FINAL REPORT

1 General Information

DFG reference number: 684928

Project number: 501131738

Project title: „Feinblasen für biokatalytische Prozesse: Mikroskalige Phänomene und neuartige Anwendungen“

Name(s) of the applicant(s): Prof. Dr. rer. nat. Liese, Prof. Dr.-Ing. Schlüter

Official addresses:
Technische Universität Hamburg (TUHH), Institut für Technische Biokatalyse (ITB), Denickestraße 15, 21073 Hamburg
Technische Universität Hamburg (TUHH), Institut für Mehrphasenströmungen (IMS), Eißendorfer Straße 38, 21073 Hamburg

Name(s) of the co-applicants:

Name(s) of the cooperation partners:
Prof. Koichi Terasaka as Mercator-Fellow

Reporting period (entire funding period): 01.04.2022 – 31.10.2025

2 Zusammenfassung

Um das Potenzial und die Grenzen der Feinblasenbegasung für mehrphasige biokatalytische Anwendungen besser zu verstehen, wurden im Rahmen des Projekts sowohl globale als auch lokale Studien durchgeführt. Global betrachtet lassen sich die Auswirkungen der Feinblasenbelüftung hinsichtlich des Stofftransports und der Reaktionseffizienz einer biokatalytischen Reaktion in einem Rotating Bed Reaktor (RBR) quantifizieren. Die Immobilisierung des Enzyms Glucoseoxidase (GOx) auf epoxidfunktionalisierten Trägern ermöglicht die Wiederverwendung der Enzyme und erhöht somit die Nachhaltigkeit und maximale Zykluszahl biokatalytischer Reaktionen signifikant. Als Modellreaktion dient die Glucoseoxidation durch GOx-Enzyme. Die Reaktion konsumiert Sauerstoff, der durch Begasung mit Luft in das System eingebracht wird. Die Studie zeigt, wie die Ausbeute der biokatalytischen Reaktion durch die Verwendung einer Begasung mit Feinblasen gesteigert wird, da der Stoffaustausch zwischen Gas und Flüssigkeit beschleunigt stattfindet. Des Weiteren wird eine fluiddynamische Charakterisierung des RBR durchgeführt. Auf lokaler mikroskopischer Ebene lassen sich Potenziale und Grenzen fundamentaler untersuchen, was zu einem tieferen Verständnis der makroskopisch global beobachteten Phänomene beiträgt. Mittels Lichtschnittfluoreszenzmikroskopie (LSFM) werden die lokalen Stofftransportphänomene von Feinblasen untersucht. Diese Untersuchungen zeigen eine starke Abhängigkeit des Stofftransports von den in der Flüssigkeit gelösten gasförmigen Spezies.

Summary

To deepen our understanding of the potentials and limits of fine bubble aeration for multiphase biocatalytic applications, both global and local studies were conducted within the scope of the current project. On a global scale, the effects of fine bubble aeration can be quantified with respect to the mass transfer performance and reaction efficiency of a biocatalytic reaction in a rotating bed reactor (RBR). The successful immobilisation of the enzyme glucose oxidase (GOx) on epoxy-functionalised carriers enables a reuse of the enzymes, which increases the sustainability and total turnover number of biocatalytic reactions significantly. As a model reaction, glucose oxidation by GOx enzymes is used. The reaction consumes oxygen, which is brought into the system via fine bubble aeration by sintered spargers. The study shows how the yield from the biocatalytic reaction is increased when using fine bubble aeration, due to the acceleration of the diffusion of gas into the liquid by increased mass transfer. Furthermore, a fluid dynamic characterisation of the RBR is conducted. On a local microscopic scale, on the other hand, the potentials and limits can be studied more fundamentally, helping to deepen our understanding of

the globally macroscopically observed phenomena. Light Sheet Fluorescence Microscopy (LSFM) is used to study the local mass transfer phenomena of fine bubbles. These studies show a high dependence of the mass transfer performance with respect to the dissolved gaseous species present in the liquid bulk.

3 Progress Report

3.1 Characterisation of an Enzyme Reaction in a Rotating Bed Reactor Utilising Fine Bubbles

One objective of the project is the optimisation of a model reaction operated within a Rotating Bed Reactor (RBR) utilising fine bubble aeration. For this purpose, the mass transfer performance, reaction yield, enzyme recycling and fluid dynamics in the SpinChem® RBR are considered. Details to the results and methods used can be found in the published papers [A2-3] or summarized in the thesis [1]. In the following, the main findings along with unpublished results and methods are summarised shortly.

To enable a recycling of the glucose oxidase (GOx) and to increase sustainability, immobilisation on epoxy-functionalized carriers serving as packing material of the RBR was established at the ITB. The covalent immobilised procedure of GOx on epoxy-functionalised carriers reached an activity yield of over 98% [1], demonstrating a successful immobilization of the biocatalyst. The oxidative biotransformation, catalysed by the immobilised GOx, yielded an average conversion of 96.2 ± 2.5% using the established enzyme carrier system within the SpinChem® RBR [1]. The Michaelis-Menten constants of the free and immobilised GOx were determined to be 164 ± 34 mM and 187 ± 54 mM, respectively. In comparison to the immobilised GOx, which exhibited an enzyme-specific activity of 6.65 ± 0.62 U mg_{GOx}^{-1}, the free form of GOx demonstrated a higher activity, with an enzyme-specific activity of 8.08 U mg_{GOx}^{-1} [1]. The reaction rate equation was extended by implementing the biocatalyst deactivation constant and effectiveness factor, enabling quantitative estimation of reactor performance under diffusion and stability limitations. Detailed kinetic parameters were determined [1]. A laboratory-scale techno-economic assessment, based on experimentally validated kinetics and quantified recyclability, confirmed that GOx represents the primary cost driver of the process. The total cost was reduced by half utilizing the immobilized biocatalyst in repetitive batches under fine bubble aeration. The integration of fine bubble aeration in within the RBR setup resulted in a 7.7-fold improvement in the performance of the reactor, with doubling of the production rate as well as catalyst productivity [1]. The SpinChem® RBR vessel with integrated fine bubble aeration at repetitive batch processing further exhibited greater enzyme stability at higher substrate concentrations. The still observed decline in enzyme stability was most probably attributed to the accumulation of by-products, namely hydrogen peroxide [1].

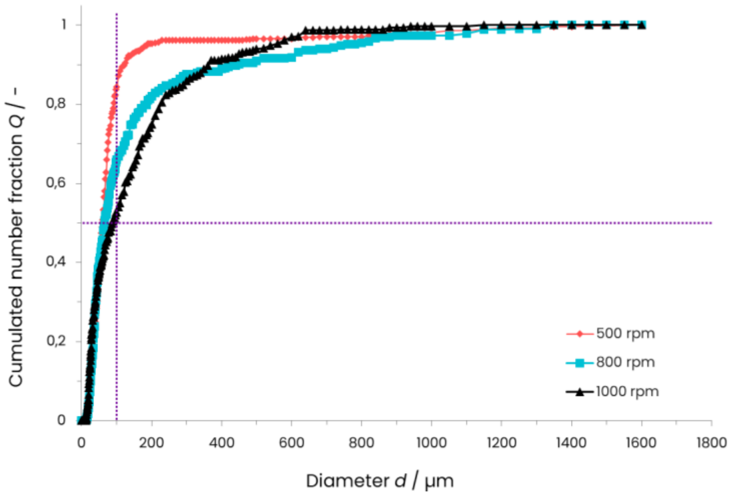

Figure 1: Cumulated bubble size distributions in the SpinChem® vessel for n = 500, 800 and 1000 rpm.

As the project aimed to study the effect of fine bubble aeration on the efficiency of the named model reaction, a method for the production of fine bubble in the SpinChem® vessel had to be established. In consequence, fine bubbles were generated using sintered frit spargers with pore sizes of 2 µm. The bubble size distribution (BSD) in the vessel was measured at the IMS using a SOPAT probe in sodium-acetate buffer for RBR frequencies of $n = 500, 800 \text{ and } 1000$ rpm. Analysis of the images was conducted manually. Figure 1 shows the measured cumulated BSD in the SpinChem® vessel for the named RBR frequencies. For all RBR frequencies, at least 50% of the bubbles have diameters smaller than $d = 100$ µm, as indicated by the dashed lines in Figure 1, and are thus classified as fine bubbles.

To assess the effect of fine bubble aeration on the mass transfer performance in the RBR, the volumetric mass transfer coefficient was measured at the ITB and was found to be 67.3 h^{-1} for fine bubble aeration. This is three times that of the macrobubble aeration (22.5 h^{-1}) at an equal volume-specific aeration rate in the carried-out experiments [1]. The influence of the positioning of the sintered frit sparger within the SpinChem® reactor on the volumetric mass transfer coefficient was also investigated. In the first configuration, the sparger was located beneath the RBR. In the second configuration, sparger was positioned at an elevated level inside the RBR. The mass transfer performance of the SpinChem® vessel was quantified under both configurations, yielding volumetric mass transfer coefficients of 67.5 ± 3.7 h^{-1} for position 1 and 61.8 ± 1.6 h^{-1} for position 2. To further elucidate the effect of sparger positioning, a greater vertical distance between the RBR and the sintered frit sparger would be required. However, such a modification is not technically feasible with the SpinChem® vessel V2 used in this study [1].

The highest specific enzyme activity achievable under fine bubble aeration was 5.82 ± 0.23 U $mgGOx^{-1}$. This corresponds to 12.9 increase in reaction rate compared to macrobubble aeration under identical conditions at a substrate concentration of 600 mM. Biocatalyst productivity increased by a factor of 3.5 at an identical operating time of 21 h, reaching 373 ± 5 $g_{product}\ g_{immobilizedGOx}^{-1}$; compared to 107 ± 8 $g_{product}\ g_{immobilizedGOx}^{-1}$ under macrobubble aeration [1]. These results clearly highlight the significant influence of the bubble size on mass transfer performance. Furthermore, at comparable volumetric mass transfer coefficients, fine bubble aeration (1 vvm) reduced oxygen consumption by 87.5% compared to a macrobubble aeration (0.125 vvm), while maintaining similar enzyme-specific reaction rates [1].

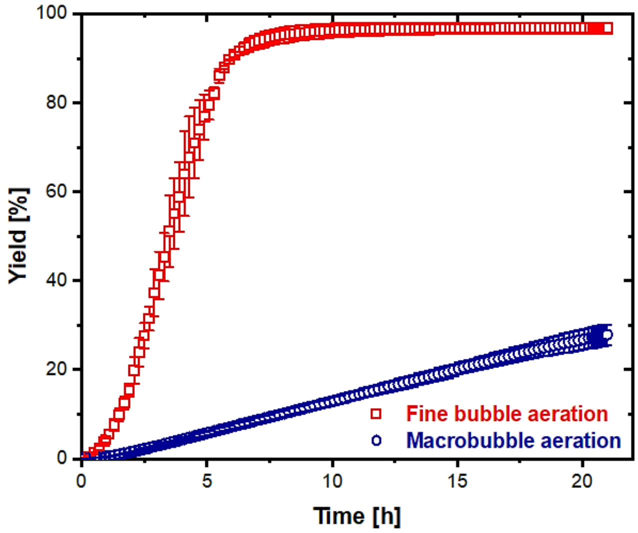

Figure 2: Experimental conditions 8.2 g carrier, 35 ± 1°C, 1000 rpm, 7.52 mgGOx gcarrier-1, 600 mM glucose in 10 mM NaAc buffer pH 5.5 ± 0.2, initial volume before titration 200 mL, 1 vvm, 0.28 ± 0.06 mg mL-1, antifoam 204, error bars show the standard deviation obtained from duplicate measurements.

The main fluid dynamic challenge for the optimisation of a RBR poses the high pressure drop through the packing. A high specific surface area is essential to immobilize large amounts of enzyme, however, this requirement conflicts with the need for high macroscopic porosity to ensure sufficient liquid flow rate through the packed bed. One solution is to improve the flow through the packed bed reactor is the utilisation of carriers with larger diameters. Alginate beads were prepared and evaluated at the ITB, however, they were found to be thermally unstable at 37°C after 2 h, resulting in the termination of further quantitative investigations at the ITB. In consequence, the commercial carrier ReliZymeTM HFA 403 M was applied. Carriers with larger diameters than those used are not commercially available, currently. Thus, it would be necessary to manufacture stable carriers with larger diameters and appropriate functional groups in collaboration with commercial suppliers for further investigation [1].

Fluid dynamically, the effect of the high pressure drop can be seen in the mixing time in the SpinChem® vessel. For this reasoning, the mixing time was determined at the IMS using residual stocks of larger enzyme carriers from the ITB (Table 1). The global

Table 1: Enzyme carriers used for the characterisation of the mixing time

Carriers	Sauter diameter d_{32}	Porosity ϵ
SepaBeads EC ReliZymeTM HFA 403 M	0.32 mm	0.398
Purolite Lifetech ECR 1090 M	0.43 mm	0.398
Purolite Lifetech ECR 8209 M	0.46 mm	0.399
Zeolith particles	4 mm	0.368

mixing time in the SpinChem® vessel was determined optically by recording the decolourisation of a neutralisation reaction, as described in [2]. Figure 3 shows the mixing time numbers in the SpinChem® vessel for the enzyme carriers from Table 1 and the impeller frequencies $n = 500, 800, 1000, 1100$ rpm.

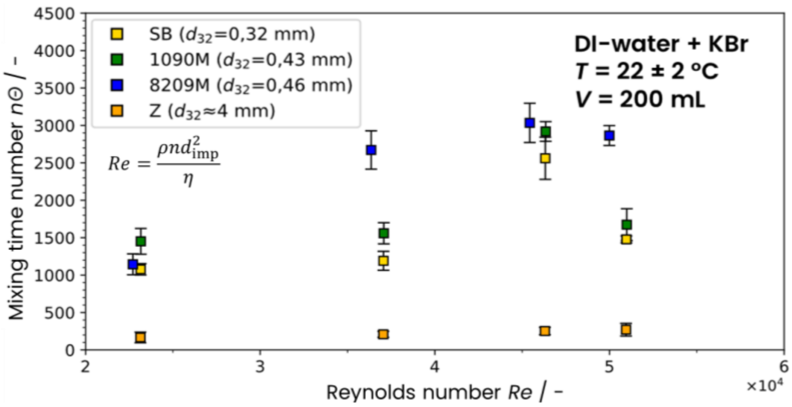

Figure 3: Mixing time number in the RBR for different enzyme carriers and impeller frequencies. KBr was dissolved in the liquid phase to adjust the refractive index of the liquid improving optical accessibility.

The mixing time numbers, essentially describing the number of impeller revolutions needed to reach the mixing time, are highest at the impeller frequency of $n = 1000$ rpm. A comparison with literature values [3] demonstrates that the mixing efficiency in the SpinChem® vessel is inferior to that of a stirred tank reactor (STR), regardless of the impeller type. Although the largest enzyme carriers approach the mixing regime of STRs, their markedly reduced specific surface area available for enzyme immobilisation decreases substantially with increasing particle size, rendering such large carriers unsuitable for the intended application.

To study the liquid volume flow through the RBR for further fluid dynamic characterisation, particle image velocimetry (PIV) experiments were conducted at the IMS. Because of a challenging optical access to the inside of the SpinChem® vessel, a transparent phantom of the vessel was constructed using SILGLAS25 in combination with a liquid of matching refractive index. To enable a broader range of Reynolds numbers, PIV experiments were conducted in two vessel set-ups and liquid systems: a conventional vessel containing 96.5 wt% glycerol in a glycerol bath and the transparent phantom with 56 wt% of glycerol. In the first one, the liquid system and size of the carriers were selected to match the pressure drop of the carriers and liquid system in [1]. The same carriers were employed in the phantom experiments. PIV measurements were conducted below the RBR, i.e., in the region where the liquid is expected to enter the RBR. The results showed that as the viscosity of the liquid approaches that of water, the fluid increasingly follows the rotational motion of the impeller, thereby reducing the effect of the RBR acting as a radial pump. A diffusion limitation of the process is, therefore, conceivable.
Finally, to complete the fluid dynamic characterisation of the SpinChem® vessel, the power input was determined at the IMS. The method for the determination of the power input is described in [4]. The power input is characterised using the Newton number, measured for a range of impeller Reynolds numbers (Figure 4). The Newton number indicates that the SpinChem® vessel behaves

like a baffled stirred tank reactor (STR) with a turbulent flow regime for an impeller frequency of $n = 1000$ rpm. Furthermore, the aeration rate does not have a significant influence on the power input.

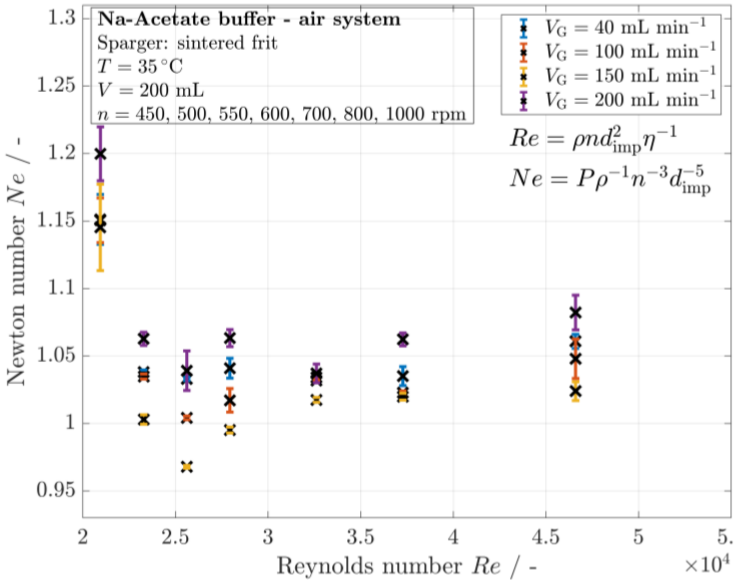

Figure 4: Newton number in dependence of the impeller Reynolds number in the SpinChem® vessel for different aeration rates and impeller frequencies.

3.2 Local Phenomena

The methods and results describing the local mass transfer phenomena of fine bubbles studied at the IMS are published in [A1], [O1-5], [P1] and a further article on the local Sherwood numbers is currently in preparation. Using LSFM, the concentration boundary layer of small bubbles could be resolved in space and time [A1], showing for the first time locally how heavily the diffusive and convective mass transfer of fine bubbles depend on the dissolved species present in the liquid phase [O1-4]. Figure 5 exhibits this for diffusive mass transfer of oxygen in water saturated with different gaseous species. As the mass transfer of gas from the bubble to the liquid, and vice versa, progresses in time, the partial pressure of oxygen in the bubble decreases, evidently lowering the mass transfer performance. For industrial reactors with high residence times of the bubbles, this can have a major influence on the achievable mass transfer performance.

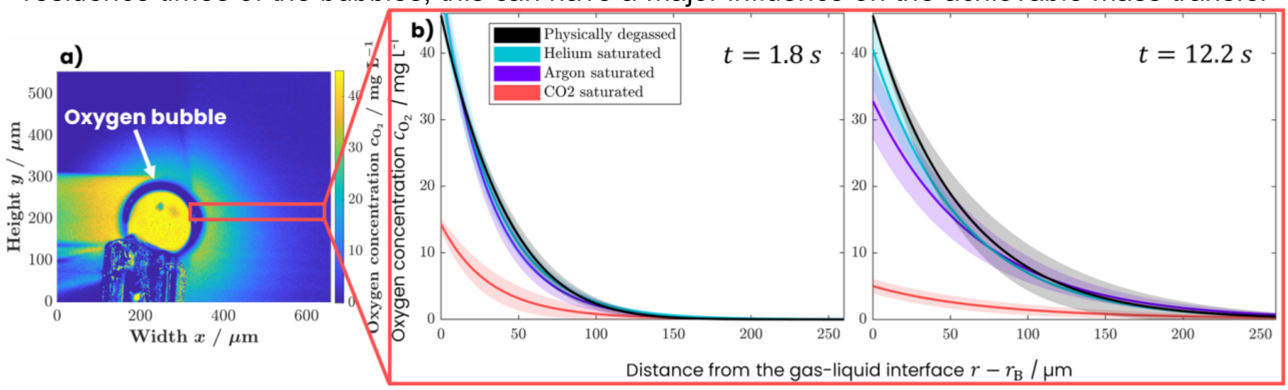

Figure 5: a) Exemplary concentration field of dissolved oxygen in degassed water measured with LSFM [A1]. b) Temporal evolution of the dissolved oxygen concentration in physically degassed (black), helium-saturated (blue), argon-saturated (purple) and carbon dioxide-saturated (red) water [O2].

From the biocatalytic perspective investigated at the ITB and detailed in Perçin's dissertation [1], local mass transfer effects were evident through internal diffusion limitations within the immobilised enzyme carriers. This was reflected by reduced effectiveness factors compared to free GOx. Incorporating deactivation kinetics into the reactor model further demonstrated that locally formed hydrogen peroxide contributes to activity loss, thereby linking microscale transport phenomena to overall reactor performance.

In summary, the biocatalytic oxidation catalysed by glucose oxidase (GOx), employed as model reaction, was successfully intensified, resulting in enhanced catalytic productivity. At the same time, the required aeration rate was reduced by the application of fine bubbles. Whilst it was not possible to improve the liquid flow rate through the RBR by decreasing the pressure drop, the applied enzyme carriers enabled an efficient immobilisation and recycling of the GOx. As GOx represents the primary cost driver of the process, recyclisation should be considered a main achievement and weighted accordingly in the overall assessment. Consequently, the optimisation of the model reaction through the implementation of fine bubble aeration can be regarded successful. However, the local mass transfer studies conducted within the scope of the project indicate that the application of fine bubbles should be reconsidered when scaling the system up, as the improvements in mass transfer performance have strong limitations that have not been accounted for before.

3.3 Data handling, open access research data and software:

At the IMS, research data is handled according to FAIR guidelines. Raw data and corresponding meta data are stored on hard disks. Every disk is copied and both copies are stored for a minimum of 10 years at two different buildings. Evaluations and project management related files are stored on an IMS-internal server. At the ITB, the research data is stored on backed up hard discs and kept for a minimum of 10 years. Project reports and files are stored at the TUHH backed up servers. Data related to publications of both partners is published on TORE (TUHH Open Research, https://tore.tuhh.de/home) with their own DOI and respective software on GitLab (see Section 4.2).

3.4 Mercator fellow and Scientific events:

Within the duration of the project, Mercator Fellow Prof. Terasaka visited the TUHH once a year for one week to discuss the project. The project benefited greatly from his expertise in the field of fine bubbles. Every year, exchange students between Keio University and TUHH worked on the project. In 2025, the 3rd International Workshop on Applications of Fine and Ultrafine Bubbles (IWFB) was held, funded by the project. The workshop took place in Matsue, Japan, after the 5th MMPE conference (12.09.2025). This way, many experts in the field as well as interested participants from the MMPE took part in the workshop. All in all, the 3rd IWFB was a success with

54 participants, including 14 oral presentations and 17 poster presentations. Because of the great interest within the community, the 4th IWFB will be hosted by the TUHH in 2027.

3.5 Bibliography:

[1] Perçin, Zeynep, Technische Universität Hamburg, Institute of Technical Biocatalysis. Enabling Oxygen-dependent Biocatalysis Under Fine Bubble Aeration in a Rotating Bed Reactor. Hamburg, 2025. https://doi.org/10.15480/882.14932.

[2] Fitschen, J.; Hofmann, S.; Wutz, J.; von Kameke, A.; Hoffmann, M.; Wucherpfennig, T.; Schlüter, M. (2021). Novel evaluation method to determine the local mixing time distribution in stirred tank reactors. Chemical Engineering Science: X. https://doi.org/10.1016/j.cesx.2021.100098

[3] Zlokarnik, M. (2001). Stirring. Wiley-VHC Verlag GmbH. ISBN: 3-527-29996-3.

[4] Fitschen, J.; Maly, M.; Rosseburg, A.; Wutz, J.; Wucherpfennig, T.; Schlüter, M. (2019). Influence of Spacing of Multiple Impellers on Power Input in an Industrial-Scale Aerated Stirred Tank Reactor. Chemie Ingenieur Technik. 10.1002/cite.201900121

4 Published Project Results

4.1 Category A – Articles in peer-reviewed journals, contributions to peer-reviewed conferences or to anthology volumes, and book publications

Journal Articles:

[A1] Kursula, L.; Kexel, F.; Hoffmann, M.; Epping, N.-M.; Bubenheim, P.; Terasaka, K.; Liese, A.; Schlüter, M. (2026). Experimental studies on microscopic, multiphase transport phenomena: Novel applications of Light Sheet Fluorescence Microscopy. Journal of Visualization. (accepted)

[A2] Perçin, Z.; Kursula, L.; Löfgren, E.; Byström, E.; Kexel, F.; Bubenheim, P.; Schlüter, M.; Liese, A. (2024). Intensification of a Biocatalytic Oxidation under Fine Bubble Aeration in a Rotating Bed Reactor. Biochemical Engineering Journal. https://doi.org/10.1016/j.bej.2024.109333 (open access)

[A3] Perçin, Z.; Bubenheim, P.; Schlüter, M.; Liese, A. (2022). Process Intensification of Biocatalysts with a Fine-Bubble Aerator, Chem. Ing. Tech., 94, No:9, 1213-1214. https://doi.org/10.1002/cite.202255239

A selection of oral contributions at conferences:

Full list of conference contributions including [O2-5] and [O7] is provided in Section 5.

[O1] Lotta Kursula, Sayaka Takagi, Sumika Haga, Felix Kexel, Marko Hoffmann, Andreas Liese, Koichi Terasaka and Michael Schlüter: Microscale Experimental Studies on Mass

Transfer Performance of Single Oxygen Bubbles, 5th International Symposium on Multiscale Multiphase Process Engineering, Matsue, Japan (2025)

[O6] Zeynep Perçin, Lotta Kursula, Erik Löfgren, Emil Byström, Felix Kexel, Paul Bubenheim, Michael Schlüter and Andreas Liese: Sustainable Biocatalysis by Fine Bubble Aeration, 14th European Congress of Chemical Engineering and 7th European Congress of Applied Biotechnology, Berlin, Germany (2023)

A selection of posters at conferences:

Full list of conference contributions including [P3-6] is provided in Section 5.

[P1] Sayaka Takagi, Lotta Kursula, Felix Kexel, Marko Hoffmann, Koichi Terasaka and Michael Schlüter: Novel Experimental Set-up for Studies of Microscopic Transport Phenomena at Gas-Liquid Interfaces, 5th International Symposium on Multiscale Multiphase Process Engineering, Matsue, Japan (2025)

[P2] Zeynep Perçin, Lotta Kursula, Paul Bubenheim, Marko Hoffmann, Erik Löfgren, Emil Byström, Michael Schlüter and Andreas Liese: Fine Bubble Aeration in Applied Biocatalysis, Himmelfahrtstagung on Bioprocess Engineering, Weimar, Germany (2023)

4.2 Category B – Any other form of published results

Data and software:

- Kursula, L.; Kexel, F.; Hoffmann, M.; Epping, N.-M.; Bubenheim, P.; Terasaka, K.; Liese, A.; Schlüter, M. (2026). Data for publication: Experimental studies on microscopic, multiphase transport phenomena: Novel applications of Light Sheet Fluorescence Microscopy. TUHH Open Research. https://doi.org/10.15480/882.15833 (accepted)
- Kursula, L.; Kexel, F.; Hoffmann, M.; Epping, N.-M.; Bubenheim, P.; Terasaka, K.; Liese, A.; Schlüter, M. (2026). Supplementary Information for publication: Experimental studies on microscopic, multiphase transport phenomena: Novel applications of Light Sheet Fluorescence Microscopy. TUHH Open Research. https://doi.org/10.15480/882.16054 (accepted)
- Kursula, L. (2026). Codes for publication: Experimental studies on microscopic, multiphase transport phenomena: Novel applications of Light Sheet Fluorescence Microscopy. GitHub. https://collaborating.tuhh.de/v5/reactive-bubbly-flows/lotta-kursula/codes-for-lsfm-evaluation
- Perçin, Z.; Kursula, L.; Löfgren, E.; Byström, E.; Kexel, F.; Bubenheim, P.; Schlüter, M.; Liese, A. (2024). Data for publication: Intensification of a Biocatalytic Oxidation under Fine Bubble Aeration in a Rotating Bed Reactor. TUHH Open Research. https://doi.org/10.15480/882.16627

4.3 Patents (applied for and granted)

Not applicable.