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Short running title: CFD predicted pH gradients in LAB cultivations

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Abstract

The formation of pH gradients in a 700 L batch fermentation of *Streptococcus thermophilus* was studied using multi-position pH measurements and computational fluid dynamic (CFD) modelling. To this end, a dynamic, kinetic model of *S. thermophilus* and a pH correlation were integrated into a validated one-phase CFD model, and a dynamic CFD simulation was performed. First, the fluid dynamics of the CFD model were validated with NaOH tracer pulse mixing experiments. Mixing experiments and simulations were performed while multiple pH sensors, which were placed vertically at different locations in the bioreactor, captured the response. A mixing time of about 46 s to reach 95 % homogeneity was measured and predicted at an impeller speed of 242 rpm. The CFD simulation of the *S. thermophilus* fermentation captured the experimentally observed pH gradients between a pH of 5.9 and 6.3, which occurred during the exponential growth phase. A pH higher than 7 was predicted in the vicinity of the base solution inlet. Biomass growth, lactic acid production, and substrate consumption matched the experimental observations. Moreover, the biokinetic results obtained from the CFD simulation were similar to a single-compartment simulation, for which a homogeneous distribution of the pH was assumed. This indicates no influence of pH gradients on growth in the studied
bioreactor. This study verified that the pH gradients during a fermentation in the pilot-scale bioreactor could be accurately predicted using a coupled simulation of a biokinetic and a CFD model. In order to support the understanding and optimization of industrial-scale processes, future biokinetic CFD studies need to assess multiple types of environmental gradients, like pH, substrate, and dissolved oxygen, especially at industrial scale.

**Keywords:** Computational fluid dynamics (CFD); transient CFD simulation; dynamic simulation; heterogeneities; pH gradients; lactic acid bacteria (LAB) fermentation

**Introduction**

Heterogeneities of culture parameters like substrate concentrations, pH, and dissolved oxygen concentrations are regarded as mainly responsible for productivity loss in large-scale bioreactor cultivations. Transport limitations occur at large scale due to insufficient mixing, and cells are consequently exposed to fluctuating conditions. Non-limiting substrate concentrations in the range of 0.3 – 2 g L⁻¹ are reported in feeding zones during fed-batch processes, while there are substrate-limited conditions further away from the feeding position (Bylund et al., 1998; Larsson et al., 1996). pH values might also be outside of physiological ranges next to acid or base addition points (Langheimrich and Nienow, 1999; Lara et al., 2006). Mixing times of large-scale bioreactors for microbial cultures exceed 100 s to reach 95 % homogeneity, and the circulation time of the cells, which is proportional to the mixing time, is consequently in the magnitude of 10 s and longer depending on the stirring conditions (Delvigne et al., 2006; Nagata, 1975). Cells might adapt to continuously changing environments while they move through the bioreactor. Biomass and product yield reduction are reported for several different strains and processes when a process is
scaled up to large scale (Bylund et al., 1998; Enfors et al., 2001; George et al., 1998; Xu et al., 1999). This is most likely related to heterogeneities, because microorganisms are exposed to fluctuating environmental conditions at large scale, which might affect the metabolic activity. pH gradients have shown an influence on the transcriptional response and enzyme activity in bacteria, and may therefore lead to decreased biomass growth and product formation as shown in scale-down studies (Amanullah et al., 2001; Cortés et al., 2016; Onyeaka et al., 2003).

Computational fluid dynamic (CFD) modelling is capable of representing the fluid dynamic conditions in bioreactors. It was already applied for process optimization by improving the impeller configuration for an increased oxygen transfer rate (Yang et al., 2012; Zou et al., 2012). Moreover, biokinetic models are coupled with fluid dynamics to analyze environmental gradients during fermentations (Schmalzriedt et al., 2003; Wang et al., 2015). Either compartment models can be built and coupled with a biokinetic model or a biokinetic model is directly integrated into a CFD model. Compartment models, which are based on the knowledge about the fluid dynamics in the bioreactor obtained from CFD models, reduce the number of spatial elements and decrease the computational demand (Vrábel et al., 2001). If biokinetic models are directly integrated into CFD, both the Euler-Euler approach (Bannari et al., 2012; Elqotbi et al., 2013) and the Euler-Lagrange approach combined with a population balance model (Haringa et al., 2016; Lapin et al., 2004; Lapin et al., 2006; Morchain et al., 2013) are commonly applied. The fluid is treated as a continuum in both approaches, but the biological phase is treated as a continuum in the Euler-Euler approach and as a discrete phase in the Euler-Lagrange approach. The latter allows tracking single cells there. So far, studies have mainly been focused on substrate and oxygen gradients in aerobic nutrient-limited fed-batch processes. Furthermore, their...
relevance is questionable because many of the aforementioned works use CFD models that were not experimentally validated e.g. by comparing the model response to mixing experiment data. There is therefore a considerable lack of scientific literature focusing specifically on dynamic CFD simulations of biokinetic models integrated into validated CFD models with the intention to simulate the formation of pH gradients in pilot and large-scale bioreactors.

The objective of this study was to predict the pH gradients, which occur in a 700 L bioreactor during a *Streptococcus thermophilus* fermentation, by coupling CFD and kinetic modelling in a CFD simulation. This tool, which combines fluid dynamics and microbial kinetics, will be used to study pH heterogeneities at pilot scale. To this end, first a one-phase CFD model of a 700 L bioreactor for a *S. thermophilus* fermentation was set up. Tracer pulse experiments with a NaOH solution and multi-position pH monitoring validated the fluid dynamic model predictions of the bioreactor. Then a kinetic model describing the biomass growth, lactic acid synthesis, and lactose consumption of *S. thermophilus* was integrated into the validated CFD model to simulate a pH controlled batch cultivation. An algebraic equation was applied to calculate the pH value based on the lactic acid and ammonia concentrations.

Materials and Methods

Bioreactor geometry and settings

A stirred tank bioreactor (Chemap AG, Switzerland) equipped with three 6-blade Rushton turbines was used (Fig. 1) and filled to a liquid height of \( H_L = 1.92 \) m, corresponding to a volume of 700 L, for both the mixing time experiment and the fermentation. The stirrer speed was 242 rpm (\( P/V = 0.79 \) kW m\(^{-3}\)) for the mixing time.
determination and 132 rpm (P/V = 0.13 kW m⁻³) for the fermentation. The stirrer speed was measured with a testo 477 LED stroboscope (Testo SE & Co. KGaA, Germany). The Reynolds number (Re) was defined as:

\[
Re = \frac{N \cdot D_i^2 \cdot \rho_{H_2O}}{\mu_{H_2O}}
\]

(1)

where \(N\) represents the stirrer speed, \(D_i\) the impeller diameter, \(\rho\) the fluid density, and \(\mu\) the dynamic viscosity of the fluid.

The power input (P) was calculated:

\[
P = N_p \cdot \rho \cdot N^3 \cdot D_i^5
\]

(2)

where \(N_p\) is the power number. \(N_p\) was assumed to be 5.5 (Doran, 1995; Ruston et al., 1950) for each Rushton turbine as \(Re > 10^5\) (see the results section). The power input could unfortunately not be measured in the studied bioreactor.

Mesh generation and simulation settings

The bioreactor geometry was designed in SolidWorks (Dassault Systèmes, France). The sparger ring, the gas inlet pipe, and a supporting structure, which holds the shaft, were omitted. The bioreactor consisted of a stationary tank domain and three rotating impeller domains. Only half of the bioreactor volume was modelled applying a rotational periodicity plane. Meshes with 6-sided hexahedral elements were defined for both domains in ANSYS ICEM CFD 17.1 (ANSYS, Inc., US-PA). The stationary domain consisted of approximately 2,000 mesh elements per liter. Each rotating domain, with a height of \(H_i = 0.075\) m and diameter \(D_i = 0.32\) m, was defined with about 95,000 elements per liter. The complete mesh consisted of 1.6 million nodes. The interface between the rotating and stationary domains was defined as Frozen-
Rotor interfaces. CFD simulations were performed in ANSYS CFX 17.1 with the k-ε turbulence model (Supplementary Material). The top boundary was assumed a flat surface with a free-slip wall. The liquid density was assumed to be $\rho = 997 \, kg \, m^{-3}$ and the dynamic viscosity $\mu = 8.9 \cdot 10^{-4} \, kg \, m^{-1} \, s^{-1}$ in both the tracer pulse and fermentation simulation.

Tracer pulse simulations

An additional variable was specified for the tracer pulse experiments in all domains with a diffusion coefficient $D_{tracer} = 5.17 \cdot 10^{-9} \, m^2 \, s^{-1}$ corresponding to the characteristics of the tracer compound $OH^-$ (Cents et al., 2005). Six monitoring points were located at different positions, which corresponded to the sensor positions (Fig. 1). Vertical positions of the sensors were, with respect to the bottom of the bioreactor: 0.10 m, 0.35 m, 0.60 m, 0.95 m, 1.25 m, and 1.60 m. The horizontal distance to the bioreactor wall was 0.10 m. A transient (time-dependent velocity field) simulation was performed with a physical time scale of a step time of 0.1 s and a root mean square (RMS) residual target of $10^{-4}$. The RMS is a measure to validate the convergence. The tracer pulse was simulated by starting the simulation with 1 mol of the tracer variable in a cylindrical volume with a height of 0.20 m and a width of 0.10 m, which was at the center at the top of the liquid phase.

Tracer pulse experiments

The bioreactor was filled with tap water. A NaOH solution (27 %, Novadan ApS, Denmark) was used as tracer substance for the pulse experiments. When dosing a pulse, 80 mL of NaOH was poured into the liquid at the center of the bioreactor within 1 s, from 0.30 m above the liquid level. Experiments were carried out at 35 °C.
within a pH range of 5.0 to 6.0 to ensure that the mixing time is measured without interference of the slow reverse reaction of the carbonate dissociation (Einsele, 1976). The pH was reduced with H₃PO₄ (75 %, Novadan ApS, Denmark). The pulses were performed in three replicates. Six pH sensors (CPS471D, Endress+Hauser AG, Switzerland) mounted on a lance measured the pH at different positions every second. The positions were equivalent with the monitoring points in the tracer pulse simulation with the exception of the top placed sensor, which failed to record the data (Fig. 1).

Mixing time calculation

Mixing times were calculated after normalizing the pH measurements according to Paul et al. (2003):

\[ pH'_{i,exp}(t) = \frac{pH_{i,exp}(t) - pH_{i,exp}(t=0)}{pH_{i,exp}(t=\infty) - pH_{i,exp}(t=0)} \]  

(2)

where \( pH'_{i,exp} \) is the normalized pH output of the i-th sensor in the experiment, \( pH_{i,exp} \) is the experimental pH value measurement, and \( pH_{i,exp}(t = \infty) \) are the average pH measurements measured between 4.5 and 5 minutes after the pulse. The normalized response of all sensors was plotted with the logarithmic squared deviation with respect to the normalized upper bound 1 in order to determine the mixing time:

\[ log D^2 = log \left[ \frac{1}{n} \cdot \sum_{i=1}^{n} (pH'_{i,exp}(t) - 1)^2 \right] \]  

(3)

where \( n \) is the number of sensors. \( log D^2 = -2.6 \) when 95% homogeneity was achieved, \( log D^2 = -2 \) and \( log D^2 = -1.65 \) at 90% and 85 % homogeneity, respectively. The simulated tracer pulse concentrations were normalized by eq. (2), in which the pH values were replaced by the tracer concentrations.

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Streptococcus thermophilus fermentation and analysis

The batch fermentation of the homolactic \textit{S. thermophilus} strain (provided by Chr. Hansen A/S, Hørsholm, Denmark) was carried out in the aforementioned 700 L stirred tank bioreactor at a stirring speed of 132 rpm, 40 °C, and with N\textsubscript{2} headspace gassing. The pH was controlled by adding 24 % (w/v) ammonia solution (NH\textsubscript{4}OH) through a pipe, which was located 0.1 m above the bottom in the center of the vessel, to maintain pH = 6.0. The pH-value was measured by means of a sensor, which was located 0.3 m above the bottom of the bioreactor close to the reactor wall. The initial pH was 6.8. The medium contained 70 g L\textsuperscript{-1} lactose, 10 g L\textsuperscript{-1} casein hydrolysate, 12 g L\textsuperscript{-1} yeast extract, 11.5 mM K\textsubscript{2}HPO\textsubscript{4}, 36.6 mM sodium acetate, 8.2 mM trisodium citrate, 0.8 mM MgSO\textsubscript{4}, and 0.3 mM MnSO\textsubscript{4}. The pH was monitored every second at 4 of the 6 previously mentioned positions at heights of 0.10 m, 0.60 m, 1.25 m, and 1.60 m. Dry cell weight was determined from centrifuged, washed (with 0.9 % NaCl), and dried (at 70 °C for 24 h) cell broth. Sugars and organic acids were quantified from filtered (0.2 µm) samples in an HPLC system (Dionex UltiMate 3000, Thermo Fisher Scientific, Waltham, MA) and a refractive index detector (ERC RefractoMax 520), with an Aminex® HPX-87H column (Bio-Rad Laboratories, Hercules, CA) using 5 mM H\textsubscript{2}SO\textsubscript{4} at a flow rate of 0.6 mL min\textsuperscript{-1} at 50 °C according to suppliers instructions.

Biokinetic and pH simulation in the CFD model

An unstructured kinetic model of \textit{S. thermophilus}, which described the lactose consumption, biomass growth, and lactic acid synthesis, was integrated into the CFD model (eq. (4-7)). Effects of the lag-time, substrate limitation and inhibition (Haldane, 1930), pH (Schepers et al., 2002), and lactate inhibition (Aghababaie et al., 2015)
were considered in the growth function. A simplified version of the Luedeking-Piret equation (Luedeking and Piret, 1959) was applied to describe the lactic acid synthesis.

\[
(1 + Y_{gal}) \cdot \text{lactose} \overset{q_X}{\rightarrow} \text{biomass} + Y_{gal} \cdot \text{galactose}
\]  

\[
(1 + Y_{gal}) \cdot \text{lactose} \overset{q_P}{\rightarrow} \text{lactic acid} + Y_{gal} \cdot \text{galactose}
\]

\[
q_X = \mu_{max} \cdot \left(1 - e^{-t/t_{lag}}\right) \cdot \frac{C_S}{C_S + K_s + \frac{C_S^2}{K_i}} \cdot e^{-\left(\frac{(pH_{opt} - pH)^2}{a^2}\right)}
\]

\[
\frac{1}{1 + e^{K_{p,La}(C_{p,La} - K_{La})}} \cdot C_X
\]

\[
q_P = \alpha \cdot q_X
\]

where \(q_X\) and \(q_P\) are the volumetric growth and lactic acid production rates, respectively. Lactose (\(C_S\)), biomass (\(C_X\)), and lactic acid (\(C_P\)) were listed as additional variables, and their rate equations were defined as expressions in the CFX expression language. Initial concentrations were \(C_{S,t=0} = 70 \text{ g L}^{-1}, C_{X,t=0} = 0.025 \text{ g L}^{-1}\), and \(C_{P,t=0} = 0 \text{ g L}^{-1}\). The kinetic parameters as listed in Table I were derived from a parameter estimation, which was based on 2 L lab-scale fermentations with the aforementioned medium at 300 rpm (two 6-blade Rushton turbines with a diameter = 53 mm) and 40 °C at different pH values (in the range of 5.5 – 7.0) and initial lactose concentrations (20 and 70 g L\(^{-1}\)) (Spann et al., 2018). It must be considered in the evaluation of the model that the supplemented yeast extract contains ca. 6 g L\(^{-1}\).
carbon, which is not included in the model. However, this is only partially taken up by
the cells and the dynamic model accounts for it by under-predicting the galactose
concentration. The biomass, lactic acid, and lactose concentrations, which are crucial
in this study, are predicted accurately (Spann et al., 2018).

An algebraic linear correlation for the pH calculation based on the lactic acid and
ammonia concentrations was obtained based on experiments performed at 2 L scale
(Supplementary Material):

\[
pH = -0.44 \cdot (C_p - 5.29 \cdot C_{NH_3}) + 7.00 \tag{8}
\]

The dynamic simulation with a time step of 1 s and an RMS residual target of
\(1 \cdot 10^{-5}\) was carried out using a steady state result as initialization state. Continuity,
momentum, and energy equations were derived from a steady state solution, and thus
assumed constant. They were therefore not solved in the dynamic simulation in order
to reduce the computational time. The impeller speed was set to 200 rpm for the
steady state velocity profile in the fermentation simulation in contrast to 132 rpm in
the experimental fermentation. This modification was necessary in order to represent
the mixing behaviour in the fermentation simulation (with a steady state velocity
profile) as the predicted mixing times differed when applying a steady state or
transient velocity profile (see the Results and the Discussion sections for further
details). The pH was controlled by adding ammonia at the same position as in the
experiment. Control was conducted with a P-controller, which was using the step
function:

\[
NH_{3,add} = step(6 - pH) \cdot (6 - pH) \cdot 11900 \text{ g h}^{-1} \tag{9}
\]
where the pH is calculated at the monitoring point 35 cm above the bottom of the bioreactor. The kinetic model was also implemented in MATLAB (The MathWorks, Natick, MA) and solved with the ode 15s solver. There, the fermentation broth was modelled as a single compartment with a homogeneous distribution of the pH and all state variables, i.e. no gradients were considered.

Results

A one-phase CFD model of a 700 L bioreactor for a *S. thermophilus* fermentation was set up and tracer pulse experiments with NaOH and multi-position pH monitoring validated the fluid dynamic model predictions. A kinetic model of *S. thermophilus* was integrated into the validated CFD model in order to predict pH gradients during the fermentation.

The velocity profile of the bioreactor

A steady state solution of the CFD model was initially obtained, which predicted the macroscopic flow profile of the bioreactor. It clearly revealed six recirculation loops, which were generated by the Rushton turbines (Fig. 2). A turbulent flow regime was assumed, because the Reynolds number was $2.2 \times 10^5$ at 242 rpm. The fluid velocity was highest behind the turbine blades, which turned with 2.8 m s$^{-1}$ tip speed at 242 rpm. Low velocities were observed close to the bioreactor wall and especially around the baffles. The steady state solution converged with respect to the RMS values of the velocity components, while the velocities were unstable at the monitoring points. Further analysis revealed that the velocity profile of the bioreactor had a transient (time-dependent) nature (Supplementary Movie 1). The four recirculation loops
between the impellers were changing in size and moving up- and downwards. The tracer pulse simulation was therefore performed with a transient velocity field.

**Tracer pulse simulation and experiments**

Fast radial and slower axial mixing were predicted in the tracer pulse simulations (Fig. 3 and Supplementary Movie 2). It took several seconds until the tracer passed to a subsequent recirculation loop after the simulated injection from the top. The monitoring points in the CFD model and pH sensors in the experiment were positioned in each anticipated recirculation loop in order to be able to follow the dynamic distribution of the tracer.

**The dynamic response at all sensor locations**

The dynamic responses of the monitoring points during tracer pulse simulations were captured with the intention to understand the fluid flow dynamics when e.g. an acid or a base solution is added to regulate the pH value in a cultivation. The two monitoring locations 1 and 2 at the upper part of the liquid phase showed an overshooting response before they reached a stable value, whereas the other monitoring points 3 – 6, which were located farther away from the injection point, responded with sigmoid curves (Fig. 4). In order to validate the tracer pulse simulations, these results were compared with the experimental measurements. Both the shapes and order of magnitude of the dynamic trends obtained from the predictions agreed with the measurements obtained at the different positions. Nevertheless, oscillations of the pH signal and the initial high overshoot of sensor 2 predicted by the simulation were not captured by the measurements.
**Determination of the mixing time**

To assess the progress of reaching homogeneity, the logarithmic squared deviation of all sensors was evaluated. All experimental curves followed the predicted trend until 95 % homogeneity was achieved (Fig. 5). The variance of the replicates increased for homogeneities higher than 95 %. The predicted and measured mixing times at the levels of 85 %, 90 %, and 95 % homogeneity matched very well (Table II). 95 % homogeneity was reached after about 46 seconds.

The dynamic response at all locations and the mixing time prediction gave considerable evidence that the fluid flow in the bioreactor was well described by the applied CFD model.

**Simulated and measured pH gradients in the *S. thermophilus* fermentation**

As a next step, the CFD and biokinetic models were combined in a dynamic simulation in order to predict the pH gradients during the fermentation. Therefore, an unstructured non-segregated kinetic model of *S. thermophilus* and a pH correlation were integrated into the validated CFD model. A dynamic simulation was performed with the purpose of predicting the pH gradients during the batch fermentation. A steady state velocity profile was applied, which decreased the computational demand in contrast to solving the fluidic profile for the entire fermentation time. The mixing time at the fermentation conditions of 132 rpm was 85 s according to a tracer pulse simulation with a transient velocity profile. The impeller speed had to be set to 200 rpm in the fermentation simulation in order to represent the same mixing time with a steady state velocity profile (Supplementary Fig. S1). Expected biomass growth,
substrate consumption, and lactic acid production profiles of a Monod type kinetic model were observed and in accordance with the measurements (Fig. 6). A final biomass concentration of 6 g L\(^{-1}\) was reached after 5 h when 34 g L\(^{-1}\) lactic acid seemed to inhibit growth completely. The observed biomass yield was similar to 2 L lab-scale experiments, where instantaneous mixing was assumed (Supplementary Fig. S2). As the applied time step was crucial in order to solve the differential equations in the CFD model, a time step of 1 s was chosen. An increased time step led to larger deviations of the kinetic profiles (data not shown). The obtained kinetic results from the CFD simulation were very similar to the single-compartment simulation performed in MATLAB, in which a homogeneous distribution of the pH and all state variables was assumed (Supplementary Fig. S3). The pH predictions were in close agreement with the measurements in all locations (Fig. 7). In the beginning of the fermentation, the pH dropped from 6.8 to the controlling pH value 6, when the pH controller started in both the simulation and experiment. A minimum pH of 5.9 was attained in the top zone of the bioreactor during the exponential growth phase. In the bottom zone, where ammonium hydroxide was added, pH values of up to 6.3 were measured and simulated at the sensor positions. Close to the base injection, pH values larger than 7 were predicted (Fig. 8). As the applied pH correlation is only valid up to a pH of 7, a more accurate pH prediction was not possible in this case.

Discussion

In order to simulate the pH gradients of a \textit{S. thermophilus} fermentation in a 700 L bioreactor, a one-phase CFD model was first validated and then coupled with a biokinetic model and a pH correlation. Multi-position pH monitoring in tracer pulse experiments validated the fluid dynamic model predictions of the one-phase CFD...
The CFD model predicted the mixing time of around 46 s to reach 95 % homogeneity at an impeller speed of 242 rpm and forecasted the dynamic response of all sensors in the tracer pulse experiments. The dynamic simulation of the non-aerated *S. thermophilus* batch fermentation predicted both the biokinetic profiles and the pH gradients matching the experimental observations. Rather large pH gradients between pH values of 5.9 and higher than 7 were predicted in the bioreactor while the fermentation was controlled at pH 6.

The simulated flow profiles showed six recirculation loops generated by the three Rushton turbines consistent with literature data (Vrabel et al., 2000). pH sensors and monitoring points were placed so that conditions in all six recirculation loops were monitored in tracer pulse experiments and simulated accordingly. The dynamic pH response of the pH sensors was well represented by the simulated data. It is important to stress that the CFD model relied among other criteria on physical and chemical properties, empirical equations, and the mesh structure. Importantly, no parameter estimation/model calibration of the CFD model was performed in order to fit experimental data. However, the predicted oscillating behavior of the pH and the initial overshoot of sensor 2 was not measured. This can likely be attributed to the response time of the applied ISFET pH sensors, which is in a range of 4 - 8 s to reach ±0.02 of the final pH value in the relevant pH range. This response time was determined in own measurements, and is in accordance with vendor specifications.

Furthermore, there was a discrepancy between the predicted and measured homogeneity when 95 % homogeneity was reached 60 s after the pulse, which could be caused by the fluctuating sensor output (±0.01), while the model asymptotically approaches 100 % homogeneity.
It was shown that the recirculation loops were dynamically changing, and hence a transient velocity profile was required. Dynamic velocity changes that might have caused the dynamic behaviour of the recirculation loops have been already observed for Rushton turbines (Hartmann et al., 2004; Nikiforaki et al., 2003). However, the velocities have not yet been experimentally validated for the studied system.

Both observed and simulated mixing times were consistent with results from Delvigne et al. (2006). They reported similar mixing times between 20 and 53 s to reach 85 % homogeneity in stirred tank bioreactors with a working volumes of 350, 1200, and 1800 L with a comparable power input to the present study. However, as no power input measurements were available for our 700 L bioreactor, the theoretical power input could not be validated in this study. With regard to the definition of mixing time in CFD simulations, Larsson (2015) concluded that there is no consistent definition so far. Instead, there exist several possibilities to determine the mixing time from observing the CFD system at one or several points, up to detecting the concentration on flat surface planes covering a larger area of the CFD system. In contrast to previous studies, which only used one position to calculate the mixing time, six points, which were distributed over the whole liquid phase, were used in this study in order to improve reproducibility and accuracy of the results. Overall, it should be noted that both the experimental setup as well as the way of treating and interpreting the data still lead to uncertainties. For example, the location of the top sensors and their monitoring points affects the accuracy of the measurements and predictions of the overshoot after the tracer pulse. A sensitivity analysis of the sensor locations in the simulation could support the assessment of the accuracy of the model. Up to now, this study has proven that the CFD model achieved a good prediction of the fluid dynamics in the bioreactor.
**Discussion of the results from the combined CFD and biokinetic model**

Since heterogeneities at large scale affect the productivity of many chemical and biochemical processes, a tool to couple fluid dynamics and reaction kinetics is highly demanded. Dynamic simulations of biokinetic models integrated in the fluidic profile simulated by a CFD model can pave the way for enhanced understanding of microbial behavior in large-scale bioreactors. Consequently, it is a basic requirement that the CFD simulation provides accurate results.

To achieve an affordable computational time for the biokinetic CFD simulation, a steady state velocity profile was required. The necessary manipulation of the stirrer speed (to 200 rpm) in the steady state simulation was necessary because of the general transient fluid dynamics in the bioreactor as discussed above. It could also be considered to apply other turbulent models in future. However, a tuning of the CFD model to fit the experimental data should be generally avoided, and the computational development might allow in future using the transient velocity profile for the biokinetic CFD simulation within an acceptable time frame.

Due to the higher computational demand, while solving the differential equations in all nodes of the CFD model mesh (about 1.6 million nodes in this study), discretization errors are likely when selected time steps are inappropriate. The same issue occurs if RMS targets are too high. Applying a time step of 1 s resulted in a similar biological growth as observed in the experiment, while larger time steps led to larger deviations between measurements and predictions. This is most likely due to the accumulation of numerical errors. However, a smaller time step might have reduced numerical errors further, but will also increase computational burden. The similarity between the single-compartment simulation – where completely mixed
conditions were assumed – and the CFD simulation results might be caused by three reasons: (i) either the pH gradients had a very small effect on the culture performance in the present study, or (ii) the biokinetic model was not sensitive to pH changes, or (iii) the small differences arose from the aforementioned numerical errors in the CFD simulation due to the coarse time step.

pH gradients between 5.9 and 6.3 were predicted and observed between the top and the bottom zone of the bioreactor, respectively. A pH higher than 7 was predicted for the vicinity of the base solution inlet. Even though the pH measurements and predictions matched, the uncertainties in the applied pH correlation need to be considered. The fast production of lactic acid led to a small decrease of the pH at the top of the bioreactor, whereas the addition of ammonium hydroxide caused a pulse-wise increase of the pH at the bottom of the bioreactor. Langheinrich and Nienow (1999) reported pH gradients of 0.8 units due to alkali addition in an 8 m³ reactor for mammalian cell cultures. pH gradients have a noticeable effect on the productivity. Aghababaie et al. (2015) reported that the growth of *S. thermophilus* was reduced by 20 % when cultivated 0.3 pH units away from the optimal pH conditions. However, cells are not constantly exposed to unfavorable environmental conditions while moving through a large-scale bioreactor. Cortés et al. (2016) and Amanullah et al. (2001) showed in two-compartment scale-down studies of *E. coli* and *B. subtilis*, in which they mimicked oscillating pH conditions up to a delta pH of 0.9, that growth was not statistically significantly affected. However, the organic acid metabolism changed, and *E. coli* responded on the transcriptional level to the alkaline stress. The extracellular pH affects the intracellular pH of lactic acid bacteria (Cachon et al., 1998; Hansen et al., 2016) and by this the enzymatic activity. *Lactobacillus* sp. maintain their intracellular pH with the energy consuming Na⁺/(K⁺)/H⁺ antiporters.
(Sawatari and Yokota, 2007). The additional energy requirements could lead to altered culture performance in large-scale fermentations. The remaining open question is how fast the cells are affected by pH changes and how fast they adapt to them. In the immediate vicinity of the inlet for base addition, the cells are exposed to unfavorable pH values that might lead to viability loss (Hansen et al., 2016). Cells that are moving through the bioreactor and have suffered in an unfavorable environment before will not function in an optimal manner immediately, when they enter a more favorable zone, as they need to adapt to the new conditions again (Löffler et al., 2016; Nieß et al., 2017). Further studies like Vanrolleghem et al. (2004), who studied and modelled the dynamic response to substrate pulses in wastewater treatment plants, are required to understand the adaptation processes of microorganisms under oscillating conditions better. This knowledge about metabolic phenomena, e.g. the dynamic response of growth to changing substrate availability, could then expand the biokinetic models coupled with CFD models (Delvigne and Noorman, 2017).

Coupling biokinetic and fluid dynamic modelling will open the way for the understanding and optimization of large-scale processes. To predict gradients at large scale is of utmost interest, because measurements during fermentations at large scale are either very difficult or even impossible to perform due to the size of the bioreactors, the costs of a single fermentation run, and the GMP regulations at production sites. Scale-down systems (Oosterhuis, 1984) could be designed based on the CFD predictions, and mimic the gradients in lab-scale experiments (Lara et al., 2006; Neubauer and Junne, 2016). They allow to study the response mechanisms upon external oscillating conditions, and can be integrated in the scale-up process (Neubauer et al., 2013). This will reduce the risk of failure when scaling up processes.
Conclusion

The present study was designed to predict pH gradients in a 700 L lactic acid bacteria fermentation by applying a dynamic CFD simulation. It gave evidence that pH heterogeneities existed in the studied 700 L bioreactor. More importantly, it proved that pH gradients could be quantitatively predicted with the CFD simulation. pH gradients between 5.9 at the top and above 7 close to the alkali inlet at the bottom of the bioreactor were predicted. The high pH in the alkali inlet zone could cause cell damage and an undesired production loss in large-scale bioreactors. Therefore, the results could support fine-tuning of the stirring rate when reaching the maximum growth rate in order to distribute the base faster. In summary, these findings suggest that coupling a biokinetic model and a fluid dynamic model is a very useful tool to predict gradients in bioreactors. However, to predict the effect on microorganisms growing under oscillating conditions was beyond the scope of this study. The validation of the applied CFD model with multi-position pH monitoring during mixing experiments is a promising outcome of this study, which should be performed in further CFD studies of bioprocesses as well. Future work should include multiple environmental gradients in the dynamic CFD simulations. Besides pH, also substrate, oxygen, carbon dioxide, and temperature gradients are of high interest for batch, fed-batch, and continuous cultivations since most of them are regarded to contribute to productivity loss at large scale.

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Figures

Figure 1. The geometry of the stirred tank bioreactor with dimensions in cm. The bioreactor is equipped with three 6-blade Rushton turbines and four baffles. Six monitoring points were positioned in the bioreactor. The reactor was filled up to 1.92 m liquid height both in the pulse experiment to determine the mixing time and in the fermentation.
Figure 2. Steady state solution of the 700 L stirred tank bioreactor for a stirrer speed of 300 rpm. Left: velocity streamlines with velocity in stationary frame. Right: contour plot with the circumferential velocity in stationary frame.
Figure 3. Concentration fields of the tracer during the transient simulation of the pulse in the 700 L stirred tank bioreactor at 242 rpm. The tracer was injected at 0 s at the top of the liquid phase and snapshots are taken at different time points.
Figure 4. Normalized pH response of the five pH sensors (Position 2-6, as shown in Fig. 1) in the pulse experiment and six monitoring points in the transient simulation performed at 242 rpm. Experimental values (symbols) and simulated values (solid lines). The pH showed an overshoot close to the injection point at the top of the bioreactor before it leveled out. The pH increased gradually at the lower positioned sensors and monitoring points.
Figure 5. Logarithmic squared deviation of the pH values in the tracer pulse experiments and simulation considering all monitor points. Three tracer pulse experiments (dotted lines) and the CFD simulation (solid line) at 242 rpm are shown. 95% homogeneity was reached at $\log D^2 = -2.6$. 

![Graph showing logarithmic squared deviation of pH values](image)
Figure 6. Dry cell weight, lactose, and lactic acid concentrations as measured and predicted in the *S. thermophilus* batch fermentation. The fermentation was carried out in the 700 L bioreactor at 132 rpm, 40 °C, and the pH controlled at pH = 6. Dry cell weight (circles) with standard deviation, lactose (squares), lactic acid (triangles), and the CFD simulation result (solid line).
Figure 7. pH values measured and predicted every second at different positions during the *S. thermophilus* fermentation. Fermentation (A) and CFD simulation (B). In the beginning of the fermentation, the pH dropped from 6.8 to the controlling pH value 6, when the pH controller started. The pH was controlled at pH = 6 using the measurement of sensor 5 by adding NH₄OH at the bottom of the bioreactor. pH sensors and monitoring points were placed at position 1, 2, 4, and 6 in the bioreactor as shown in Fig. 1. The pH dropped down to pH = 5.9 in the top zone of the bioreactor, whereas a maximum of around pH = 6.3 was measured and predicted at position 6 in the bottom zone of the bioreactor.
Figure 8. Simulated pH gradients during the *S. thermophilus* fermentation in the 700 L bioreactor after 4 h 40 min of cultivation time. The pH was higher than 7 close to the alkali inlet at the bottom of the bioreactor and around 5.9 in the top zone of the bioreactor. As the applied pH correlation is only valid up to a pH of 7, a more accurate pH prediction was not possible.
Table I. Kinetic parameters of the integrated *S. thermophilus* model.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Value</th>
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<tbody>
<tr>
<td>$K_I$</td>
<td>substrate inhibition parameter</td>
<td>164 g L$^{-1}$</td>
</tr>
<tr>
<td>$K_S$</td>
<td>substrate limitation parameter</td>
<td>0.79 g L$^{-1}$</td>
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<tr>
<td>$K_{La}$</td>
<td>lactate inhibition parameter</td>
<td>21.1 g L$^{-1}$</td>
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<td>$K_{P,La}$</td>
<td>2. lactate inhibition parameter</td>
<td>0.2 L g$^{-1}$</td>
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<td>$K_{P,pH1}$</td>
<td>LA inhibition pH parameter</td>
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</tr>
<tr>
<td>$K_{P,pH2}$</td>
<td>2. LA inhibition pH parameter</td>
<td>7</td>
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<tr>
<td>$pH_{opt}$</td>
<td>optimal pH in the pH function</td>
<td>6.22</td>
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<tr>
<td>$t_{lag}$</td>
<td>lag-time coefficient</td>
<td>0.38 h</td>
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<tr>
<td>$Y_{gal}$</td>
<td>galactose yield</td>
<td>0.63 g g$^{-1}$</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>growth related production coefficient of lactic acid</td>
<td>5.59 g g$^{-1}$</td>
</tr>
<tr>
<td>$\mu_{max}$</td>
<td>maximum specific growth rate</td>
<td>2.16 h$^{-1}$</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>spread parameter in the pH function</td>
<td>1.09</td>
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Table II. Experimental and CFD predicted mixing times for different levels of homogeneity at 242 rpm ($P/V = 0.79 \text{ kW m}^{-3}$).

<table>
<thead>
<tr>
<th>Level of homogeneity</th>
<th>Mixing time for the tracer pulses [s]</th>
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<tr>
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<td>Experiments</td>
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<tr>
<td>85 %</td>
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<td>30</td>
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<td>90 %</td>
<td>32</td>
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<td></td>
<td>36</td>
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<tr>
<td>95 %</td>
<td>42</td>
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