Biochemical Engineering Journal 58–59 (2011) 87–95

Contents lists available at SciVerse ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regime analysis of a Baeyer–Villiger bioconversion in a three-phase (air–water–ionic liquid) stirred tank bioreactor

R. Melgarejo-Torres[a], D. Torres-Martínez[a], M. Gutiérrez-Rojas[a], A. Gómez de Jesús[a], G.J. Lye[b], S. Huerta-Ochoa[a,*]

[a] Departamento de Biotecnología, Universidad Autónoma Metropolitana-Iztapalapa, Av. San Rafael Atlixco 186, Col. Vicentina, 09340, México D.F., Mexico
[b] The Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, London, WC1E 7JE, United Kingdom

ARTICLE INFO

Article history:
Received 16 March 2011
Received in revised form 3 August 2011
Accepted 29 August 2011
Available online 3 September 2011

Keywords:
Regime analysis
Multiphase bioreactor
Ionic liquid
Bioconversion
Mass transfer
Oxygen transfer

ABSTRACT

The aim of this work was to conduct a regime analysis on a three-phase (air–water–ionic liquid) stirred tank bioreactor of the Baeyer–Villiger bioconversion process, using [MeBuPyrr][BTA] ionic liquid as the dispersed phase. The regime analysis based on characteristic times of the different mechanisms involved (mixing, mass transfer, reaction) can yield a quantitative estimate of bioreactor performance. The characteristic time obtained for oxygen uptake rate ($54\,s^{-1}$) was among the characteristic times determined for oxygen transfer ($13–129\,s^{-1}$) under different operating conditions, suggesting that the oxygen transfer rate under certain operating conditions could be a limiting step in the bioconversion process. Further enhancement of oxygen transfer rates requires proper selection of the bioreactor operational conditions, and improved design of the ionic liquid used as oxygen transfer vector.

1. Introduction

The potential for microbial transformation through Baeyer–Villiger (BV) oxidation of linear or cyclic ketones (one of the major oxidation reactions in organic chemistry) has attracted the attention of scientists interested in microbial screening for the detection of new activities [1]. Flavin-containing Baeyer–Villiger monooxygenases employ NADPH and molecular oxygen to catalyse the insertion of an oxygen atom into the carbon–carbon bond of a carbonyl substrate. Given the wide utility of Baeyer–Villiger reactions in synthetic organic chemistry, these enzymes can potentially be exploited in a variety of biocatalytic applications [2,3]. An isolated thermostable Baeyer–Villiger monooxygenase (phenylacetone monooxygenase, PAMO) in the presence of ionic liquids was first reported by Rodríguez et al. [4] enhancing the *E*-value in the oxidation of racemic benzylketones and increasing the optimal substrate concentration for performing Baeyer–Villiger oxidation. However, substrate inhibition of recombinant cyclohexanone monooxygenase (CHMO) in oxidative biocatalysis has been reported. In fact, Doig et al. [5], using *Escherichia coli* TOP10 (pQR239) to study the oxidation of bicyclo[3.2.0]-hept-2-en-6-one to its two corresponding lactones, observed substrate inhibition up to 0.2–0.4 g L^{-1}. More recently, Doo et al. [6] using *Corynebacterium glutamicum* to study cyclohexanone oxidation, reported CHMO substrate inhibition up to 4 g L^{-1}. Substrate inhibition problem may be overcome by using two-liquid-phase partitioning bioreactors, which have demonstrated significant potential for enhancing the productivity of many bioprocesses [7]. For example, water immiscible ionic liquids can be used as biocompatible solvents in whole-cell biocatalysis, as evidenced by the increased yield of chiral alcohol synthesis [8]. Recently, Bräutigam et al. [9] successfully applied twelve water immiscible ionic liquids with adequate distribution coefficients, which acted as substrate reservoirs and in situ extractants for a product catalysed by a recombinant *E. coli* strain whole-cell biocatalyst. However, the productivity of oxidative biocatalysis in multiphase systems can also be limited by mass transport (oxygen and substrate). Mass transfer rates depend on fluid physical properties, temperature, pressure, media composition, mixing, air superficial velocity and configuration of the bioreactor. In particular, mass transfer across an interfacial liquid area depends on the Sauter mean droplet diameter (d_{32}), volumetric oxygen transfer coefficient (k_La), and substrate and product partition coefficients. Although it is possible to increase the interfacial area and k_La by increasing the agitation and aeration rates, this is associated with increased power consumption and operational costs. In addition, high agitation rates present a limitation when sensitive cells to hydrodynamic stress are used. In fact, maintenance of a constant interfacial area per unit volume

* Corresponding author.
E-mail address: sho@xanum.uam.mx (S. Huerta-Ochoa).

1369-703X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bej.2011.08.017

Nomenclature

A	liquid–liquid interfacial area available for mass transfer in the bioreactor ($m^2\ m^{-3}$)
a_{Cell}	interfacial area of the Lewis Cell ($m^2\ m^{-3}$)
C_S	substrate or product aqueous phase concentration in the Lewis Cell at time t ($g\ L^{-1}$)
C_S^*	substrate or product aqueous phase equilibrium concentration in the Lewis Cell ($g\ L^{-1}$)
C_{S_0}	substrate or product aqueous phase initial concentration in the Lewis Cell ($g\ L^{-1}$)
C_{O_2}	dissolved oxygen concentration in the bioreactor ($mg\ L^{-1}$)
$C_{O_2}^*$	saturated oxygen concentration ($mg\ L^{-1}$)
d_i	droplet diameter ORM determination (μm)
D_i	Rushton turbine diameter (cm)
D_T	bioreactor tank diameter (cm)
d_{32}	Sauter mean droplet diameter (μm)
H_L	liquid height in the bioreactor (cm)
k_e	oxygen electrode constant (s^{-1})
k	substrate or product mass transfer coefficient ($m\ s^{-1}$)
k_La	volumetric oxygen transfer coefficient in the bioreactor (h^{-1})
kA	substrate or product volumetric mass transfer coefficient in the bioreactor (h^{-1})
k_P	product mass transfer coefficient in the Lewis cell ($m\ s^{-1}$)
k_PA	product volumetric mass transfer coefficient in the bioreactor (h^{-1})
k_S	substrate mass transfer coefficient in the Lewis cell ($m\ s^{-1}$)
k_SA	substrate volumetric mass transfer coefficient in the bioreactor (h^{-1})
$\mathcal{R}_{O_2}$	oxygen uptake rate ($g\ L^{-1}\ h^{-1}$)
$\mathcal{R}_S$	substrate uptake rate ($g\ L^{-1}\ h^{-1}$)
$[S]_0$	initial substrate concentration in the bioreactor ($g\ L^{-1}$)
T	time
t_m	mixing characteristic time (s)
t_{O_2}	oxygen mass transfer characteristic time (s)
t_P	product mass transfer time characteristic time (s)
t_S	substrate mass transfer time characteristic time (s)
$t_{\mathcal{R}_{O_2}}$	oxygen consumption characteristic time (s)
$t_{\mathcal{R}_S}$	substrate consumption characteristic time (s)
X	biomass concentration ($g\ L^{-1}$)
$Y_{S/P}$	substrate–product yield ($g\ g^{-1}$)

Greek letters

ϕ	volume fraction of the dispersed phase ($L\ L^{-1}$)

is a key parameter for the successful scale-up of two-liquid-phase bioconversion processes [10].

Regime analysis, combined with small-scale experiments (Fig. 1), is a powerful tool for the development of scale-down strategies [11,12]. Law et al. [13] illustrated a regime analysis technique applied to a whole-cell Baeyer–Villiger bioconversion process catalysed using a recombinant *E. coli* strain. Their results demonstrated that implementation of bioconversion processes are practically limited by substrate and product inhibition, adequate supply of oxygen and biocatalyst longevity. Regime analysis can also be based on the characteristic times of the different mechanisms involved (mixing, mass transfer, reaction), which can provide a quantitative estimate of bioreactor performance [11,12]. Characteristic times have been defined as the ratio of capacity to flow. In other words, a low characteristic time implies a fast mechanism; while a high value indicates a slow mechanism. Thus, characteristic times can also be used to determine if there is a single rate-limiting mechanism (pure regime), or whether more than one mechanism (mixed regime) is responsible.

The aim of this work was to conduct a regime analysis on a three-phase (air–water–ionic liquid) stirred tank bioreactor of the Baeyer–Villiger bioconversion process using [MeBuPyrr][BTA] ionic liquid as the dispersed phase.

2. Materials and methods

2.1. Chemicals

Bicycle cetone Bicyclo[3.2.0]hept-2-en-6-ona (≥98%) and bicycle lactone (1S, 5R)-(−)-2-oxabiciclo[3.3.0]oct-6-en-3-ona (≥99.0%) (Fluka, Switzerland) were used as substrate and product standards, respectively. A buffer solution consisting of 50 mM phosphate pH 7.0 supplemented with 10 g glycerol L^{-1} was used as the aqueous phase for the bioconversion media and the extractive phase for mass transfer determinations. The ionic liquid butylmethylpyrrolidinium bis (trifluoromethylsulfonyl) imide [MeBuPyrr][BTA] (>98%), obtained from Solvent Innovation GmbH (Köln, Germany), was used as the dispersed phase. [MeBuPyrr][BTA] was selected among three ionic liquids based on its favorable partition coefficient (25.8), relative low viscosity (40 cp) and biocompatibility (45% viability of *E. coli* in 9%, v/v ionic liquid; 1 h at 30 °C) [14]. It is worth to mention that [MeBuPyrr][BTA] has been reported as a Newtonian fluid [15].

2.2. Analysis

Gas chromatography (GC) was used to quantify the concentrations of bicycle[3.2.0]hept-2-en-6-one and its corresponding regioisomeric lactones. 1 μL samples were injected into an XL gas chromatograph (PerkinElmer, Norwalk, CT) fitted with a CYCLOSILB 113-6632 capillary column (30 m × 530 μm) (J&W Scientific), and concentrations were determined using an external calibration curve. The GC Injector temperature was set at 250 °C. The GC temperature program used was as follows: the initial oven temperature of 100 °C was held 1 min and increased at 10 °C/min up to 150 °C, which was then held 3 min.

2.3. Microorganism

The *E. coli* strain TOP10 pQR239 was kindly provided by Professor John M. Ward (University College London, London, United Kingdom) for research and academic purposes, and is referred to hereafter simply as *E. coli*. To prepare inoculums for bioconversion experiments, *E. coli* cells were cultured in Erlenmeyer flasks containing 70 mL of mineral media (in $g\ L^{-1}$): glucose 2.5, NH_4Cl 0.92, Na_2SO_4 0.1, $MgSO_4$ 0.1 and $CaCl_2$ 0.03, in phosphate buffer 50 mM pH 7.0, supplemented with 10 g L^{-1} glycerol at pH 7.0. Culture media was sterilised in an autoclave at 115 °C for 15 min and supplemented with 100 mg L^{-1} ampicillin (previously filter sterilised using a 25 μm filter). Erlenmeyer flasks were incubated at 250 rpm for 16 h at 37 °C. After this 16 h growth period, cyclohexanone monooxygenase expression was induced by adding the necessary amount of arabinose solution (100 g L^{-1}) to reach a final concentration of 2 g L^{-1}. Cells were harvested after 3 h of induction by centrifugation at 10,000 rpm for 20 min.