Calorimeter-Fermentor Combination for Investigations on Microbial Steady State Cultures

H. P. Leiseifer* and G. H. Schleser

Abteilung Biophysikalische Chemie, ICH Kernforschungsanlage Jülich GmbH, Postfach 19 13, D-5170 Jülich 1

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The combination of a 3-liter fermentor with a flow-through calorimeter is described. Minimum time-lags are less than 10 s corresponding to maximum thermal peak disturbances of ~ 4 µW. The set up shows an excellent long time stability. Aerobic growth of *Escherichia coli* K12 in batch cultures was used to test the equipment. Precision is ± 2.1% with regard to the total heat release of 4.94 kJ • l⁻¹ and ± 2.5% with respect to the maximum heat flow of 609 mW • l⁻¹. The accuracy is comparable with calorimeters using commercial flow mixing cells.

1. Introduction

The investigation of microbial cultures using calorimetric techniques represent nowadays an important tool in biophysical chemistry [1–4].

However, calorimetric measurements with continuous cultures performed so far are relatively few e.g. [5–10] in spite of the fact that the corresponding thermodynamic data are necessary for biophysical studies of open systems and for certain problems in biotechnology.

The present study therefore centers on the design and testing of a combination of a flow through calorimeter and a fermentor which allows to measure the heat production of aerobic chemostat cultures.

The separation of fermentor and calorimeter largely avoids the transfer of thermal disturbances from the cultivation system to the calorimeter. Furthermore the culture is easily accessible during the cultivation process.

As a consequence, however, the heat production of a volume element leaving the steady state of the chemostat culture might strongly decrease during its passage to the calorimetric measuring tube [4, 11–13]. Our experiments (see Fig. 1) indeed show that the heat production within the chemostat can only be evaluated by successive measurements at different but sufficiently high and constant flow rates allowing extrapolation to flow rates "∞".

Therefore, one important feature of the described combination represents its possibility of operating at high flow rates (up to 540 ml • h⁻¹) without loosing too much of its sensitivity and precision.

![Fig. 1. Steady state heat production \( \dot{Q} \) in the calorimetric measuring tube versus residence time \( t' \). Results obtained with aerobic steady state (chemostat) cultures of *E. coli* K12 at different dilution rates \( D \), i.e. specific growth rates. Temperature of the culture medium: \( T = 37 \) °C. Inflowing glucose concentration: \( S_e = 318.2 \) mg • l⁻¹. The residence time \( t' \) represents the time which a volume element needs to reach half of the volume of the calorimetric measuring tube after leaving the culture.](image-url)
In our case very little information is available about the heat effects associated with microbial growth in chemostat cultures, which implies that the latter cannot be used to test the apparatus.

Therefore detailed theoretical considerations are reported to show that growth of *E. coli* K12 in aerobic batch culture constitutes a suitable 'standard process' to test the combination against systematic errors and to estimate its accuracy. Test runs of the described combination are reported using batch cultures, by heeding the theoretical considerations.

2. Experimental set-up

The connection of the calorimeter (type “Microcalorimeter System 2107”, LKB Produkter AB, Bromma, Sweden) to the fermentor (type “BioStat”, Fa. Braun Melsungen AG, W. Germany) is schematically shown in Fig. 2.

The calorimeter is equipped with two nearly identical flow through tubes, one being for the sample fluid (bacterial suspension) and the other for a reference fluid (sterile medium, without carbon source). The set up is operated in a so called twinflow mode: Sample and reference pass the same precisely controlled thermal environment, namely a prethermostatic waterbath, before entering the calorimeter. Since the outputs of the tube thermopiles are connected in opposition, signals of thermal disturbances cancel out. This situation is illustrated in Fig. 3: at position 1 the calorimetric signal is recorded, using just the reference channel, after the prethermostatic bath has been stimulated to large undamped temperature oscillations. At 2 the twin flow mode is selected, resulting in a strongly reduced amplitude of the thermo-voltage $U$ and a nearly drift free signal. The twinflow method reduces the peak to peak amplitude of thermal disturbances by a factor of 10 independent of the flow rate. Moreover it leads to a precisely assessable mean value of the calorimetric signal.

The culture vessel (19) of the fermentor has a volume of 5.31 (culture volume 2.6–3.1) and is immersed into a prethermostatic waterbath (11) to approximately 2/3. The latter consists of a plexiglass container which extends into the air bath (2) of the calorimeter by means of a plexiglass duct (14) (internal length: 268 mm, $\delta_1 = 17$ mm, wall strength: 2.5 mm). In order to have the sample and reference capillaries (8) embedded into the same thermal environment, a circulating pump (12) sucks continuously water, at a rate of approximately $5 \cdot \text{min}^{-1}$ from the prethermostat through (14) and through the internal heat exchanger (16). Through tube (17) the water reaches the prethermostatic bath again. Thorough mixing is achieved by the forced convection of pump (12) and a stirrer (25). Agitation of the culture is likewise achieved by means of a stirrer (20).

A precision temperature controller type PTC 40 (Tronac Inc. Orem, Utah/USA) in conjunction with a combined heater cooler (18) [14] adjusts the temperature of the prethermostatic waterbath (11). It

![Fig. 2. Coupling of calorimeter and fermentor. For explanation see text.](image-url)
contains 25 l of water. The stability of the bath temperature is better than $\pm 10^{-3} ^\circ\text{C}$ from the established steady state value over a period of at least 72 h (Control by a calorimeter thermometer after Berthelot-Mahler with 1/100 °C division, Fa. A. Amarell, Wertheim, W. Germany). The necessary room temperature stability is $\pm 1 ^\circ\text{C}$. The sample and reference capillaries (8) consist of 24 carat gold (length = 320 mm, $\phi_1 = 1$ mm, $\phi_2 = 1.4$ mm) and are parallelly running along the axis of the duct (14). Before sample and reference reach the flow through tubes 3 and 6 (spiralled gold tubes, $\phi_1 = 2$ mm, volume of the sample tube = 0.6866 ml) they enter the internal heat exchangers (4 and 5) via teflon tubings (7) of equal length ($\phi_1 = 1$ mm, $\phi_0 = 1.4$ mm). Due to a hydrostatic pressure upon the culture medium and reference fluid the multichannel peristaltic pump (23) (type mp-ge Ismatec SA, Zürich, Switzerland) operates as a limiter for the sample and reference flow rates. Two tubes connected with Y-pieces of glass were used at the exit of each cell. Deviations from a fixed flow rate are usually within the range of $\pm 2.0\%$ as related to the mean value of the two.

The gold capillaries are connected to the fermentor via two transition pieces of silicon tubing (10) ($\phi_1 = 1$ mm, $\phi_0 = 3$ mm) which carry a T piece and a cross piece. The latter allows sterilizing and rinsing via a coupling (26).

To achieve identical temperatures for sample and reference at their entries (8a, 8b) into the tubes (10), the reference (22) passes the heat exchanger (21) inside the culture vessel (19).

By means of pinchcocks (9a, 9b, 9c) which act upon the tubes (10) it is possible to select the flow-modes 'reference against reference' and 'sample against reference'. The cocks are operated via 3 stainless steel rods ($\phi = 2$ mm) which protrude out of the water bath. The total volume of the connection between fermentor and the entrance of the measuring tube for the sample side amounts to only 1.306 ml. In case of batch cultures, sample fluid is routed back into the culture vessel. Reference fluid is wasted into a vessel (24). The differential voltage signal of the thermopiles is amplified by a Keithley

![Fig. 3. Reduction of thermal disturbances and drift by the twin flow method. Flow rate $f = 395$ ml $\cdot$ h$^{-1}$. For explanation see text.](image)

![Fig. 4. Batch version of the fermentor with its main peripheric components. 27, 28: Electrically heated sterility filters; 29: water column; 30: cooling trap; 31: Polarographie oxygen electrode; 32: Rota flow.](image)
150 B Microvolt Ammeter (Keithley Instr. Inc. Cleveland, Ohio/USA) and fed to a 1-channel recorder.

Fig. 4 shows the batch version of the fermentor. The chemostat version is equipped with a relay triggered volume control and an automatic medium supply unit.

3. Methods

3.1. Colorimetric methods

Calibration of the calorimeter follows from liberating a known amount of JOULE heat \( Q_j \) in the near neighbourhood of the sample tube resulting in a recorder deflection \( \Delta L_j \). The calibration current is provided by a commercial unit type “LKB 2107-310”.

To illustrate the method, Fig. 5 shows a typical measuring run for a steady state chemostat culture. Measurements at different constant flow rates during the exponential growth phase of batch cultures are performed similarly. Denoting the ratio of \( \dot{Q}_j \) and \( \Delta L_j \) by \( \varepsilon (\varepsilon = \dot{Q}_j / \Delta L_j) \), the unknown heat production \( \dot{Q} \) is given by

\[
\dot{Q} = \varepsilon \cdot \Delta L.
\]

\( \varepsilon \) is called the calibration “constant” of the calorimeter.

From Fig. 6 it can be concluded, that \( \varepsilon \) is a linear function of \( f \), with positive segment on the ordinate, in the range of \( 0 \leq f \leq 540 \text{ ml} \cdot \text{h}^{-1} \). Since the sample flow rate in mode II was usually slightly different from that in mode I, \( \varepsilon \) for mode II was corrected via a regression line obtained from values in mode I with different flow rates. In experiments \( \varepsilon \) proved to be independent of whether reference fluid or bacterial suspension ran through the sample tube.

Test measurements were also performed with a blank solution, namely sterile medium, instead of bacterial suspension, to determine possible blank steady state differences between modes I and II as a function of flow rate. If necessary, the measured heat effects were corrected for the corresponding, usually small blank effect.

Flow rates through the sample tube were determined volumetrically, recorder deflections by using a precision slide gauge.

3.2. Microbial methods

All experiments were performed with \( E. coli \) K12 [15] at 37 °C. A mineral salt medium of the following composition was used: \( \text{KCl: } 2.237 \text{ g} \).
MgCl$_2$ \cdot 6$H_2$O: 0.203 g, Na$_2$HPO$_4$ \cdot 2$H_2$O: 16.020 g, 
Na$_2$H$_2$PO$_4$ \cdot H$_2$O: 4.114 g, (NH$_4$)$_2$SO$_4$: 1.980 g, with 
either glucose or succinic acid of 636.4 mg filled up 
to 1 l with demineralized water. The pH of the whole 
medium was between 7.0 — 7.5 throughout 
the experiments, requiring no corrections.

Identification of the bacterial strain and tests of 
contamination were performed after Knothe et al. 
[16], pre-cultivation and sterilization of apparatus 
and medium were achieved as described in [17].

Dry weight of the culture was determined by cen­
trifuging samples of 65 ml for 30 min at 20000 $g$, 
removing the supernatant and drying at 80 °C. 
Weighing followed drying to constant weight. Op­
tical density was measured at 400 nm with a pho­
tometer.

![Fig. 7. Thermograms of batch cultures with equal initial 
conditions taken at different flow rates $f$ through the 
sample tube of the calorimeter. The thermograms are 
centered to the first maximum of heat production. $S_{01}$, 
$S_{02}$, $S_{03}$: initial concentration of glucose in each experiment.](image)

![Fig. 8. Growth of E. coli K12 in aerobic batch culture. 1. 
Heat production; 2. optical density; 3. oxygen partial-
presure in the culture.](image)

Fig. 9. Semi steady state measurements performed at di­
ferent constant flow rates $f$ during the exponential phase of 
growth of E. coli K12 in batch cultures.

### 4. Experimental Results

As has already been pointed out, the set-up was 
tested by employing growth in batch cultures. Three 
thermograms with $f = 101, 243$ and $172$ ml \cdot h$^{-1}$ were 
taken at first. The initial culture conditions remained­
changed in these experiments, the thermo­
grams of which are shown in Fig. 7. Fig. 8 exhibits 
the behaviour of the culture in terms of pO$_2$, op­
tical density and heat production ($f = 172$ ml \cdot h$^{-1}$). 
A further thermogram was taken at $T = 29$ °C.

Total heat evolved and maximum heat flow are 
practically independent of the flow rates tested, as 
can be seen from Table I.

The doubling time of the calorimetric signal and 
optical density expressed as $\mu_{max}$ and $\mu_{max}$, respec­
tively, as well as heat flow, optical density and pO$_2$ 
are strongly correlated in the course of time ac­
cording to Fig. 8. Therefore, it seems justified to 
assume that for $f \approx f_{min} = 101$ ml \cdot h$^{-1}$ and the 
chosen culture conditions the biological processes as 
measured in the culture vessel are identical to those 
recorded in the sample tube of the calorimeter.
Table I. Characterization of culture conditions and thermal behaviour of growth in batch culture for different flow rates through the calorimeter.

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Organic carbon source and initial concentration ( S_0 ) ( [\text{mg} \cdot \text{L}^{-1}] )</th>
<th>Temperature ( T ) ( [°C] )</th>
<th>Maximum dry weight ( \text{[mg} \cdot \text{L}^{-1}] )</th>
<th>( \mu_{\text{max}} ) ( [\text{h}^{-1}] )</th>
<th>( \mu_{\text{o.D.}} ) ( [\text{h}^{-1}] )</th>
<th>Flow rate through calorimeter ( Q_{\text{St/\text{max}}} ) ( [\text{ml} \cdot \text{h}^{-1}] )</th>
<th>Maximum heat flow ( Q_{\text{St/\text{max}}} ) ( [\text{mW} \cdot \text{L}^{-1}] )</th>
<th>Total heat evolved ( Q_{\text{total}} ) ( [\text{kJ} \cdot \text{L}^{-1}] )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GL/636.4</td>
<td>37</td>
<td>229.43a</td>
<td>0.738</td>
<td>0.73</td>
<td>101</td>
<td>599.01</td>
<td>5.033b</td>
</tr>
<tr>
<td>2</td>
<td>GL/636.4</td>
<td>37</td>
<td>229.43a</td>
<td>0.706</td>
<td>0.71</td>
<td>243</td>
<td>600.49</td>
<td>4.825b</td>
</tr>
<tr>
<td>3</td>
<td>GL/636.4</td>
<td>37</td>
<td>229.43a</td>
<td>0.767</td>
<td>0.71</td>
<td>172</td>
<td>626.29</td>
<td>4.955b</td>
</tr>
<tr>
<td>Mean values ( \pm \text{s. D. of experiments} )</td>
<td>( 0.737 \pm 0.031 ) ( (\pm 4.1%) ) ( 0.717 \pm 0.012 ) ( (\pm 1.6%) )</td>
<td>( 608.60 ) ( \pm 15.34 ) ( (\pm 2.5%) )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>S/636.4</td>
<td>37</td>
<td>233.5a</td>
<td>0.496</td>
<td>0.47 ± 0.02a</td>
<td>221 – 469</td>
<td>n. d.</td>
<td>n. d.</td>
</tr>
<tr>
<td>6</td>
<td>GL/636.4</td>
<td>29</td>
<td>219.48</td>
<td>0.338</td>
<td>0.33</td>
<td>215</td>
<td>502.21</td>
<td>n. d.</td>
</tr>
</tbody>
</table>

\( a \) Measured parallel in separate batch cultures.

\( b \) Gravimetrically integrated, integration interval ranges from inoculation to 6.7 h after occurrence of the first heatflow maximum.

\( Q_{\text{St/\text{max}}} = \frac{\dot{Q}_{\text{St}}}{\text{1000}} \cdot \frac{1}{0.6866} \), and both heat fluxes given in mW.

Since it is very time consuming, to produce complete thermograms with this set-up, semi steady state measurements were performed during the exponential phases of two further batches to test the set-up at the remaining, higher flow rates. The results are shown in Fig. 9.

Based on these experimental results a rigorous approach concerning the accuracy of our set-up is given below.

5. Discussion

For flow through calorimeters, even the localization of a JOULE heater within the sample fluid streaming at a constant rate through the sample tube does not guarantee an accuracy “per design and mode of operation” as is the case with properly designed batch calorimeters [18]. In fact, the results of Gustafsson and Lindman [19] indicate, that for flow calorimeters the measured heat effects strongly depend upon the type of measuring tube employed. Hence, theoretical, as well as experimental investigations are necessary to give a reasonable estimate of the accuracy to be expected.

Such an estimate requires measurements of heat effects liberated within the sample fluid by a well defined standard process \( \text{St} \). The known heat effect \( \dot{Q}_\text{St} \) of \( \text{St} \) is to be compared with the corresponding heat effect \( \dot{Q}_\text{St} \) obtained from the electrical calibration. The ratio \( \Gamma = \dot{Q}_\text{St}/\dot{Q}_\text{St} \) represents a measure for the accuracy of the apparatus.

Furthermore, the standard process should be as similar as possible to the actual process \( P \) to be studied, such that \( \Gamma \) remains valid for \( P \). The microbial growth of \( E. \text{coli} \) K12 in batch cultures was used as standard process in order to fulfill the requirement of similarity between \( \text{St} \) and \( P \). Moreover, a growth in batch culture appears to be slowly enough for quasi steady state measurements during the exponential phase of growth.

An oxygen deficiency of sample fluid passing through the tube is avoided by sufficiently low residence times \( \tau' \), oxygen partial pressures near to the saturation level within the culture and carefully chosen microbial densities. Under these conditions, the microbial processes taking place in the sample tube are expected to be almost identical with those in the culture vessel independent of the flow rates employed. This is confirmed by the results of our batch experiments.

Besides the many advantages of batch cultures if taken as standard processes, their main drawback represents the fact, that the absolute values of \( \dot{Q}_\text{St} \) are unknown. This severe problem is solved by
evaluating the exponential time behaviour of \( \hat{Q}_{St} \). To do so, it is assumed that during a certain time interval, \( \hat{Q}_{St} \) exhibits the following form:

\[
\hat{Q}_{St} = A \exp(z t) = G(t), \quad t_1 \leq t \leq t_2.
\]

(1)

As far as the experiments are concerned, Eqn. (1) has been established by numerous experiments performed with microbial batch cultures in batch calorimeters [3, 9, 20–23]. It should be noted, that apart from the restrictions discussed above (\( f \equiv f_{min} \), etc.), and the fact that \( \hat{Q}_{St} \) contains the volume of the sample tube as a scaling factor, \( A \) and \( z \) are independent of the calorimetric apparatus and the conditions (e.g. flow rate) under which St is performed. The experimental results show that the electrically calibrated heat effects \( \hat{Q}^{el}_{St} \) which correspond to \( \hat{Q}_{St} \) have the form:

\[
\hat{Q}^{el}_{St} = B \exp(\beta t), \quad t_1 \leq t \leq t_2
\]

(2)

where \( B \) and \( \beta \) may depend on the flow rate, the calorimetric apparatus etc.

From (1) and (2) the existence of a mapping \( W \) is derived, namely

\[
\hat{Q}^{el}_{St} = B \exp(\beta t) = W(\hat{Q}_{St}) = W(A \exp(z t)) = W(G(t))
\]

(3)

with the property

\[
W(0) = 0.
\]

Differentiation of Eqn. (3) with respect to time yields a differential equation of \( W \) in \( G \), the solution of which expressed in \( \hat{Q}_{St} \) and \( \hat{Q}^{el}_{St} \), reads as

\[
\dot{\hat{Q}}^{el}_{St} = \frac{B}{A^{\beta/2}} \cdot (\hat{Q}_{St})^{\beta/2}.
\]

(4)

From Eqn. (4) it follows that

\[
\alpha = \beta, \quad f_{min} \leq f \equiv f_{max}
\]

(5a)

and

\[
B = \text{const.}, \quad f_{min} \equiv f \equiv f_{max}
\]

(5b)

constitute sufficient conditions for \( \Gamma \) to be constant in the range of the indicated flow rates. To verify these conditions \( \alpha \) and \( \beta \) have to be determined. In the case of a standard process St, the following relation holds for a fixed \( f \):

\[
U_{St} = \frac{\hat{Q}_{St}}{\delta + \gamma \cdot f}
\]

(6)

(for details see appendix).

Hence, the constant \( \alpha \) for St is identical with that computed from the corresponding signal \( \Delta U_{St} \), provided (6) is at least true in a small interval of flow rates containing the flow rate at which St is performed.

To verify (6), \( \hat{Q}_{St} \) is taken to be the maximum heat flow in the 3 batches (1, 2, 3, see Table I). Thus, \( \hat{Q}_{St} \) is unknown but constant so that a plot of the corresponding \( \Delta U_{St}^{el} \) versus \( f \) should yield a straight line.

Fig. 10 shows the reciprocal plot of the maximum recorder deflections that correspond to the maximum calorimetric signals \( \Delta U_{St}/\max \) in the three experiments according to Eqn. (6). From these results it is concluded that Eqn. (6) is experimentally verified and \( z \) amounts to \( \mu_{\max} = 0.74 \pm 0.03 \text{h}^{-1} \). Condition (5a) is immediately verified in the range from 101 to 243 ml·h\(^{-1}\) since due to the linear feature of \( \hat{Q} \), the \( \hat{Q}^{el}_{St} \) values are computed from the corresponding \( \Delta U_{St} \) values simply by multiplying them with a constant factor. Since the initial conditions of the three batches were slightly different, the following arguments have to be used for the verification of (5b): Due to the fact that the maximum heat flows \( \hat{Q}^{el}_{St}/\max \) are independent of the flow rate, it is reasonable to assume that the same is true for all heat flows in the range \( 0 \leq \hat{Q}^{el}_{St} \leq 609 \mu \text{W} \). Thus, if we had had identical initial conditions in all three experiments, all values \( \hat{Q}^{el}_{St} \) of the exponential phases would lie on one straight line if semilog-
rhythmically plotted because of the identical slopes obtained from experiments 1, 2, and 3 and the exactly identical initial heat productions.

If $B$ was not constant, the result would be a set of straight lines parallely shifted but with equal slopes. For the verification of (5a) and (5b) in the range from 221 to 469 ml·h$^{-1}$, the semi steady state measurements (Fig. 9) were evaluated. This type of experiment is characterized by identical initial conditions for all measurements of $\hat{Q}_{st}$.

For each experiment, either with glucose or succinic acid as organic carbon source, the electrically calibrated values $\hat{Q}_{st}$ of the corresponding exponential phase lie on one straight line, the slope of which is identical with $a$, thereby proving (5a) and (5b).

Since St experiments in the range of $0 \leq f \leq 101$ ml·h$^{-1}$ are not possible, the linear behaviour of $\varepsilon$ in the range $0 \leq f \leq 541$ ml·h$^{-1}$ is taken to be sufficient for the conclusion that $I$ is constant from 0 to 101 ml·h$^{-1}$ with unaltered value. Thus, in the range from $0 \leq f \leq 541$ ml·h$^{-1}$ the set-up produces at the most a constant, flow rate independent, absolute error. At $f = 0$ ml·h$^{-1}$ the flow calorimeter equals a batch calorimeter the accuracy of which is primarily dependent on the outer shape of the sample tube used. Since the latter is almost identical with the well established commercial flow mixing tubes [24, 25], the accuracy of our apparatus should be comparable to those equipped with flow mixing tubes, for which Johnsson and Biltonen [24] report an accuracy of better than 2%, and Monk and Wadsö [26] an accuracy of around 1%.

Acknowledgements

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Appendix

Derivation of $\Delta U = \hat{Q}/(\delta + \gamma \cdot f)$

Let the measuring tube be a straight tube of length $l$, internal diameter $d$ and constant internal wall temperature $T_B$ (temperature of the calorimetric block). The steady state heat flow balance for the interior of the tube reads:

$$\hat{Q} = \hat{Q}_{\text{cond}} + \hat{Q}_{\text{conv}}.$$  \(i\)

where $\hat{Q}$ is the heat flow produced by the sample within the tube, $\hat{Q}_{\text{cond}}$ the heat loss due to heat conduction to the calorimetric block and $\hat{Q}_{\text{conv}}$ the heat loss due to convection. In particular, we assume that

$$\hat{Q}_{\text{cond}} = \pi \cdot d \cdot \alpha \int_0^l (T_B - T(x)) \, dx$$  \(ii\)

and

$$\hat{Q}_{\text{conv}} = c f \cdot (T_{\text{in}} - T_{\text{out}})$$  \(iii\)

which implies, that the temperature distribution within the tube only depends upon the scalar coordinate $x$, $0 \leq x \leq 1$, $[T_{\text{in}} = T_{(x=0)}, T_{\text{out}} = T_{(x=1)}]$, with axial and radial heat conduction being neglected. $\alpha$ is the heat transfer coefficient from the surface of the sample fluid to the internal surface wall of the tube, $c$ the constant heat capacity of the fluid and $f$ the constant flow rate. Let furthermore the one dimensional tube be covered with thermoelements of constant density $\varrho_{\text{Em}}$ the "cold" junctions of which are exposed to the temperature $T_B$. The resulting thermovoltage $U$ then reads:

$$U = \int_0^l \varrho_{\text{Em}} \psi (T_B - T(x)) \, dx$$  \(iv\)

with $\psi$ being a combined material and apparatus constant. Combining (ii), (iii) and (iv), leads to:

$$\hat{Q} = \pi \cdot d \cdot \alpha (\varrho_{\text{Em}} \psi)^{-1} U + c f \cdot (T_{\text{in}} - T_{\text{out}})$$

with the unknown temperatures $T_{\text{in}}$ and $T_{\text{out}}$.

For elimination of $T_{\text{in}}$ it is assumed that reference fluid flows through the measuring tube, being identical to the sample fluid, except for the heat effects to be studied ("ideal reference"). This yields:

$$\hat{Q}^R = \pi \cdot d \cdot \alpha (\varrho_{\text{Em}} \psi)^{-1} U^R + c^R f^R \cdot (T_{\text{in}}^R - T_{\text{out}}^R).$$  \(v\)

Immediately thereafter, sample fluid is introduced to flow through the measuring tube, resulting in:

$$\hat{Q}^S = \pi \cdot d \cdot \alpha (\varrho_{\text{Em}} \psi)^{-1} U^S + c^S f^S \cdot (T_{\text{in}}^S - T_{\text{out}}^S).$$  \(vi\)

The condition "ideal reference" is equivalent to

$$c^R = c^S = c \quad \text{and} \quad \hat{Q} = \hat{Q}^S - \hat{Q}^R.$$  \(vii\)

Assuming "ideal operational conditions" leads to:

$$f^R = f^S = f$$  \(viii\)

and "ideal prethermostatisation" of reference and sample leads to:

$$T_{\text{in}}^R = T_{\text{in}}^S = T_{\text{in}}.$$  \(ix\)
The combination of equations (v) to (ix) results in:

\[ AU = U^S - U^R \]

\[ = (\pi \cdot d \cdot \alpha)^{-1} \theta_{Em} \psi (\hat{Q} - c \int \{ T_{out} - T_{out}^S \} ) . \]

To eliminate the unknown temperatures \( T_{out}^S \) it should be noted that in contrast to our initial assumption, the measuring tube consists of a bifilar spiralled gold tube, embedded into a cell body of nearly two dimensional shape. It seems therefore reasonable to assume that the tube is “thermally well mixed” and no mixing occurs of the volume elements which pass through. The condition “thermally well mixed” is equivalent to:

\[ T(x) = T = T_{out} : 0 \leq x \leq 1 . \]

Combining (iv) and (xi) results in:

\[ T_B - T = (\theta_{Em} \cdot \psi \cdot l)^{-1} U . \]

Introducing (xii) into (x) and rearrangement gives the desired result:

\[ \Delta U = \hat{Q} / (\delta + \gamma \cdot f) \] with \( \delta, \gamma \) being

\[ \delta = \pi \cdot d \cdot \alpha \cdot (\theta_{Em} \psi)^{-1} \]

\[ \gamma = (\theta_{Em} \cdot \psi \cdot l)^{-1} c . \]