

Effects of Hydrocarbon Additions on Gas-liquid Mass Transfer Coefficients in Biphase Bioreactors

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Abstract The effects of aliphatic hydrocarbons (*n*-hexadecane and *n*-dodecane) on the volumetric oxygen mass transfer coefficient ($k_L a$) were studied in flat alveolar airlift reactor and continuous stirred tank reactors (CSTRs). In the flat alveolar airlift reactor, high aeration rates (>2 vvm) were required in order to obtain efficient organic-aqueous phase dispersion and reliable $k_L a$ measurements. Addition of 1% (v/v) *n*-hexadecane or *n*-dodecane increased the $k_L a$ 1.55- and 1.33-fold, respectively, compared to the control (superficial velocity: 25.8×10^{-3} m/s, sparger orifice diameter: 0.5 mm). Analysis of the gas-liquid interfacial area a and the liquid film mass transfer coefficient k_L suggests that the observed $k_L a$ increase was a function of the media's liquid film mass transfer. Addition of 1% (v/v) *n*-hexadecane or *n*-dodecane to analogous setups using CSTRs led to a $k_L a$ increase by a factor of 1.68 and 1.36, respectively (superficial velocity: 2.1×10^{-3} m/s, stirring rate: 250 rpm). These results propose that low-concentration addition of oxygen-vectors to aerobic microbial cultures has additional benefit relative to incubation in purely aqueous media.

Keywords: flat alveolar airlift bioreactor, CSTR, oxygen-vector, $k_L a$, dodecane, hexadecane

INTRODUCTION

Aerobic microorganisms need oxygen for growth, cell maintenance, and product formation. To maintain metabolic activities of microbial cells, the oxygen supply rate must match the overall oxygen consumption rate under equilibrium conditions [1]. When oxygen is limited, microorganisms significantly decrease their metabolic rate and the culture may respond adversely to the resulting stress, decreasing the process efficiency.

Increased stirrer speed and/or air supply may help overcome these problems, but the added agitation can mechanically damage sensitive organisms such as microalgae [2] and lead to uncontrollable foam formation in the bioreactor. Alternative approaches with improved volumetric mass transfer coefficients ($k_L a$) are needed to ensure economic fermentation processes.

By adding an organic phase with a higher affinity for oxygen (oxygen-vector), larger amounts of oxygen are removed from the aeration gas stream, being retained in the two-phase system. Whereas no more than the saturation

concentration of oxygen can be dissolved in the aqueous phase, the supply of oxygen to the aqueous phase from the gas stream may be supplemented by equilibrium partitioning of dissolved oxygen from the organic phase to the aqueous phase. Importantly, aqueous-organic dispersions have undergone increased application through two-phase partitioning bioreactors for the biodegradation of toxic and poorly water-soluble compounds, as evidenced by recent reviews [3,4].

Several research groups have tried to enhance oxygen supply and biomass production in various cultures systems [5-7] by experimenting with different oxygen vectors like hydrocarbons [6,8,9-12], perfluorocarbons [7,13,14], and vegetable oils [15].

The solubility of oxygen in hydrocarbons or fluorocarbons exceeds that of pure water, and because hydrocarbons are cheaper than fluorocarbons, their application in large-scale fermentation is increasingly recognized for its economic benefit.

Various oxygen transfer mechanisms have been proposed in the literature, the most plausible being hydrocarbons being adsorbed into the bubble surface, possibly followed by the formation of a continuous film, and subsequent oxygen transport from the air to the microorganisms in the aqueous phase via an oxygen-vector. Various

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studies have shown that the main obstacle in the oxygen transfer is the diffusion from the hydrocarbon-aqueous phase interface through the aqueous boundary layer. This, however, is somewhat compensated by an increase of the gaseous interfacial area of oxygen transfer. The aqueous film-related gas-liquid transfer coefficient, $k_L a$, can hence be used to describe the oxygen transfer in these systems [12,16]. Separation of the gas-liquid transfer coefficient into k_L and a is the first step toward a better understanding of the underlying phenomena [17].

The aim of the present work was to study the effect of low concentrations of *n*-dodecane and *n*-hexadecane on the oxygen transfer in the two most widely used microbial fermentation systems – the alveolar air-lift bioreactor and the continuous stirred tank reactor (CSTR). Our analysis sets itself apart from previous work on flat alveolar airlift reactors by separately considering the hydrocarbon effect on the liquid film mass transfer, k_L , and the specific interface area, a .

MATERIALS AND METHODS

Airlift Measurements

An acrylic, flat alveolar airlift bioreactor was a parallelepiped, incorporating a lower mixing chamber (width/length: 9.3×6.3 cm), a central draft tube ($3 \times 1,065$ mm), and a top mixing chamber (20×6.3 cm) with a degassing zone (Fig. 1). The total length of the bioreactor was 120 cm.

The effect of the organic phase on the gas-liquid transfer coefficient was analyzed by individually considering the liquid film mass transfer coefficient, k_L , and the specific interface area, a . The latter is given by:

$$a = \frac{6}{d_{32}} \cdot \frac{\varepsilon_g}{1 - \varepsilon_g} \quad (1)$$

where ε_g is the gas hold-up and d_{32} is the Sauter mean bubble diameter defined by:

$$d_{32} = \frac{\sum_{i=1}^n d_{eqi}^3}{\sum_{i=1}^n d_{eqi}^2} \quad (2)$$

where n is the number of bubbles and d_{eqi} is the diameter of the volume-equivalent sphere derived from the major and minor axes, E and e , in two-dimensional projection:

$$d_{eqi} = (E^2 e)^{\frac{1}{3}} \quad (3)$$

The bubble diameter was determined photographically with an Olympus digital camera C-5050 ZOOM (Melville, NY, USA) exposing for 1/1,000 sec at aperture f2.0

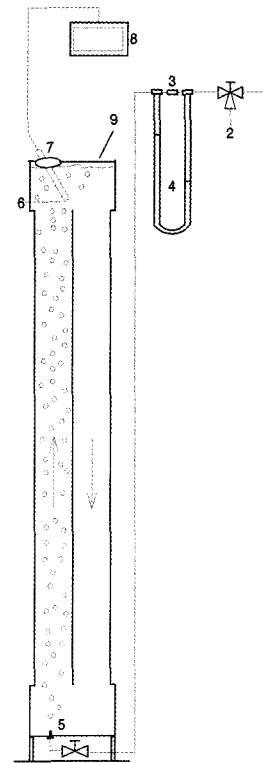


Fig. 1. Experimental setup of the flat alveolar airlift reactor. 1; air, 2; connection to the vacuum pump, 3; capillary, 4; water manometer, 5; sparger, 6; oxygen probe, 7; O-ring, 8; oxygen meter, 9; cover.

under 500-W halogen lighting. All data were acquired with a DT500 Series 3 data logger (Datataker, Rowville, Australia). For any given hydrodynamic condition (sparger orifice diameter and superficial velocity), >35 bubbles were randomly selected from the bottom, the middle, and the top of the bioreactor draft tube. The gas hold-up ε_g was determined by visually measuring the aerated liquid height relative to the gas-free liquid level. Values for k_L were obtained by calculating the $k_L a$ -to- a ratio. The $k_L a$ was determined at orifice diameters 0.5, 1, 2, and 3 mm and superficial gas velocities of 7.4×10^{-3} , 11×10^{-3} , and 14.7×10^{-3} m/s (corresponding to 0.6, 0.9, and 1.3 vvm, respectively). Because these superficial gas velocities did not promote efficient water-hydrocarbon mixing, higher superficial velocities had to be used (18.4×10^{-3} , 22.1×10^{-3} , and 25.8×10^{-3} m/s, corresponding to 1.6, 1.9, and 2.2 vvm).

CSTR Measurements

A two-liter CSTR glass vessel with a 1.5-L working volume was fitted with two equally spaced baffles and a four-blade Rushton 4.8-mm diameter turbine. The stirrer was placed on the shaft, 5.2 mm from the bottom of the vessel. The sparging system consisted of a 5-hole (0.5-mm diameter) single-ring sparger with 6-cm diameter situated 2.2 mm above the bottom of the vessel (Fig. 2). Measurement of the $k_L a$ were done at 250 and 500 rpm.

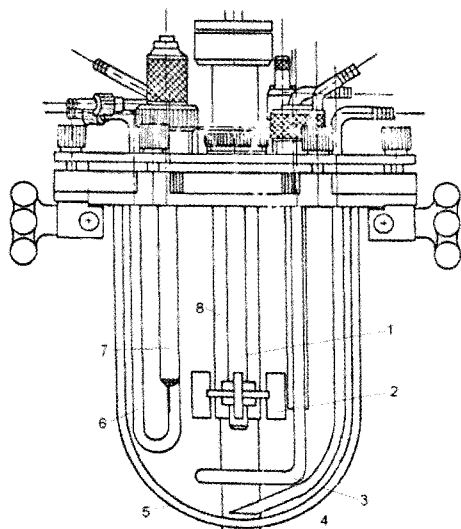


Fig. 2. Experimental setup of the continuous stir tank reactor (CSTR). 1; shaft, 2; Rushton disk turbine, 3; sparger, 4; feed inlet, 5; vessel wall, 6; baffle and re-circulating heating water, 7; rack arm.

The gas superficial velocity was kept between 1.1 and 2.1×10^{-3} m/s, corresponding to 0.5 to 1 vvm.

$k_L a$ Measurements

The dissolved oxygen concentration in the flat alveolar airlift was measured with a CELLOx 325 probe (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany) and a polarographic probe (Mettler Toledo, Columbus, OH, USA). Because the $k_L a$ was consistently less than 0.1 1/s, the response of the oxygen electrode was sufficiently rapid to detect any changes in the dissolved oxygen concentration [18]. Data were acquired with DT 500 Series 3 software (Datataker). The gas-liquid transfer coefficient was measured by the dynamic pressure method as previously described [19] based on the principle of a quick change in the vessel total pressure and the recording of the oxygen probes response in liquid phase [20].

All experiments were conducted at 25°C.

The following equation was used to calculate the $k_L a$:

$$\frac{dC}{dt} = k_L a \cdot (C^* - C) \quad (4)$$

where C represents the dissolved oxygen concentration in the aqueous phase, C^* represents the dissolved oxygen concentration at equilibrium with the oxygen concentration in the gas phase (*i.e.*, the oxygen saturation concentration in the aqueous phase). The $k_L a$ is often reported as a the product of a and k_L because the two components are not easily separable experimentally. In the present work, a and k_L were determined individually under different airlift bioreactor conditions.

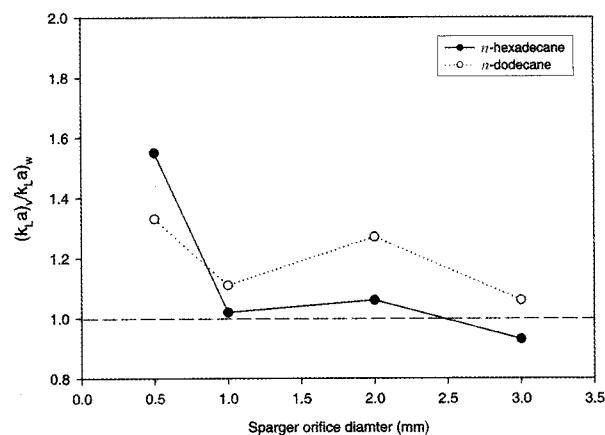


Fig. 3. Effect of *n*-hexadecane and *n*-dodecane on $k_L a$ in the flat alveolar airlift bioreactor.

Organic Phases

Previous work [21] has shown that addition of 1% (v/v) *n*-dodecane to fed-batch fermentations with the marine heterotrophic microalgae *Cryptocodinium cohnii* increased the biomass concentration, docosahexaenoic acid content, and total fatty acid production. Higher concentrations of *n*-dodecane or *n*-hexadecane gave rise to cell lysis. We hence added 1% (v/v) *n*-dodecane and *n*-hexadecane (oxygen solubility 54.9 and 64.7 mg/L, respectively, at 35°C and atmospheric air pressure; Merck, Darmstadt, Germany) to the water.

RESULTS AND DISCUSSION

Airlift Reactor Experiments

The Gas-liquid Transfer Coefficient

Galaction *et al.* [21] previously described increased oxygen transfer in the presence of oxygen-vectors with an amplification factor, defined as the ratio between $k_L a$ in the presence of an oxygen-vector ($(k_L a)_v$), and in the absence of an oxygen-vector for similar experimental conditions ($(k_L a)_w$), as an indicator of oxygen transfer enhancement. We used the same method to describe the ratios for Sauter mean bubble diameters ($[d_{32}]_v/[d_{32}]_w$), gas hold-ups ($[\varepsilon_g]_v/[\varepsilon_g]_w$), gaseous interfacial areas (a_v/a_w), liquid film mass transfer coefficients ($[k_L]_v/[k_L]_w$), and gas liquid transfer coefficients ($[k_L a]_v/[k_L a]_w$). Fig. 3 shows how the $(k_L a)_v/(k_L a)_w$ ratio varied for different sparger orifice diameters. The dashed line at unity delineates the boundary where no additional benefit was gained by further addition of oxygen-vectors. Aeration rates below 25.8×10^{-3} m/s generated unexpectedly high $(k_L a)_v/(k_L a)_w$ ratios (data not shown), which was attributed to inefficient mixing of the organic-aqueous phase. Presumably, the organic phase droplets were heterogeneously distributed in the liquid column and especially concentrated near the liquid surface. As the hydrocarbon oxygen solubility exceeds that of water, dissolved oxygen

Table 1. Gas-liquid mass transfer coefficient $k_L a$ (1/h) for the flat alveolar airlift bioreactor at a superficial velocity of 25.8×10^{-3} m/s and a hydrocarbon volumetric fraction of 1%

$k_L a$ (1/h)	$\delta = 0.5$ mm	$\delta = 1.0$ mm	$\delta = 2.0$ mm	$\delta = 3.0$ mm
<i>n</i> -hexadecane	59.7	29.9	29.1	22.5
<i>n</i> -dodecane	50.1	31.5	28.7	28.1

was apparently higher in this area, explaining the elevated $(k_L a)_v / (k_L a)_w$ ratios at lower air flow rates. Analyses of the organic phase effects on $k_L a$, d_{32} , ε_g , and a was hence done only at the highest superficial velocity (25.8×10^{-3} m/s, Fig. 3), where a visually discernible, white emulsion reconfirmed homogeneous organic-aqueous phase mixing. With an orifice diameter of 0.5 mm, the $(k_L a)_v / (k_L a)_w$ peaked at 1.55 and 1.33 for *n*-hexadecane and *n*-dodecane, respectively. Orifice diameters larger than 0.5 mm had a negative effect on the $(k_L a)_v / (k_L a)_w$ ratio for both organic solvents, albeit *n*-dodecane performed slightly better. This decrease was chiefly related to a reduction in the interfacial area a and a concomitant increase in the gas bubble diameter (as determined by the sparger orifice diameter) which in turn reduced the $k_L a$.

Table 1 lists the gas-liquid mass transfer coefficients for the airlift bioreactor at a superficial velocity of 25.8×10^{-3} m/s and different orifice diameters.

The highest $k_L a$ values (59.7 and 50.1 1/h for *n*-hexadecane and *n*-dodecane, respectively) were obtained at the smallest sparger orifice diameter ($\delta = 0.5$ mm). For diameters greater than 0.5 mm, the *n*-dodecane $k_L a$ was almost always higher. Interestingly, *n*-dodecane also outperformed *n*-hexadecane as a control and anti-foaming agent in *Aspergillus terreus* cultures [11].

Gas Hold-up, Sauter Mean Bubble Diameter, Specific Interface Area, and Liquid Film Mass Transfer Coefficients

Changes in the gas hold-up, Sauter mean bubble diameter, gas-liquid interface area, and the liquid film mass transfer coefficient ratios in response to addition of 1% (v/v) *n*-hexadecane or *n*-dodecane and different orifice diameters (constant superficial velocity of 25.8×10^{-3} m/s) are shown in Fig. 4. The gas hold-up ratio $(\varepsilon_g)_v / (\varepsilon_g)_w$ was almost unchanged (~ 1.01) for orifice diameters between 0.5 and 3 mm. Ratios $(d_{32})_v / (d_{32})_w$ and $(a)_v / (a)_w$ behaved similarly, except for the smallest orifice diameter. In fact, at $\delta = 0.5$ mm, *n*-hexadecane lowered the $(d_{32})_v / (d_{32})_w$ ratio to a level consistent with a 10% bubble diameter reduction compared to the control, which effectively increased the specific interface area a by 13%. This is in agreement with Rols *et al.* [16], who reported that the Sauter mean bubble diameter (measured photographically) was reduced by 15% when the *n*-dodecane fraction was equal to 23% (v/v). The same study also reported a proportional increase in the gaseous interfacial area, leading the authors to conclude that the effect of the vector on the gaseous dispersion constitutes only a weak contribution to the observed increase of $k_L a$.

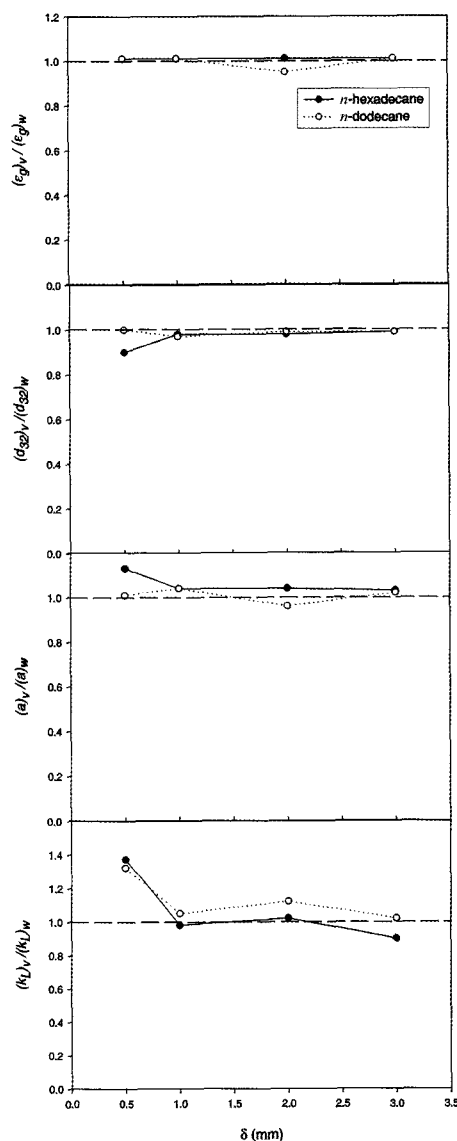


Fig. 4. Effect of the sparger diameter on the gas hold-up $(\varepsilon_g)_v / (\varepsilon_g)_w$, Sauter mean bubble diameter $(d_{32})_v / (d_{32})_w$, gas-liquid interface area $(a)_v / (a)_w$, and the liquid film mass transfer coefficient $(k_L)_v / (k_L)_w$ ratios in the flat alveolar airlift bioreactor.

Rols *et al.* [16] have measured the oxygen transfer coefficients k_L for gas-water, gas-vector, and vector-water interfaces and found that the gas-vector k_L was higher than the corresponding value for gas-water interfaces. They concluded that the limiting resistance within a four-phase emulsion transfer (gas/oxygen-vector/water/cells) is the water boundary layer, in other words, either in the gas-water or in the vector-water interface. In the present work, the calculated k_L should correspond to the mean k_L of the gas-water and gas-vector systems. The liquid film mass transfer coefficient ratio $(k_L)_v / (k_L)_w$ dropped as the orifice diameter was increased (Fig. 4). At 0.5 mm, both oxygen-vectors increased the k_L significantly in comparison to water (37 and 32% for *n*-hexadecane and *n*-dodecane, respectively). These results confirm that the

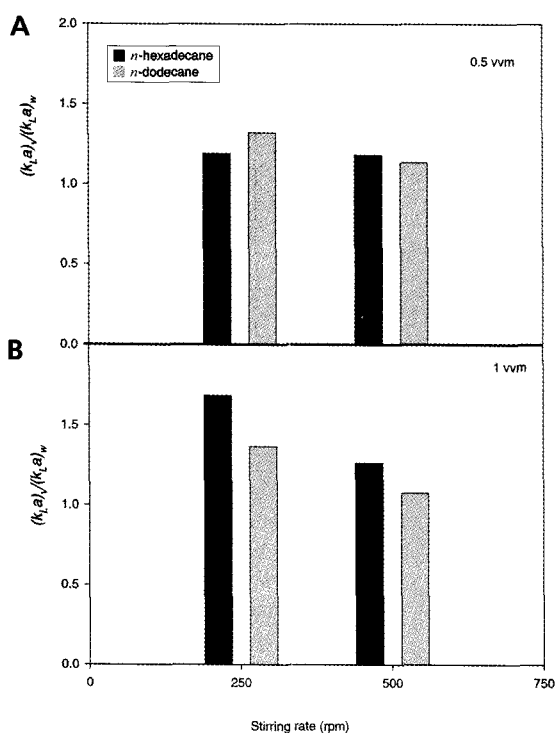


Fig. 5. Effect of *n*-hexadecane and *n*-dodecane on the $k_L a$ in the continuous stir bioreactor with (A) 0.5 and (B) 1-vvm aeration.

oxygen vectors improved the $k_L a$ by controlling the media's liquid film mass transfer, which was, in turn, strongly influenced by the corresponding oxygen diffusion coefficient, D [16,22]. It has been demonstrated theoretically and experimentally that k_L is proportional to $D^{2/3}$ for rigid surface bubbles (assumed for oxygen-vectors). The high oxygen resistance between the vector and the water phase can be compensated for by a large vector-water interface area, which seems to be implicated in the overall $k_L a$ changes. In fact, it is thought that the droplet size of the vector plays a crucial role in this process [1,16,21].

CSTR Experiments

Fig. 5 shows the $(k_L a)_v / (k_L a)_w$ ratios obtained for a CSTR with flow rates of (A) 0.5 and (B) 1 vvm (*i.e.*, superficial velocities of 1.1×10^{-3} and 2.1×10^{-3} m/s, respectively). An increase in the stirring rate (250 to 500 rpm) led to a reduction in the $(k_L a)_v / (k_L a)_w$ ratio for both hydrocarbons, particularly at the stronger aeration rate (1 vvm) where fluid turbulence was highest. Even in the absence of an oxygen-vector, enhanced agitation and, consequentially, turbulence should result in increased interface surface between the gas and the liquid phase. Oxygen transfer in the presence of hydrocarbons, however, includes hydrocarbon droplet adsorption into the bubble surface with or without coalescing with the superficial film resulting in a continuous oxygen-vector film. Increased agitation may then create hydrodynamic instabili-

Table 2. - Gas-liquid mass transfer coefficient $k_L a$ (1/h) for the CSTR bioreactor

Oxygen vector	Superficial velocity			
	1.1×10^{-3} m/s		2.1×10^{-3} m/s	
	250 rpm	500 rpm	250 rpm	500 rpm
<i>n</i> -hexadecane	79.1	116.8	147.2	175.4
<i>n</i> -dodecane	87.9	121.1	110.4	149.4

ties that lead to disruption of the oxygen-vector film or removal of oxygen-vector droplets giving rise to reduced oxygen diffusion rate from the air to the aqueous phase – an effect previously reported by Galaction *et al.* [22].

The highest $(k_L a)_v / (k_L a)_w$ ratios (1.68 and 1.36 for the *n*-hexadecane and *n*-dodecane, respectively) were found at 250 rpm and 2.1×10^{-3} m/s (1 vvm). Under almost all conditions, *n*-hexadecane fared better than *n*-dodecane as an oxygen-vector.

The highest $k_L a$ (175.4 1/h) was obtained in the presence of *n*-hexadecane, 500 rpm stirring, and an aeration rate of 2.1×10^{-3} m/s (1 vvm, Table 2). It has been reported that addition of 0.5% (v/v) *n*-hexadecane to *Penicillium chrysogenum* Wisconsin 54-1255 cultures enhanced the oxygen transfer and partially relieved the oxygen limitation. No further improvements in fermentation were observed when >2% *n*-hexadecane was added to the media [5].

The $k_L a$ increased with the aeration and agitation, and, as expected, the $k_L a$ values for the CSTR were higher than those obtained for the airlift bioreactor due to a more efficient gas dispersion.

CONCLUSIONS

Addition of 1% (v/v) oxygen-vectors to alveolar flat airlift bioreactors and CSTRs increased the $k_L a$, suggesting that even low concentrations of oxygen-vectors to aerobic microbial cultures have additional benefit compared to aqueous phase media. Moreover, despite the relatively high cost of hydrocarbons, their use as oxygen-vectors at low concentrations in fermentations may be of economic importance in industrial scale processes.

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NOMENCLATURE

a	specific interfacial area (1/m)
C^*	oxygen saturation concentration of the aqueous phase (g/L)
C	dissolved oxygen concentration of the aqueous

	phase (g/L)
d_{32}	Sauter mean bubble diameter (m)
d_{eqi}	diameter of the volume-equivalent sphere (m)
D	impeller diameter (m)
E	bubble major axis in two-dimensional projection (m)
e	bubble minor axis in two-dimensional projection (m)
k_L	liquid film mass transfer coefficient (m/h)
$(k_L)_v$	k_L in the presence of an oxygen-vector (organic phase) (m/h)
$(k_L)_w$	k_L in the absence of an oxygen-vector (aqueous phase) (m/h)
$k_L a$	volumetric mass transfer coefficient (1/h)
$(k_L a)_v$	$k_L a$ in the presence of an oxygen-vector (organic phase) (1/h)
$(k_L a)_w$	$k_L a$ in the absence of an oxygen-vector (aqueous phase) (1/h)
N	impeller speed (1/s)
P	power (W)
t	time (h)
V	liquid volume (m ³)

Greek symbols

δ	sparger orifice diameter (mm)
ε	gas hold up (dimensionless)
v_s	superficial gas velocity (m/s)

REFERENCES

- Ju, L.-K. and C. S. Ho (1989) Oxygen diffusion coefficient and solubility in *n*-hexadecane. *Biotechnol. Bioeng.* 34: 1221-1224.
- Märk, H. and R. Bronnenmeier (1985) Mechanical stress and microbial production. pp. 369-392. In: H.-J. Rehm and G. Reed (eds.). *Biotechnology*. VCH, Weinheim, Germany.
- Déziel, E., Y. Comeau, and R. Villemur (1999) Two-liquid-phase bioreactors for enhanced degradation of hydrophobic/toxic compounds. *Biodegradation* 10: 219-233.
- Malinowski, J. J. (2001) Two-phase partitioning bioreactors in fermentation technology. *Biotechnol. Adv.* 19: 525-538.
- Ho, C. S., L.-K. Ju, and R. F. Baddour (1990) Enhancing penicillin fermentations by increased oxygen solubility through the addition of *n*-hexadecane. *Biotechnol. Bioeng.* 36: 1110-1118.
- Jia, S., M. Wang, P. Kahar, Y. Park, and M. Okabe (1997) Enhancement of yeast fermentation by addition of oxygen vectors in air-lift bioreactor. *J. Ferment. Bioeng.* 84: 176-178.
- Menge, M., J. Mukherjee, and T. Scheper (2001) Application of oxygen vectors to *Claviceps purpurea* cultivation. *Appl. Microbiol. Biotechnol.* 55: 411-416.
- Wei, D. Z. and H. Liu (1998) Promotion of L-asparaginase production by using *n*-dodecane. *Biotechnol. Tech.* 12: 129-131.
- Giridhar, R. and A. K. Srivastava (2000) Productivity enhancement in l-sorbose fermentation using oxygen vector. *Enzyme Microb. Technol.* 27: 537-541.
- Jialong, W. (2000) Enhancement of citric acid production by *Aspergillus niger* using *n*-dodecane as an oxygen vector. *Process Biochem.* 35: 1079-1083.
- Lai, L. S., T. H. Tsai, and T. C. Wang (2002) Application of oxygen vectors to *Aspergillus terreus* cultivation. *J. Biosci. Bioeng.* 94: 453-459.
- Galaction, A. I., D. Cascaval, M. Turnea, and E. Folescu (2005) Enhancement of oxygen mass transfer in stirred bioreactors using oxygen-vectors. 2. *Propionibacterium shermanii* broths. *Bioprocess Biosyst. Eng.* 27: 263-271.
- MacLean, G. (1977) Oxygen diffusion rates in organic fermentation broths. *Process Biochem.* 12: 22-28.
- Lowe, K. C., M. R. Davey, and J. B. Power (1998) Perfluorochemicals: their applications and benefits to cell culture. *Trends Biotechnol.* 16: 272-277.
- Zhao, S., S. Kuttuva, and L. Ju (1999) Oxygen transfer characteristics of multi-phase dispersions simulating water-in-oil xanthan fermentations. *Bioprocess Eng.* 20: 313-323.
- Rols, J. L., J. S. Condoret, C. Fonade, and G. Goma (1990) Mechanism of enhanced oxygen transfer in fermentation using emulsified oxygen-vectors. *Biotechnol. Bioeng.* 35: 427-435.
- Alves, S. S., C. I. Maia, and J. M. T. Vasconcelos (2004) Gas-liquid mass transfer coefficient in stirred tanks interpreted through bubble contamination kinetics. *Chem. Eng. Process.* 43: 823-830.
- Özbek, B. and S. Gayik (2001) The studies on the oxygen mass transfer coefficient in a bioreactor. *Process Biochem.* 36: 729-741.
- Linek, V., P. Benes, J. Sinkule, and T. Moucha (1993) Non-ideal pressure step method for $k_L a$ measurement. *Chem. Eng. Sci.* 48: 1593-1599.
- Moucha, T. V., V. Linek, E. Prokopová (2003) Gas hold-up, mixing time, and gas-liquid volumetric mass transfer coefficient of various multiple-impeller configurations: Rushton turbine, pitched blade, and techmix impeller and their combinations. *Chem. Eng. Sci.* 58: 1839-1846.
- Silva, T. L., A. Mendes, R. L. Mendes, V. Calado, S. S. Alves, J. M. T. Vasconcelos, and A. Reis (2006) Effect of *n*-dodecane on *Cryptocodium cohnii* fermentations and DHA production. *J. Ind. Microbiol. Biotechnol.* 33: 408-416.
- Galaction, A. I., D. Cascaval, C. Oniscu, and M. Turnea (2004) Enhancement of oxygen mass transfer in stirred bioreactors using oxygen-vectors. 1. Simulated fermentation broths. *Bioprocess Biosyst. Eng.* 26: 231-238.

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