Journal of Biotechnology 157 (2012) 253–257

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

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

Short communication

Evaluation of parallel milliliter-scale stirred-tank bioreactors for the study of biphasic whole-cell biocatalysis with ionic liquids

Danielle Dennewald, Ralf Hortsch, Dirk Weuster-Botz*

Lehrstuhl für Bioverfahrenstechnik, Technische Universität München, Boltzmannstr. 15, 85748 Garching, Germany

ARTICLE INFO

Article history:
Received 17 June 2011
Received in revised form 19 October 2011
Accepted 24 October 2011
Available online 3 November 2011

Keywords:
Ionic liquid
Whole-cell
Biocatalysis
Milliliter-scale
Energy dissipation
High-throughput

ABSTRACT

As clear structure–activity relationships are still rare for ionic liquids, preliminary experiments are necessary for the process development of biphasic whole-cell processes involving these solvents. To reduce the time investment and the material costs, the process development of such biphasic reaction systems would profit from a small-scale high-throughput platform. Exemplarily, the reduction of 2-octanone to (R)-2-octanol by a recombinant *Escherichia coli* in a biphasic ionic liquid/water system was studied in a miniaturized stirred-tank bioreactor system allowing the parallel operation of up to 48 reactors at the mL-scale. The results were compared to those obtained in a 20-fold larger stirred-tank reactor. The maximum local energy dissipation was evaluated at the larger scale and compared to the data available for the small-scale reactors, to verify if similar mass transfer could be obtained at both scales. Thereafter, the reaction kinetics and final conversions reached in different reactions setups were analysed. The results were in good agreement between both scales for varying ionic liquids and for ionic liquid volume fractions up to 40%. The parallel bioreactor system can thus be used for the process development of the majority of biphasic reaction systems involving ionic liquids, reducing the time and resource investment during the process development of this type of applications.

Ionic liquids gain more and more attention as potential alternative to commonly used organic solvents. Their negligible vapour pressure, their large electrochemical window, as well as their high thermal and chemical stability make them suitable for applications in many areas. Previous works have proven the successful application of ionic liquids in biphasic whole-cell biotransformations, where a non-water miscible ionic liquid serves as substrate reservoir and *in situ* product extractant. This effectively protects the biocatalyst from toxic substrates and/or products, and permits to increase the substrate availability in the reaction system for substances of low water solubility (a.o. Bräutigam et al., 2007, 2009; Cull et al., 2000; Pfruender et al., 2004, 2006; Schroer et al., 2007; Wang et al., 2009). The enormous multitude of possible anion/cation combinations – generating according to estimations up to 10^{18} different compounds (Carmichael and Seddon, 2000) – leads to a large choice of possible ionic liquids. In the absence of clear structure–activity relationships predicting the biocompatibility of the solvent or the partitioning behavior of given substances in the biphasic reaction setup, the choice for one ionic liquid best suited for a given process is still difficult. Choosing a solvent therefore often makes time intensive preliminary tests and screening procedures necessary.

Screening of different reaction setups is preferentially made at the small scale and, if possible, in parallel reaction setups, in order to limit both the time and the resource investment. To date, screening experiments for reaction systems including ionic liquids are mostly performed in shake flasks or other magnetically stirred vials (e.g., Arai et al., 2010; Cull et al., 2000; He et al., 2009; Kratzer et al., 2008; Lou et al., 2009; Pfruender et al., 2004, 2006; Wolfson et al., 2006). However, in such common small-scale reaction systems, sufficient mass transfer is not always assured when liquids of large viscosity – such as ionic liquids – are involved. In addition, the scalability with respect to the larger L-scale stirred-tank reactor used subsequently for production purposes is often not given (Hortsch and Weuster-Botz, 2010b).

To overcome the general issue of scalability between small-scale and large-scale vessels, a miniaturized stirred-tank bioreactor system was developed that allows the parallel operation of up to 48 reactors at the mL-scale (Puskeiler et al., 2005; Weuster-Botz et al., 2005). The system is equipped with magnetically driven impellers and shows similar process engineering characteristics compared to standard laboratory- and production-scale reactors (Hortsch and Weuster-Botz, 2010a). This way, a reliable and robust scale-up of the processes from the small scale to a larger scale is assured. Previous studies demonstrated the usefulness of this tool for

* Corresponding author. Fax: +49 0 89 289 15714.
E-mail address: D.Weuster-Botz@lrz.tu-muenchen.de (D. Weuster-Botz).

0168-1656/$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.jbiotec.2011.10.008

"high-throughput bioprocess design" (Hortsch and Weuster-Botz, 2011; Knorr et al., 2007; Kusterer et al., 2008; Vester et al., 2009).

In the present study, the suitability of the described high-throughput platform is studied for the process development of reactions involving non-water miscible ionic liquids. For the scale-up of the biphasic reactions, the maximum local energy dissipation is determined at the 200 mL scale, and compared to the data available from literature for the small-scale reactors. The results obtained for biotransformations at small scale are then compared to previously performed larger-scale experiments to determine if the small-scale high-throughput platform can be used for the process design of these types of reactions.

1. Materials and methods

The process exemplarily considered here is the process integrated biotransformation of 2-octanone to (*R*)-2-octanol using a recombinant *Escherichia coli* in a biphasic reaction setup ionic liquid/buffer.

The biocatalyst used – *E. coli* BL21 (DE3) T1r(pET24a-$adh_{L.\ brevis}$-$fdh_{C.\ boidinii}$) – was kindly provided by Julich Chiral Solutions (Jülich, Germany). It contains the *Lactobacillus brevis* alcohol dehydrogenase (ADH) for the reduction of the ketone to the chiral alcohol and the *Candida boidinii* formate dehydrogenase (FDH) for the internal cofactor regeneration (NADH). The cultivation of the biocatalyst was performed in fed-batch mode in a stirred-tank reactor of 7.5 L nominal volume (Labfors, Infors AG, Bottmingen, Switzerland) as described previously (Bräutigam et al., 2009).

The ionic liquids were kindly provided by Merck KGaA (Darmstadt, Germany). 2-Octanone (>98%), 2-octanol (>97%), (*R*)- and (*S*)-2-octanol (99%) were purchased from Sigma–Aldrich (Schnelldorf, Germany). The biotransformations were performed at varying ionic liquid phase fractions. Each 200 mL reaction system contained 8 g_{DCW} biocatalyst in the aqueous phase and an initial substrate quantity of 24 mmol 2-octanone in the ionic liquid phase. The reaction systems at smaller scale contained the equivalent amount corresponding to the reduced volume. The experimental procedure was as described previously (Bräutigam et al., 2009).

Larger-scale biotransformations were carried out at room temperature in a stirred-tank reactor with a nominal volume of 400 mL (Profors, Infors AG, Bottmingen, Switzerland) equipped with a magnetically driven six-blade Rushton turbine (Weuster-Botz et al., 2002). The experiments were carried out as technical replicates. The results presented are the average values of three measurements and the error bars indicate the corresponding standard deviation. Small-scale biotransformations were performed in the "bioreaction block" according to Kusterer et al. (2008), at a reaction volume of 14 mL in each bioreactor. Three parallel reactors were used for each reaction setup. The results presented are the average values obtained in the three parallel experiments and the error bars indicate the corresponding standard deviation. Stirring was set to 600 min^{-1} in the 200 mL stirred-tank reactor and to 1500 min^{-1} in the mL-scale bioreactors.

The maximal local energy dissipation was determined by applying the clay/polymer flocculation system described earlier (Hortsch and Weuster-Botz, 2010a).

2. Results and discussion

The mass transfer in emulsion forming reaction systems is predominantly a function of the interfacial area and hence, of the drop size of the dispersed phase in the continuous phase. For a robust scale-up of processes from small scale to larger scale, the drop sizes should be similar at both scales for the same reaction conditions (stirrer speed, phase ratio etc.). The drop size distribution within a given system is determined by the hydrodynamic forces, and the maximum drop diameter (d_{max}) of the dispersed phase is related to the maximum local energy dissipation (ε_{max}) (Arai et al., 1977; Hinze, 1955).

Table 1
Ionic liquids used as second phase for the biotransformation of 2-octanone to (*R*)-2-octanol in the biphasic ionic liquid/water system and their corresponding abbreviation.

Ionic liquid	Abbreviation
1-(2-Ethoxyethyl)-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide	[(EO2E)MPL][NTF]
1-Hexyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide	[HMPL][NTF]
n-Hexylpyridinium bis(trifluoromethylsulfonyl)imide	[HPYR][NTF]
n-Ethyl-n,n-dimethyl-2-methoxyethylammonium bis(trifluoromethylsulfonyl)imide	[(NEMM)EO2E][NTF]

In the present work, ε_{max} was measured for the 200 mL system at different impeller speeds (Fig. 1), and compared to the ε_{max} values measured in the mL reactors presented in literature (Hortsch and Weuster-Botz, 2010a). When comparing both sets of data, it can be seen that the maximum local energy dissipation reached in each reaction system is within the same range. This means that the drop size, and consequently also the mass transfer is very similar in both reactor types, assuring a good reproducibility of this variable at both scales. The data also shows the advantage of miniaturized stirred-tank reactors compared to shaken systems like MTP or shake flasks: significantly lower maximum local energy dissipations are measured in shake flasks (Peter et al., 2004), and consequently different drop size distributions are obtained. Furthermore, active mixing of highly viscous media, such as ionic liquids, can become a problem in shaken systems, as so called "out of phase" conditions can occur (Büchs et al., 2000, 2001). Data available on the volumetric power consumption at both scales as a function of the impeller speed (Amidjojo, 2004; Hortsch and Weuster-Botz, 2010a) also shows that the volumetric power consumptions reached in the range of operational impeller speeds is within the same order of magnitude at both scales (Fig. 1).

The scalability of the small-scale parallel bioreactor system for biphasic whole-cell biotransformations involving ionic liquids was verified by comparing the conversion reached in larger-scale devices with the results obtained in the parallel mL-reactors. The biotransformations at the 200 mL scale were performed at an impeller speed of 600 min^{-1}. According to Fig. 1, the maximal local energy dissipation provoked would correspond to an impeller speed of ~1100 min^{-1} at the mL-scale. At this impeller speed, the dispersion of the relatively dense ionic liquid (1.34 kg dm^{-3} at 25 °C) – settled at the bottom of the reactor at the beginning of the reaction – was however relatively slow. In order to reach a more rapid homogenisation of the reaction medium, the impeller speed was increased to 1500 min^{-1}. This does not influence the evolution of the reaction. Indeed, it was shown previously that the biotransformation was not mass transfer limited when the ionic liquid was homogeneously dispersed in the aqueous phase at a maximal local energy dissipation ≥9 W L^{-1} (Dennewald et al., 2011). A direct translation in terms of maximal local energy dissipation/power consumption from the larger scale to the milliliter-scale was not possible here, because such a translation is only valid in homogeneous mixing conditions – a condition not satisfied at the beginning of the reaction when the two phases of the system are not yet mixed.

In a first approach, four different ionic liquids were used at a volume ratio of 20% ionic liquid (Table 1). The reaction kinetics and final conversion of 2-octanone for the four different ionic liquids are in good agreement between the mL-scale and the 20-fold larger stirred-tank reactor (Fig. 2). This proves the usability of the