supposed to attribute to the proliferation of the cells. From this, it is possible to consider the specific rate of growth, \( r \), and that of substrate uptake, \( s \), over the whole volume of reactor which are substituted with \( \frac{dN}{dt} \) and \( \frac{dS}{dt} \), respectively.

\[
\frac{dN}{dt} = \mu N \tag{3}
\]

\[
\frac{dS}{dt} = \nu N \tag{4}
\]

If the yield is constant during the culture and defined as

\[
Y = \frac{N - N_0}{S - S_0} \tag{5}
\]

then, Eqs. (3) and (4) are combined using Eq. (5) to get

\[
\frac{dN}{dt} = Y \frac{dS}{dt} \tag{6}
\]

3.1. Biological reaction control

When the substrate uptake rate and microbial growth rate are wholly dependent on the microbial reaction rate, application of Monod equation in Eq. (3) gives

\[
\frac{dN}{dt} = \frac{\mu_m S N}{K_s + S} \tag{7}
\]

When \( N \) does not reach its limit value, arranging Eqs. (3) and (4) using Eqs. (5)–(7) gives

\[
\frac{dN}{dt} = \frac{\mu_m N(N_0 - N + S_0 Y)}{N_0 - N + Y(K_s + S_0)} \tag{8}
\]

and

\[
\frac{dS}{dt} = \frac{\mu_m S(S - S_0 - N_0/Y)}{K_s + S} \tag{9}
\]

3.2. Transport control

Eqs. (8) and (9) are only available in the range of \( 0 \leq t \leq t_c \). The critical time, \( t_c \), can be obtained from the point where the microbial reaction rate of substrate consumption equals the transport rate of substrate to the microbes. If the \( t_c \) is infinite, the microbial reaction rate is entirely responsible for the degradation of substrate in oil phase. When the microbial reaction rate exceeds the transport rate, Eqs. (8) and (9) are no more available.

In such a condition, the rate of substrate uptake by microorganism is defined by mass transfer considerations. Let us assume the uniform substrate concentration in bulk oil drop at the start of culture. The difference in substrate concentration between the oil surface and the inner membrane of cell \( S_c \) is thought to be the driving force of transport through the cell wall. And the substrate concentration of inner membrane is in equilibrium with substrate concentration at inner membrane adjacent to the cell wall, as shown in Fig. 2. Since the concentration of substrate in the aqueous phase is negligible, only the rate of transport of substrate from the oil phase to the surface of the microorganism is to be considered. Thus, the available area of transport will be confined to the projection area of attached cells. Introducing proportionality, \( \alpha \), is necessary since the whole projection area of the attached cells cannot serve for transport. The contact area between the surfaces of oil and cell is related with the geometry of the surfaces of oil and cells and with the conformational factor of the arrangement of cells. With these assumptions, we can write the transport of substrate from the oil phase to the cell,
\[
\frac{ds}{dt} = J_s \alpha n
\]
and the mass flux to the cell is given by
\[
J_s = k_L(s - s'_c) = k_L(s - s_c)
\]
Replacing Eq. (11) into Eq. (10) and rearranging over the whole reactor gives
\[
\frac{dS}{dt} = -k_L \alpha A_e N(S - S_c)
\]
Thus we finally get
\[
\frac{dS}{dt} = -k_L \alpha A_e (S - S_c) \{N_0 + Y(S_0 - S)\}
\]
and
\[
\frac{dN}{dt} = k_L \alpha A_e N \{N_0 - N + Y(S_0 - S_c)\}
\]
Eqs. (13) and (14) describe the degradation rate and growth rate under the transport limitation, respectively. They are valid only in the range of \(t \geq t_c\), with the initial condition of \(dS/dt\) reaction = \(dS/dt\) transport = 0.

4. Results and discussion

4.1. Characteristics of the isolated microorganism

Eight bacteria which could grow on crude oil hydrocarbons were isolated from the activated sludge of a municipal waste water treatment plant and two of them were found to degrade hydrocarbons of Arabian light crude oil at high percentage after three days of culture. Finally, a Gram-negative type coccoid bacillus, which showed such a high degradability after one day of culture, was isolated. The isolate was identified tentatively as belonging to the genus *Acinetobacter*. The isolated *Acinetobacter* spp. grew on crude oil of the initial concentration of 0.1% (v/v) and degraded n-alkanes from C13 to C30 simultaneously (data not shown). With increasing number of carbons, their degradation rate decreased. No difference of the degradation rate of the n-alkanes between odd and even numbered carbons was observed. No detectable accumulations of intermediates were observed during cultivation.

The chromatogram of crude oil components before and after cultivation is shown in Fig. 3. The remaining three large peaks in Fig. 3(b) after 20 h of culture in the range of 10–15 min of retention time did not correspond to n-alkanes. These three peaks are likely to be branched hydrocarbons or aromatics. The third one was referred to as pristane, from the comparison of the retention time of pristane. The isolate could grow on each n-alkane, not only on the liquid alkanes but also on the solid alkanes at room temperature, as the sole carbon source with no detectable accumulation of a shorter n-alkane, while it failed to grow on aromatic compounds. Though branched alkanes are highly persistent to microorganisms, long chain of branched alkane can be degraded [8]. On the basis of the result in Fig. 3, where many unidentified components other than n-alkanes were degraded, the isolate was thought to degrade the long chain of the branched hydrocarbons. However, pristane was persistent.

4.2. Examination of experimental model system

To predict microbial growth and substrate degradation by the direct contact of cells with hydrophobic oil drops, model parameters were obtained from the experiment using fermentor, in which the complete mixing was expected by adopting dual impellers with a revolution speed of 600 rpm. n-Heneicosane and pristane were used as sole carbon source and dispersed oil phase, respectively. The attachment of microbial cells to the surface of pristane drop was observed microscopically during cultivation (data were not shown).

Heneicosane was selected as a representative hydrocarbon for its stability as a solid at room temperature and insolubility in water. It could be degraded by the pure culture of the isolate as a sole carbon and energy source despite of its existence as solid particles during cultivation (data not shown). This fact revealed that the successful degradation of solid particles could be achieved by the direct contact of cells on the surface of particles. Such solid particles dissolved in oil droplet can represent water-insoluble oil contamination in environment.

Pristane was a nondegradable hydrocarbon to the *Acinetobacter* sp. and was able to dissolve the desired amount of heneicosane. Used pristane had ca. 2% of impurities, and one of the major impurities was identified as heneicosane. Fig. 4 shows the gas chromatogram of the three major impurities of pristane and their variations with time in the cultivation on 1% (v/v) of pristane in MSM without...
the addition of a carbon source. One of them, identified as heneicosane, was degraded, while another two components were persistent till the end of cultivation. The concentration of heneicosane existing as an impurity in the pristane was quantitated as 5.0 kg m$^{-3}$ pristane. This value was considered as a background substrate in the cultivation.

The microscopic observation estimated the oil droplet size in the fermentor tentatively as in the range of 10–20 μm in diameter (data were not shown), though the size distribution and its effect on the degradation rate remained to be studied. The portion of oil phase, pristane, which contained desired amount of heneicosane in the system was set as 1% (v/v). This value was selected from the consideration of available maximum value, where the oil phase could be dispersed stably fully enough to confirm that there exists no coalescence of the oil drops [9]. Thus, this volume fraction of 1% of oil was thought to be rational to maintain hydrodynamic constant interfacial area between oil and aqueous phase.

4.3. Parameter estimation from the experimental data

Fig. 5 gives the Lineweaver–Burk plot of the Eq. (7) from the experimental data in early stage of growth. Linearity of plot was obtained as a correlation coefficient, $r^2 = 0.97$. The values of the saturation constant, $K_s = 0.086$ kg m$^{-3}$ ($=8.6$ kg heneicosane m$^{-3}$ pristane), and the maximum growth rate, $\mu_m = 0.60$ h$^{-1}$, were obtained. The yield was obtained as $2.7 \times 10^{15}$ cell kg$^{-1}$. With these, estimated parameters are shown in Table 1. It should be noticed that an exceptionally large value of $K_s$ was observed. However, the present approach allowed a prediction that a larger value was to be expected, because the effective area-to-substrate of the cells would be much smaller than that for dissolved substrates in bulk water. Merchuk and Asenjo [10] reported the cases of such high values with the considerations of mass transfer. Verkooyen and Rietema [11] reported nearly the same order of the $K_s$ value as in this study for $n$-alkane as 15 kg m$^{-3}$ oil from the cultivation of yeast on light Iranian crude oil. Thus, it was concluded that the model oil system

<table>
<thead>
<tr>
<th>$\mu_m$ (h$^{-1}$)</th>
<th>$K_s$ (kg m$^{-3}$)</th>
<th>$Y$ (cell kg$^{-1}$)</th>
<th>$A_c$ (m$^2$ cell$^{-1}$)</th>
<th>$k_L$ (m h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_0 = 0.30$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_0 = 0.55$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_0 = 1.0^b$</td>
<td></td>
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</tbody>
</table>

$^a \pi d^2/4$, diameter of cell, $d = 0.7$ μm.

$^b$ Total concentration of heneicosane including the heneicosane contained in the used pristane as an impurity, kg m$^{-3}$.

Fig. 4. Chromatogram of impurities of used pristane and their variations with time by the culture of Acinetobacter sp.: 1000 ml of mineral salts medium in fermentor was supplemented with 10 ml of pristane with no addition of other carbon source.

Fig. 5. Lineweaver–Burk plot for the determination of model parameters: $\mu_m = 0.60$ h$^{-1}$; $K_s = 0.086$ kg m$^{-3}$ ($=8.6$ kg heneicosane m$^{-3}$ pristane); correlation coefficient, $r^2 = 0.97$. 


Table 1
Parameters estimated from the degradation of heneicosane
in this study, composed of heneicosane and pristane, was valid enough to predict microbial growth and substrate degradation on crude oil.

However, the theoretical growth curve based on this initial growth rate exceeded the measured data. This inferred the existence of the rate limit switching, from reaction control to transport control. The increase of cells in the system might inhibit the further adherence of cells on oil surface. Assuming the constant property of cell surface, we can suppose the decrease of attached cell fraction with the decrease of the available surface area of oil phase during cultivation. The change of the ratio of adhered cells to total cells is shown in Fig. 6. The attached cell fraction was monitored by sufficient centrifugal force where the free cells might be harvested completely. As predicted, the attached cell fraction decreased with growth, though the absolute number of attached cells was in the increase. The collision of cells with reduced available oil surface and the collision between the cells were thought to lower the attached cell fraction. The plateau of attached cell fraction observed in the stationary phase of growth was due to the shortage of substrate or oil surface. However, it would be generally considered that the value of cell fraction responsible for the actual contact with the oil drops might exceed that of attached cell fraction measured by centrifugation, where the weakly or partially attached cells would be liberated from the oil surface. Thus, the capability of attachment of the cells to oil surface was enough to support the degradation of oil components by direct contact of cell.

The permeability coefficient, \( k_L \), and the proportionality, \( \alpha \), could not be measured directly, owing to indefinable and variable property of the interface between the surface of attached cells and oil drops. Instead of separated values of \( k_L \) and \( \alpha \), the value of \( k_L \alpha \), was estimated for the different initial substrate concentrations (Table 1). These values were about one order lower than those reported from the soluble substrate in aqueous phase [12]. The proportionality, \( \alpha \), was considered to be inversely proportional to the existing cell number and to be able to have an appropriate average value in the range of \( 0 < \alpha < 1 \) over the time during transport controlled growth.

4.4. Evaluation of the hydrocarbon degradation model

Fig. 7 showed the results of predicted microbial growth and substrate degradation with experimental data, using the parameters obtained from the above results. The model prediction was in good agreement with the experimental data. The pecked line and dash-dotted line in each plot represent the result of biological reaction control and mass transfer control, respectively. The initial exponential growth rate was varied slightly with the initial concentration of substrate, while Aiba et al. [13] reported the constant growth rate regardless of the initial concentration of oil. When considering the relatively low value of the concentrations of heneicosane compared with that of \( K_s \) in this study, the maximum growth rate could not be expected. When the solubility or solubilization of oil is negligible, the direct contact of microorganisms with oil phase is responsible for the biodegradation of oil. This will be the case in the environment due to low solubility of oil and low population of degraders capable of solubilization. In such circumstances, the growth of microorganisms is greatly dependent on the substrate concentration in oil, or degradable fraction of oil.

As shown in Fig. 7, the substrate was not degraded completely. A certain amount of heneicosane persisted in the culture broth. As it was assumed in Section 3 that the difference in substrate concentration between the bulk oil and adjacent cell was the driving force of transport, there would be no more mass transfer thus no more degradation of substrate when the substrate concentration in bulk oil reached the substrate concentration in cell. The remainder fraction of substrate after cultivation was considered to reflect the existence of hydrocarbon inclusions in the cell mass, one of energy pool for maintenance. This hydrocarbon pooling is thought to be characteristic of a variety of hydrocarbon-oxidizing microorganisms and also the case in Acinetobacter species [14]. Since the substrate concentration in the inner membrane could not be measured directly, the remainder fraction due to inclusions was used to estimate the value of \( s_c \). The substrate concentration of cell surface was considered to be in equilibrium with that of inclusions at the end of cultivation. The remainder substrate concentration might be attributed to the summation of the amount of substrate in oil droplet and in cell at the end of cultivation. The ratio of remainder substrate concentration
to the initial substrate concentration in culture broth, $S_c/S_0$, was obtained from the experimental data for each initial substrate concentration (Table 2). The value of the ratio of $S_c/S_0$ was assumed to be same as that of $s_c/s_0$ and constant during cultivation. With these assumptions, the concentration of the remaining portion could be taken equal to the substrate concentration at the inner membrane adjacent to the cell wall, $S_c$, which might have a major role in mass transfer as shown in Eqs. (13) and (14).

Thus, the validity of the model developed by assuming direct contact of microorganisms with oil surface could be elucidated from the experiment on model oil system using heneicosane and pristane.

### 5. Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_c$</td>
<td>projection area of an attached cell (m² cell⁻¹)</td>
</tr>
<tr>
<td>$J_s$</td>
<td>mass flux (kg m⁻² h⁻¹)</td>
</tr>
<tr>
<td>$k_L$</td>
<td>permeability coefficient (m h⁻¹)</td>
</tr>
<tr>
<td>$K_s$</td>
<td>saturation constant (kg m⁻³)</td>
</tr>
<tr>
<td>$n$</td>
<td>concentration of the cells over the oil phase (cell m⁻³ pristane)</td>
</tr>
<tr>
<td>$N$</td>
<td>concentration of the cells (cell m⁻³)</td>
</tr>
<tr>
<td>$N_0$</td>
<td>initial concentration of the cells (cell m⁻³)</td>
</tr>
<tr>
<td>$r_n$</td>
<td>growth rate over the oil phase (cell h⁻¹ m⁻³ pristane)</td>
</tr>
<tr>
<td>$r_N$</td>
<td>growth rate (cell h⁻¹ m⁻³)</td>
</tr>
<tr>
<td>$r_s$</td>
<td>substrate uptake rate over the oil phase (kg h⁻¹ m⁻³ pristane)</td>
</tr>
<tr>
<td>$r_s$</td>
<td>substrate uptake rate (kg h⁻¹ m⁻³)</td>
</tr>
<tr>
<td>$s$</td>
<td>substrate concentration over the oil phase (kg m⁻³ pristane)</td>
</tr>
<tr>
<td>$S$</td>
<td>substrate concentration (kg m⁻³)</td>
</tr>
<tr>
<td>$s_c$</td>
<td>substrate concentration at the outer side of inner membrane over the oil phase (kg m⁻³ pristane)</td>
</tr>
<tr>
<td>$S_c$</td>
<td>substrate concentration at the outer side of inner membrane (kg m⁻³)</td>
</tr>
<tr>
<td>$s'_c$</td>
<td>substrate concentration in the inner membrane over the oil phase (kg m⁻³ pristane)</td>
</tr>
<tr>
<td>$s_0$</td>
<td>initial substrate concentration over the oil phase (kg m⁻³ pristane)</td>
</tr>
<tr>
<td>$S_0$</td>
<td>initial substrate concentration (kg m⁻³)</td>
</tr>
<tr>
<td>$t$</td>
<td>time (h)</td>
</tr>
<tr>
<td>$t_c$</td>
<td>critical time where the reaction rate equals the transport rate (h)</td>
</tr>
<tr>
<td>$Y$</td>
<td>yield (cells kg⁻¹)</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>$S_0$ (kg m⁻³)</th>
<th>$S_c/S_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05⁺</td>
<td>0.10</td>
</tr>
<tr>
<td>0.30</td>
<td>0.14</td>
</tr>
<tr>
<td>0.55</td>
<td>0.17</td>
</tr>
<tr>
<td>1.0</td>
<td>0.29</td>
</tr>
</tbody>
</table>

⁺ Obtained from the background concentration of heneicosane in the cultivation on the pristane without addition of heneicosane.

Fig. 7. Growth of *Acinetobacter* sp. on heneicosane and degradation of heneicosane dissolved in pristane: symbols, pecked lines, and dash-dotted lines are indicating the experimental data sets, microbial reaction control, and mass transfer control, respectively. The critical time was estimated as $t_c = 8$ h. Initial additions of heneicosane were 0.25 (a), 0.50 (b), and 1.0 (c) kg m⁻³. Cells grown on LB medium for 15 h were harvested and washed twice with mineral salts medium prior to inoculation.
Greek letters

$\alpha$  the ratio of contact area to projection area
$\mu$  specific growth rate (h$^{-1}$)
$\mu'$  specific growth rate over the oil phase (h$^{-1}$)
$\mu_m$  maximum specific growth rate (h$^{-1}$)
$\nu$  specific substrate uptake rate (h$^{-1}$)
$\nu'$  specific substrate uptake rate over the oil phase (h$^{-1}$)

References