Modelling of a hybrid culture system with a stationary layer of liquid perfluorochemical applied as oxygen carrier

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Dedicated to Prof. Ryszard Pohorecki on the occasion of his 80th birthday

A mathematical model of a hybrid culture system supported with a stationary layer of liquid perfluorochemical (PFC) as a source of O_2 for cells which grow in the aqueous phase of culture medium has been developed and discussed. The two-substrate Monod kinetics without inhibition effects, i.e. the Tsao-Hanson equation, has been assumed to characterise the biomass growth. The Damköhler number which relates the growth rate to the mass transfer effects has been used to appraise the regime (i.e. diffusion-limited or kinetics) of the whole process. The proposed model predicted accurately previously published data on the submerged batch cultures of *Nicotiana tabacum* BY-2 heterotrophic cells performed in a culture system supported with a stationary layer of hydrophobic perfluorodecalin as a liquid O_2 carrier. Estimated values of the parameters of the model showed that the process proceeded in the kinetics regime and the growth kinetics, not the effects of the mass transfer between aqueous phase and liquid PFC, had essential influence on the growth of biomass.

Keywords: liquid perfluorochemical (fluorocarbon), liquid gas carrier, mathematical modelling, cell/tissue culture, hybrid culture system

1. INTRODUCTION

Oxygen is the critical nutrient in all *in vitro* cultures of those cells which perform aerobic metabolic pathways, i.e. many microbial organisms, as well as all cells isolated from plants, insects and animals, due to the fact that in aerobic metabolism energy is generated by substrate (organic carbon source, mainly) oxidation. Difficulties in achieving a sufficient level of dissolved oxygen, which is close to its physiological range for cells, motivate investigators to search for bioprocess solutions which intensify the mass transfer between the key elements of culture system: an oxygenation agent, a culture medium and continuously metabolizing cells (Garcia-Ochoa et al., 2010; Martin et al., 2010; Suresh et al., 2009).

Water-immiscible and biochemically inert, fully synthetic liquid highly-fluorinated compounds, known as perfluorochemicals (synonym: perfluorocarbons; PFCs), which are characterised by O_2 solubility much higher than that in water, are alternatives to conventional aeration systems applied in small-scale bioreactors as well as in mililiter- and microliter-scale cell culture systems (Lowe, 2002; Ntwampe et al., 2010; Pilarek, 2014; Riess 2006).

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There are two ways of liquid PFCs application in submerged culture systems. One of them depends on the flow of an aerated PFC applied as a continuous layer or droplets dispersed in culture medium. From the bioengineering point of view such systems are analogical to typical culture systems with barbotage-based aeration and mathematical models of these bioprocesses have been previously published, e.g. by Amaral et al. (2007), Dragoi et al. (2011) or van Sonsbeek et al. (1992). The second type of PFC-supported cell culture systems utilises a liquid perfluorinated oxygen carrier as a stationary layer in a hybrid culture system developed with two immiscible liquid phases: aqueous-based culture medium and hydrophobic PFC. Such culture systems are exceedingly suitable when a 3-D structure of fragile aggregates, spheroids or sheets of cells, as well as cell-consisting biomaterial-based implant constructs must be preserved to eliminate negative effects caused by hydrodynamic shear forces generated in typical intensively-stirred/agitated culture systems (Davey et al., 2005; Hillig et al., 2014; Pilarek et al., 2013; Pilarek et al., 2014). It should be emphasised that the data available in open literature for such a system are still rather limited, with most quantitative data just omitted.

The aim of our study is to develop and discuss a mathematical model of a hybrid culture system supported with a stationary layer of liquid PFC as a source of O_2 for heterotrophic cells which grow in the aqueous phase of culture medium. To our knowledge, this is the first report which quantitatively characterises such type of culture system being increasingly used for cultures of plant and animal cells or tissues, as well as microalgae cells. This is probably also one of the first reports on the modelling of cell culture systems utilising liquid PFCs in general.

2. MODEL OF A HYBRID CULTURE SYSTEM WITH STATIONARY PHASE OF LIQUID PFC

2.1. Scheme of a hybrid culture system

A scheme of a hybrid culture system supported with stationary layer of liquid PFC taken into consideration in this study is presented in Fig. 1. Cells grow only in the aqueous phase of culture medium with a volume of V_{aq}. The separate phase of liquid PFC has a volume of V_{PFC}. It is assumed that mixing inside both contacting liquid phases is ideal.
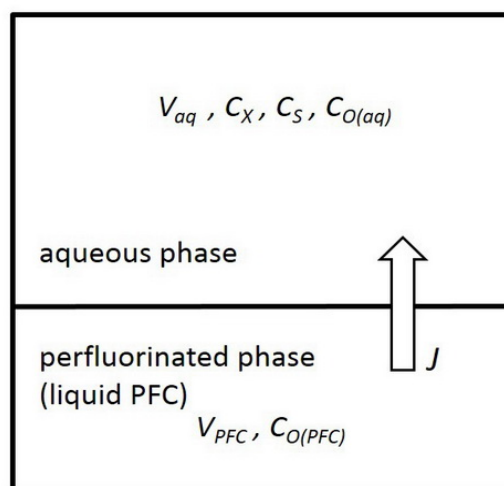

Fig. 1. A scheme of a hybrid culture system supported with stationary layer of liquid PFC

O_2 is soluble in a phase of liquid PFC and is transferred into the aqueous phase. The concentration of O_2 in the phase of liquid PFC equals to $C_{O(PFC)}$. The growth of cells is limited by two critical factors: concentration of a carbon source (C_S) and concentration of O_2 ($C_{O(aq)}$) soluble in aqueous phase.

2.2. Kinetics and balances

The rate of mass flux transferred between both liquid, i.e. aqueous and perfluorinated, phases (J) is characterised by the following equation:

$$J = (k_L a)V_{aq}(C_{O(PFC)} - HC_{O(aq)}) \tag{1}$$

where the O_2 partition coefficient (H) describes the equilibrium between concentration of O_2 dissolved in aqueous and perfluorinated phase:

$$C^*_{O(PFC)} = HC^*_{O(aq)} \tag{2}$$

The cells grow only in the aqueous phase of culture medium. The specific growth rate (μ) is characterised by the Tsao-Hanson equation, i.e. the two-substrates Monod kinetics is assumed:

$$\mu = \mu_{max} \frac{C_S}{K_{m,S} + C_S} \frac{C_{O(aq)}}{K_{m,O} + C_{O(aq)}} \tag{3}$$

where:

$$C_S \gg K_{m,S} \tag{4}$$

$$C_{O(aq)} \gg K_{m,O} \tag{5}$$

as it typically occurs.

The oxygen balance in aqueous phase is characterised by the following equation:

$$V_{aq} \frac{dC_{O(aq)}}{dt} = (k_L a)V_{aq}(C_{O(PFC)} - HC_{O(aq)}) - Y_{OX}V_{aq}\mu_{max} \frac{C_S}{K_{m,S} + C_S} \frac{C_{O(aq)}}{K_{m,O} + C_{O(aq)}} C_X \tag{6}$$

whilst the oxygen balance in perfluorinated phase is characterised as:

$$V_{PFC} \frac{dC_{O(PFC)}}{dt} = -(k_L a)V_{aq}(C_{O(PFC)} - HC_{O(aq)}) \tag{7}$$

The biomass balance is described as:

$$V_{aq} \frac{dC_X}{dt} = V_{aq}\mu_{max} \frac{C_S}{K_{m,S} + C_S} \frac{C_{O(aq)}}{K_{m,O} + C_{O(aq)}} C_X \tag{8}$$

The balance of a carbon substrate, as the growth limiting factor, can be expressed as follows:

$$V_{aq} \frac{dC_S}{dt} = -\frac{1}{Y_{XS}}V_{aq}\mu_{max} \frac{C_S}{K_{m,S} + C_S} \frac{C_{O(aq)}}{K_{m,O} + C_{O(aq)}} C_X \tag{9}$$

The initial conditions ($t = t_0 = 0$) for Eqs. (6) - (9) are as follows :

$$C_{O(aq)} = C^*_{O(aq)} \tag{10}$$

$$C_{O(PFC)} = C^*_{O(PFC)} \tag{11}$$

$$C_X = C_{X,0} \tag{12}$$

$$C_S = C_{S,0} \tag{13}$$

Initial conditions (10) and (11) state that at the beginning of the culture both liquid phases, aqueous as well as perfluorinated, are saturated with O_2.

2.3. A dimensionless model

Introducing the following dimensionless variables:

$$c = \frac{C_{O(aq)}}{C^*_{O(aq)}} \tag{14}$$

$$p = \frac{C_{O(PFC)}}{C^*_{O(PFC)}} \tag{15}$$

$$x = \frac{C_X}{Y_{XS}C_{S,0}} \tag{16}$$

$$s = \frac{C_S}{C_{S,0}} \tag{17}$$

as well as a dimensionless time:

$$\tau = t\mu_{max} \tag{18}$$

and dimensionless saturation constants:

$$K_O = \frac{K_{m,O}}{C^*_{O(aq)}} \tag{19}$$

$$K_S = \frac{K_{m,S}}{C_{S,0}} \tag{20}$$

the system of balance equations, i.e. Eqs. (6) - (9), results in a dimensionless form:

$$\frac{dc}{d\tau} = \frac{1}{Da}(p-c) - C_0 \frac{s}{K_S+s}\frac{c}{K_O+c}x \tag{21}$$

$$\frac{dp}{d\tau} = -\frac{1}{Da}\varphi(p-c) \tag{22}$$

$$\frac{dx}{d\tau} = \frac{s}{K_S+s}\frac{c}{K_O+c}x \tag{23}$$

$$\frac{ds}{d\tau} = -\frac{s}{K_S+s}\frac{c}{K_O+c}x \tag{24}$$

where

$$Da = \frac{\mu_{max}}{H(k_L a)} \tag{25}$$

is the Damköhler number relating the growth rate to the mass transfer rate.

The dimensionless parameter C_0 defined as:

$$C_0 = \frac{Y_{XS}Y_{OX}C_{S,0}}{C^*_{O(aq)}} \tag{26}$$

may be recognised as the relative initial concentration of the carbon substrate. C_0 relates the ratio of an amount of O_2 assimilated by cells in the case of total carbon substrate consumption and the initial concentration of O_2 soluble in aqueous phase of culture medium.

The dimensionless parameter φ relates the volume of aqueous phase to the volume of liquid PFC:

$$\varphi = \frac{V_{aq}}{HV_{PFC}} \tag{27}$$

Hereby, φ may be recognised as "the relative volume of an aqueous phase" because it is the ratio of a volume of aqueous phase and the volume of water containing the same amount of oxygen as phase of liquid PFC.

Taking onto account the dimensionless variables, the initial conditions (i.e. when $\tau = \tau_0 = 0$) for Equations (21) - (24) take the following forms:

$$c = 1 \tag{28}$$

$$p = 1 \tag{29}$$

$$s = 1 \tag{10}$$

$$x = x_0 = \frac{C_{X,0}}{Y_{XS}C_{S,0}} \tag{31}$$

3. DISCUSSION OF THE RESULTS

3.1. Simulation of the process

A system of dimensionless Eqs. (21) - (24) has been numerically solved. The classic fourth-order Runge-Kutta method with a constant integration step has been applied to integrate the model equations. Parameters of the simulation were: the Damköhler number Da, the relative volume of aqueous phase φ, the inoculum concentration $C_{X,0}$ and the relative initial carbon substrate concentration C_0. The following values of dimensionless saturation constants: $K_S = 0.01$ and $K_O = 0.1$, have been assumed for calculations. This corresponds to typical bioprocesses, where the initial concentration of carbon substrate is much higher than the saturation constant. In such conditions cells grow with a constant specific growth rate.

The influence of C_0 on the course of culture has been presented in Fig. 2. C_0 determines the type of cell growth limitation. For small values of C_0, the initial concentration of a carbon substrate is the critical factor which limits the final biomass concentration (Fig. 2a). This affects the consumption of a carbon substrate and partially the assimilation of O_2. Large values of the parameter C_0 indicate an excess of a carbon substrate relative to the amount of O_2 in a culture system. As a result, O_2 is totally assimilated by cells while the consumption of a carbon substrate is only partial (Fig. 2b).

Tu sum up, the critical value of the parameter ($C_{0,critical}$) corresponding to total consumption of both ingredients of a culture medium, i.e. a carbon substrate as well as O_2, is defined by the following relationship:

$$C_{0,critical} = 1 + \frac{1}{\varphi} \tag{32}$$

The influence of φ on the cell growth has been presented in Fig. 3. Results of a model calculations presented here, refers to value of C_0 equal to 5.0, i.e. to the case of the cell growth limited by O_2 concentration in the culture system.

According to the theoretical predictions and our experimental results published previously (Pilarek and Szewczyk, 2008; Sykłowska-Baranek et al., 2013), increasing the volume of perfluorinated phase, i.e.

decreasing φ, leads to increased biomass growth. In the case of low values of C_0, i.e. low initial concentration of carbon substrate, the ratio of aqueous phase to perfluorinated one has low impact on the course of culture.

The courses of cultures shown in Fig. 2 and Fig. 3 refer to small values of the Damköhler number, i.e. to the situation when the growth rate is lower than the rate of mass transfer between aqueous phase and liquid PFC. The course of culture in the case of large value of the Damköhler number has been presented in Fig. 4.
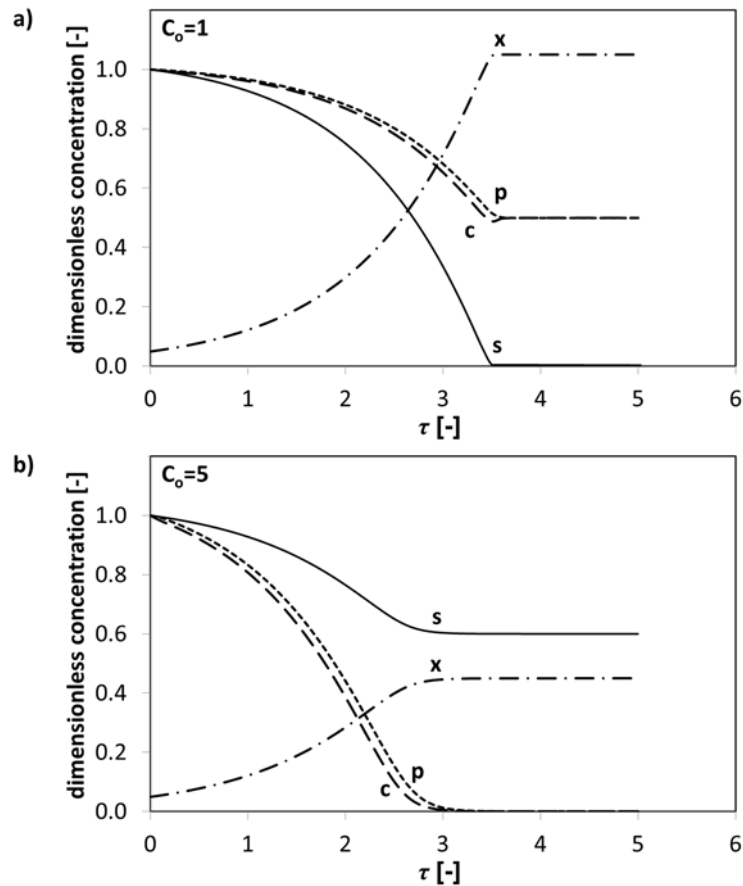

Fig. 2. The influence of the relative (dimensionless) initial substrate concentration (C_0) on the course of culture: $C_0 = 1$ (a) and $C_0 = 5$ (b), where: c - dimensionless concentration of O_2 in aqueous phase, p - dimensionless concentration of O_2 in perfluorinated phase, s - dimensionless concentration of carbon substrate, x - dimensionless concentration of biomass
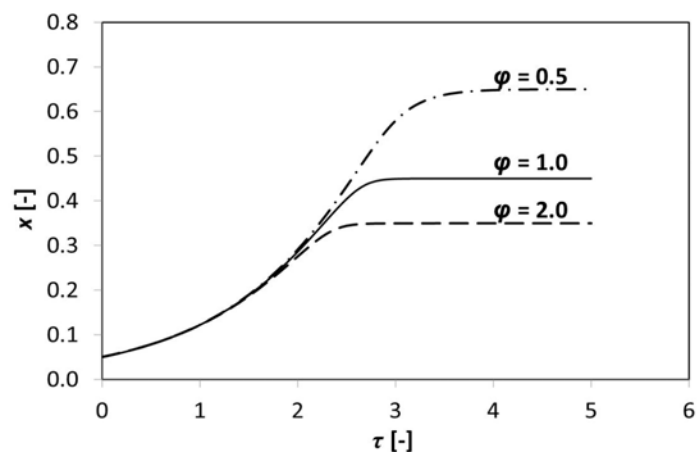

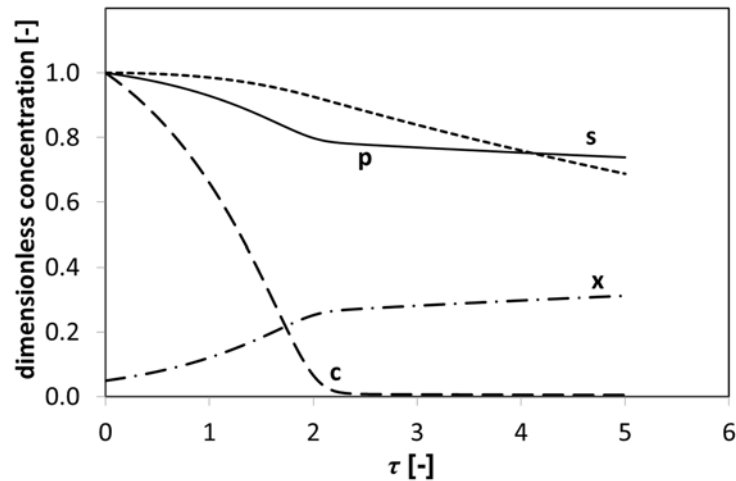Fig. 3. The influence of the relative volume of aqueous phase (φ) on the cell growth

Fig. 4. The simulation of the course of culture for large value of the Damköhler number ($Da = 10$), where: c - dimensionless concentration of O_2 in aqueous phase, p - dimensionless concentration of O_2 in perfluorinated phase, s - dimensionless concentration of carbon substrate, x - dimensionless concentration of biomass

If the process proceeds in the diffusion-limited regime, i.e. the mass transfer resistances have significant influence and cannot be neglected, two characteristic stages of a culture are clearly observed. First of them is the exponential growth of cells, during which the assimilation of O_2 takes place, and the rate of cell growth depends on the growth kinetics. When the concentration of O_2 in aqueous phase is reduced considerably, the rate of biomass growth decreases and further growth of cells is limited by the rate of O_2 transfer from liquid PFC into the aqueous phase of culture medium.

The curves of the cell growth for various values of the Damköhler number have been presented in Fig. 5. The influence of the values of the Damköhler number lower than 0.1 on the course of culture is minor. However, the increase in the value of this parameter, e.g. above 1.0, reduces significantly the rate of cell growth.
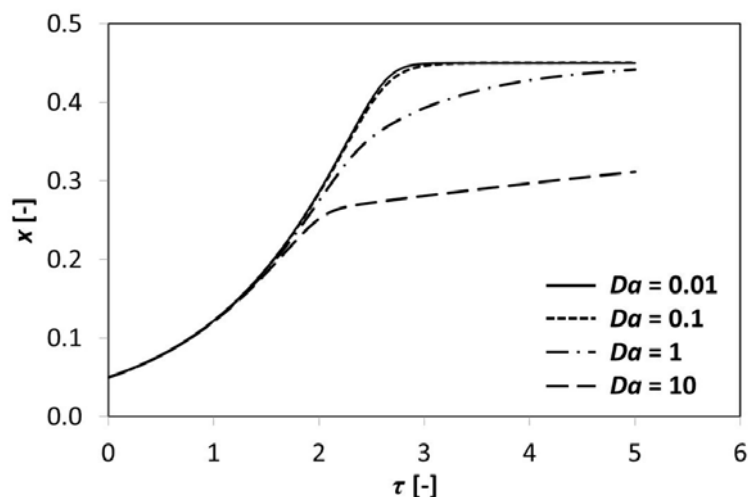

Fig. 5. The influence of the Damköhler number (Da) on the cell growth

3.2. Estimation of values of the model parameters for a plant cell culture

Estimation of values of the model parameters was performed for the submerged batch cultures of *Nicotiana tabacum* BY-2 heterotrophic cells supported with the stationary layer of liquid PFC, based

on data which have been previously published by Pilarek and Szewczyk (2008). The relevant culture system contained 100 cm³ of culture medium and was supported with air-saturated hydrophobic perfluorodecalin ($C_{10}F_{18}$) as the liquid carrier of O_2, and volume of liquid PFC ranged from 20 % to 60 % of aqueous phase volume. The value of the O_2 partition coefficient for the water-perfluorodecalin system, $H = 16.1$, has been calculated based on the values of $C^*_{O(aq)} = 2.2$ mM and $C^*_{O(PFC)} = 35.3$ mM. Hence, the relative volume of aqueous phase φ varied in the range of 0.1 - 0.3.

Values of the Damköhler number for growth of BY-2 cells have been calculated based on k_La and μ, which have been determined previously (Pilarek and Szewczyk, 2008). The value of the volumetric liquid-side mass transfer coefficient was close to 0.5 h⁻¹, and the specific growth rate of BY-2 cells was from 0.023 h⁻¹ to 0.043 h⁻¹. Therefore the values of the Damköhler number vary from 0.28×10^{-3} to 5.2×10^{-3}. Thus, rather low values of Da mean that the process proceeded in the kinetics regime.

Based on the previously published values of the yield of biomass from substrate coefficient (Y_{XS}) and yield of oxygen consumption by unit of biomass coefficient (Y_{OX}), which equal 0.5 g/g and 0.3 mmol/g respectively, as well as taking into account the initial concentration of sucrose, i.e. the carbon substrate, in culture medium which was from 20 to 30 g/dm³, the value of the dimensionless, i.e. relative, initial concentration of carbon substrate $C_{S,0}$ has been determined as equal to 1.36 - 2.05.

4. CONCLUSIONS AND OUTLOOK

The proposed model, based on the two-substrate Monod kinetics without inhibition effects, predicts accurately previously published data on the cultures of *N. tabacum* BY-2 cells performed in a hybrid culture system with a stationary layer of liquid PFC. Estimated values of the model parameters clearly show that the process proceeded in the kinetics regime. The growth kinetics, not the effects of the mass transfer between aqueous phase and liquid PFC, had essential influence on the growth of biomass.

However, for full identification of growth kinetics of plant cell/tissue submerged cultures, some other factors characterising other gaseous compounds should be discussed. Not only O_2 dissolved in culture medium has crucial influence on the process, but also concentrations of such gases as CO_2 and ethene (synonym: ethylene; C_2H_4) dissolved in aqueous phase of an *in vitro* culture system have substantial impact on plant cell metabolism. Both of them, i.e. CO_2 and ethene, are continuously generated in plant cells. CO_2 acidifies the culture medium and changes its osmomolarity. As one of the plant growth regulators (often called "phytohormones"), ethene causes a variety of metabolic responses of plant cells. Similarly, in the case of O_2, its higher concentrations in a culture system have detrimental effects on plant cell biomass resulting in a non-physiological high level of free radicals inside cells. To sum up, all of these remarks indicate the need for further studies on modelling of the growth of biomass in plant cell culture *in vitro* systems utilising a liquid PFC, and a perfluorinated phase should be taken into consideration as a liquid carrier of O_2, CO_2 as well as ethene.

SYMBOLS

c	dimensionless concentration of O_2 in aqueous phase
C_0	relative (dimensionless) initial concentration of the carbon substrate
$C_{O(PFC)}$	molar concentration of O_2 in liquid PFC, mol dm⁻³
$C^*_{O(PFC)}$	molar concentration of O_2 in liquid PFC which is in equilibrium with the O_2 partial pressure in gas phase, mol dm⁻³
$C_{O(aq)}$	molar concentration of O_2 in aqueous phase, mol dm⁻³

$C^*_{O(aq)}$	molar concentration of O_2 in aqueous phase which is in equilibrium with the O_2 partial pressure in gas phase, mol dm^{-3}
C_S	molar concentration of carbon substrate, mol dm^{-3}
$C_{S,0}$	initial concentration of carbon substrate, mol dm^{-3}
C_X	mass concentration of biomass, g dm^{-3}
$C_{X,0}$	initial mass concentration of biomass, g dm^{-3}
Da	the Damköhler number, Eq. (25)
H	the O_2 partition coefficient for the water - liquid PFC system
J	mass flux transferred between aqueous phase and liquid PFC, kg m^{-2} s^{-1}
k_La	volumetric liquid-side mass transfer coefficient, h^{-1}
$K_{m,O}$	saturation constant relative to O_2, g dm^{-3}
$K_{m,S}$	saturation constant relative to carbon substrate, g dm^{-3}
K_O	dimensionless saturation constant relative to O_2
K_S	dimensionless saturation constant relative to carbon substrate, g dm^{-3}
p	dimensionless concentration of O_2 in aqueous phase
s	dimensionless concentration of carbon substrate
s_0	dimensionless initial concentration of carbon substrate
t	time, h
V_{PFC}	volume of liquid PFC, dm^{-3}
V_{aq}	volume of aqueous phase, dm^{-3}
x	dimensionless concentration of biomass
Y_{OX}	yield of oxygen consumption by unit of biomass coefficient, mmol g^{-1}
Y_{XS}	yield of biomass from substrate coefficient, g g^{-1}

Greek symbols

φ	the relative volume of aqueous phase
μ	specific growth rate, h^{-1}
μ_{max}	maximum specific growth rate, h^{-1}
τ	dimensionless time

Superscripts

$*$	equilibrium with prevailing value in the other phase
BY-2	*Nicotiana tabacum* cv. BY-2 (cultivar Bright Yellow No. 2 of the tobacco plant)
PFC	perfluorochemical (synonym: perfluorocarbon)

Subscripts

0	initial
max	maximum
O	oxygen
PFC	(perfluorinated) phase of liquid PFC
S	carbon substrate
aq	aqueous phase
X	biomass

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