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Mathematical model of a three phase partitioning bioreactor for conversion of ketones using whole cells

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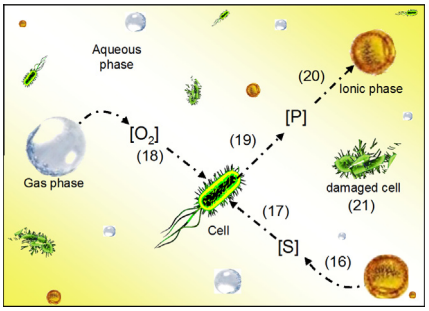
HIGHLIGHTS

- We developed a three-phase mathematical model for bicyclic ketone bioconversion.
- The model accounted for mass transfer, kinetics and loss of cell viability.
- The model fitted adequately bioconversion at different ionic-liquid fractions.
- Ionic liquid fraction seemed to cause cell deactivation.
- The model promises to be useful for design and optimization of these bioconversions.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study we developed a pseudo heterogeneous mathematical model for the oxidation of bicyclic ketone bicyclo[3.2.0]hept-2-to-6-one to bicyclic lactone (1S,5R)-(-)-2-oxabiciclo[3.3.0]oct-6-en-3-ona using whole cells of *E. coli* strain TOP10 pQR239 in a three-phase partitioning bioreactor (TPPB). The pseudo heterogeneous TPPB model accounted for mass transfer mechanisms occurring in the air–water and water–ionic liquid phases along with bioconversion and loss of cell viability caused by ionic liquid – trioctylmethylammonium bis(trifluoromethylsulfonyl)imide ([OMA][BTA]). The development of the model was based on reactor engineering principles and, hence, experiments with and without bioconversion were carried out in order to characterize thermodynamic (K_{ps} and K_{pp}), transport (k_s, k_p *and* A) and kinetic (k_i *and* k_i') parameters along with the loss of cell viability (k_{in}) parameter for the mechanisms involved. The model described adequately the bioconversion of experimental data with two different ionic liquid fractions, namely 5% (v/v) and 12.5% (v/v). A parametric sensitivity analysis of the model was conducted to obtain information on the effect of oxygen transport rate on bioconversion. The development of the TPPB model led to the following findings: (i) 5% (v/v) ionic liquid fraction caused less cell deactivation; (ii) presence of ionic liquid decreased the oxygen transport rate; and (iii) a higher oxygen transport rate lead to a higher bioconversion but also cell inactivation.

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Nomenclature

A	interfacial area per unit volume (m^2/m^3)
d_{32}	Sauter mean diameter of the ionic phase (μm)
A	interfacial mass transfer area of the drop of ionic phase (m^{-1})
k_L	oxygen mass transfer coefficient ($m\ h^{-1}$)
k_S	substrate mass transfer coefficient ($m\ h^{-1}$)
k_P	product mass transfer coefficient ($m\ h^{-1}$)
k_La	overall oxygen mass transfer coefficient (h^{-1})
k_SA	overall substrate mass transfer coefficient (h^{-1})
k_PA	overall product mass transfer coefficient (h^{-1})
k_{in}	inhibition constant due to ionic liquid fraction (h^{-1})
N_T	enzyme concentration ($g\ g^{-1}$ of biomass)
$\tilde{N}_T$	total enzyme concentration ($g\ L^{-1}$)
$[P]$	product concentration ($g\ L^{-1}$)
$[O_2]$	oxygen concentration ($g\ L^{-1}$)
$[O_2^*]$	saturated oxygen concentration in *ap* ($g\ L^{-1}$)
$[O_{20}]$	initial dissolved oxygen concentration in *ap* ($g\ L^{-1}$)
$[S]$	substrate concentration ($g\ L^{-1}$)
r_i	reaction rate without cell deactivation ($g\ L^{-1}\ h^{-1}$)
r_i'	reaction rate with cell deactivation ($g\ L^{-1}\ h^{-1}$)
k_j	forward kinetic constant of the reaction ($L\ g^{-1}\ h^{-1}$)
k_j'	backward kinetic constant of the reaction (h^{-1})
K_P	partitioning coefficient between aqueous and ionic phase (–)
$[X_V]$	viable biomass concentration ($g\ L^{-1}$)
$[X_{Vi}]$	initial viable biomass concentration ($g\ L^{-1}$)

Notations

ag	gas phase
ap	aqueous phase
dp	dispersed or ionic phase
CHMO	cyclohexanone monooxygenase
IL	ionic liquid

Greek symbols

φ	ionic or dispersed fraction (–)
θ_E	free enzyme fraction (–)
θ_{EO2}	enzyme-oxygen complex fraction (–)
θ_{EO2S}	enzyme-oxygen-substrate complex fraction (–)
θ_{EO2SS}	substrate inhibition complex fraction (–)
θ_{O2EO2}	oxygen inactivation complex fraction (–)
θ_X	viable biomass fraction (–)

1. Introduction

The Baeyer–Villiger (BV) oxidation reaction, including the conversion of cyclic ketones into their corresponding lactones, was observed more than 100 years ago [1]. Enzymatic BV oxidation has been efficiently applied to synthetic organic chemistry since there are many enzymes that are being exploited in the last decade [2]. Particularly, in the BV oxidation reaction, cyclohexanone monooxygenase (CHMO) from *Acinetobacter calcoaceticus* NCIMB 9871 is one of the best-known enzymes [3]. In the last decade, an increase of the rate of oxidation of cyclic ketones has been obtained when the CHMO gene is cloned into an expression vector to give the biocatalyst strain *E. coli* TOP10 (pQR239) [4]. Nevertheless, substrate/product inhibition was identified for this conversion with the whole cell-mediated conversion using bicyclo[3.2.0]hept-2-en-6-one [5]; particularly, the reaction is inhibited by ketone concentrations above 0.4 g/L and by combined regioisomeric lactones with concentrations above 3.5 g/L.

A number of solutions have been recommended to decrease the substrate/product inhibition; for instance: the proposal of adsorbent resins [6]; *in situ* supply of the substrate and removal of lactone product, maintaining both substrate and product at sub-inhibitory levels in the environment of the biocatalyst [7]; and encapsulation of biocatalyst to prevent CHMO oxidation [8]. However, one of the main limitations of these strategies is the difficulty in controlling mass transfer of substrate, product and oxygen that directly impacts on the productivity and the cost-benefit ratio.

The conversion of ketones using whole cells using a pseudo three-phase (air–water–ionic liquid) partitioning bioreactor (TPPB) seems a promising proposal since this technology leads to an increment on consumption rates [9] avoiding substrate/product inhibition and, mainly, giving a better control of the mass transfer phenomena [10].

In a previous work from our research group, the use of a TPPB maintaining the biocatalyst in the aqueous phase and using ionic liquid (IL) as immiscible phase for substrate reservoir and *in situ* product removal was attempted [11]. Specifically, a regime analysis for the oxidation of bicyclic ketone bicyclo[3.2.0]hept-2-to-6-one to bicyclic lactone (1S,5R)-(-)-2-oxabiciclo[3.3.0]oct-6-en-3-ona using whole cells based on *E. coli* strain TOP10 pQR239, using trioctylmethylammonium bis(trifluoromethylsulfonyl)imide ([OMA][BTA]) as IL, allowed to observe an increment on consumption rates avoiding substrate/product inhibition. From this end, since the establishment of design, scale up or scale down, optimization and operational guidelines ensuring optimal conversions require the understanding of process performance [12], modeling of this TPPB accounting for the interaction between mass transport phenomena and kinetics along with loss of cell viability is mandatory. To date there are few studies [13] explaining through mathematical modeling the complex interaction between mass transport phenomena along with kinetics and loss of cell viability in a TPPB considering the inclusion of a heavy organic solvent in the system. For example, Mahanty et al. [14] proposed a kinetic model for degradation of pyrene in a partitioning bioreactor; however, they did not account for the mass transfer mechanisms involved in Bordel et al. [12] modeled gas–liquid VOC transport in a partitioning bioreactor and described adequately the mass transport mechanisms but kinetic modeling was not considered. For treating polluted air streams in a continuous stirred tank bioreactor, Mohammad [15] described the simulation trends of the model but failed to compare with experimental data.

The aim of this study was to develop a pseudo heterogeneous mathematical model for bioconversion of bicyclic ketone bicyclo[3.2.0]hept-2-to-6-one to bicyclic lactone (1S,5R)-(-)-2-oxabiciclo[3.3.0]oct-6-en-3-ona using whole cells based on *E. coli* strain TOP10 pQR239 in a TPPB using 5% and 12.5% (v/v) of IL fraction ([OMA][BTA]). The model was based on independent studies in abiotic bioconversion and loss of cell viability conditions, which in turn were used to determine the mass transport, kinetic and deactivation parameters respectively, that characterize the corresponding phenomenon involved in the TPPB. Finally, the developed pseudo heterogeneous TPPB model was used to carry out a parametric sensitivity study with a goal to observe the effect of oxygen transport rate on bioconversion.