From Coiled Flow Inverter to Stirred Tank Reactor – Bioprocess Development and Ontology Design

Julia Grühn[1,‡,*], Alexander S. Behr[1,‡,*], Talha H. Eroglu[1], Valentin Trögel[1], Katrin Rosenthal[2], and Norbert Kockmann[1]

Miniaturized bioreactors, such as the coiled flow inverter (CFI), offer several benefits within process development such as lower time and cost factors. In this study, we demonstrate continuous flow experiments in a CFI and transferred them to experiments in a batch reactor by using the oxygen transfer coefficient $k_L a$ as a key parameter. In order to simplify the parameter transfer and at the same time develop a basis for future data handling according to the FAIR data principles, an equipment and process ontology was developed for these examples.

1 Introduction

Microreactors are characterized by an enhanced heat and mass transfer due to their large specific surface and controlled flow conditions [1]. Good mixing, short residence time as well as a narrow residence time distribution result in high yield and selectivity, which makes microreactors interesting for chemical and biochemical process industry [2]. Microreactors, in particular miniaturized bioreactors have gained acceptance as a tool for obtaining process-relevant data in the early stages of process development [3, 4]. These devices are an attractive alternative in bioprocess development and intensification because of a high degree of control over process variables and the possibility to reduce time and cost factors [5, 6]. Data obtained from these devices are of high quality, when integrated with sensor technology, and are invaluable for scaling up [7]. One of the major challenges in bioprocesses is to enable rapid and successful development. This requires obtaining data relevant to production scale at minimal labor and cost. In addition to process variables, data on productivities must be collected. It is estimated that thousands of experiments are required for a bioprocess development project from screening of primary biocatalysts to pilot-scale experiments. The necessary experiments are typically performed using laboratory-scale bioreactors. Although this results in reliable and information-rich data, it is labor intensive and expensive, while the number of experiments that can be performed simultaneously is limited.

1.1 Capillary Microreactors for (Bio)Process Development

Micro- and millifluidic systems are relevant for faster process development and optimization, as long as the data are representative. Nevertheless, bioprocess design, development, and implementation may exhibit variations that depend on intrinsic characteristics of the reactor. Therefore, an adequate and comprehensive approach must be defined for each individual process, including the identification and definition of key parameters required for proper scale-up [8]. These are, e.g., hydrodynamics and mass transfer-oriented parameters such as oxygen mass transfer coefficient ($k_L a$) and volumetric power input (P/V).

There are four predominant gas/liquid flow patterns occurring in microchannels or capillaries with a circular cross section. These are bubbly flow, slug flow, churn flow and ring flow [9]. The slug flow, also called Taylor flow or segmented flow, is the most widely used flow pattern for two-phase flows in capillaries due to its stable flow regime and good mixing characteristics [10, 11]. In the liquid slugs, internal circulations, called Taylor vortices, lead to higher mixing and thus to an enhanced mass transfer. Kashid et al. [12] confirmed the enhanced mass transfer in their experimental studies on liquid-liquid extraction systems in straight capillaries with slug flow. Fig. 1a shows a gas/liquid flow pattern in a straight capillary. The liquid slugs contain

[1]Julia Grühn, Alexander S. Behr, Talha H. Eroglu, Valentin Trögel, Prof. Dr.-Ing. Norbert Kockmann
julia.gruehn@tu-dortmund.de; alexander.behr@tu-dortmund.de
TU Dortmund University, Department of Biochemical and Chemical Engineering, Lab of Equipment Design, Emil-Figge-Straße 68, 44227 Dortmund, Germany.

[2]Dr.-Ing. Katrin Rosenthal
TU Dortmund University, Department of Biochemical and Chemical Engineering, Chair for Bioprocess Engineering, Emil-Figge-Straße 66, 44227 Dortmund, Germany.

[‡]These authors contributed equally.
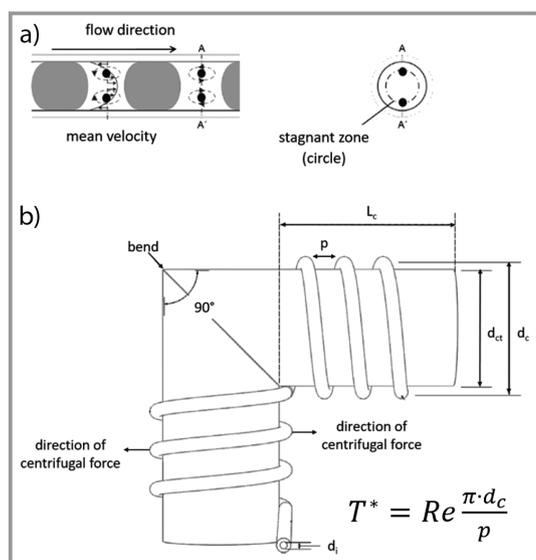
Figure 1. a) Longitudinal view of a slug flow with Taylor-vortex (left) and circular cross section A-A' with stagnant zone (right) of a straight capillary (adapted from [13]). b) CFI with one 90°-bend and equation for the modified torsion number T^* (adapted from [14]).

stagnant zones, where mixing is controlled by diffusion [13].

A break-up of these stagnant zones is required to further enhance mass transfer and can be achieved by using a coiled capillary device. The centrifugal force in a coiled capillary leads to a secondary flow profile called Dean vortices [15]. Experimental studies of a liquid-liquid extraction system (water/acetone/butyl acetate) performed by Kurt et al. [2] and based on results of Kashid et al. [12] have shown 20 % higher extraction efficiency in helically coiled capillaries than in straight capillaries. Furthermore, helically coiled capillaries revealed mass transfer coefficient values twofold higher than the values for straight capillaries. A coiled flow inverter or CFI is a helically coiled capillary with alternating 90°-bends. These 90°-bends redirect the entire flow pattern in the capillary and lead therefore to a further increase of radial mixing [15]. Fig. 1b shows a CFI with one 90°-bend on the right side, where d_{ct} is the coil tube diameter, d_c describes the coil diameter and d_i is the inner tube diameter. The pitch distance between two turns is p and L_C is the length of a single coil. Kurt et al. [13] investigated the cobalt(II)-catalyzed air oxidation of sodium sulfite as gas-liquid test system in a straight capillary, helically coiled capillary and CFI under identical process conditions. The results have shown a conversion that is 14 % higher for the test reaction in a CFI than in a helically coiled and straight capillary. Accordingly, in this work the CFI is used as a promising microreactor for process development and scale-up of a biocatalytic reaction by using the mass transfer coefficient $k_L a$ as critical to quality parameter.

This miniaturization results in spatially and temporally resolved analytics and thus intensive data handling. In addi-

tion, experiments are performed much faster under different conditions, so a high data density is expected. Thus, data handling is essential to cope with high data density while also assuring comparability of data. To help this, ontologies can be used to provide a structured way of storing data.

1.2 Fundamentals of Ontologies

The application of different catalysts, reactor configurations and process flows longs for the use of a certain knowledge. Most of the time, knowledge can only be properly understood if the context of the knowledge is clear and understandable. For example, a table of concentrations within a reactor can only be of use, if the user is aware of the concept of concentration. Often there is no general, formal conceptualization of the knowledge presented, so that the data can only be understood in the context of the presentation.

An ontology can be defined according to [16] as an explicit specification of a conceptualization. In other words, ontologies are used to represent relations among terms offering denser representations for the relationships among concepts. [17]

Thus, an ontology can describe conceptional knowledge in an explicit way. Description logic can then be used to formalize this knowledge and store it in a machine and human readable way. Ontologies are applied to ease the exchange and reuse of knowledge between different actors, whether they are human or artificial. Ontologies thus combine two key aspects that help to enhance data by semantic knowledge: On the one hand, they allow information processing by a computer by defining formal information semantics, on the other hand, they define semantics of the real world based on consensual terminologies. In this way, machine-processable content couples to meaning for humans. [18]

Thus, ontologies can be used not only to reproduce knowledge (i.e., data), but also to reuse this knowledge in, e.g., in silico applications such as simulations.

1.3 Scope and Objective

This study presents the investigations of the CFI as a tool for process development of biocatalytic gas/liquid reactions. For this purpose, an operating point is first determined by the means of a design of experiment (DoE). The DoE considers design parameters as well as reaction parameters to maximize the reaction rate of an enzyme-catalyzed reaction as target value. Experiments with continuous flow in a CFI are mapped onto experiments in a batch reactor. It is assumed as working hypothesis that same values for the oxygen mass transfer coefficient $k_L a$ result in the same reaction rate in both reactor configurations. Hence, $k_L a$ is the key parameter for the transfer from continuous flow to

batch mode as well as for scale-up and is treated as critical to quality attribute.

In addition, based on the ontologies OntoCAPE (ontology for chemical process engineering) [19] and REX (ontology of physico-chemical processes) [20], an advanced ontology is created to describe the investigations on the CFI. Expanding this advanced ontology in turn by actual data sets up a knowledge graph of the DoE conducted in the experiments. Afterwards, a python script is programmed to derive meta data and querying the ontology to allow for a connection of the ontology with experimental data recorded during the experiments.

2 Methods

In the following, the reaction system as well as the experimental setup and experimentation of the continuous flow in the CFI and the batch-mode in a batch reactor are presented. General remarks on the ontology design are subsequently provided.

2.1 Enzymatic Reaction System

The oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Merck KGaA, Darmstadt, Germany) was chosen as an easy to quantify model reaction. The used enzymes laccase (E.C. 1.10.3.2) from *Trametes versicolor*, glucose oxidase (E.C. 1.1.3.4) from *Aspergillus niger* and horseradish peroxidase (E.C. 1.11.1.7) were purchased from

Merck (Merck KGaA, Darmstadt, Germany) as well. For ABTS oxidation, either laccase or glucose oxidase and peroxidase were used (Fig. 2). In the first step of the reaction with glucose oxidase, glucose is oxidized to gluoconolactone with hydrogen peroxide formation, which is subsequently used as an oxidant for the oxidation of ABTS by the peroxidase.

2.2 Continuous Flow Experiments in a CFI

First, the CFI design is presented and both the experimental setup and the experimentation for the continuous flow mode, including the design of experiment to set an operating point.

2.2.1 CFI Design

The CFI consists of a capillary coiled around a supporting structure. The capillary is made of fluorinated ethylene propylene (FEP) and has an inner diameter of $d_i = 1.6$ mm and an outer diameter of $d_o = 3.2$ mm. FEP is transparent and therefore offers the possibility for an optical evaluation method. The support structure was designed in *Autodesk®Inventor* and printed with an Ultimaker S5 3D printer (Ultimaker, Utrecht, Netherlands). The printing material is polylactid acid (PLA). The support structure of the CFI consists of two components: a connector and a helically tube. Fig. 3 shows the computer-aided design (CAD) of the helically tube (a) and the connector (c) and the printed version of helically tube (b) and connector (d). With
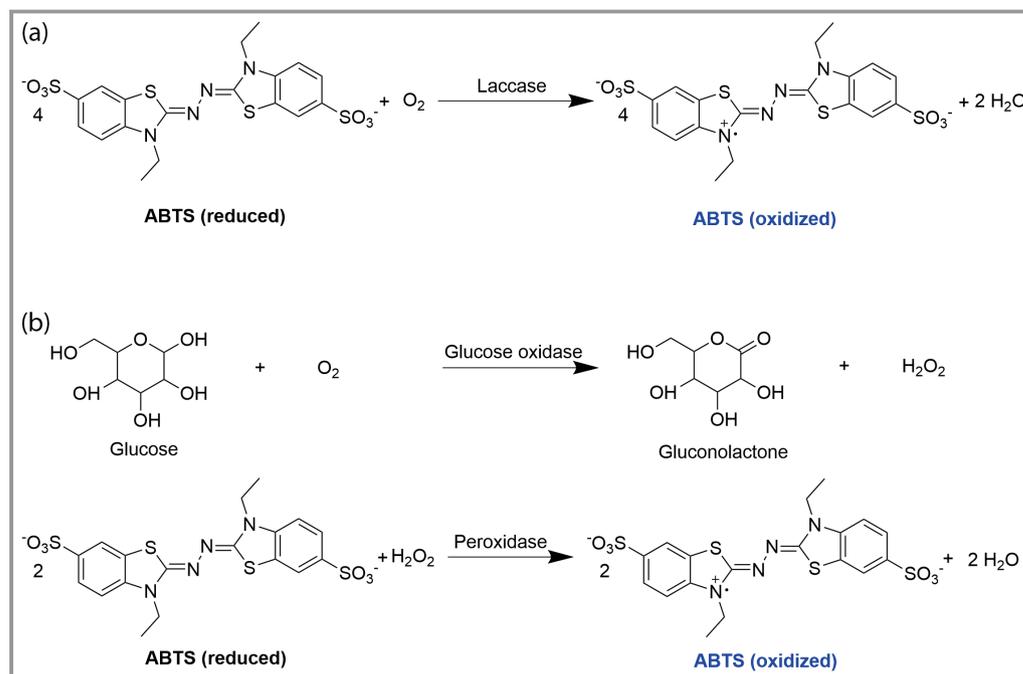
Figure 2. Reaction scheme of ABTS oxidation reactions with either (A) laccase or (B) glucose oxidase and peroxidase. Both reaction systems are well-described model oxidations [21, 22].
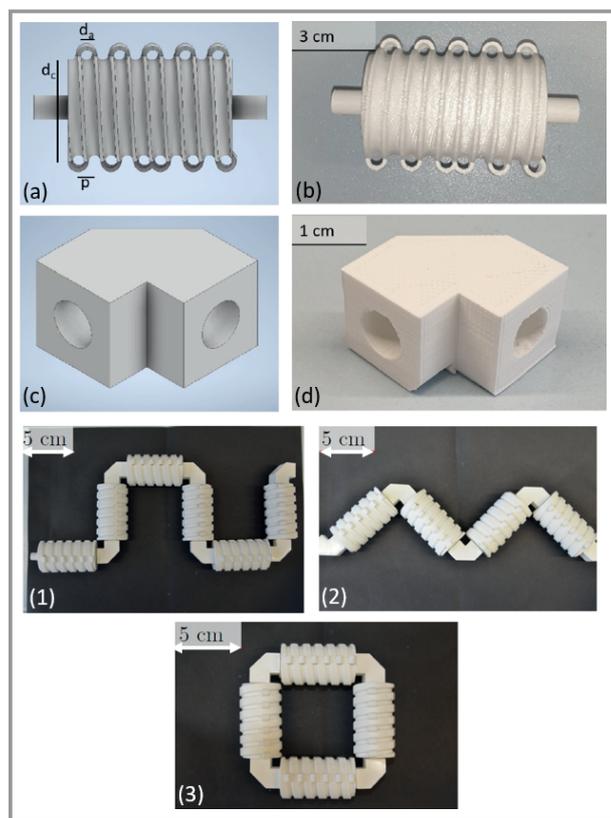
Figure 3. Upper part: CAD and printed version of the helically tube (a, b) and the connector (c, d). Lower part: stepwise (1), zigzag (2) and frame-wise (3) construction of the CFI according to Kurt et al. [2].

these two components, a module-wise construction of the CFI is possible, e.g., stepwise (1), zigzag (2) or frame-wise (3) as shown in Fig. 3 on the right side according to Kurt et al. [2].

2.2.2 Design of Experiment

The three different CFI constructions as well as design parameter such as number of turns or bends, or reaction parameter like concentration of enzyme or oxygen result in different mixing efficiencies and thus to varying reaction rates. The parameters for the operating point are set with a design of experiment (DoE) in which the target value is a maximum reaction rate r. The simple test reaction system used for the DoE is the oxidation of ABTS to the blue product $ABTS_{ox}$ by the enzyme laccase as shown in Fig. 2. The reaction rate r is calculated as the amount of oxidized ABTS divided by the residence time τ:

$$r = \frac{ABTS_{ox,\,out} - ABTS_{ox,\,in}}{\tau} \tag{1}$$

where $ABTS_{ox,in}$ and $ABTS_{ox,out}$ are the amounts of oxidized ABTS at the inlet and outlet of the CFI. The parameters entering the DoE are the number of turns n_{turns} and the con-

centrations of enzyme $c_{laccase}$ and oxygen c_{O2}. In order to calculate the oxygen mass transport across the phase boundary of gaseous to liquid phase, oxygen limited conditions are required. If oxygen is transported from the gas phase into the liquid faster than the reaction can convert, no reliable correlation between the mass transport and the reaction can be determined, since the oxygen concentration in the aqueous phase cannot be measured. Therefore, a set of experiments was done with varying oxygen and enzyme concentrations in a CFI. The pitch distance p and the coil diameter d_c are parameters that are not varied based on the following consideration: the smaller the coil diameter d_c, the higher is the Dean number De and a better mixing is achieved. For this reason, the coil diameter d_c is chosen as small as possible, which correspondents to the minimum bending radius of FEP. In principle, the modified torsion number T^* (cf. Fig. 1) increases with a higher pitch distance p. In this work the pitch distance p was chosen so that is was easy printable and space-saving. The DoE with its single experiments is shown in Tab. 1.

Table 1. DoE to determine the operating point with maximum reaction rate r. Each experiment is performed in triplet.

Experiment	n_{turns} [−]	$c_{laccase}$ [g L^{-1}]	c_{O2} [%]
1	3	0.2	3
2	10	0.2	3
3	3	0.8	3
4	10	0.8	3
5	3	0.2	7
6	3	0.2	10
7	10	0.2	7
8	10	0.2	10
9	3	0.8	7
10	3	0.8	10
11	10	0.8	7
12	10	0.8	10

2.2.3 Experimental Setup and Experimentation

The result of the DoE represents the operating point for the further investigations on the glucose-oxidase system presented in Fig. 2. The experimental setup for continuous flow is shown in Fig. 4 and includes the following components: synthetic air with an oxygen content of 20 vol % is mixed with nitrogen (1) via a Y-mixer (3) to obtain the desired oxygen content in the gas phase with the help of two mass flow controller (MFC) (Bronkhorst, Kamen, Germany) (2). A computer (4) controls the MFC with the program Lab-VIEW® (Hitec Zang, Herzogenrath, Germany). The reaction vessel (5) is tempered with a water bath at 38 °C. The
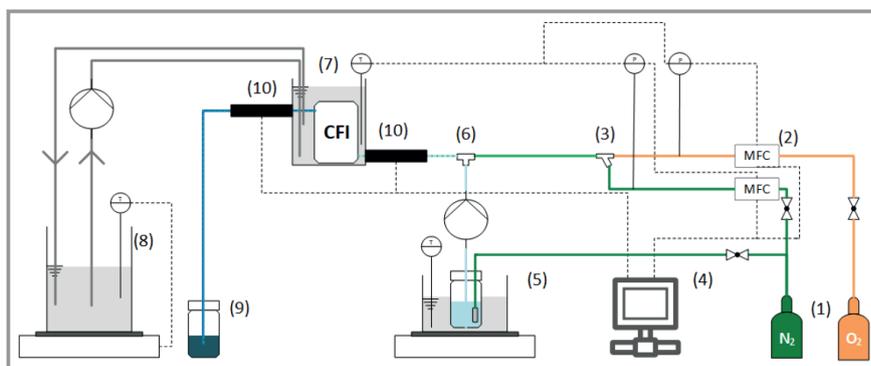
Figure 4. Experimental setup of the continuous flow experiments with a CFI.

Germany) and the gas phase is introduced into the reactor by a sinter filter. In order to improve mass transport, a stirring bar submerged in the liquid stirs the content of the 1000-mL batch reactor. The reaction medium is gassed with nitrogen at the surface to ensure that it does not react with oxygen from the environment. A thermostat (Huber® CC 304, Offenburg, Germany) tempered the reaction medium (6). For the optical evaluation, the reaction medium flows through an LDR sensor (8), which delivers a concentration dependent signal to the computer (5). The composition of the reaction medium corresponds to that of the continuous flow experiments.

reaction solution is inertized with nitrogen via a sintered filter (VitraPor®, ROBU®, Hattert, Germany) (6). A peristaltic pump (Ismatec ISM597, Grevenbroich, Germany) transports the reaction medium through a T-mixer (6), in which the gas/liquid contacting takes place, into the capillary of the CFI (7). The CFI is tempered with a water bath (8) also at 38 °C. Reaction medium leaving the CFI is collected in a waste vessel (9). Two LDR sensors (light-dependent resistor) (LUNA Optoelectronics, Camarillo, USA) record the amount of oxidized medium at the inlet and outlet of the CFI (10).

The experiments are performed in a CFI with 3 turns, 13 bends, and a reactor length of 4 m. The reactor volume is 8 mL. For these experiments the enzymes glucose oxidase ($c_{GO} = 0.001\,\mathrm{g\,L^{-1}}$) and horseradish peroxidase ($c_{HRP} = 0.002\,\mathrm{g\,L^{-1}}$) are used. The substrates are glucose ($c_{Glucose} = 1\,\mathrm{g\,L^{-1}}$), ABTS ($c_{ABTS} = 0.3\,\mathrm{g\,L^{-1}}$) and oxygen ($c_{O2} = 3\,\mathrm{vol\,\%}$). Furthermore, the reaction medium contains 1 vol % sodium acetate as buffer for pH 5.3, and 1 vol % Tween®80 (Merck KGaA, Darmstadt, Germany) to ensure a stable gas/liquid flow in the capillary. The reaction temperature is 38 °C. The liquid flow rate is $5\,\mathrm{mL\,min^{-1}}$ and the flow rate of the gaseous phase is $3\,\mathrm{mL\,min^{-1}}$. The residence time is 64 s.

2.3 Batch Experiments

The experimental setup for the experiments in a batch reactor is shown in Fig. 5. Corresponding to the experiments in continuous flow, synthetic air and nitrogen are mixed to obtain the desired concentration of oxygen in the gas phase ((2)–(5)). The batch reactor (1) is a double-walled wide-necked laboratory bottle (200 mL and 1000 mL, Duran group now DWK life sciences, Wertheim,

2.4 Analytics

The oxidation of ABTS leads to the blue product ABTS$_{ox}$. There is a linear correlation between color intensity and concentration of the product that makes an optical evaluation method possible. For this purpose, an LDR sensor is used. It measures the product concentration at the inlet and outlet of the CFI. With this information, the reaction rate can be calculated as formed product per time. The sensor converts incident light into an electrical signal [23, 24] as shown in Fig. 6.

The light source is a light emitting diode and the sensor is a light dependent resistor (LDR). The higher the light intensity is, the more free charge carriers are formed on the photo resistor, where the electrical resistance decreases. The
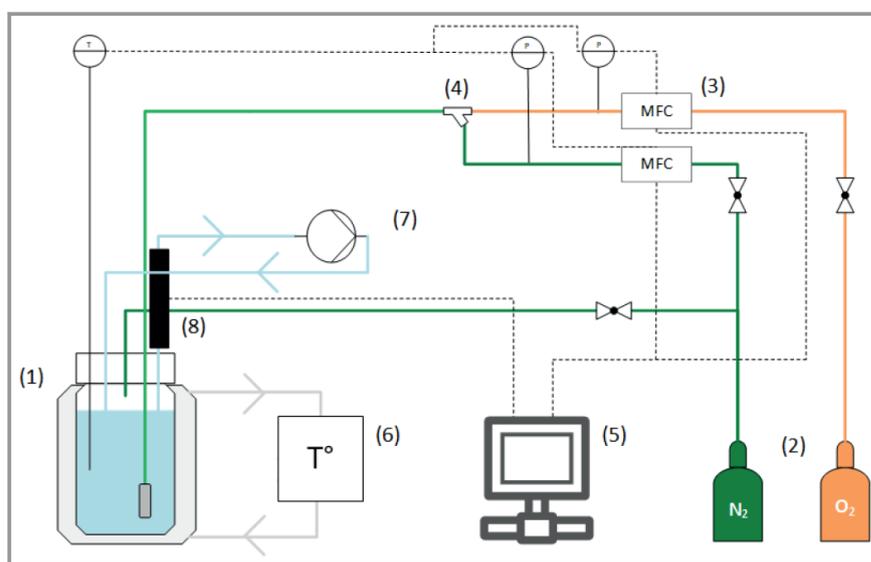
Figure 5. Experimental setup of the experiments in batch mode.
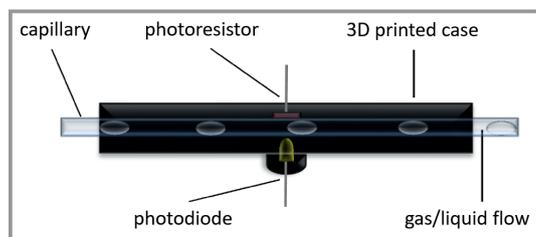
Figure 6. LDR sensor scheme according to [23].

3D printed case protects the sensor from stray light. The product concentration is calculated with the Lambert-Beer law.

2.5 Ontology Design

Ontologies base on description logic, which predominantly uses triplets of subject, predicate, and object to describe real-world concepts. Information objects that are to be described in an ontology are called entities and can be used as a collective term for different objects. These entities encompass concrete objects such as reactors, as well as abstract objects such as organizations. [25] If such entities share a common characteristic, they are grouped under a class in an ontology.

Applying a hierarchically structuring to classes yields a taxonomy where subordination relations indicate subclasses and superclasses. Entities belonging to a particular class are referred to as instances of that class. After defining a taxonomy, properties of classes are defined using relations. They can be further specified by relation properties to specify explicit relationships between classes or individuals. Attributes are used to describe properties, characteristics, or parameters of a class and its instances. Finally, axioms describe a logical statement that is always true. They are used to define the semantics of an ontological concept, verify consistency of the knowledge represented in the ontology, or derive new knowledge from explicitly stated facts. [19]

The modeling of ontologies with logical languages or description logics allows for using inference engines called reasoners. With these reasoners, it is examined whether the accomplished extensions of the ontology were set up logically correct.

Creating an ontology from scratch requires a lot of work by both domain experts and ontology experts. Thus, it is desirable to find and reuse already existing ontologies. Already existing ontologies and semantic artefacts in the domain of catalysis science from catalyst data to process design are presented in [26]. Though some ontologies and semantic artefacts relevant to catalysis science already are present, none of them is able to describe knowledge of the experiments presented at full extend.

The experiments carried out are mainly in the field of process engineering. OntoCAPE [19] is an ontology in the field of process engineering. Built in a modular approach, it consists of different sub ontologies. They encompass, e.g., concepts for chemical process systems, used materials or process units. This provides the advantage the ontology is easily adapted to new concepts by adding new or advancing existing modules. OntoCAPE is used as a starting point for the ontology design to advance it by other ontologies and new concepts relevant for the conceptual modeling of the experiments presented. While the concept of catalysis is not represented in detail in OntoCAPE, it is defined to some extent in the ontology REX [20].

Both OntoCAPE and REX are available and formatted in the web ontology language (OWL). Protégé is a free ontology editor for OWL ontologies, whose source code is freely available. It supports editing and creation of ontologies in OWL, providing an editing interface that allows access to commonly used OWL constructs. Furthermore, Protégé supports the reasoning of ontologies to allow inference of information and knowledge. Moreover, it is possible to represent classes, individuals, and relations of the ontology graphically to get a better visualization of the created ontology. Protégé is a widely used and established editor with a large user base. Accordingly, the support of this software is mature and many features, as well as successful applications, are available.[27] Thus, Protégé is used as ontology editor in this work.

3 Results and Discussion

Results of the laboratory experiments are presented in the following. The reactor and scale-up, reaction rates, and $k_L a$ values are considered. In order to obtain a more reusable way of storing the resulting data, the extension of existing ontologies to a knowledge graph with the experimental data are discussed.

3.1 Reactor and Scale-up

In order to calculate the oxygen mass transport across the phase boundary of gaseous to liquid phase, oxygen limited conditions are required. If oxygen is transported from the gas phase into the liquid faster than the reaction can convert, no reliable correlation between the mass transport and the reaction can be determined, since the oxygen concentration in the aqueous phase cannot be measured. Therefore, a set of experiments was done with varying oxygen and enzyme concentrations in a CFI with 6 turns and 9 bends and a residence time of 64 s. For these experiments, laccase was used to keep the reaction system as simple as possible. The oxygen concentration in the gaseous phase was first varied between 0 vol % to 21 vol % and the enzyme concentration between $0.2\,\mathrm{g\,L^{-1}}$, $0.6\,\mathrm{g\,L^{-1}}$ and $0.8\,\mathrm{g\,L^{-1}}$. The ABTS concentration was $0.5\,\mathrm{g\,L^{-1}}$.

While the influence on the reaction rate can be clearly seen up to an oxygen concentration of 10 vol %, only slight differences are found for measurements above 10 vol % (Fig. 7). It can be assumed that a mass transfer limitation occurs at concentrations up to 10 vol % in the gas phase. As the reaction rate also increases for values above 10 %, it can be assumed that the influence of the oxygen content continues to exist, but to a much lower extent. Furthermore, it is observed an increasing enzyme concentration leads to an increasing reaction rate, as expected. To ensure an oxygen limitation, the DoE considered oxygen concentrations of 3 vol %, 5 vol % and 10 vol %. According to the results of the DoE, further experiments were performed with an oxygen concentration of 3 vol % in the gas phase. Furthermore, the DoE leads to $n_{turns} = 3$. With a fixed reactor length of 4 m a lower number of turns leads to a higher number of 90° bends. The results show that the influence of the redirection of the flow pattern (90° bends) on mixing efficiency is higher than the influence of the Dean vortices that occur due to centrifugal force. This is consistent with literature data from Kurt et al. [13]. In addition, experiments with a lower enzyme concentration of $c_{laccase} = 0.2$ g L^{-1} result in a higher reaction rate. This is contrary to expectations. A possible explanation is that the oxygen near to the bubbles reacts so quickly that the mass transport into the liquid phase plays a subordinate role.
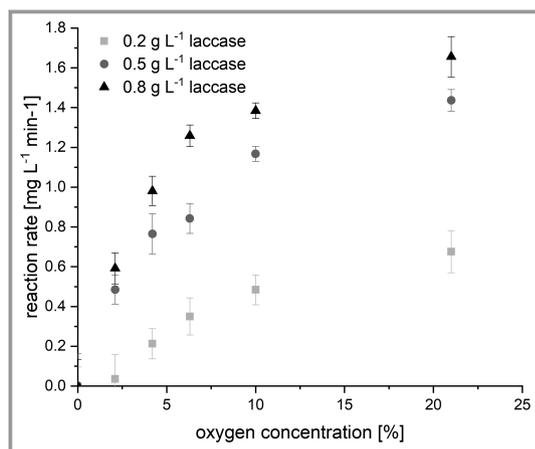
Figure 7. Reaction rates of ABTS oxidation as a function of the oxygen concentration in the gas phase for laccase concentrations of about 0.2 g L^{-1}, 0.5 g L^{-1} and 0.8 g L^{-1}. Experiments were performed with a CFI with 6 turns and 9 bends, with a reactor length of 4 m and 8 mL reactor volume. Error bars are a result of three independent experiments.

3.1.1 Calculation of Reaction Rates from CFI Experiments

To realize the transfer from the CFI to a batch reactor, the reaction rate of ABTS oxidation was determined. A CFI with 3 turns and 13 bends was used with a length of 4 m and a volume of 8 mL. The gas and liquid flow rates were set to 3 and 5 mL min^{-1}, respectively, to obtain a regular Tay-

lor bubble flow. The enzyme concentrations were 0.1 mg L^{-1} for the glucose oxidase and 2 mg L^{-1} for the horseradish peroxidase. In the two-step reaction, it can be assumed that the first step of the reaction is rate-determining since the peroxidase (250–2000 U mg^{-1}) has a substantially higher activity than the glucose oxidase (168 U mg^{-1}). The oxygen concentration was set to 3 vol % in order to obtain oxygen limited conditions. The substrate concentrations were 0.3 g L^{-1} ABTS and 1 g L^{-1} glucose. Using the described CFI setup, a specific activity of 38.3 U mg^{-1} was achieved, determined by the ABTS oxidation rate. This correlates with an ABTS oxidation rate of 25.48 µmol min^{-1}L^{-1} and an oxygen consumption rate of 12.74 µmol min^{-1}L^{-1}. These results demonstrate that the two-step biocatalytic reaction with glucose oxidase and horseradish peroxidase is feasible in the CFI.

3.1.2 Calculation of $k_L a$ Values

In order to transfer the reaction from the continuous flow CFI to a batch reactor, key parameters as critical for quality attributes have to be determined for an adequate scale-up. In this case, the oxygen mass transfer coefficient ($k_L a$) was calculated and used as transfer metric. It is assumed as guiding hypothesis that the same reaction rate will be achieved in both systems if the $k_L a$ value is successfully transferred. The $k_L a$ is composed of the mass transfer coefficient k_L and the specific phase interface a and can be represented by the film theory. For simplicity, it is assumed that the transport of a gas through the gas/liquid phase interface is the velocity-determining step (2).

$$k_L a = \frac{dc_{O_2,L}}{dt} \frac{1}{\left(c_{O_2}^* - c_{O_2,L}\right)} \qquad (2)$$

In Eq. (2), $c_{O_2,L}$ is the oxygen concentration in the liquid phase, $c_{O_2}^*$ is the equilibrium concentration at the phase interface or, expressed differently, the maximum solubility of oxygen in water, and t is the time. The expression $dc_{O_2,L}/dt$ is determined via the previously determined oxygen consumption rate of about 12.74 µmol min^{-1}L^{-1}. The oxygen concentration in the liquid phase $c_{O_2,L}$ can be neglected as it is assumed that any oxygen is immediately consumed by the reaction. The equilibrium concentration $c_{O_2}^*$ can be determined with the Henry's law constant $K_{H,38°C}$ at 38 °C and the oxygen partial pressure p_{O_2} (0.03 atm, corresponding to 3039 Pa)

$$c_{O_2}^* = K_{H,\,38\,°C} p_{O_2} \qquad (3)$$

The Henry's law constant $K_{H,38°C}$ at 38 °C is dependent on the temperature with the Henry's law constant $K_{H,25°C}$ at 25 °C (1.3 · 10^{-3} mol L^{-1}atm^{-1}), the constant C of oxygen (1700 K), the temperature T (311.15 K) and the standard temperature T_n (298.15 K), Eq. (4).

$$K_{H,\,38\,°C} = K_{H,\,25\,°C} \exp\left(C\left(\frac{1}{T} - \frac{1}{T_n}\right)\right) \qquad (4)$$

The values shown in Tab. 2 are derived from the given values and Eqs. (2)–(4) for the CFI. The specific phase interface a was calculated from the total number of bubbles in the CFI and the bubble surface area per bubble that was determined by photographing and measuring the bubble diameters.

Table 2. Calculated values for the oxygen mass transfer coefficient ($k_L a$), mass transfer coefficient k_L and the specific phase interface a of the CFI reactor. The specific phase interface was calculated from the total number of bubbles in the CFI (449 bubbles) and the bubble surface area (20.1 mm^2) per bubble.

Parameter	Value
$k_L a$ [s^{-1}]	$7.4 \cdot 10^{-3}$
a [m^{2}m^{-3}]	1121
k_L [m s^{-1}]	$6.6 \cdot 10^{-6}$

As expected, the mass transfer coefficient k_L was lower (two orders of magnitudes) compared to batch reactors, bubble columns or gas-liquid millichannels, because of the reduced oxygen supply of 3 vol % used to set oxygen limitation [28]. The reduced oxygen availability leads to a limited oxygen transfer rate across the phase boundary, resulting in a comparatively lower $k_L a$.

3.1.3 Transfer to Batch Reactor with the Aid of $k_L a$ Values

The oxidation of ABTS via glucose oxidase is transferred from the continuous flow in a CFI to batch reactor. With an oxygen content of 3 vol % the formed product concentration of ABTS$_{ox}$ is 0.0155 g L^{-1} after 4 m reactor length, which corresponds to 63 s residence time. With a gas flow of 18 mL min^{-1} through a sinter filter the desired product concentration is formed after 42 s in a 200-mL batch reactor. The phase boundary with an interface of $A_{200mL} = 0.007$ m^2 was calculated by multiplying the reaction time with the averaged bubble surface. The specific interphase is then $a_{200mL} = 370.8$ m^{-1}. The mass transport coefficient is calculated to $k_{L,200mL} = 2.95 \cdot 10^{-5}$ m s^{-1}. These values are consistent to literature data [29]. The same $k_L a$ values are aimed for batch reactor as in the CFI. It is assumed that the dissolved oxygen in the reaction medium can be neglected. The desired $k_L a$ is $7,4 \cdot 10^{-3}$ s^{-1}. The residence time is $\tau = 63$ s and the necessary interphase per time is $A/\tau = 0.048$ m^{2}min^{-1}. With the help of the captured images the averaged diameter and surface of the gas bubbles is determined to $d_{bubble} = 1.2$ mm. At least the needed gas flow rate is calculated to $V_{gas} = 9.7$ mL min^{-1}. The experiments show that the set point of $ABTS_{ox} = 0.0155$ g L^{-1} was reached after 63.6 s ± 7.9 s. Hence, it has successfully been shown that a transfer of a biocatalytic reaction from a continuous flow in a CFI to a batch reactor is possible.

The scale-up to a 1000-mL batch reactor was according to the proceeding for the 200-mL reactor. Due to the limitations of the mass flow controller, the use of a stirrer was necessary. With a stirrer-speed of 400 rpm the reaction time needs 64.3 s until the desired reaction rate is reached. Since power input via a stirrer is required for the batch reactor at higher volumes, the dimensionless power input P/V might be another critical to quality parameter. More experiments are to be conducted. In principle, a scale-up could be carried out by using the mass transfer coefficient as transfer metric and critical to quality attribute.

3.2 Advanced Ontology and Knowledge Graph

To get a structured way of storing the experimental data in a reusable way, existing ontologies were merged and extended. Then, the experimental data were inserted and structured in this way by the regulated vocabulary of the ontology. While constructing the ontology, focus of conceptualization lies on the experimental data of the CFI experiments and the online concentration measurements with LDR sensors.

The ontology consisted of a new OntoCAPE module, which itself consisted of already existing OntoCAPE modules, advanced by concepts from REX regarding catalysis and concepts specific to the experiment conducted. This combination of existing ontologies and extension with REX concepts was necessary, because the experimental setups to be described were complex, not standardized, and also required different reactors, catalysts, etc. Thus, a tailored ontology is necessary to describe the concepts needed. For example, concentrations are measured as time series of a voltage, thus knowledge of respective calibration(s) and the conversion to concentrations is essential to interpret the measured raw data. The ontology will then be used to deduce the respective meta data, such as calibration data for a certain dataset of a LDR sensor, of the conducted laboratory experiments.

In order to be able to describe the laboratory experiments with an ontology, all physical components of the experimental setup and all functional aspects of the experiments must be represented. This results in a collection of concepts that are subsequently differentiated into classes (e.g., chemical species), relations (e.g., *contains*) and individuals (e.g., ABTS). Furthermore, data properties can be assigned to individuals (e.g., ABTS *has amount value* 0.5 g/L). Fig. 8 displays a first hierarchy created from the identified classes among those objects.

The documentation of OntoCAPE [19] was analyzed and usable modules identified. The chemical_process_system module was used to describe the DoE laboratory tests. In order to describe a technical system, which produces a material, the class ChemicalProcessSystem of the module chemical_process_system is used. The module consists of seven submodules, four of which are core modules, each describing an aspect of the chemical process system.

Functional specifications, such as chemical processes or reactions, are described in the functional aspect system
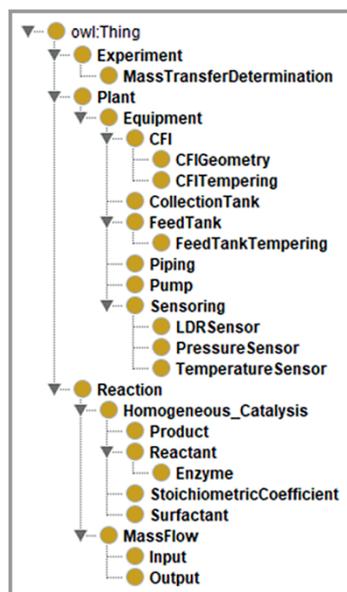
Figure 8. Class hierarchy of concepts required to describe DoE data of CFI laboratory experiments.

CPS_function. The realization aspect system CPS_realization deals with the technical realizations of a system, such as piping or measuring instruments. In the behavior aspect system CPS_behavior, the behavior of the chemical process system is described, and a qualitative and quantitative empirical characterization of the process behavior is possible. For the DoE experiments, this aspect system mostly is neglected because the characterization of the process behavior is time consuming and not necessary for describing the ChemicalProcessSystem class. The performance aspect system CPS_performance in turn is used to evaluate the economic performance of a system. It is also neglected for the laboratory tests since the tests were not performed under an economic aspect. Furthermore, the process_units module describes basic process units like a chemical reactor or heat transfer unit. To describe geometry of the reactor and materials used, the modules material and geometry are also imported.

The modules described provide a good basis of concepts to describe the DoE laboratory experiments performed. However, they do not adequately describe the concept of catalysis. Only the aspect system CPS_performance contains a class Catalyst, which solely describes the costs of an applied catalyst.

In REX ontology [20], concepts for catalysis and oxidation are modeled. These concepts were imported in the chemical_process_system module as a subclass of the Reaction class. This applies to the considered concepts of catalysis and oxidation in the laboratory experiments. Therefore, the classes REX:Catalysis and REX:Oxidation were inserted as subclasses of the OntoCAPE class Reaction.

This modified chemical_process_system module was used as a basis to build a new module called CFILabTrials. The creation of this module pursues the goal of describing laboratory experiments for the comparison of different CFI

geometries and reaction conditions. To achieve this, an individual chemical process system is set up in which the enzyme laccase catalyzes the homogeneous catalysis of ABTS with oxygen in a defined experimental system to $ABTS_{ox}$ and hydrogen peroxide. Thus, a functional, a realization, and a performance aspect system are created to describe these experiments in the module CFILabTrials. Fig. 9 shows a snippet of the advanced CFILabTrials module created. The class CFIComparison is a subclass of the ChemicalProcessSystem class defined in OntoCAPE and has the functional aspect of MassTransferDetermination and the realization aspect of a CFIComparisonPlant. Among other things, MassTransferDetermination has the relation *has direct subsystem* REX:HomogeneousCatalysis. This describes that in order to determine the mass transfer analyzed in the laboratory experiments, the concept of homogeneous catalysis is applied.

The inserted classes describe concepts that are necessary for the laboratory experiments. Since the laboratory experiments are a use case, these classes alone cannot model them. Individuals and attributes are needed to describe the individual mass transfer determinations of the 12 experiments performed. Thus, the respective individuals for each of the experimental setups conducted are inserted into the CFILabTrials module.

Fig. 10 compares the metrics of the CFILabTrials ontology module before and after the extension. It can be seen how many classes, relations, individuals, and attributes are present in the ontology before (gray bars) and after (black bars) the extension. The difference in values of the respective bars represent the number of respective concepts that were newly created to describe the CFI experiments. The imported modules from OntoCAPE are composed of 496 classes, 213 relations, 163 individuals, and 31 attributes. In total, 4289 axioms are used in these modules to describe the existing concepts. After the extension of the ontology, there are 522 classes, 217 relations, 320 individuals, and 40 attributes in the ontology and 5226 axioms are used. In conclusion, 26 classes, 4 relations, 157 individuals, and 9 attributes were added to the ontology in the course of the extension. In addition, 937 new axioms are used to describe the concepts of the laboratory experiments.

In order to check if the performed extensions of the ontology have been modeled logically correct and consistent, HermiT version 1.4.3.456 [30] is used as a reasoner to process the ontology.

In an overall data flow depicted in Fig. 11, the data obtained from the experiments is described via the ontology presented. Then the ontology gets evaluated via a python script to obtain the data from the experiments together with their corresponding meta data derived from the ontology. Additionally, a database was created from the generated experimental data using the software tool DB Browser for SQLite [31]. In the database, the generated tables of the laboratory experiments were stored and assigned database IDs. These IDs are linked to individuals of the ontology.
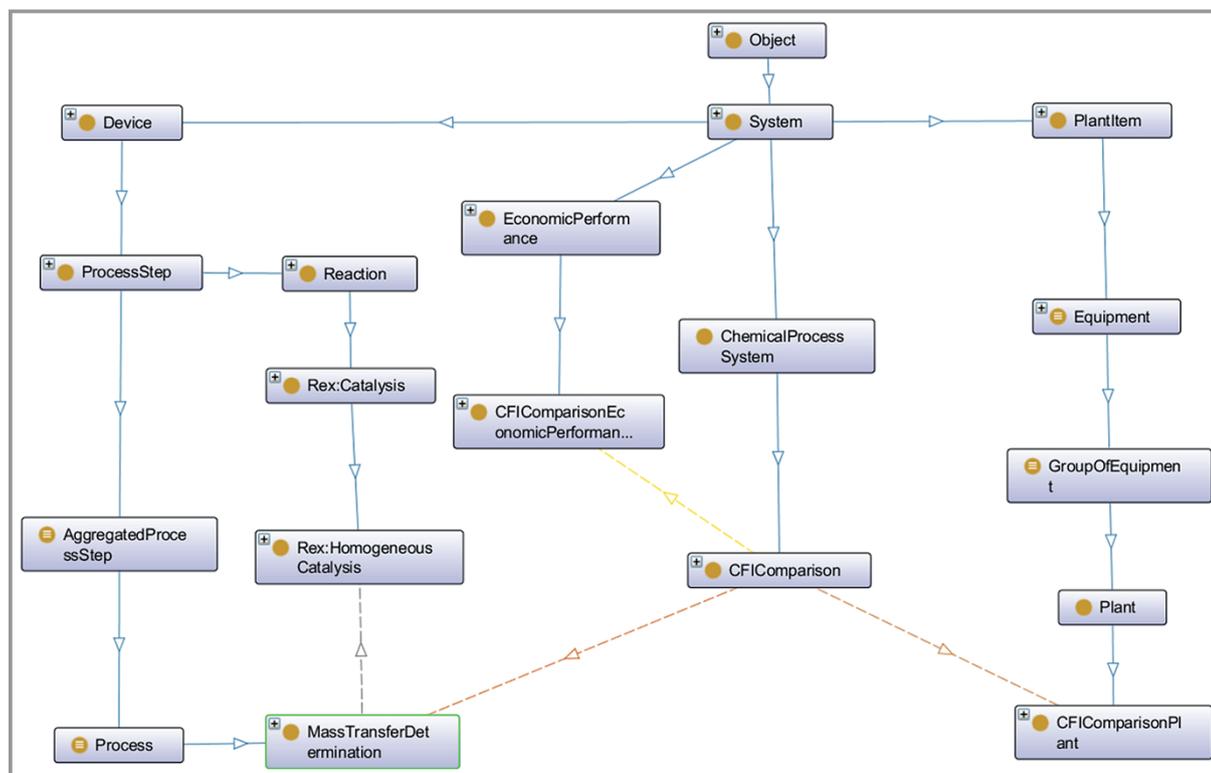
Figure 9. Extract from Protégé for visualization of class relationships. Among other things, the inserted class CFIComparison and the according aspect systems are shown. Blue arrow: has subclass, gray arrow: has direct subsystem, yellow arrow: has performance aspect, light brown arrow (left): has functional aspect and dark brown arrow (right): has realization aspect.
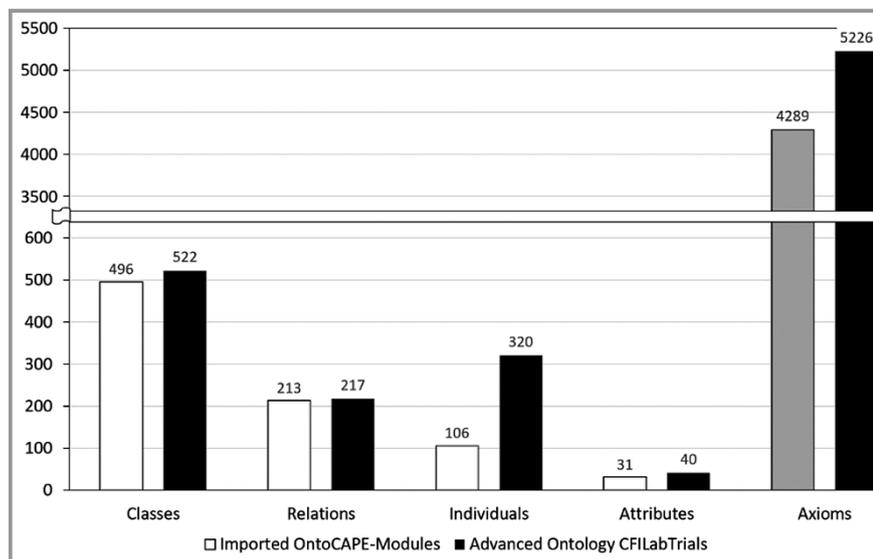
Figure 10. Comparison of the numbers of classes, relations, individuals, attributes and axioms before and after the extension of the ontology. Light gray columns represent the imported OntoCAPE modules while black columns represent the extended ontology.

performs queries of the ontology using the query language SPARQL. The SPARQL query can filter the search results by various criteria, e.g., by experiments, in which a volume fraction of ten percent oxygen is present in the reactant stream. The results of the SPARQL query are stored as variables, so that the meta data of the queried experiments, e.g., number of capillary turns, 90° deflections or the pitch distance, can be output additionally. The database IDs of the corresponding raw files of the laboratory experiments are stored in the ontology via the attribute hasFileID. These IDs can therefore be output via the SPARQL query, too. The SQLite database is searched for entries with the corresponding IDs from the ontology and the associated raw files of the laboratory experiments are saved in an export folder. Thus, a connection between data and meta data of the conducted lab experiments and a search based on the meta data is possible.

To test the implementation, a python script based on the python package Owlready2 [32] was used to search the ontology for user defined criteria. Then it returns the corresponding raw data of the experiments from the SQLite database fitting to these criteria. It imports the .owl files and

With this data storage structure and workflow, existing lab data can be retrieved for later scale-up studies. Critical
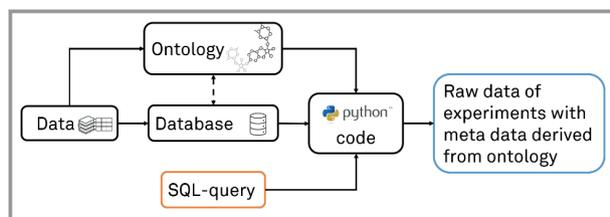
Figure 11. Overall data flow from raw data to structured queries on ontologies to gain meta data.

to quality attributes can be retrieved, too, and guide future process development in biocatalysis. The overall approach also can be applied to different sets of data on different reactor scale-up studies. Data obtained by querying the knowledge graph could be used to set up further simulations or experiments.

4 Summary and Outlook

The capillary CFI is presented as a process development tool for bio-catalytic gas/liquid reactions. Therefore, it is an interesting device for the chemical and biochemical process industry in both, research and development. The mass transport coefficient $k_L a$ has turned out to be a suitable transfer metric (critical for quality parameter) for the transfer of reactions in continuous flow to batch reactor. Furthermore, this work presents a way to gather and store structured knowledge from the experiments conducted using ontologies. To achieve this, two already existing ontologies were combined and advanced. Afterwards, the advanced ontology and raw data from the laboratory experiments were used to set up a knowledge graph, which connects raw data and ontology in dependence of queries posed on the ontology. FAIR data principles are achieved with this approach. Data produced within the experiments can be accessed in a more structured way, and, e.g., reused in future simulations more easily.

Further investigations regarding other reactions in the CFI are desirable to apply the process development on industry-related reactions or make them applicable for lab use. More experiments regarding different capillary reactors and reaction rates can give a deeper insight into the correlations between reaction system, continuous flow experiments and the transfer to a batch reactor. Here, the ontology can help to set up a unified way of storing and comparing the data obtained in those experiments.

Regarding the ontology, a further description of the concepts used to conduct the scale-up is necessary in order to get a more holistic knowledge base on the experiments. This can lead to a more unified data structuring and acquisition for future laboratory experiments. In addition, the ontology could be used to automate and enrich the meta data generation for the generated experimental data. This can be of certain use for an ontology-based database, which poses one of the scopes of the German research project NFDI4Cat. An

ontology-based database allows for more enhanced information searches and knowledge queries regarding the experiments conducted already now and in the future.

Symbols used

a	[mm^2]	specific surface
c	[mol L^{-1}]	concentration
d	[mm]	diameter
E	[–]	extinction
H	[M Pa^{-1}]	Henry constant
I	[W m^{-2}]	intensity
k_L	[m s^{-1}]	mass transfer number
L	[m]	length
n	[–]	number
P	[W]	mechanical power input
p	[mm]	pitch distance
r	[μmol min^{-1}]	reaction rate
t	[s]	time
T^*	[–]	modified torsion number

Greek symbols

τ	[s]	residence time

Sub- and superscripts

c	coil
ct	coil tube
i	inner
o	outer
ox	oxidized

Abbreviations

ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
CAD	Computer-aided design
CFI	Coiled flow inverter
DoE	Design of experiment
FEP	Fluorinated ethylene propylene
GO	Glucose oxidase
HCC	Helically coiled capillary

HRP	Horseradish peroxidase
LDR	Light-dependent resistor
MFC	Mass flow controller
OntoCAPE	Ontology for chemical process engineering
OWL	Web ontology language
PLA	Polylactid acid
REX	Ontology of physico-chemical processes
RTD	Residence time distribution
SC	Straight capillary
SPARQL	SPARQL protocol and RDF query language
SQL	Structured query language

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