Assessment of the limiting step of mass transfer in \( n \)-hexadecane biodegradation in a bubble column reactor

Guillermo Quijano, Sergio Huerta-Ochoa and Mariano Gutiérrez-Rojas

**ABSTRACT**

A mathematical model was developed to assess limiting step of mass transfer in the \( n \)-hexadecane (HXD) biodegradation by a microbial consortium. A double Monod kinetic (oxygen and HXD) for biomass production was successfully used to describe the experimental data. Good fitting \((r^2 = 0.92)\) between the model solution and experimental data was obtained. The overall mass transfer coefficients for HXD, \(k_La_{\text{HXD}}\), and oxygen, \(k_La_{\text{O}_2}\), were experimentally determined and biosurfactant production was indirectly determined through surface tension measurements in the aqueous phase. It was observed that a surface tension reduction from 65 (0 h of culture) to 47 mN m\(^{-1}\) (240 h of culture) was related to a decrease of 52% in the HXD droplet diameter and to an increase of 63% in \(k_La_{\text{HXD}}\), respect the initial values. Conversely, \(k_La_{\text{O}_2}\) was repressed up to 37% compared to the initial value. The maximum rate analysis based on the mathematical model showed that HXD transfer was up to 5-fold lower than its consumption. On the contrary, oxygen transfer was always higher than its consumption. Thus, the limiting step under the working conditions was the HXD transfer to the aqueous phase. However, slight reductions in \(k_La_{\text{O}_2}\) could result in oxygen transfer limitations during the last 60 h of the cultures.

**Key words** | biosurfactants, limiting step, mass transfer, mathematical model, \( n \)-hexadecane

**NOMENCLATURE**

- \(C^*_{\text{HXD}}\) : HXD saturation in aqueous phase (mg L\(^{-1}\))
- \(C^*_{\text{O}_2}\) : Oxygen saturation in aqueous phase (mg L\(^{-1}\))
- \(C_{\text{HXD} \text{tot}}\) : Total HXD concentration (g L\(^{-1}\))
- \(C_{\text{HXD} \text{aq}}\) : Aqueous HXD concentration (mg L\(^{-1}\))
- \(C_{\text{O}_2 \text{aq}}\) : Aqueous oxygen concentration (mg L\(^{-1}\))
- \(Y_{x/s}\) : Growth yield (g g\(^{-1}\))
- \(K_{\text{HXD}}\) : Half-saturation constant for HXD (mg L\(^{-1}\))
- \(K_{\text{O}_2}\) : Half-saturation constant for oxygen (mg L\(^{-1}\))
- \(m\) : Maintenance coefficient (mg g\(^{-1}\) h\(^{-1}\))
- \(Q_{\text{O}_2}\) : Specific oxygen consumption rate (mg g\(^{-1}\) h\(^{-1}\))
- \(k_La_{\text{O}_2}\) : Oxygen mass transfer coefficient (h\(^{-1}\))
- \(k_La_{\text{HXD}}\) : Overall mass transfer coefficient of HXD (h\(^{-1}\))
- \(R_{\text{HXD} \text{cons}}\) : Maximum HXD consumption rate (mg L\(^{-1}\) h\(^{-1}\))
- \(R_{\text{HXD} \text{transfer}}\) : Maximum HXD transfer rate (mg L\(^{-1}\) h\(^{-1}\))
- \(R_{\text{O}_2 \text{cons}}\) : Maximum oxygen consumption rate (mg L\(^{-1}\) h\(^{-1}\))
- \(d_{32}\) : Sauter mean of HXD droplet diameter (\(\mu\)m)
- \(\mu_{\text{max}}\) : Maximum specific growth rate of microbial consortium (h\(^{-1}\))

**INTRODUCTION**

Many studies have been devoted to analyze and eventually overcome oxygen transfer limitations in bioreactors. However, during microbial degradation of hydrocarbons is common to find carbon sources less soluble than oxygen in aqueous phases (Volke-Sepulveda et al. 2006). This is the case of the alkanes, the main constituent of oil and refined products, which have very limited solubility in water, ranging from \(10^{-7}\) to \(10^{-3}\) mg L\(^{-1}\) for \(C_9\) and above (Wu & Ju 1998). Due to its low solubility in water, alkanes tend to
separate out and form hydrophobic phases with low bioavailability for microorganisms. According to Beal & Betts (2000), three main alkane consumption mechanisms can be considered. The first mechanism describes direct consumption of dissolved alkanes in the aqueous phase, although consumption by this mechanism seems to be restricted by the low solubility of most alkanes. The second mechanism proposes that alkanes are transported into the cells by direct contact between alkane droplets and microbial cells. However, the occurrence of this mechanism is related to the alkane/aqueous interfacial area available for cell attachment. Moreover, the alkane/aqueous interfacial area depends on the mixing characteristics of the bioreactor (Rols et al. 1990) and thus, it is difficult to estimate a priori the relevance of this consumption mechanism. The third mechanism proposes alkane consumption mediated by biosurfactants produced by the microorganisms. The presence of biosurfactants allows the formation of biosurfactant micelles in which the alkane is solubilized. Direct consumption of the alkane contained in biosurfactant micelles is well documented (Bury & Miller 1993; Ratledge 1988). Moreover, this emulsification process, also called pseudosolubilization, improves the alkane transfer towards the aqueous phase by increasing the alkane/water interfacial area and consequently increasing the area for potential cell attachment to the alkane. Several authors reported that pseudosolubilization may be the most important alkane consumption mechanism used by microorganisms capable of producing biosurfactants (Sekelsky & Shreve 1999; Ron & Rosenberg 2002).

On the other hand, aerobic alkane biodegradation is an oxygen-intensive metabolic process due to the highly reduced state of alkanes (Wei et al. 2005), thus it is expected that oxygen demand increases if alkane pseudosolubilization occurs. For instance, the oxygen requirement in the HXD biodegradation is up to 2.5-fold higher respect to glucose (Clarke & Correia 2008). Since alkane pseudosolubilization is related to biosurfactant production, it is not clear if the limiting step is the alkane or the oxygen transfer under such dynamic conditions. The aim of this work was to assess the limiting step of mass transfer in the aerobic biodegradation of HXD. In order to achieve the goal, the effect biosurfactant production on both the volumetric mass transfer coefficient of HXD and on the HXD droplet diameter was investigated. A mathematical model to describe HXD and oxygen consumption, as well as biomass production was developed. The model was solved using mass transfer coefficients experimentally determined. Moreover, from the seven kinetic parameters included in the model only two of them were fitted and the rest were experimentally determined.

Model development

Data on mathematical modeling and experimental validation of alkane biodegradation is scarce in the literature. From the available models, two key limitations can be identified: (i) oxygen is not considered as potential limiting substrate, and (ii) the model required up to four parameters to be fitted, including the mass transfer coefficients (Sekelsky & Shreve 1999; Cruickshank et al. 2000). Thus, an improved and more general model for the aerobic HXD biodegradation should include: dual-substrate growth kinetics to account for potential oxygen limitations and experimental determination of the mass transfer coefficients. A general model can be made from mass balances for HXD, oxygen and biomass. Since HXD is not toxic for the cells (Quijano et al. 2009) a double Monod kinetic can be used to account for potential oxygen limitations. Then, the time course of biomass concentration can be described by

\[
\frac{dX}{dt} = \mu_{\text{max}} \left( \frac{C_{\text{HXD,aq}}}{K_{\text{HXD}} + C_{\text{HXD,eq}}} \right) \left( \frac{C_{\text{O2,aq}}}{K_{\text{O2}} + C_{\text{O2,eq}}} \right) X
\]

where \( \mu_{\text{max}} \) is the maximum specific growth rate of the microbial consortium. \( C_{\text{HXD,eq}} \) is the HXD concentration in the aqueous phase considering both pseudosolubilized (biosurfactant-alkane micelle) and soluble HXD. However, as the HXD solubility in water is around \( 10^{-7} \text{mg L}^{-1} \), the amount of solubilized alkane can be neglected. \( C_{\text{O2,eq}} \) is the oxygen concentration in the aqueous phase. \( K_{\text{HXD}} \) and \( K_{\text{O2}} \) are the half-saturation constants for HXD and oxygen, respectively. The necessary steps for HXD pseudosolubilization and further transport to the aqueous phase are lumped in the overall HXD transfer coefficient, \( k_{\text{L,aHXD}} \). The HXD/aqueous interfacial area in the model is thus included in \( k_{\text{L,aHXD}} \), resulting in an easier experimental
determination of this parameter. Therefore, decreases in the HXD droplet diameter must be reflected in \( k_L a_{HXD} \) increases. The mass balance for pseudosolubilized HXD in the aqueous phase can be written as

\[
\frac{dC_{HXD_{aq}}}{dt} = k_L a_{HXD} \left( C^*_{HXD} - C_{HXD_{aq}} \right) - R_{cons} \tag{2}
\]

where \( C^*_{HXD} \) is the saturation concentration of HXD in the aqueous phase and \( R_{cons} \) is the HXD consumption rate by the microorganisms defined as

\[
R_{cons} = \frac{\mu_{max} X}{Y_{X/a}} \frac{C_{HXD_{aq}}}{K_{HXD} + C_{HXD_{aq}}} \frac{C_{O2_{aq}}}{K_{O2} + C_{O2_{aq}}} + mX \tag{3}
\]

where \( Y_{X/a} \) is the growth yield of the microbial consortium and \( m \) is the maintenance coefficient. Any decrease in the total HXD concentration (pseudosolubilized and non-soluble organic HXD), \( C_{HXD_{tot}} \), is assumed to be due only to microbial consumption and therefore it can be expressed as

\[
\frac{dC_{HXD_{tot}}}{dt} = -R_{cons} \tag{4}
\]

In the absence of HXD consumption, Equation (2) can be written as

\[
\frac{dC_{HXD_{aq}}}{dt} = k_L a_{HXD} \left( C^*_{HXD} - C_{HXD_{aq}} \right) \tag{5}
\]

whose integration gives

\[
\ln \left( 1 - \frac{C_{HXD_{aq}}}{C^*_{HXD}} \right) = -k_L a_{HXD} \cdot t \tag{6}
\]

Thus, the value of \( k_L a_{HXD} \) can be obtained by means of Equation (6) if HXD consumption is inhibited. Finally, the mass balance for oxygen in the aqueous phase can be written as

\[
\frac{dC_{O2_{aq}}}{dt} = k_L a_{O2} \left( C^*_{O2} - C_{O2_{aq}} \right) - Q_{O2}X \tag{7}
\]

where \( k_L a_{O2} \) is the volumetric oxygen transfer coefficient, \( C^*_{O2} \) is the saturation concentration of oxygen in the aqueous phase, and \( Q_{O2} \) is the specific oxygen consumption rate. Additional model assumptions are; (i) HXD pseudosolubilization is the preferential consumption mechanism, (ii) surface tension only decreases due to biosurfactant production, and (iii) oxygen is consumed only in the aqueous phase.

### Limiting step assessment

When consumption and mass transfer rates reach its maximum value, the mathematical model can be used to determine the limiting step of mass transfer. First, the maximum HXD consumption rate \( \left( \frac{R_{HXD}}{Q_{cons}} \right) \) may be defined as the consumption rate in which none limitation neither of HXD nor oxygen takes place in the aqueous phase. This means that \( C_{HXD_{aq}} \gg K_{HXD} \) and \( C_{O2_{aq}} \gg K_{O2} \), thus for these conditions Equation (3) can be expressed as:

\[
q_{HXD_{cons}} = \frac{\mu_{max} X}{Y_{X/a}} + mX \tag{8}
\]

In the same way, when \( Q_{O2} \) gets its maximum value \( \left( Q_{O2}^{max} \right) \) the maximum oxygen consumption rate \( \left( q_{O2_{cons}} \right) \), can be expressed as

\[
q_{O2_{cons}} = Q_{O2}^{max} X \tag{9}
\]

Finally, the maximum transfer rates for HXD and oxygen \( q_{HXD_{transfer}} \) and \( q_{O2_{transfer}} \), respectively) are given by

\[
q_{HXD_{transfer}} = k_L a_{HXD} \cdot C^*_{HXD} \tag{10}
\]

\[
q_{O2_{transfer}} = k_L a_{O2} \cdot C^*_{O2} \tag{11}
\]

### MATERIALS AND METHODS

#### Bacterial consortium and culture medium

The bacterial consortium was composed of *Achromobacter (Alcaligenes) xylosidoxidans*, *Bacillus cereus*, *Bacillus subtilis*, *Brevibacterium luteum* and *Pseudomonas pseudoalcaligenes*. The consortium was obtained from the rizosphere of *Cyperus laxus*, a native plant able to grow in weathered oil-contaminated sites (Díaz-Ramírez et al. 2003). The minimal culture medium was (g L\(^{-1}\)): 6.75, NaNO\(_3\); 2.15, K\(_2\)HPO\(_4\); 1.13, KCl; 1.1, MgSO\(_4\)-7H\(_2\)O, initial pH 6.5;
\(n\)-hexadecane (Sigma, Mexico) 1.67% (v/v) was used as carbon and energy source equivalent to a total initial concentration of 13 g L\(^{-1}\).

**Equipment and operation mode**

The bioreactor consisted of a 0.4 m glass jacketed column with an inner diameter of 0.062 m provided by a stainless steel gas sparger (7 orifices, 1 mm diameter). Temperature of 25°C and superficial gas velocity of 1.1 \(\times\) \(10^{-2}\) m s\(^{-1}\) were maintained during the cultures. The bioreactor containing 1 L of the minimal culture medium was inoculated with the bacterial consortium and the microorganisms were acclimated during 21 days under the operating conditions. After that, sequential 240-hour cultures were started by draining 0.7 L of the culture broth. The remaining 0.3 L was used as inoculum for the next cycle by restoring the initial volume with fresh minimal medium (1 L) and the initial HXD concentration (13 g L\(^{-1}\)). The bioreactor behavior (final HXD concentration and biomass production) was roughly constant after 4 cycles of 240 h. Thus, all reported measurements were recorded from the 5th cycle and onwards. The initial biomass concentration in the three sequential cultures was 0.41 \(\pm\) 0.23 g L\(^{-1}\).

**Surface tension, biomass and HXD measurements**

Samples of 15 mL were taken at different times and centrifuged at 15,000 rpm during 20 min at 4°C. HXD solidified at the centrifugation temperature and it was rapidly removed after centrifugation. Once HXD was completely removed, the supernatant and the pellet were used for surface tension and biomass determination, respectively. Biosurfactant production was indirectly measured through surface tension measurements of the aqueous phase as described by Beal & Betts (2000). Biomass pellets were washed with distilled water and measured by dry weight. Additional samples of 15 mL were taken from the bioreactor and a liquid–liquid extraction with hexane (1:1 v/v) was made. The organic fraction was separated and analyzed by gas chromatography in order to determine the total HXD concentration (pseudosolubilized and non-soluble organic HXD).

**Kinetic parameters of the microorganisms**

Cultures of the microbial consortium with minimal medium and HXD as sole carbon source were performed in 250-mL flasks. Three initial HXD concentrations were tested (4, 8 and 13 g L\(^{-1}\)), the flasks were incubated at 25°C and 150 rpm for 96 h. Biomass production and HXD consumption were measured as described above. From these experiments, three kinetic parameters (\(\mu_{\text{max}}, Y_{x/s}\) and \(K_{\text{HXD}}\)) were obtained.

**Mass transfer coefficients**

The values of \(k_{\text{Lao}}\) and \(Q_{\text{O}}\) were determined in the bioreactor according to the dynamic method described by Badino et al. (2000). Additional experiments in the bioreactor were carried to determine \(k_{\text{LaHXD}}\). In these experiments HXD consumption was inhibited through chloramphenicol addition (180 mg L\(^{-1}\), equivalent to 50 mg g\(^{-1}\)biomass). Visible cell count on Petri dishes confirmed growth inhibition for all bacterial strains. The remaining HXD was removed by centrifugation as above described and fresh HXD was added to perform pseudosolubilization kinetics according to Equation (6). The value of \(C_{\text{HXDaq}}\) was determined according to Bodour et al. (2004). In brief, aeration in bioreactor was interrupted for 2 min which allowed phases separation and then a sample of 10 mL was taken from the aqueous phase. The value of \(C_{\text{HXDaq}}\) was obtained after HXD extraction with hexane from an aliquot of the aqueous phase sample and subsequent analysis by gas chromatography. The maximum value of \(C_{\text{HXDaq}}\) observed during the three sequential cultures was considered as \(C_{\text{HXDaq}}^*\). The values of \(k_{\text{Lao}}\) and \(k_{\text{LaHXD}}\) were determined at 0, 96 and 240 h of culture, the error in the measurements being lower than 10% (experiments in triplicate). Polynomial equations describing the time course of \(k_{\text{Lao}}\) and \(k_{\text{LaHXD}}\) were included as auxiliary equations to consider the dynamic nature of the mass transfer coefficients in the model.

**HXD droplet diameter**

The HXD droplet diameter was measured during the three sequential cultures with an optical reflectance measurement.
system (Particle Scan 2008, MTS, Düsseldorf, Germany). Calibration of the equipment and automatic determinations were performed according to Torres-Martínez et al. (2009). The Sauter mean diameter \(d_{32}\) was obtained from two thousand automatic measurements using Equation (12)

\[
d_{32} = \frac{\sum \frac{d_i^3}{n_i}}{\sum \frac{d_i^2}{n_i}}
\]

where \(n_i\) represents the number of droplets with an equivalent diameter \(d_i\).

**Analytical procedures**

The dissolved oxygen concentration was measured with a polarographic probe (Applikon, USA). HXD concentration was determined in a gas chromatograph (Varian 3900, Palo Alto, CA, USA) coupled with a flame ionization detector and equipped with a DB-Petro Narrow bore column (J&W Scientific). The oven was heated at 120°C (30°C min\(^{-1}\)), then the temperature raised to 150°C at 10°C min\(^{-1}\) and then to 170°C at 15°C min\(^{-1}\). Helium was used as the carrier gas at 30 mL min\(^{-1}\). Surface tension measurements were performed with a ring tensiometer (Fisher Scientific, model 20, USA). Biomass concentration was determined by dry weight according to Wei et al. (2005).

**Mathematical model solution**

The model was solved using the fourth-order Runge-Kutta method. The numerical method was programmed in Mathematica software. Nonlinear parameter optimization (for \(K_{O_2}\) and \(m\)) was performed using the Levenberg-Marquardt method.

**RESULTS AND DISCUSSION**

**HXD consumption and surface tension**

HXD consumption and the surface tension of the aqueous phase were measured during three 240-h sequential cultures (Figure 1). A fast reduction in the surface tension was observed during the first 96 h of cultures, while from 96–240 h of culture the surface tension remained roughly constant around 47 mN m\(^{-1}\). Surface tension reduction is a typical consequence of biosurfactant production by microorganisms growing with HXD as sole carbon source (Bai et al. 1997; Beal & Betts 2000; Inakollu et al. 2004). According to Lang & Wullbrandt (1999), when the biosurfactants produced by *Pseudomonas aeruginosa* reach the critical micellar concentration (CMC), the surface tension values of the aqueous phase ranges from 25 to 40 mN m\(^{-1}\). In our work, the steady value of the surface tension reached after 96 h of culture is very similar to that reported by these authors, suggesting that the CMC of biosurfactants produced by the microbial consortium was reached at this stage of culture. Once the CMC was reached, a maximum pseudosoluble HXD concentration of 57 ± 9 mg L\(^{-1}\) was recorded and this value was considered as \(C^*_{HXD}\). This value is in agreement with Bai et al. (1997). These authors found that HXD pseudosolubilization mediated by the biosurfactants produced by *Pseudomonas aeruginosa* may result in \(C^*_{HXD}\) values of 48 mg L\(^{-1}\).

Additionally, it was observed that HXD consumption was strongly associated to the surface tension reduction. This clearly evidenced the important role of biosurfactant production for HXD consumption by the microbial consortium. A maximum HXD consumption of 95% respect the initial value was reached at 240 h of culture, which represents a consumption rate of 50 mg L\(^{-1}\) reactor h\(^{-1}\). A similar HXD consumption rate (50 mg L\(^{-1}\) reactor h\(^{-1}\)) was found by Sekelsky & Shreve (1999) during cultures of *Pseudomonas aeruginosa* growing with HXD as sole carbon source.
Mass transfer coefficients

The mean values of the HXD droplet diameter ($d_{32}$) and the mass transfer coefficients are summarized in Table 1. As anticipated from the mathematical model development, a reduction in $d_{32}$ was expected due to the biosurfactant production. Thus, the reduction of 52% in $d_{32}$ observed at 240 h of culture clearly indicates that HXD pseudosolubilization occurred. The reduction of 52% in $d_{32}$ represents a 2-fold increase in the HXD/aqueous interfacial area which was related to a 1.6-fold increase in $k_La_{HXD}$. The enhancement in $k_La_{HXD}$ is also consistent with the model assumptions since it was proposed that reductions in $d_{32}$ should be reflected on $k_La_{HXD}$. These results agree with Srivastava et al. (2000) who found that improvements in the mass transfer coefficient of a poorly water-soluble compound, which is transferred from the organic to the aqueous phase, were related to reductions in $d_{32}$. The authors also found that for a 100% increase in the organic/aqueous interfacial area the corresponding increase in the mass transfer coefficient was 80%. Additionally, a decrease in the alkane/water interfacial area due to HXD consumption should be reflected in $k_La_{HXD}$ decrease. Indeed, a decrease in the $k_La_{HXD}$ value was observed at the end of the cultures.

Unlike $k_La_{HXD}$, the value of $k_La_{O2}$ was depressed throughout the cultures. A maximum reduction of 37% was observed at 96 h, while at 240 h the repression on $k_La_{O2}$ was approximately of 14% respect the initial value. This complex behavior of $k_La_{O2}$ could be due to the combined effect of biosurfactant production and the presence of HXD. Several authors (Nielsen et al. 2003; Clarke et al. 2006) have observed that $k_La_{O2}$ is repressed in presence of HXD, regardless of the agitation rate used. Moreover, Clarke & Correia (2008) reported that $k_La_{O2}$ is usually depressed in presence of HXD and other long-chain alkanes. The increase in $k_La_{O2}$ observed at 240 h can be associated to the HXD consumption. The remaining concentration of HXD at 240 h was 5% with a correspondent reduction of 14% on $k_La_{O2}$, while the remaining concentration of HXD at 96 h was around 50% with a correspondent decrease of 37% on $k_La_{O2}$. This suggests that the amount of HXD in the culture medium influenced $k_La_{O2}$ depression. In addition, Vasconcelos et al. (2005) reported that surface active compounds (e.g. synthetic antifoams) can repress $k_La_{O2}$ by contaminating the gas/aqueous interface. Gomes et al. (2007) also found that the presence of a surfactant (Tween 80) repressed the value of $k_La_{O2}$ in oil-in-water dispersions. In our system, the CMC was reached at 96h of culture (Figure 1), when the higher $k_La_{O2}$ repression is observed. Thus, the decrease of $k_La_{O2}$ can be also related to biosurfactant production (likely due to gas/water interface contamination). Finally, the slight increase in $k_La_{O2}$ at the end of the cultures could be attributed to HXD depletion from the culture medium.

Mathematical model validation

Figure 2 shows the experimental data versus the model solution. The experimental data for dissolved oxygen was roughly constant in the three sequential cultures. Fast oxygen consumption was observed after the first 24 h of culture, matching with both the exponential growth of the biomass and a fast HXD consumption. In the last 72 h of culture the dissolved oxygen concentration reached stable lectures around 4 mg L$^{-1}$.

In order to gain accuracy in the mathematical model solution, only two kinetic parameters included in the model were obtained by fitting ($K_{O2}$ and $m$). Independent experiments in flasks were carried out to determine $\mu_{max}$, $Y_{x/s}$ and $K_{HXD}$. Different biomass production patterns were observed at each initial alkane concentration. Biomass production increased as the initial HXD concentration increased in the same culture time. This suggests that the pseudosolubilization process in the flasks was different in each initial HXD concentration. Moreover, different alkane/water interfacial areas also should be expected with different HXD concentrations. Table 2 summarizes the values of the seven kinetic parameters used in the model.

![Table 1](https://iwaponline.com/wst/article-pdf/62/4/906/446458/906.pdf)
Although the kinetic parameters are clearly a function of both the microorganisms used and the carbon source, the values of the fitted parameters in our work ($K_{O_2}$ and $m$) were comparable with previous data available in the literature. For instance, values of $K_{O_2}$ range from 0.048 to 6.2 mg L$^{-1}$ for carbon sources such as glucose, phenol and methyl tert-butyl ether, while values of $m$ range from 1 to 7.8 mg$_{substrate}$ g$_{biomass}$ h$^{-1}$ for substrates such as glucose and phenol (Cruickshank et al. 2000; Fortin et al. 2001; Beyenal et al. 2003).

The mathematical model was able to fit the overall behavior of the system. Moreover, the complex evolution of the dissolved oxygen with time was well described by the model. Conversely, the model solution for the HXD consumption was fairly accurate at 96 h, especially in the first sequential culture. In order to have quantitative information on the goodness of fit, a weighted sum of squares between the model solution and experimental data was evaluated according to Quijano et al. (2009b). The resulting value for the overall correlation factor, $r^2$, was 0.92. Therefore, the key assumption regarding pseudosolubilization as main consumption mechanism is supported by the experimental data.

### Limiting step assessment

Once the mathematical model was validated, the mass transfer limiting step was determined by means of the maximum transfer and consumption rates given by Equations (8–11). Figure 3 depicts the model simulations of the maximum consumption and transfer rates for HXD and oxygen. In the first sequential culture the HXD consumption rate was lower than the transfer rate during approximately 70 h of culture (Figure 3(a)). Similarly, during the first 24 h of the second and third sequential

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
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<tbody>
<tr>
<td>$M_\mu_{max}$</td>
<td>0.02 ± 0.01</td>
<td>h$^{-1}$</td>
</tr>
<tr>
<td>$M_{Y_{x/s}}$</td>
<td>0.39 ± 0.06</td>
<td>g g$^{-1}$</td>
</tr>
<tr>
<td>$M_{K_{HXD}}$</td>
<td>4.03 ± 0.35</td>
<td>mg L$^{-1}$</td>
</tr>
<tr>
<td>$M_{K_{O_2}}$</td>
<td>0.17</td>
<td>mg L$^{-1}$</td>
</tr>
<tr>
<td>$F_m$</td>
<td>2.80</td>
<td>mg g$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$M_{Q_{O_2}}$</td>
<td>26.80 ± 4.93</td>
<td>mg g$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$M_{Q_{O_2}}$</td>
<td>51.20 ± 6.07</td>
<td>mg g$^{-1}$ h$^{-1}$</td>
</tr>
</tbody>
</table>

Note: $^M$ Measured; $^F$ Fitted.
cultures both mass transfer and consumption rates were very similar. However, as cultures progressed the HXD transfer rate was up to 5-fold lower than the consumption rate. This means that as biomass increased a high HXD consumption capacity was reached in the bioreactor but the HXD transfer was limiting the biodegradation capacity of the system. In contrast, the oxygen transfer rate was higher than the consumption rate during the three sequential cultures (Figure 3(b)). During the first 180 h of the cultures, the transfer rate was up to 27-fold higher than the consumption rate. Nevertheless, the transfer and consumption rates reached very similar values in the last 60 h of each sequential culture. This suggests that slight decreases in $k_{L}a_{O_2}$ could result in both oxygen and HXD transfer limitations.

The analysis of the maximum transfer and consumption rates indicates that, under the working conditions, the HXD transfer was the limiting step of the aerobic biodegradation of HXD. Our results globally agree with Sekelsky & Shreve (1999), who reached the same conclusion using a different modeling approach. These authors concluded that the HXD transfer was the limiting step in the aerobic biodegradation of HXD by *P. aeruginosa*. Furthermore, their assumption on HXD droplet diameter reduction as a result of biosurfactant production was confirmed experimentally in our work. However, quantitative information on potential oxygen limitations can be also obtained with the mathematical model herein presented. In addition, the parameter-fitting task was avoided as possible in this work, while the mass transfer parameters and practically all the kinetic parameters of the microbial consortium were experimentally determined, enhancing the certainty of the model simulations.

**CONCLUSIONS**

A mathematical model able to assess the mass transfer limiting step of the HXD biodegradation was developed. The good fitting ($r^2 = 0.92$) between the model solution and experimental data supported the key assumption regarding pseudosolubilization as main consumption mechanism. Additionally, the behavior of $k_{L}a_{HXD}$ during the cultures was consistent with the model assumptions since it was proposed that reductions in $d_{32}$ should be related with $k_{L}a_{HXD}$ enhancements. The limiting step under the working conditions was the HXD transfer. However, slight reductions in $k_{L}a_{O_2}$ could result in further oxygen transfer limitations during the last 60 h of the sequential cultures. As the limiting step was the HXD transfer to the aqueous phase, $k_{L}a_{HXD}$ seems to be a good criterion for the scale-up of processes where the biodegradation of alkanes or other oils is involved.

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