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Research Article

Measurement of heat transfer coefficients in stirred single-use bioreactors by the decay of hydrogen peroxide

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Single-use bioreactors are barely described by means of their heat transfer characteristics, although some of their properties might affect this process. Steady-state methods that use external heat sources enable precise investigations. One option, commonly present in stirred, stainless steel tanks, is to use adjustable electrical heaters. An alternative are exothermic chemical reactions that offer a higher flexibility and scalability. Here, the catalytic decay of hydrogen peroxide was considered a possible reaction, because of the high reaction enthalpy of -98.2 kJ/mole and its uncritical reaction products. To establish the reaction, a proper catalyst needed to be determined upfront. Three candidates were screened: catalase, iron(III)-nitrate and manganese(IV)-oxide. Whilst catalase showed strong inactivation kinetic and general instability and iron(III)-nitrate solution has a pH of 2, it was decided to use manganese(IV)-oxide for the bioreactor studies. First, a comparison between electrical and chemical power input in a benchtop glass bioreactor of 3.5 L showed good agreement. Afterwards the method was transferred to a 50 L stirred single-use bioreactor. The deviation in the final results was acceptable. The heat transfer coefficient for the electrical method was 242 W/m²/K, while the value achieved with the chemical differed by less than 5%. Finally, experiments were carried out in a 200 L single-use bioreactor proving the applicability of the chemical power input at technical relevant scales.

Keywords: Heat transfer coefficient / Hydrogen peroxide / Single-use technology / Stirred tank reactor

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1 Introduction

1.1 Motivation

Object of this work are stirred, single-use bioreactors, equipped with a water-filled jacket. This class of bioreactors is mainly used for cell culture applications in the biopharmaceutical industry [1]. They consist of two major parts: a reusable hardware assembly and a disposable bag. Whilst the general process engineering

characterization is widely established by means of mixing times, power input and $k_L a$ [2], the specific implications of this single-use technology regarding heat transfer are not described well yet. In this context, the plastic film layer must be mentioned. Although being thin, the low heat conductivity of these materials is expected to act as a significant additional heat barrier. Therefore, it is important to determine the capabilities of such systems and, thereby, avoid limitations during real fermentations.

Due to their slow growth, commonly used cell culture strains, such as CHO cells, only produce small amounts of heat. In contrast, the metabolic heat produced by microbial strains, like *E. coli*, can easily reach 50 W/L [3, 4], thus being magnitudes higher compared to mammalian cells.

Thus, aim of this work was to develop and apply a method that allows steady-state heat transfer measurements to single-use bioreactors at different scales that represents an exothermic fermentation process and limits the need for modifying the process equipment.

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Abbreviations: CHO, Chinese hamster ovary; TOC, temperature oscillation calorimetry

1.2 Chemical reaction as heat source

When representing a fermentation as described above, an adjustable heat source is required. With electrical heaters, the desired power can simply be set according to the power expected during real bioprocesses [5]. Further, electrical heaters can be used under process conditions, or even during fermentations [3,6]. On the other hand, there are some disadvantages. First, a heater must be built specifically for a given bioreactor. When investigating different types and scales of vessels, a set of dedicated heaters is necessary. Further, there are wave mixed single-use bioreactors that are hardly accessible by electrical power input. The chemical power input should overcome some of these disadvantages, allowing the direct use independent from scale and geometry. A chemical method would also have its specific drawbacks, but offers an improved flexibility and comparability for the characterization heat transfer capabilities of such systems.

Therefore, an exothermic chemical reaction is needed alternatively. One promising candidate is the catalytic decay of hydrogen peroxide, due to its high reaction enthalpy of -98.2 kJ per mole and harmless products: water and oxygen (Eq. (1))

Because of the release of oxygen, this reaction is already present for different applications in biotechnology [7–9]. Further, there are some protocols available making use of the high reaction enthalpy to investigate the thermal behavior of chemical reactors. These protocols mainly focus on educational purposes in small scales. In this work, it is aimed to evaluate the usability in larger, technical scale, single-use bioreactors. The main difference between mass and heat transfer experiments utilizing this reaction lies in the amount of hydrogen peroxide that is needed for a steady state over a given time. Since one liter 30%(w) hydrogen peroxide solution contains 4.9 mole pure oxygen, the needed flow rates are comparably low for applications that aim to utilize this oxygen. On the other hand, for a stable introduction of 1000 W 3.7 L/h are necessary of the same solution. The characteristics of the experimental setup must be capable of managing such high loads. When implementing this reaction for the desired purpose, the first challenge is to select a proper catalyst among the large variety of possible candidates. Catalyst species divide into homogenous solutions and heterogeneous suspensions. Further, there are biological catalysts, which often exceed their inorganic counterparts by means of reaction rate. At the other hand, stability and costs are an issue [10]. Selecting the proper catalyst is one objective of the present work and will be discussed later.

1.3 Determination of the overall heat transfer coefficient

The process of heat transfer within a technical apparatus is very complex but on a basic physical level there are only three types of how heat can be transferred: conductive, convective and by radiation. In the specific case of a bioreactor, only conduction and forced convection are relevant, which are represented by Eqs. (2)

and (3), respectively [11]. The left-hand side of both equations represent the heat flow q , through a given heat transfer area, A

$$\frac{q}{A} = \frac{\lambda_w}{s_w} \cdot (T_{w,1} - T_{w,2}) \quad (2)$$

$$\frac{q}{A} = \alpha_1 \cdot (T_{\infty,1} - T_{w,1}) \quad \text{with } \alpha = \frac{\lambda_l}{\delta_{lam}} \quad (3)$$

The tendency of matter to conduct heat is represented a material property, λ . Together with the thickness of this material, s , a thermal resistance is derived, which results from the division of both parameters. Whilst the concept of heat conduction is straightforward, the convective heat transfer coefficient, α , fuses the complex actions taking place at the fluid–solid interface. Simplified, it adapts principles of conduction to the convective process by stating that heat is transferred either way by conduction through the fluids laminar boundary layer of length, δ_{lam} . Consequently, this means α is a function of fluid and flow properties in the near-wall region. For the simplest case of conjugate heat transfer through a plain wall, i.e. liquid–wall–liquid, the thermal resistances of three sub-processes sum up to a representative value, the overall heat transfer coefficient, U (Eq. (4)) [12]

$$\frac{1}{U} = \frac{1}{\alpha_r} + \frac{s_w}{\lambda_w} + \frac{1}{\alpha_j} \quad (4)$$

For bag-based single-use bioreactors, a second heat conduction resistance must be introduced, originating from the plastic film layer. Further effects, like a thin air layer between film and steel wall, could be present, acting as additional insulation. These aspects become important when a detailed evaluation of the single terms contributing to the overall heat transfer coefficient is desired, e.g. for optimization purposes. Otherwise, U lumps up all resistances that are present during the experiment.

The experimental determination of heat transfer coefficients strongly depends on the given apparatus. Starting point is a heat balance, in this case around a bioreactor, which in detail is the combination of two balance spaces: reactor and jacket. For bioreactors, there are extensive balances available in the literature, considering all major heat flow terms [13]. For the setup used in this work, the heat balance is adapted according to Eq. (5) for the reactor side and Eq. (6) for the jacket side

$$m_r \cdot c_{p,r} \cdot \frac{dT_r}{dt} = q_p + q_s - q_{rj} + q_{in,r} - q_{out,r} - q_{loss,r} \quad (5)$$

$$m_j \cdot c_{p,j} \cdot \frac{dT_j}{dt} = q_{rj} + q_{in,j} - q_{out,j} - q_{loss,j} \quad (6)$$

The left sides of the equations represent the accumulation terms under transient conditions, with the mass of the content, m , i.e. liquid and installations, the mean heat capacity, c_p , and the time derivative of the corresponding temperatures, dT/dt , which must only be considered when performing transient experiments. Further, for the reactor side (Eq. (5)), the equations right side contains a heat source, q_p , the stirrer power input, q_s , the power transferred to the jacket, q_{rj} , heat of material flows entering and exiting the reactor $q_{in,r}$ and $q_{out,r}$, respectively, and heat loss to the environment, $q_{loss,r}$. The source term is realized by the chemical reaction or electrical power input. For the latter, the balance further simplifies, because there is no steady flow of

liquids into or from the reactor. On the other hand, the right side of Eq. (6) also contains the term q_{rj} , but with a positive sign, because both balance spaces are coupled at this point. Since a steady flow of cooling liquid through the jacket, there is also an inlet and outlet term, $q_{in,j}$ and $q_{out,j}$, as well as a heat loss term $q_{loss,j}$. To determine the overall heat transfer coefficient, the jacket transfer term must be regarded (Eq. (7))

$$q_{rj} = U \cdot A_j \cdot \Delta T_{log} \quad (7)$$

with A_j being the heat exchange area and ΔT_{log} the mean logarithmic temperature difference, according to Eq. (8)

$$\Delta T_{log} = \frac{(T_r - T_{in,j}) - (T_r - T_{out,j})}{\ln \left(\frac{T_r - T_{in,j}}{T_r - T_{out,j}} \right)} \quad (8)$$

Further, the flow terms $q_{in,j}$ and $q_{out,j}$ are represented by Eq. (9)

$$q_j = q_{in,j} - q_{out,j} = \dot{m}_j \cdot c_{p,j} \cdot (T_{in,j} - T_{out,j}) \quad (9)$$

A major advantage of steady-state experiments is that the accumulation term equals zero (Eq. (10))

$$m \cdot c_p \cdot \frac{dT}{dt} = 0 \quad (10)$$

Both, transient [14] and steady-state [5, 15, 16] strategies are found in the literature when determining heat transfer coefficients. Some authors use both techniques, depending on scale [17]. Often, large scales are hardly accessible by steady-state measurements, since the required power input increases accordingly. Therefore, transient experiments are easier to carry out at full scale. On the other hand, the exact consideration of temperature changes of all relevant mechanical components, as well as the temperature-dependent fluid properties, can be an extensive and error prone task. Another important technique that is used to determine heat transfer coefficients is temperature oscillation calorimetry (TOC), which has elements of both, transient and steady-state operation [18, 19]. However, the prerequisite of a uniform jacket temperature is not given here, because of a significant temperature difference between inlet and outlet and thus, TOC can not be applied. Finally, a steady-state experiment represents a real fermentation process well, i.e. the final application of the investigated single-use bioreactors.

Considering Eq. (6) to (10), the overall heat transfer coefficient under steady-state conditions is calculated by Eq. (11)

$$UA_j = \frac{\dot{m}_j \cdot c_{p,j} \cdot (T_{out,j} - T_{in,j}) - q_{loss,j}}{\Delta T_{log}} \quad (11)$$

This parameter then describes the heat transfer characteristics present at the jacketed surface. Hence, for determining the overall heat transfer coefficient, it is sufficient to consider the jacket side heat transfer. It shall be noted, that in this work the product UA_j is used at some points, and not the transfer coefficient alone, since the definition of the exchange area for complex geometries is somehow arbitrary. This fact is discussed in a later section. To estimate the variation of results based on measurement uncertainty, Gauss' propagation of error algorithm was applied to Eq. (11) with the following deviation values: $\Delta \dot{m}_j = 0.01 \dot{m}_j$ and $\Delta T = 0.1K$ (same for all temperatures). Since these were

the major contributors to error, other influences were neglected (i.e. $\Delta c_{p,j}$ and $\Delta q_{loss,j}$).

2 Materials and methods

2.1 Chemicals and analytics

The used hydrogen peroxide was technical grade, 30%(w) stabilized solution (Carl Roth, CP26). For the catalyst screening three candidates were evaluated: Catalase from bovine liver (lyophilized powder, > 10,000 units/mg, Sigma-Aldrich, C40), Iron(III)-nitrate nonahydrate (Carl Roth, CN84) and Manganese(IV)-oxide (Carl Roth, 7751).

To determine the present peroxide concentration within the liquid, an analytical titration procedure was carried out. First, a pre-solution was prepared, consisting of 32 mL 0.24 M potassium iodide solution acidified with 4 mL muriatic acid (32%w). Depending on the peroxide concentration of the sample, a specific amount was added to the solution, e.g. for a concentration of less than 0.05 mole/L about 4 mL sample were added. Here, the color changed from clear to dark orange. The solution was then titrated using 0.1 M sodium thiosulfate solution until decolorization occurred. Finally, 0.8 mL 1%(w) starch solution were added to colorize last traces of elementary iodine which then could be accurately titrated further using the thiosulfate solution. The peroxide concentration then was calculated from the consumed volume of thiosulfate considering the stoichiometry.

2.2 Vessels and bioreactors

The catalyst screening was performed in simple 250 mL beaker wrapped in insulation to reduce heat loss. Mixing was performed by a magnetic stirrer bar and temperature was logged by a Testo 435-2 (Testo SE & Co. KGaA, Lenzkirch). The first scale-up step was carried out in a UniVessel® 5 L glass bioreactor (Sartorius Stedim Biotech GmbH, Göttingen), with a single six blade Rushton impeller and baffles. The technical scale trials were performed in a BIOSAT STR® 50 and 200 (Sartorius Stedim Biotech GmbH, Göttingen), containing a Flexsafe® bag equipped with a 2 x three blade segment impeller configuration, both operated at 1.5 m/s tip speed, which corresponds to 200 rpm (STR® 50) and 126 rpm (STR® 200), respectively. The vessel bulk temperature was measured with the dedicated Pt100 sensor according to the used bioreactor control unit, while the jacket in- and outlet temperatures were measured with flow-through Pt100 sensors. The signal was converted using MINI MCR-SL-PT100-UI (Phoenix Contact GmbH, Blomberg) enabling the connection to analogue inputs of the control tower. Jacket flow was measured using a magnetic-inductive sensor FEH300 (ABB, Göttingen).

2.3 Setup

First, the catalyst screening was performed in batch mode, i.e. a given amount of hydrogen peroxide was added to a catalyst solution or vice versa and the time course of the decreasing

hydrogen peroxide concentration was monitored by titration of samples. Batch experiments were only performed initially using a beaker. The final application required the continuous addition of a peroxide stream. For the beaker and small scale bioreactor trials a 205 U peristaltic multichannel pump was used and for the technical scale two 520 U pumps, respectively (Watson Marlow GmbH, Rommerskirchen). The peroxide consumption was monitored with Midrics® 1 balances (Sartorius Labs Instruments GmbH, Göttingen).

Alternatively to the chemical power input, electrical heaters were used (Wema Elektronik GmbH, Illmensee). The cylindrical heaters were introduced through the top of the bioreactor and consisted of a cold and hot area, since power input is only allowed when immersed into liquid. The power was then adjusted by a thyristor power controller. The setpoint of the bioreactor temperature was set to 35°C and controlled by a digital control unit. The schematic setup can be seen in Fig. 1. It is important to note that while the small scale glass vessel is fully covered by a jacket, the larger single-use bioreactors are only jacketed partially, at the bottom. This affects especially the heat losses of the single-use bioreactors. While the process liquid of the fully jacketed glass vessel has no direct contact to the environment, there are significant heat loss areas for the single-use bioreactors,

which increases with filling volume. Therefore, with increasing scale and filling volume a higher portion of the introduced electrical or chemical heat is directly transferred to the environment, instead of the jacket. However, this has no effect on the calculation of the heat transfer coefficient, since U_j only describes the jacketed portion of the wall (Fig. 2).

All waste solutions were collected and disposed separately. Large amounts of manganese(IV)-oxide solutions were first filtered by Sartopore® 2 MidiCaps® (Sartorius Stedim Biotech GmbH, Göttingen) for volume reduction.

3 Results and discussion

3.1 Catalyst evaluation

3.1.1 Batch mode

As mentioned before, there is a variety of possible catalysts available. Here, it was decided to evaluate a catalyst of each class, namely iron(III)-nitrate (homogenous), manganese(IV)-oxide (heterogeneous) and catalase (biological). The initial characterization took place in batch mode to study the general reaction behavior to a point where the layout of a steady-state process

Figure 1. Schematic of the experimental setup, consisting of containers for fresh hydrogen peroxide supply and a waste container, connected via pumps to the bioreactor. Either the reactor is connected to the containers or equipped with a heater, in case of electrical power input. A digital control unit performed control of the reactor temperature, T_r , and was connected to the jacket. Cooling water was supplied by a cooling unit.

Figure 2. Schematic of the full heat balance for the different bioreactor geometries including the relevant heat flow terms according to Eqs. (5) and (6). An electrical heater represents the heat source. While the small-scale glass vessel (A) is fully covered by a jacket over its complete height, the single-use bioreactors (B) are only jacketed at the bottom. The overall heat transfer coefficient, U_j , only refers to the jacketed part of the vessel wall. For the single-use bioreactors, a significant portion of the electrically or chemically introduced heat is lost to the environment, which further is dependent on the filling volume.

was possible. Clarification of the detailed underlying reaction kinetics was not object of this work, since the catalyst would be applied in excess and should only provide a sufficient activity over a given period. All of the following experiments were carried out in a liquid volume of 100 mL.

Catalase. In the first trial, 0.5 mg catalase was added to 100 mmole hydrogen peroxide. The amount of catalase was calculated considering the activity of 10.000 units/mg with additional excess. Thus, a complete turnover was expected but after 40 min only 84% of the substrate was consumed. The strong exothermic behavior resulted in a temperature increase of 14 K. On the other hand, the maximum temperature was below 30°C and thus, below a level that was considered critical for denaturation. To further investigate the incomplete turnover, a second experiment with re-feeding of catalase was performed, starting with initially 150 mmole hydrogen peroxide. The first load of catalase converted 64 mmole hydrogen peroxide within 50 min, followed by a re-feeding of again 0.5 mg catalase, which converted about 69 mmole hydrogen peroxide, too. The reaction rate of the second phase was increased significantly compared to the first phase, probably because of the higher temperature inside the vessel. However, even a re-feeding did not result in a complete turnover. Finally, an experiment with reduced hydrogen peroxide of initially 50 mmole hydrogen peroxide was performed. This time, a complete turnover was achieved. Afterwards, another 100 mmole hydrogen peroxide was added of which only 33 mmole were converted.

Summarizing this, about 75 mmole hydrogen peroxide per mg catalase can be converted, making this catalyst unsuitable for high peroxide loads present during heat transfer measurements. This is due to an underlying inactivation of the enzyme [20], which is not investigated further, since these results already are sufficient to exclude catalase as proper catalyst.

Iron(III)-nitrate. Iron(III)-ions are known catalysts for the decomposition of hydrogen peroxide. Here, it is used as nitrate-salt. The influence of different concentrations (2, 5 and 15 g/L) on the reaction rate with an initial amount of 100 mmole hydrogen peroxide was investigated. In contrast to catalase trials, a complete turnover was achieved over the complete concentration range. Further, the reaction rate increased slightly corresponding to the applied concentration. While for the 15 g/L, all hydrogen peroxide was consumed after three minutes, it took about 12 minutes for the lowest concentration. Although it is reported that acidification with nitric acid supports the reaction, this statement did not hold true in own experiments. Acidification suppressed the reaction strongly. The results showed the general usability of iron(III)-nitrate in an applicable concentration range with satisfactory reaction rate. However, solutions had a pH of < 2, which gave safety concerns when working with large quantities. Attempts to neutralize the pH value had negative impact on activity and stability of solutions and thus, failed (Data not shown).

Manganese(IV)-oxide. Following the approach above, again, a broad concentration range of the insoluble catalyst suspension was investigated, representing different magnitudes from 10^{-1} to 10^1 . This approach was necessary, since there was no experience present before. Only the lowest concentration of 0.1 g/L did not show a complete turnover, while concentrations of 0.9 and 8.1 g/L did (Fig. 3). Other authors reported a working concentration of

Figure 3. Manganese(IV)-oxide batch experiment at different concentrations. To ensure a sufficient fast decay of hydrogen peroxide, a concentration above 1 g/L is required. The next higher concentration investigated was 8.1 g/L, which led to an immediate reaction. Therefore, for continuous experiments the concentration must lie within this range.

0.9 g/L, but for $k_L a$ -measurements with a much lower hydrogen peroxide load [10]. Therefore, the results confirmed the good characteristics for the desired application.

A drawback arises from the inhomogeneous nature of the catalyst. To achieve a uniform distribution within a vessel a certain level of mixing is required to prevent the particles from settling. In addition, a disposing strategy is required. Here, the suspension was filtrated after use.

According to the behavior and specific properties of the evaluated catalysts, it was decided to continue with manganese (IV)-oxide.

3.1.2 Continuous mode

Since the final application requires a steady composition of hydrogen peroxide, corresponding studies were performed in continuous mode to exclude inactivation tendencies over time. Therefore, a 100 mL reaction vessel, mixed by a magnetic stir bar, was charged with a flow of 10 mL/h 30%(w) hydrogen peroxide and an initial absence of reaction educts. This flow already corresponds to a technical relevant power input of about 30 W/L. Figure 4 shows the concentration inside the vessel for different catalyst concentrations. Only the highest concentration of 4 g/L achieved a total consumption over four hours (Fig. 4). At this point it becomes obvious that, compared to hydrogen peroxide based $k_L a$ -measurements, a significant higher catalyst concentration is needed. Further, the washing-out effect must be regarded. To keep the filling level constant, an outflow steadily removed liquid from the reactor and thus, catalyst. Trials were performed with filtration probes, to reduce loss but finally it was decided to set the initial concentration in a manner that

Figure 4. Continuous mode experiments using manganese (IV)-oxide as catalyst. The hydrogen peroxide load was set according to the target for technical scale experiments (ca. 30 W/L). Only with the highest catalyst concentration of 4 g/L (grey line) it was possible to achieve a steady state over a time of four hours. Lower catalyst concentrations led to an enrichment of hydrogen peroxide over time. Thus, for further experiments, it was necessary to always keep a sufficient amount of active catalyst within the suspension and to monitor the hydrogen peroxide concentration during the experiments.

guarantees sufficient catalyst over the whole time, i.e. an initial concentration of at least 4 g/L plus a security factor that depends on the time span of the experiment and the target hydrogen peroxide load. Regarding the heat balance of the reaction vessel, the outflow decreases the net power input by about 5%, compared to power that would be expected by the reaction enthalpy.

Summarizing, a procedure was established in a model system, which allowed a steady power input over a certain time. In the next step, the procedure was transferred to a bench top bioreactor.

3.2 Small scale

Before any technical scale experiments, which consume large quantities of chemicals, the general comparability between electrical and chemical power input should be verified. Hence, experiments were performed in a 5 L jacketed glass bioreactor with 3.5 L filling volume and a manganese(IV)-oxide concentration of initially 6 g/L. The stirring frequency was set to 500 rpm to ensure sufficient mixing. The power for both, the electrical and chemical heat sources, was varied by whether a simple power controller, or by adjusting the pump rate and ranged from 20 to 70 W/L. The power input induced a temperature difference between the jacket inlet and outlet from which, according to

Figure 5. Determination of heat transfer coefficients for the small scale experiments and comparison between different heat sources. Jacket side temperature differences below 1.5 K should be avoided, because of measurement uncertainty. Above this value, there is a good agreement between electrical and chemical power input, underlining robust application of the hydrogen peroxide based method. Error bars represent Gauss error propagation applied to Eq. (10).

Eq. (10), the heat transfer coefficient was determined. Figure 5 shows the results of these trials. With temperature differences above 1.5 K, there is a good agreement between both methods. Lower differences result in high uncertainties, due to resolution of the temperature measurement, noise and other error sources. As a rule of thumb, the power input should result in a temperature difference of 2 K. When only considering the data that fulfill this requirement, the UA_j by electrical power was 21.3 W/K and 20.9 W/K by the chemical method. The plot shows an outlier with 30.4 W/K, which resulted from unsteady feeding of hydrogen peroxide at very low flow rates. As mentioned before, calculating the heat transfer coefficient U requires a clear definition of the heat transfer area, which is not trivial in case of the glass bioreactor used here. Variations may arise from the filling level of both, the reactor and jacket side. Further, since the used bench top bioreactor has a wall thickness of 5 mm, the inner and outer heat transfer area are slightly different. Here, the difference is about 4 %. In case of complete coverage of the jacket, the mean logarithmic heat transfer area is about 0.153 m².

From the bench top experiments, it was shown that the method is applicable in stirred bioreactors and is able to produce a steady state with constant power input. Although it is desirable to limit the hydrogen peroxide consumption, a jacket side temperature difference of minimum 1.5 K should be induced to ensure good data quality.

Figure 6. Temperature-time course of both technical scale single-use bioreactors, 50 L (A) and 200 L (B). For a steady state it was necessary to keep a stable chemical power input over four hours. Only the area delimited by the dotted line was used for the calculation of heat transfer coefficients.

3.3 Technical scale

Aim of this work was to transfer the method to stirred single-use bioreactors at a scale of 50 and 200 L. For the 50 L scale, again a comparison between heat sources was carried out. According to the pre trials, the manganese(IV)-oxide concentration was 6 g/L for the STR® 50 and STR® 200 at 150 L filling volume. For the STR® 200 at 200 L, a concentration of 5 g/L was used, since this corresponds to the same absolute catalyst mass as in the lower filling volume. Further, the peroxide load was the same for both filling volumes. This way the catalyst consumption was slightly reduced. Figure 6 represents the temperature curves of representative runs for both scales. For best evaluation results, the temperatures profiles should represent straight horizontal lines. Most obvious, when starting the chemical power input it takes about three hours for the systems to reach equilibrium. Maybe the time can be shortened when increasing the power according to a profile during the starting phase. However, a steady state was achieved for which heat transfer coefficients could be calculated.

The data of the technical scale experiments is summarized in Table 1. Independent of heat source type, the desired power input, q_p , was 1000 W. It can be seen, that the measured power introduced to the jacket, q_{rj} , was always below that value, especially in case of the chemical method. Reasons are the heat loss to the environment, heat loss due to the in- and outflow of liquids from the bioreactor and general measurement uncertainties. When there are distinct heat losses to the environment,

as given for the partially jacketed single-use bioreactors, only a smaller ratio of the source's power input is transferred to the jacket, which can be simplified represented by Eq. (12)

$$q_{rj} \approx q_p - q_{\text{loss},r} \quad (12)$$

This leads to a reduced jacket side temperature difference that, in this case, lies in the lower detection limits that assure confident results. Generally, for better measurement accuracy, the transfer term q_{rj} should be increased. This can be achieved whether by reducing the heat loss or increasing the power input. Further, it shall be mentioned, that the triplicate for the chemical method was done with one single batch of manganese(IV)-oxide suspension. Because of the long-term stability, the suspensions could be used multiple times under the condition, that complete turnover of hydrogen peroxide is monitored by sampling. For all the runs presented here, the hydrogen peroxide turnover was above 98%. Considering the heat exchange area, which is located only in the bottom part of the hardware assembly (Fig. 7), mean heat transfer coefficients of 242 W/m²/K for the electrical and 231 W/m²/K for the chemical method were determined. Discrepancies again might be due to differences in the absolute power input, affecting the measurement accuracy. However, the results only differ within general uncertainty and thus, the peroxide method was proven to be well applicable to technical scale single-use bioreactors. The absolute values for the heat transfer coefficients also showed good agreement with former results, received from cooling curve experiments [21].

Table 1. Summary of the data received from technical scale experiments for both heat sources and scales. For the calculation of heat transfer coefficients an exchange area of 0.33 m² for the 50 L bioreactor and 0.88 m² for the 200 L bioreactor was considered, respectively

	V_l [L]	F_j [L/h]	$T_{\text{in},j}$ [°C]	$T_{\text{out},j}$ [°C]	q_{rj} [W]	UA_j [W/K]	ΔUA_j [W/K]	U_j [W/m ² /K]
STR® 50								
Electr.	50	436.6	21.7	23.7	966.8	78.5	9.6	241.1
Electr.	50	436.6	21.8	23.7	969.8	78.9	9.6	242.2
Chem.	50	434.8	23.5	25.1	800.3	74.7	10.9	229.4
Chem.	50	436.8	23.7	25.3	793.9	75.5	11.1	232.0
Chem.	50	436.9	23.6	25.2	793.9	75.3	11.0	231.2
STR® 200								
Chem.	150	430.8	29.60	31.0	670.0	143.3	26.5	162.0
Chem.	200	430.3	30.14	31.3	558.2	131.9	28.7	149.0

Figure 7. Hardware assembly of a BIOSTAT STR® 200 (Sartorius Stedim Biotech GmbH, Göttingen). The upper part is equipped with doors to facilitate bag installation. Therefore, only the bottom part builds the jacket. The shape is determined by the bag geometry to ensure contact to the wall.

Finally, the method was performed in a 200 L system, at two different filling levels. For 150 L a heat transfer coefficient of $162 \text{ W/m}^2/\text{K}$ was calculated and a value of $149 \text{ W/m}^2/\text{K}$ at 200 L, respectively. In a conventional bioreactor, where the jacket covers the vessel over its full height, a volume increase would directly lead to an increased heat transfer area and, thus, a better heat transfer capability, represented by the product of heat transfer area and coefficient, UA_j . For the given single-use bioreactor, where there is no dependency from the filling level on the transfer area, a reverse effect on the heat transfer capability is present. This might be due to improved flow characteristics at lower filling levels, which then positively affects the heat transfer coefficient. However, the effect is less pronounced.

Comparing the two scales, the smaller bioreactor seems superior to the larger one, but the reason is probably related to the jacket side heat transfer. Since both scales of the single-use bioreactors are connected to the same control unit, the jacket flow is nearly equal. At such low flow rates as given here, it must be assumed, that there is no fully turbulent regime present in the jacket, which strongly reduces this side's heat transfer coefficient, α_j . This effect is more pronounced in the larger bioreactor, where the liquid velocity is even lower because the corresponding larger jacket. For a representative comparison of different scales, physical similarity must be guaranteed regarding the jacket side flow characteristics, which was not the case at this point. This thesis can only be evaluated in a more detailed investigation, following the procedures known from dimensionless correlations based on Nusselt numbers where the inner and outer heat transfer coefficients, α , can be calculated and set in ratio. Identifying limitations and resolve the single terms according to Eq. (4) is object of further work.

4 Concluding remarks

Characterizing single-use bioreactors by means of heat transfer in way that represents real bioprocesses is not very common. Often, simple heating or cooling curves are applied. Therefore, steady-state methods offer a better alternative, as the process parameters can be set accurately. Although the usage of electrical heaters can be found throughout the literature, an alternative approach was evaluated here first time and being applied to stirred single-use bioreactors. Finally, the exothermic decay of hydrogen peroxide was shown to be flexible and easy to use. All geometries, whether wave mixed or stirred, and material types (stainless steel/ glass vs. single-use) are accessible and no modifications to the hardware need to be made. On the other hand, to achieve sufficient data quality, a significant amount of hydrogen peroxide is consumed. For larger scales, the amount of catalyst needed must be considered, too.

Therefore, when further increasing the power input, a combination of electrical and chemical power input could be beneficial. For example in a scenario, where there is not sufficient space for introducing sufficient heaters, a baseload could be introduced electrically and a variable portion chemically, or vice versa. Since the heat source itself does not have an impact on the measurement and calculation of heat transfer coefficients that method should be applied which fulfills the specific needs of a given experimental set up. Here, a sufficiently large jacket side

temperature difference was of major concern, which can be achieved by the novel chemical method. If the exact power input is important, e.g. for complete heat balancing, an electrical power input is advantageous because it can be measured directly.

Practical application

Temperature regulation is a major concern in fermentation technology, since keeping optimal environmental conditions within a bioreactor is crucial for robust processes. Thus, having knowledge about the thermal performance is desirable concerning hardware construction as well as designing bioprocess. To gain insight into the process of heat transfer in a bioreactor heating and cooling curves are applied commonly. While being easy to perform, such trials do not represent the addressed situation of growing cells under isothermal conditions. This can be achieved by introducing external heat sources, e.g. in form of electrical heaters, which then must be designed according to a dedicated bioreactor. Further, some geometries like wave mixed bioreactors are hardly accessible this way. Therefore, using an exothermic chemical reaction that can be adjusted to the needs of an experiment is a promising alternative. The catalytic decay of hydrogen peroxide is a possible candidate to cope these requirements.

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Nomenclature

A	[m ²]	Heat transfer area
c_p	[J/kg/K]	Specific heat capacity
F	[J/kg/K]	Liquid flow
m	[kg]	Mass
$\dot{m}$	[kg/s]	Mass flow
s	[m]	Wall thickness
t	[s]	Time
T	[K]	Absolute temperature
ΔT	[K]	Temperature difference
$\Delta T_{\log}$	[K]	Mean logarithmic temperature difference
U	[W/m ² /K]	Overall heat transfer coefficient
V	[L]	Volume
q	[W]	Heat flow/ power

Greek symbols

α	[W/m ² /K]	Convective heat transfer coefficient
δ	[m]	Thickness of fluid layer
λ	[W/m/K]	Heat conductivity
ϑ	[°C]	Temperature

Indices

in	Inlet
j	Jacket
l	Fluid
lam	Laminar
loss	Heat loss
out	Outlet
p	Heat source
r	Reactor side
s	Stirrer
w	Wall
∞	Bulk

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