Can deep eutectic solvents sustain oxygen dependent bioprocesses? – Measurements of oxygen transfer rates

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ABSTRACT

The oxygen transfer rate (OTR) of dioxygen to solutions describing the transport of oxygen gas from the gaseous phase into the liquid phase of a reaction system over a given period is an important measure for biotechnological applications. The OTRs have already been described for aqueous systems and also recently for organic and non-conventional media, whereas the OTRs of a novel class of solvents, deep eutectic solvents (DESs), have not been determined yet. In this contribution, we report for the first time on the OTRs of choline chloride:ethylene glycol (ChCl:EG, ‘ethaline’) and ethylammonium chloride:ethylene glycol (EAC:EG, ‘ethethylaline’) while using water as a reference. We applied the dynamic measurement method and found up to 11-fold lower volumetric mass transfer coefficient (k_La) for ethaline and 6-fold lower for

ethethylaline when compared to water at 25°C. Furthermore, we investigated the effect temperature (35°C and 45°C) has on the k_{La} for those solvents.

INTRODUCTION

The application of Nature's catalysts 'enzymes' for the synthesis of chemicals is a key emerging field of industrial biotechnology to meet current and future needs of our society for sustainable manufacturing of chemicals.^{[1],[2],[3]} Remarkably, many enzymes catalyze processes not only in water, but also in other non-aqueous environments ('biocatalysis in non-conventional media', BNCM).^[4] The enormous potential of BNCM for industrial enzymatic synthesis relies on tackling the challenges that modern synthesis entails for water-based processes: a limited solubility of synthetically interesting substrates/products, and a resource-, time- and energy intensive and waste generating downstream processing applied to isolate diluted target compounds from aqueous media.^{[2],[3],[5]}

The scope of BNCM covers the use of solvent-free systems, organic solvents, ionic liquids (ILs),^[6] or supercritical fluids. Importantly, the selection of reaction media for biocatalysis with a particular emphasis on their 'greenness' is a main focus in the biocatalytic community.^[7] Herein, deep eutectic solvents (DESs) hold breathtaking features due to their ecological, economical and practical advantages.^{[8],[9],[10]–[12]} In fact, DESs have been coined as 'the solvents of the 21st century'.^{[8],[10],[13]} In a nutshell, the added values of most of the DESs are based on their biogenic origin, melting points below room temperature, low volatility, high thermal stability and tailored characteristics analogous to ILs – but typically with diminished toxicity. With this respect, DESs show better biodegradability, large availability at acceptable costs, and easier preparation methods (often based on simply mixing of two components under stirring).^{[13],[14]} A DES is composed of a

hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA), typically a quaternary ammonium salt (e.g. the animal feed choline chloride).^{[11],[12],[15]} Overall, biocatalysis in DESs combines the best of the two worlds: enzymes' excellent selectivities and efficiencies with environmentally-friendly and readily available features of DESs.

Redox biocatalysts, being classified in Enzyme Commission (EC) number 1, serve important roles in organic synthesis facilitating the introduction of chiral motives and selective moieties.^[16] More precisely, oxidation reactions demanding oxygen as a reaction partner are catalyzed by (i) laccases, (ii) oxidases, (iii) monooxygenases, (iv) halogenases and (v) dioxygenases. According to the outstanding reference book by Liese and coworkers,^[17] one sixth of all commercialized enzymatic processes are based on redox enzymes catalyzing oxidation processes, e.g. enantiospecific hydroxylations (ca. 40%), alcohol/amine oxidations (ca. 22%), dihydroxylations (ca. 14%), epoxidations (ca. 14%) and Baeyer-Villiger oxidations (ca. 5%).^[18] Given the importance and high interest in redox biocatalysis in organic synthesis, it is clear that accurate knowledge of oxygen (O₂) transfer is critical for the control of many bioprocesses, especially on a sustainable and economically feasible industrial scale.^[19]

The oxygen transfer rates from air into water were already broadly described in the literature,^[20] and recently, Ramesh et al. report the measurement of oxygen transfer from air into various organic solvents.^[21] The authors used the dynamic method with a novel solvent resistant optical sensor and describe for the first time online oxygen measurements (concentrations and transfer rates) in organic media (toluene, tetrahydrofuran, dimethylformamide, methyl tert-butylether, cyclohexane, heptane, isooctane and water as reference). In 2019, Brennecke and co-workers reported for the very first time the solubility and diffusivity of oxygen in ILs.^[22] The solubility of oxygen was measured gravimetrically in 16 ILs and the temperature dependence of the solubility was

determined for eight of the ILs. For those eight ILs, the authors also present other important thermodynamic data like Henry's law constants, standard enthalpies and entropies of absorption.

To the best of our knowledge, the transfer rate of dioxygen into DESs are not reported yet and thus, comprehensive data are not available until now. Within this publication, we briefly report on the first measurements of oxygen transfer from air into DESs. We used an electrochemical oxygen microsensor and determined the OTRs into choline chloride: ethylene glycol (ChCl:EG, ethaline) with a 1:2 molar ratio, ethylammonium chloride: ethylene glycol (EAC:EG, ethethylaline) with a 1:1.5 molar ratio. Additionally, the transfer rate from air into water was determined as a reference and we used the dynamic measurement method throughout all experiments. Since this field of research has gained increased attention in recent years, we believe that this publication can contribute to its successful path to sustainable and industrial applications.

RESULTS AND DISCUSSION

First, we set up the measurement reactor including a container with an electrochemical oxygen microsensor (**Figure S1**). Details on the sensor are not scope of this publication and can be found elsewhere.^[23] Initially, we evaluated the stability of the sensor in the eutectic mixtures and we found that the microsensor remain stable in the chosen DESs for at least one week proving their practicality for these measurements. Next, the oxygen sensor was calibrated at different temperatures with a simple two-point method (**Figure S2**) and subsequently, the sensor was applied in the measurement of OTR from air into DESs.

The OTRs were measured *via* the dynamic method.^[24] First, thanks to the low risk for evaporation of DESs, nitrogen bubbling was employed for the desorption of oxygen until its concentration converged to zero (**Figure 1**). After stopping the purging with nitrogen, the stirred-tank reaction vessel was opened resulting in immediate air saturation of the headspace. Consequently, the oxygen concentration within the DESs (or water) began to increase until it reached a saturation level. During the aeration, the stirring speed was kept at a constant value of 100 rpm (higher agitation rates lead to disturbance of the gas-liquid interface, data not shown). Stirring is needed to ensure that the solvent is well mixed and that no oxygen gradients form within the solvent, as the sensor only measures at a single spot.

The OTR can be described by the equation (1) below:

$$d[O_2]/dt = k_L a * ([O_2]^* - [O_2]) \quad (1)$$

Where $d[O_2]/dt$ stands for the change of oxygen concentration over time. The volumetric mass transfer coefficient is represented by $k_L a$ which is itself composed of k_L representing the mass

transfer coefficient and a representing the specific interfacial area per unit liquid volume ($\text{m}^2 \cdot \text{m}^{-3}$).^[25]

Resolving the equation (1) leads to equation (2):

$$\ln ([O_2]^* - [O_2]) = -k_L a * t + \ln [O_2]^* \quad (2)$$

According to Henry's law, the concentration of oxygen in solution in equilibrium with the gas, $[O_2]^*$, is proportional to the partial pressure of oxygen in the gas (pO_2^*):

$$[O_2]^* = H * pO_2^* \quad (3)$$

Which forms equation (2) to equation (4):

$$\ln (pO_2^* - pO_2) = -k_L a * t + \ln pO_2^* \quad (4)$$

$k_L a$ volumetric oxygen mass transfer coefficient

$[O_2]^*$ saturation concentration of oxygen

$[O_2]$ concentration of oxygen at a given time

pO_2^* saturation partial pressure of oxygen

pO_2 partial pressure of oxygen at a given time

H Henry's Law constant

In this way, the $k_L a$ value can be obtained from the change of partial pressure $\ln (pO_2^* - pO_2)$ over time (the slope of linear fit). All linear fits for all solvents are shown in the supporting information (**Figure S3 and S4**).

The $k_L a$ values for all DESs increased with increasing temperatures, indicating a higher transfer rate of oxygen from air to solvents at higher temperatures (**Table 1**). The one order of magnitude

smaller k_{La} values compared to the reference water imply a slower transfer rate of oxygen from the air to the analyzed DESs. Probably, the relatively high viscosities of the DESs are causing this effect ($> 20 \text{ mPa}\cdot\text{s}$ at room temperature compared to water with a viscosity of $0.89 \text{ mPa}\cdot\text{s}$). In fact, the use of DES glyceline for k_{La} determination was not manageable due to its very high viscosity ($339.2 \text{ mPa}\cdot\text{s}$ at 25°C) that hindered efficient stirring of the mixture.

Figure 1. Dynamic method for determination of oxygen transfer rates (OTRs) from the headspace to (a) water, (b) DES 1 (ethaline), and (c) DES 2 (ethethylaline) at three different temperatures (25, 35°C, and 45°C) (One of the duplicates is shown here).

Viscosity is an important evaluation parameter for selecting a solvent for biocatalysis, which strongly influences mass transfer rates.^[8] Bioprocess development would hence involve the use of appropriate agitators and mixing rates to alleviate the mass transfer limitations due to high viscosities of reaction media. We attribute that the relatively high viscosity of DESs have a limiting effect on OTRs. To substantiate this assumption, the relationship between viscosity and OTR was investigated. To this end, the dynamic viscosity of DESs was determined at three different temperatures. As expected, the viscosity decreased significantly with increasing temperatures (e.g., for ethaline, $42.6 \text{ mPa}\cdot\text{s}$ (25°C) vs $20.7 \text{ mPa}\cdot\text{s}$ (45°C), (**Table 1, Figure S5**). The decreased viscosities contributed to the faster OTRs, thus, the higher k_{La} values. This relationship should be

taken into account when selecting solvents for performing redox catalysis, especially since they require an oxygen supply.

Table 1. The dynamic viscosity (η), volumetric mass transfer coefficient ($k_L a$) and mass transfer coefficient (k_L) values for H₂O and DESs at different temperatures (25°C, 35°C, and 45°C).

Solvent	Components		T	Viscosity (η)	Volumetric mass transfer coefficient (k_{La})	Mass transfer coefficient (k_L)
	HBA	HBD	°C	mPa·s	h ⁻¹	m·h ⁻¹ , ×10 ⁻³
H ₂ O	/		25	0.89	2.48 ± 0.06	112.7 ± 2.5
			35	0.72	3.61 ± 0.02	163.9 ± 0.9
			45	0.60	4.53 ± 0.22	205.6 ± 10
ethaline (molar ratio)		EG	25	42.6	0.23 ± 0.01	10.4 ± 0.2
	ChCl	EG	35	28.9	0.26 ± 0.00	11.7 ± 0.1
	1	2	45	20.7	0.36 ± 0.11	16.4 ± 0.4
ethethylaline (molar ratio)		EG	25	29.8	0.42 ± 0.01	19.2 ± 0.5
	EAC	EG	35	20.9	0.59 ± 0.01	26.6 ± 0.4
	1	1.5	45	15.2	0.83 ± 0.01	37.6 ± 0.6
glyceline (molar ratio)			25	339.2	/	/
	ChCl	Gly	35	186.1		
	1	2	45	108.0		
ChCl–Choline chloride; EG–Ethylene glycol; EAC–Ethyl ammonium chloride; Gly–glycerol H ₂ O (25°C) $K_H = 4.44 \times 10^7$ hPa, 0.257 mM (theoretic value) vs (experimental value) 0.253 ± 0.001 mM H ₂ O (35°C) $K_H = 5.14 \times 10^7$ hPa, 0.217 mM (theoretic value) vs (experimental value) 0.214 ± 0.003 mM H ₂ O (45°C) $K_H = 5.70 \times 10^7$ hPa, 0.187 mM (theoretic value) vs (experimental value) 0.184 ± 0.001 mM $P = K_H \times X_{O_2}$						

The results indicate that both mass transfer coefficient (k_{La}) and dynamic viscosity (η) strongly depend on the nature of the DES i.e., individual DES components. Herein, it is worth to highlight that viscosity measurements showed 48-fold higher viscosity for ethaline and 33-fold higher for ethethylaline in comparison with water at 25 °C whereas the k_{La} values were ‘only’ 11-fold lower for ethaline and 6-fold lower for ethethylaline compared to water at 25 °C (**Figure S5**). Consequently, the freedom in the combination of hydrogen bond donor and hydrogen bond acceptor can lead to various possibilities having a DES with sufficiently high k_{La} values and not too high viscosities. The latter can be a significant advantage in bioprocess applications as diffusion limitations can be thus alleviated. In addition, small amounts of external water (up to 20% v/v) added as cosolvent to the reaction medium can significantly lower the viscosities of mixtures of DES/water.^[26] The possibility of using DESs ‘greener’ reaction media with the desired viscosities can open a new process window for sustainable (bio)process where oxygen is demanded in the process of interest. The wide range application area of DESs in (bio)catalysis but also in the valorization of biomass would broaden the process window significantly.^[27]

CONCLUSION

While the biotechnologically important OTRs from air into water or neat organic solvents were already determined, this parameter was not available for DESs yet. The here presented study demonstrated the first-time analysis of OTRs from air into DESs, new generation sustainable solvents which are nowadays widely used in bio- and chemo-catalysis, as well as for biomass valorization and downstream processing. Our experiments revealed up to 11-fold lower k_{La} values for ethaline and 6-fold lower for ethethylaline when compared to water at 25°C. This demonstrates that the nature of the DESs has a big influence on the oxygen transfer and that it is possible to tune

OTRs in this way. Consequently, DESs can have OTRs that are sufficiently high to allow biotechnological use of these environmentally friendly solvents. With this study, we aim to open possibilities for the development of new processes for the use of DESs where molecular oxygen is involved in the bioprocess.

The here presented method is not limited to oxygen, as further gaseous substances (e.g., CO₂ or H₂) are also of high interest due to their important roles in biotechnological processes. With reliable sensors becoming available the transfer rates of those gasses into green solvents like DESs could be assessed also and biotechnological processes optimized to be even more sustainable.

ASSOCIATED CONTENT

Description of Supporting information

Materials and equipment, experimental methods, and measurement results in the form of graphs

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Author Contributions

Selin Kara conceived the conception and design of this work. Ningning Zhang designed and performed the experiments including the preparation of DESs, the dynamic viscosity measurement, the construction of reactor, the OTR measurements in water and ethaline, the data analysis and manuscript writing. Fabian Steininger assisted with the design of the reactor, performed the oxygen transfer measurements in ethethylaline, and helped with the interpretation of results. Lars-Erik Meyer was highly involved in the experiment design & discussion as well as the manuscript writing. Klaus Koren participated in the experimental design, provided

infrastructure and equipment and helped discuss the results. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

DESSs, deep eutectic solvents; OTRs, oxygen transfer rates; BNCM, biocatalysis in non-conventional media; ILs, ionic liquids; HBD, hydrogen bond donor; HBA, hydrogen bond acceptor.

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