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CMA: integration of fluid dynamics and microbial kinetics in modelling of large-scale fermentations

Peter Vrábel [a,1], Rob G.J.M. van der Lans [a,*], Freija N. van der Schot [a], Karel Ch.A.M. Luyben [a], Bo Xu [b], Sven-Olof Enfors [b]

[a] *Department for Biotechnology, Delft University of Technology, Kluyver Laboratory, Julianalaan 67, 2628 BC Delft, The Netherlands*
[b] *Royal Institute of Technology, Department of Biochemistry and Biotechnology, S-10044 Stockholm, Sweden*

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Abstract

Transport limitation is regarded as one of the major phenomena leading to process yield reduction in large-scale fermentations. Knowledge of both the fluid dynamics and the microbial kinetics is needed for understanding and describing situations in large-scale production bioreactors. Microbial kinetics of *Escherichia coli* including flow metabolism was determined in lab-scale batch and fed-batch experiments. The effect of high substrate fluctuations on metabolism was quantified in scale-down experiments. This knowledge was incorporated into a flow model based on the compartment model approach (CMA). The flow model was verified by mixing time experiments on aerated reactors mixed with multiple impellers at different regimes with liquid volumes 8–22 m^3. The integral model, predicting local glucose, acetate and biomass concentrations in different parts of the reactor, was compared to three large-scale fermentations performed in two different reactors. If lab-scale kinetics was used, the biomass prediction overestimated the biomass concentration. Lab-scale kinetics modified by the results of scale-down experiments incorporating the effect of substrate fluctuations gave a rather satisfying description of biomass concentration. Glucose gradients in different parts of the reactor and acetate produced as a result of overflow metabolism were predicted on a qualitative level. The simulations show that at present the decisive factor for a successful integration of fluid dynamics and microbial kinetics is the kinetics.

Keywords: Fluid dynamics; Microbial kinetics; Compartment model; Multiple impellers; Scale-down

1. Introduction

Comparing fermentations done in large-scale reactors with lab-scale fermentations [1–4], shows that there are some typical differences: (i) the biomass yield is reduced at large-scale; (ii) more metabolic by-products like acetate and formate are observed at large-scale; (iii) limiting substrate (glucose) gradients are present at large-scale as measured at different heights in the bioreactor.

All these phenomena are classified under the term ‘scale-up effect’. Although scale-up is still regarded more as an art than a science [5], transport limitation is considered as one of the major factors responsible for phenomena observed at large-scale. In order to study this scale-up effect in bioprocesses on a quantitative level, models of (i) fluid mixing and (ii) microbial kinetics have to be developed and integrated. Such models must simulate the response of a microbial population to local variations of the environment [6].

The complexity of both model types can be very large. The Navier–Stokes equation of multiphase systems solved by direct numerical simulation (DNS) and on the other hand detailed metabolic flux models would represent the information needed for the integration. However, with respect to knowledge and computer power available at present, there is still a long way to go, to fulfil this goal. Therefore, approximate approaches incorporating the most important mechanisms describing both fluid dynamics and microbial kinetics can create an intermediate but effective way of the goal fulfilment. Combination of CMA and microbial kinetics, including overflow metabolism induced by local high glucose concentration zones, is one example of such an approach.

Integration of complex fluid dynamic models with microbial kinetics is a challenging task for the study of large aerated reactors equipped with multiple impellers. Simple Monod kinetics of sugar uptake was incorporated into CFD calculations of large reactors by Trägårdh et al. [7] and Noorman et al. [8], and this was extended to include also overflow

* Corresponding author. Fax: +31-15-278-2355.
E-mail address: vanderlans@stm.tudelft.nl (R.G.J.M. van der Lans).
[1] Present address: Faculty of Industrial Technologies, The University of Trenčin, 02032 Púchov, Slovakia.

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Nomenclature

A	acetate ($g\,l^{-1}$)
C_B	clearance between bottom impeller and bottom of the reactor (m)
C_I	clearance between impellers (m)
CF	circulation flow rate ($m^3\,s^{-1}$)
D	impeller diameter (m)
EF	exchange flow rate ($m^3\,s^{-1}$)
F	liquid flow rate ($m^3\,s^{-1}$)
g	gravitational acceleration ($m\,s^{-2}$)
H_I	height of the impeller region (m)
H_L	total liquid dispersion height (m)
IF	induced flow ($m^3\,s^{-1}$)
m_L	mass of liquid (kg)
N	stirrer speed (s^{-1})
N_I	number of impellers
O	oxygen ($g\,l^{-1}$)
P	impeller power input in non-aerated system (W)
q	specific uptake rate, or production rate (h^{-1})
S	glucose ($g\,l^{-1}$)
T	reactor diameter (m)
t	mixing time (s)
v	volume (m^3)
v_{Gs}^c	characteristic superficial gas velocity ($m\,s^{-1}$)
X	biomass ($g\,l^{-1}$)
Y	yield ($g\,g^{-1}$)

Greek letters

β	scaling exponent (Eq. (1))
ε	specific power dissipation ($W\,kg^{-1}$)

Subscripts

Feed	feed
G	aerated system (gassed)
Max	maximum
0	initial
Ox	oxidative

kinetics of *Saccharomyces cerevisiae* by Larsson et al. [1] and Hjertager et al. [9]. The differences between experiments and model predictions were mostly ascribed to insufficient verification of the mixing model at large-scale. Moreover, the microbial kinetic models were 'system dependent' [10]. According to our knowledge, an attempt to test one set of kinetic data at different reactor scales has not been reported to date.

Such an integration of a consistent fluid dynamic model based on CMA [11,12] with an overflow microbial kinetic model [3,4] was one of the goals of the European project 'Bioprocess Scale-up Strategy Based on Integration of Microbial Physiology and Fluid Dynamics'. One set of kinetic parameters was incorporated into flow models for different reactor volumes (12–30 m^3). Three large-scale fermentations of *Escherichia coli* were used as a reference. The degree of model complexity sufficient for reasonable integration of fluid dynamics and microbial kinetics will be discussed as well.

Table 1
Reactor configurations and flow regime characteristics

Configuration	A (RT/30 m^3)	B (RT/12 m^3)	C (Scaba/12 m^3)
N_I	4	3	3
T (m)	2.09	1.876	1.876
V_L (m^3)	22	8	8
Bottom impeller (D)	RT (0.33T)	RT (0.41T)	6SRGT (0.56T)
Upper impellers (D)	RT (0.33T)	RT (0.41T)	3SHP (0.61T)
H_L	3.13T	1.59T	1.59T
C_B	0.55T	0.30T	0.30T
C_I	0.70T	0.45T	0.45T
ε_G (W/kg)	1.3	2.4	3.1
t (95%)[a] (s)	165	43	15

[a] Mixing time corresponding to given total specific power consumption as measured for fermentations described in Table 2.

2. Material and methods

2.1. Reactors

Two different reactors were used for the fermentations. Their configurations (A and B) characterised by radial 6-blade Rushton turbines are given in Table 1 and Fig. 1. The third configuration, C, based on a combination of a bottom radial Scaba turbine (6SRGT) and two upper axial pumping Scaba impellers, was used for simulation of fermentation 2. The feeding pipe position varied as presented in Table 2.

2.2. Fermentations

Three large-scale experiments of *E. coli* were done in two reactors of different configurations (Table 2): Two fermentations were done in reactors of configurations A and B (see Table 1) and the third set of results used was obtained from [2] in reactor configuration B. The medium was a common mineral salts/glucose medium [4] and the fermentations were operated as fed-batch processes to assure glucose limitation. Glucose was added at a concentration of 500 or 550 g/l

Table 2
Fermentation characteristics

Fermentation	1	2	3[a]
Reactor	A	B	B
Feeding position	Top	Bottom	Bottom
S_{Feed} (mg/l)	500	500	550
$(F_0 S_{Feed}/V)_{CF_0}$ $g\,l^{-1}\,h^{-1}$[b]	4.4	5.9	–
Biomass reduction[c]	17%	17%	16%
Reference	Fig. 4	Fig. 5	Fig. 6

[a] Ref. [2].
[b] CF_0 denotes beginning of constant feeding.
[c] Reduction of the final biomass concentration in large-scale experiments in comparison to lab-scale experiments [3].

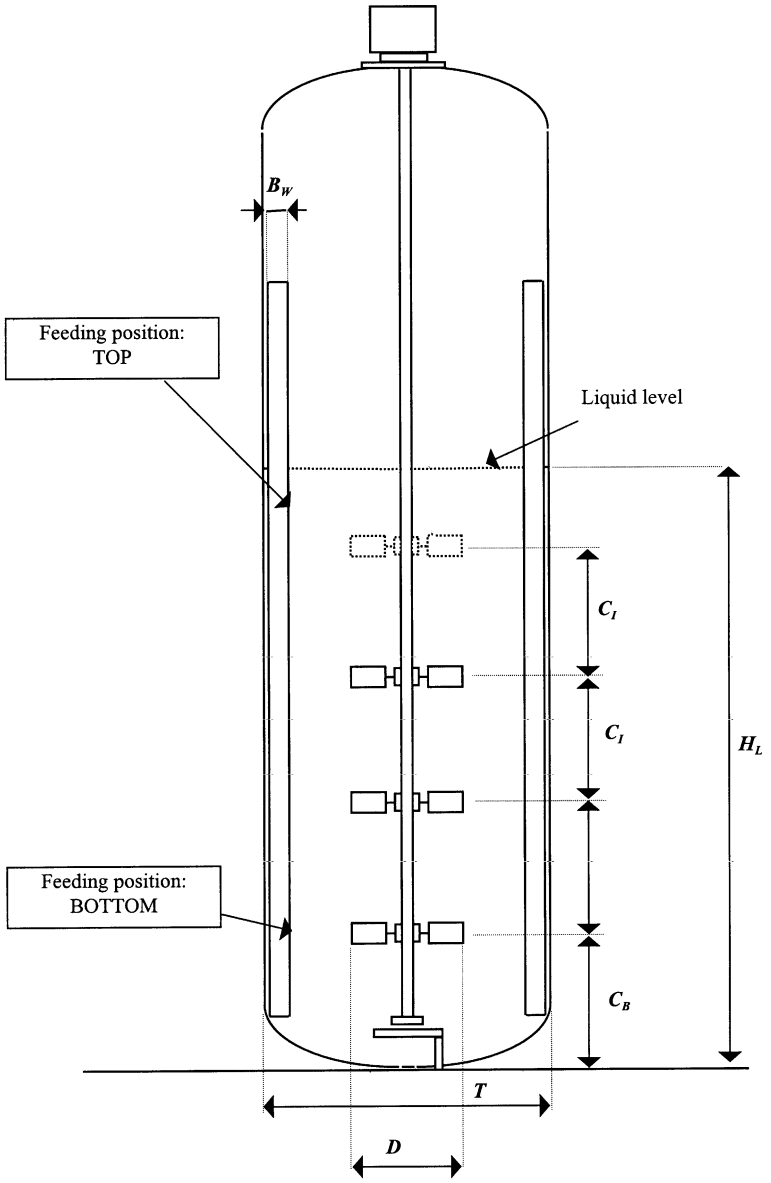

Fig. 1. Fermenter configurations. For explanation see Table 1.

(Table 2) in two phases: (i) using exponential feeding according to Eq. (1) of [3] and (ii) using constant feeding at the level of maximal glucose feeding rate, F_{Max}:

$$F(t) = F_0 \exp(\beta t) \leq F_{\mathrm{Max}} \quad (1)$$

where F is glucose feeding rate, β is an exponential factor representing the specific growth rate [3], its value in all three fermentations being $0.3\,\mathrm{h}^{-1}$. The actual feeding rate of the glucose solution is depicted in Figs. 4A–6A. The concentrations of limiting substrate (glucose), biomass and acetate are depicted in Figs. 4–6. Detailed information on fermentations 1 and 3 was published previously by Xu et al. [4] and Bylund et al. [2], respectively.

In all cases the oxygen transfer capacity was kept at a sufficient level based on the indication of two on-line DOT electrodes. DOT was kept above 20% of air saturation. The overall volumetric oxygen transfer coefficient was approximately $180\,\mathrm{h}^{-1}$ [4]. Temperature was maintained at 35°C and pH was controlled at 7.0 by adding NH_4OH at the top along the wall.

2.3. Power measurement

The electrical power supplied to the agitator motor was measured using a Fluke 41B power harmonics analyser and converted to mechanical power, P, transmitted to the fluid [11]. The total specific power input was calculated by adding the pneumatic power input component, $gv_{\mathrm{Gs}}^{\mathrm{c}}$ [13]. The total specific power contribution to the liquid in an aerated mixed vessel, ε_{G}, is calculated by

$$\varepsilon_G = \frac{P_G}{m_L} + g v_{Gs}^c \quad (2)$$

where P_G is the mechanical power consumption of the impellers under aeration, m_L the mass of liquid and v_{Gs}^c the characteristic (axial averaged) superficial gas velocity. It is calculated from a barometric gas flow rate measured by a mass flow controller.

3. Modelling

Two different model types were integrated to describe the large-scale fermentation: (i) a fluid dynamic model based on the CMA [11,12,14] and (ii) a microbial kinetic model based on Monod kinetics including glucose overflow metabolism [3].

The structure of the compartment model is based on existing knowledge of global flow patterns in vessels with multiple impellers. The model consists of interconnected ideally mixed compartments. Fig. 2 shows an example of a compartment structure of a zone stirred by a radial impeller. Two circulation flow loops created by the stirrer above and below the impeller plane are expressed by compartments connected with three types of flows: circulation flow (CF), axial exchange flow (EF) and flow induced by aeration (IF). Their values are derived from the general knowledge of hydrodynamics. Reactor geometry, impeller characteristics (power and flow numbers) and flow regime characteristics (r.p.m, aeration rate) were sufficient to describe mixing by the model with a relative standard error less than 10%. The model was verified by the pulse response technique at different reactor and impeller configurations up to liquid volumes of 22 m^3 [11,12].

The parameters of the kinetic model of *E. coli* growth and glucose overflow metabolism were proposed previously, based on sets of batch and fed-batch experiments [3]. Fig. 3 shows that the model is rather complex. The model parameter values were adopted from Table 3 in [3]. The kinetic model described satisfactorily the concentrations of biomass and acetate, and the respiration rate in lab-scale fermentations but the biomass was much underestimated when applied to large-scale experiments, in simulations that assumed homogeneity of the reactors.

4. Results

4.1. Experimental observations at large-scale fermentations

4.1.1. Limiting substrate

Glucose concentration differences found between the top and bottom part of the reactor (hereafter called concentration gradients) are a typical consequence of insufficient mixing. According to previous published data, the 95% mixing time of reactor configurations A, B and C at the given conditions was 165, 43 and 15 s, respectively (Table 1) [11,12]. These values correspond to the total specific power consumption (Eq. (2)) characteristic for the final part of the fermentation (Table 1). Therefore, it is not surprising to measure glucose concentration differences of 50 mg/l in fermentation 1 (Fig. 4A), while the gradients in the reactor with better mixing (B) are at the level of 25 mg/l (fermentation 3, Fig. 6A). There were hardly any gradients observed in fermentation 2, except for an increasing tendency at the end of the fermentation (Fig. 5A). The intensity of mixing is not the only factor responsible for local substrate profiles in fed-batch processes. Dilution rate, glucose feed concentration and actual biomass concentration also contribute to the actual glucose level. Gradients of substrate in fermentation 1 at the beginning of constant feeding would be more pronounced, if the same dilution rate would have been used as in fermentation 2 (Table 2). This also could explain the different extent of gradients in fermentations 2 and 3, which were performed in the reactor with identical mixing (configuration B). However, the dilution

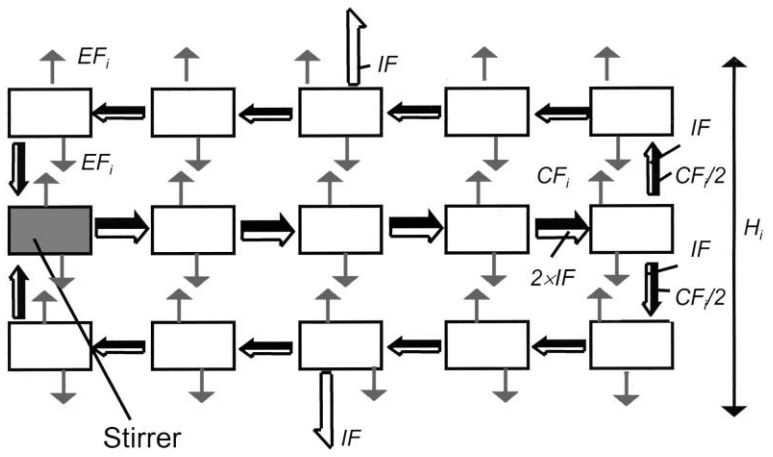

Fig. 2. Compartment structure of the region mixed by a radial impeller.

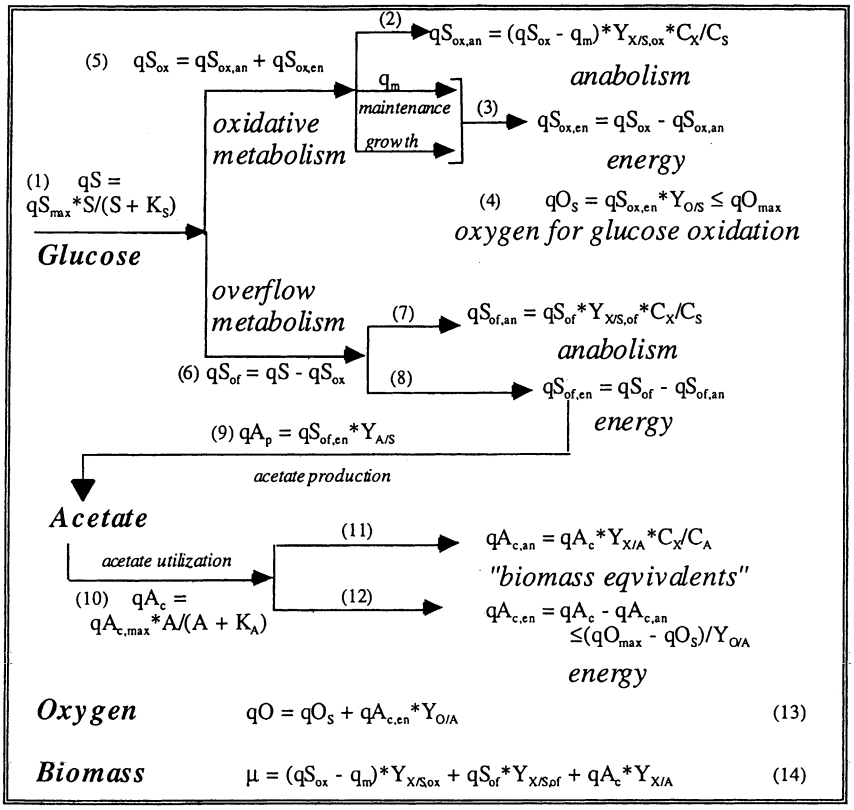

Fig. 3. A kinetic model of aerobic overflow metabolism in *E. coli*. The numbers in brackets correspond to the numbers of equations in [3]. Glucose is taken up according to Monod kinetics (Eq. (1)). At low rate all glucose enters the oxidative metabolism path where part is used for anabolism (Eq. (2)) and the rest for energy metabolism (Eq. (3)), that accounts for the oxygen consumption (Eq. (4)). If this rate at too high glucose concentration exceeds a maximal value (qO_{max}), the surplus of the glucose is metabolised via the overflow metabolism (Eq. (6)), and which is again divided into an anabolic flux (Eq. (7)) and an energy flux (Eq. (8)). From the energy flux the rate of acetate formation is obtained from the stoichiometry (Eq. (9)). Under conditions when acetate is present and the glucose concentration declines so that $qO_S < qO_{max}$, acetate is reassimilated according to Monod kinetics (Eq. (10)) and divided into an anabolic flux (Eq. (11)) and an energy flux (Eq. (12)). The specific rate of growth (Eq. (14)), sugar uptake (Eq. (1)) and net acetate production (Eqs. (9) and (10)) are inserted into corresponding mass balance equations for the simulation.

rate from fermentation 3 was not available [2]. Based on the fact that the feed concentration of glucose in fermentation 3 was 10% higher than in fermentation 2, we might guess that there was more substrate fed in fermentation 3 than 2 (Table 2). As a result, gradients of 25 mg/l were measured.

In case of fermentation 1, large (≈10–100 mg/l) and rapid (≈0.1–0.5 s^{-1}) glucose fluctuations were observed [16] at the top port close to the feeding pipe. The flow structure there was very irregular as it was determined by the pulse-response method [12] and mixing in this part of the reactor is influenced by the existence of large eddies, which might explain the large glucose concentration fluctuations. This is also observed as an increasing scattering of the concentration data at the top of the reactor (Fig. 4A). However, it is not clear why this occurred with an increasing tendency.

During constant feeding, the sugar concentration increased in the process, while the simulations predicted a slow declining concentration. Such increase was observed also in corresponding cultivations on lab-scale processes [4]. This indicates that the kinetics of the sugar uptake is changing. Such an effect can be explained by either an increasing saturation constant, a declining maximum specific uptake rate, a declining concentration of viable cells, or a combination of these. However, the adopted microbial kinetic model [3] did not take this effect into account.

4.1.2. Acetate

Acetate may be produced by *E. coli* under two different conditions: due to overflow metabolism that is induced by glucose concentrations above about 30 mg/l (strain-dependent) or due to mixed acid fermentation that is induced by oxygen limitation. In the latter case equal molar amounts of formate are produced and also some other products [4]. Production of acetate as a product of a glucose overflow metabolism and its reassimilation was characterised previously [3,15]. It was shown that acetate from overflow metabolism is produced by two mechanisms: either due to a too high feed rate that creates a too high glucose concentration in the whole bioreactor or due to insufficient mixing that creates zones of high glucose concentration at the feed inlet. The acetate in the beginning of fermentations 1–3 (Figs. 4B–6B) is caused by high glucose concentration

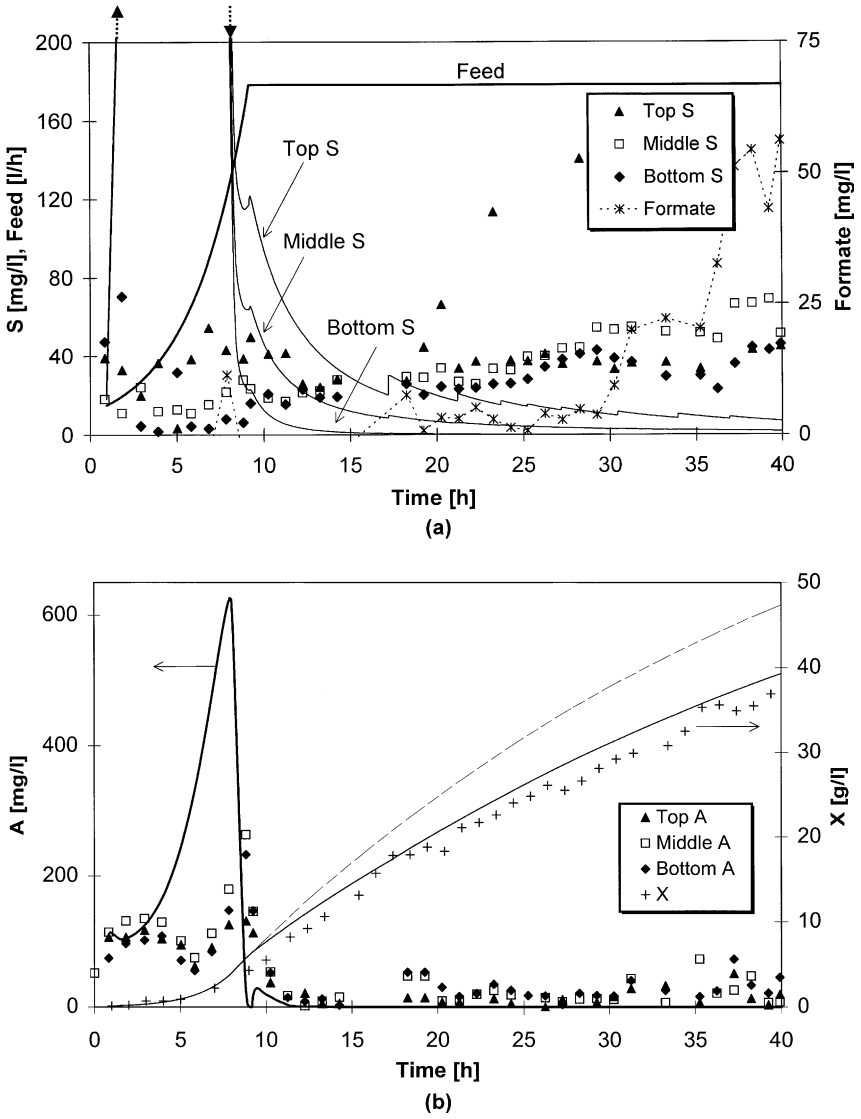

Fig. 4. (A) Glucose concentration and feeding profiles and (B) acetate, formate and biomass concentration profiles during fermentation 1 (reactor configuration A). Symbols depict experiments and solid lines depict simulations by lab-scale-kinetics [3] with $Y_{X/Sox}$ modified by factor 0.76 as a result of scale-down experiments [15]. Dashed line represents simulation of biomass by lab-scale kinetics [3].

in the whole bioreactor (overfeeding) since it was observed also in corresponding lab-scale processes [3]. However, if an acetate peak is observed at large-scale during the later stage of the fermentation, it might be explained either as a consequence of insufficient mixing, or as a consequence of a wrong feeding strategy (overfeeding), or both. In both cases a glucose overflow metabolism appears, but at high cell densities, also oxygen limitation may occur in a zone of high glucose concentration and then acetate is formed by mixed acid fermentation. This was the case in the last part of fermentation 1, during which formate accumulated (Fig. 4A). However, such locally formed acetate is quickly reassimilated in zones with lower glucose concentrations, which limits the total accumulation of acetate. This also means that the total amount of overflow metabolism/mixed acid fermentation may be much more extensive than what is indicated by the acetate analyses [4,15].

4.1.3. Biomass

Biomass yield on glucose was reduced at large-scale compared to lab-scale (Table 2). The degree of large-scale yield reduction is shown in Figs. 4B–6B by comparing the dashed lines (simulation with lab-scale yield coefficient) and the solid lines (simulation with reduced yield coefficient). If the kinetics determined at lab-scale was applied to a large-scale experiment (dashed line), the predicted data overestimated the experimentally measured biomass concentrations. It was hypothesised [4] that this was caused by excretion and reassimilation of overflow metabolism/mixed acid fermentation products.

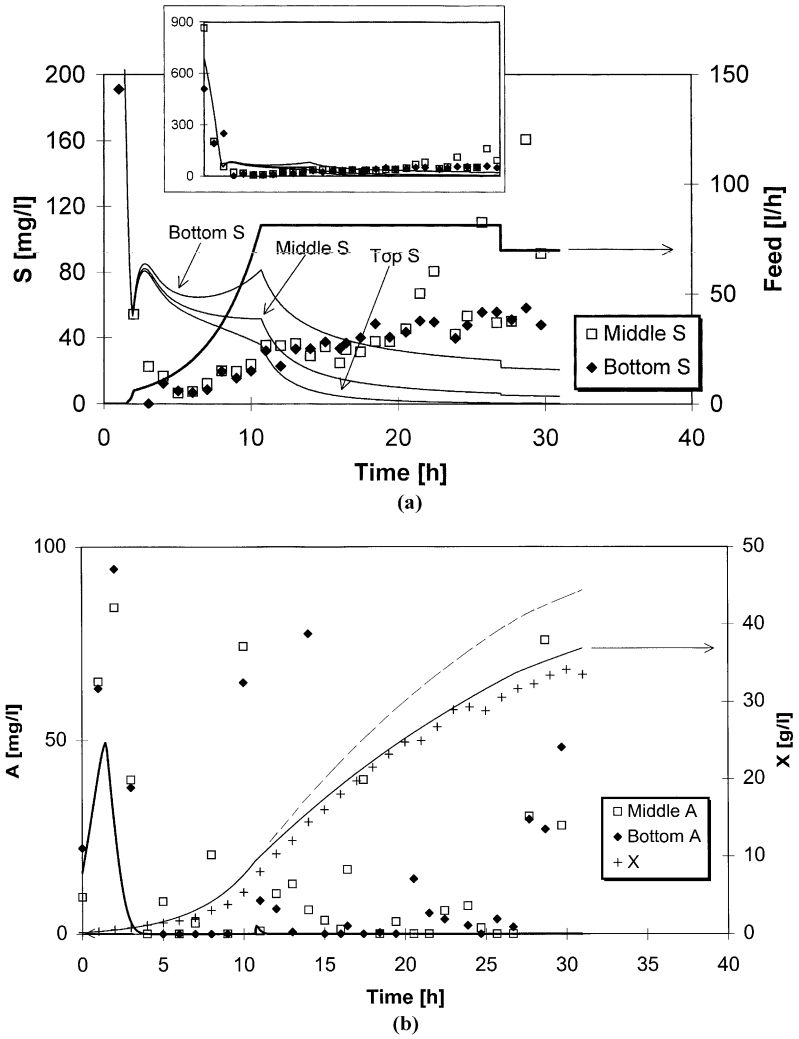

Fig. 5. (A) Glucose concentration and feeding profiles and (B) acetate and biomass concentration profiles during fermentation 2 (reactor configuration B). For explanation of symbols and lines see the legend of Fig. 4.

4.2. Modelling of large-scale experiments

The goal of complex models, integrating flow dynamics and microbial kinetics is to predict concentration profiles in space and time. A set of parameters characterizing microbial kinetics should be obtained a priori from preliminary experiments on a small-scale. Integration of these data into the fluid dynamics flow model should give reasonable predictions at desired scale and geometry.

4.2.1. Lab-scale kinetics

A microbial kinetic model of *E. coli*, based on a set of batch and fed-batch experiments, was published previously [3]. The model response on a sudden increase of substrate level was successfully tested, but only with a very low frequency ($1\,h^{-1}$). Using these kinetic data in a multi-compartment mixing model resulted in a 15–20% overprediction of biomass concentration in all large-scale experiments (Figs. 4B–6B). However, that kind of lab-scale kinetic experiment does not take into account possible high frequency fluctuations typical for large-scale. A micro-organism travelling through a large-scale fed-batch reactor experiences zones of high substrate concentrations in the neighbourhood of the feeding pipe and substrate gradients along the reactor height as well. To obtain kinetic data suitable for large-scale predictions, the design of kinetic experiment should take this aspect into account.

4.2.2. Lab-scale kinetics modified by scale-down experiments

The influence of high frequency oscillations on *E. coli* was investigated by Neubauer et al. [15] using a two-compartment scale-down experiment. A plug flow reactor with a residence time of 112 s was connected to a CSTR reactor in a recycle loop. Substrate was fed to the bottom of the PFR with a constant feed rate to simulate zones with high glucose concentrations. The yield measured during this experiment was compared to a control

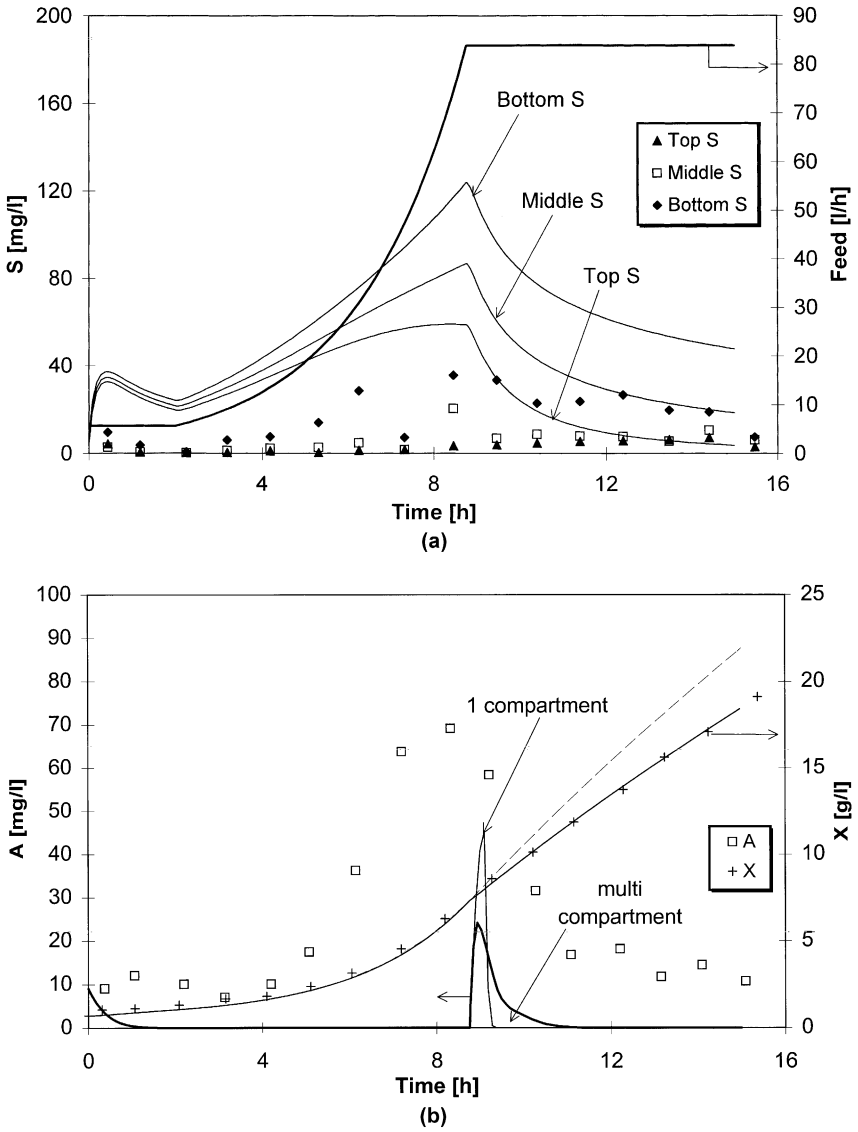

Fig. 6. (A) Glucose concentration and feeding profiles and (B) acetate and biomass concentration profiles during fermentation 3 (reactor configuration B). For explanation of symbols and lines, see the legend of Fig. 4.

experiment (fed-batch stirred reactor). It was found that the biomass yield of the process with substrate oscillations decreased from 0.50 to 0.38. Also the simulation of that experiment by fed-batch kinetics of Xu et al. [3] was successful if the original yield coefficient $Y_{X/Sox}$ (see Fig. 3) was reduced by the ratio 0.38/0.50. Based on these results it was proposed to reduce the yield coefficient in modelling of the large-scale experiments. In our case the reduction factor had a value of 0.76. More scale down experiments simulating substrate oscillations would be necessary to get a more reliable reduction factor. The residence time of the plug flow reactor used by Neubauer et al. [15] was close to the 95% mixing time of the reactor of configuration A.

4.2.3. Large-scale simulations

The approach described above was applied to simulate all three fermentations. One set of the lab-scale kinetic parameters with the biomass yield coefficient reduced by a factor of 0.76 was incorporated into the existing flow model. The experimentally observed reduction might be explained by the fact that in the same time products of mixed acid fermentations (mainly formate) were detected suggesting a change in metabolism (Fig. 4A), which was not incorporated in the kinetic model. The kinetic model was not flexible enough to simulate an observed reduction of the biomass yield. Therefore, the model parameter $Y_{X/Sox}$ was reduced artificially (by the above defined factor of 0.76) at the beginning of the constant feeding to compensate for the change of metabolism. Figs. 4–6 show the comparison of experimental data and simulations by the integrated model in the three large-scale fermentations. The description of biomass is rather satisfying (Figs. 4–6B). Glucose gradients and acetate are predicted on a qualitative level. There was an overprediction of glucose and acetate at the beginning

of fermentation 1 (Fig. 4A). This case demonstrates clearly that not the flow model, but the kinetic model was decisive with respect to the quality of the description. When an ideally mixed reactor was used, the overprediction would occur as well, because substrate prediction of an ideally mixed reactor lies between the substrate concentrations at the top and bottom. The glucose profile was well described during the initial stage of fermentations 2 and 3 (Figs. 5 and 6A).

4.2.4. Overflow metabolism

There is a slight over prediction of glucose in the case of fermentation 3 (Fig. 6A) regarding the level of substrate gradients at different reactor heights. However, the existence of the peaks following from the feeding regime and the gradient extent are predicted rather satisfactorily. The same holds for acetate. At the eighth hour, there is an acetate peak predicted at the level of 25 mg/l corresponding to a measured acetate peak of about 70 mg/l (Fig. 6B). Because both models of an ideal mixer and a non-ideal mixer predict the peak, the primary reason for the occurrence of acetate during this phase of fermentation is glucose overfeeding, not insufficient mixing. The presence of a local glucose maximum supports the previous conclusion, because it coincides with the time of acetate maximum. The acetate peak modelled for an ideal mixer is tall and narrow, while the multi-compartment model shows a small and wide peak. The reason is the non-uniformity of the overfeeding location in different parts of the reactor caused by the difference in the flow model. In fermentation 1, two peaks were simulated. The first as a residuum from accumulated acetate from the batch phase (clearly corresponding to the measured peak), but the second one was a consequence of insufficient mixing, because it was not simulated by a model of an ideally stirred reactor (Fig. 4B). An acetate peak of the same origin was predicted at the level of 5 mg/l at the 11th hour of fermentation 2 (Fig. 5B). However, its amplitude is considerably lower than the measured one. The predicted low level acetate peaks in the first two fermentations, followed from the overflow metabolism in the compartments close to the feeding point as a consequence of insufficient mixing. A model of an ideal mixer cannot simulate these peaks. Moreover, model analysis shows that the secondary acetate peak at the level of 20 mg/l, predicted in fermentation 1 as a consequence of mixing, was a result of a glucose overflow in the neighbourhood of the feeding pipe. Thirty five percent of the reactor volume was involved in acetate production, while the ideal mixer model did not show any overflow metabolism.

4.2.5. Influence of mixing on substrate gradients

It was hypothesised that besides different feeding strategies and actual glucose consumption rates, the main factor responsible for the extent of substrate gradients in a reactor is the quality of mixing. The integrated model can cope with these aspects in a way presented in Figs. 4A–6A. If the beginning of constant feeding is taken as a reference point, then gradients of 120, 40 and 80 mg/l are predicted in fermentations 1–3. This tendency corresponds to the mixing efficiency of each process (mixing time 165, 15 and 43 s, see Tables 1 and 2). If the feeding strategy had been identical, then the extent of substrate gradients in a reactor would be determined mostly by mixing. Therefore, fermentation 2 was simulated in a reactor equipped with a combination of a bottom radial and upper axial pumping up impellers (configuration C in Table 1). The mixing time at this configuration was significantly lower than at configuration B (15 s versus 43 s). The verified flow model adopted from the previous paper [11,12] was used to simulate glucose gradients in the top and at the bottom of the reactor (Fig. 7). Fifty percent reduction of the extent of substrate gradients resulted from the improved mixing. This is another aspect manifesting the usefulness of integration of fluid dynamics and microbial kinetics.

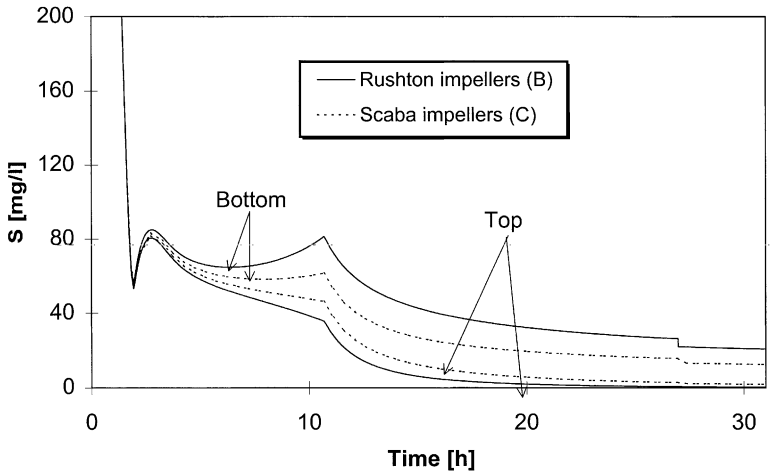

Fig. 7. Comparison of simulated glucose concentration profiles in fermentation 2, if a flow model of Rushton turbines in configuration B (solid line) or scaba impellers at equal power consumption in configuration C (dashed line) was applied. Feeding point was at the bottom of the reactor.

5. Discussion

The modelled process is very complex and both submodels are quite limited in their complexity. The applied kinetic model can treat limited changes in glucose, biomass and acetate concentrations, and was verified at limited substrate feeding strategies and for low frequencies of substrate oscillations. The influence of any other factor present at large-scale and originated by transport limitations or other causes like shear stress, gene stress responses, oxygen, temperature and pH gradients, are not treated by the applied model. The reproducibility of the fermentations was not verified either. Inspite of these facts, we believe that the transport limitations and glucose gradients as their consequence are one of the major factors that should be considered in this kind of fermentation. The integration of a CFM with a microbial kinetic model of an overflow metabolism was shown to be an effective way of handling such a complex phenomenon.

However, simple lab-scale kinetics, which includes only the acetate metabolic pathway, was not flexible enough to describe biomass yield reduction at large-scale. This was shown by simulation with a flow model with a mixing time 10 times larger than the actual mixing time in a 30 m^3 reactor of configuration A. The prediction resulted in a 4% biomass reduction only, while 16% reduction was observed experimentally. The observed metabolites (acetate, formate and lactate) of the mixed acid metabolic pathway were hypothesised to be responsible for the decrease of biomass yield [4]. However, the model did not include any mixed acid metabolism. Furthermore, Schweder et al. [17] showed that glucose gradients may induce a number of stress induced genes. This means that mRNA with very low stability

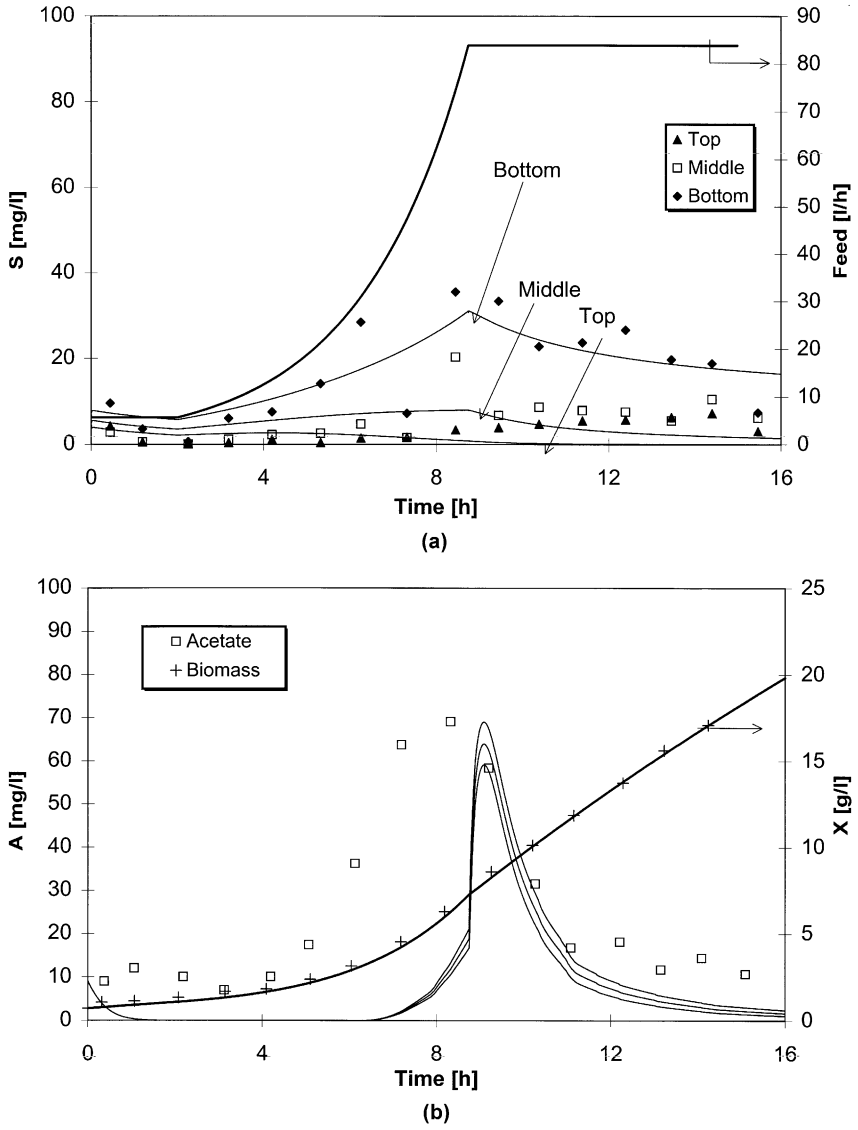

Fig. 8. (A) Glucose concentration and feeding profiles and (B) acetate and biomass concentration profiles during fermentation 3 (reactor configuration B). Solid lines represent an optimised model of Xu et al. [3].

(half-life in the range of 3 min) repeatedly may be synthesised and degraded, which may also contribute to a loss of biomass yield. Thus, the model was not flexible enough to cope with the large-scale reactor conditions; therefore, an empirical reduction of the yield coefficient at the beginning of constant glucose feeding had to be accepted in order to obtain satisfying results. This modification was based on an improved kinetic analysis using a scale-down approach [15].

In the first part of Fig. 4A, the integrated model clearly over-predicted glucose concentration. The question arises if the fluid dynamics part or microbial kinetic part of the joint model is more responsible for this failure. The influence of the fluid dynamic model on the overall calculation can be seen as follows: simulations showed that if a model of an ideally mixed reactor was used, the glucose concentration profile would almost coincide with the concentration profile predicted in the middle port by multi-compartment system (Fig. 4A). The calculation is still far from the experimental measurement. The fluid dynamic model (describing mixing non-homogeneity at the level of 165 s) applied in this calculation showed glucose gradients at the top and bottom of the vessel as symmetrically distributed lines around the concentration predicted by an ideal mixer model. If even worse mixing was introduced, the predicted concentration profile for the top of the reactor would be even further from the measured values of glucose in the top part. On the other hand, a rather satisfying description of this fermentation was achieved with a model of an ideally mixed reactor by Xu et al. [4]. In that case, several parameters of microbial kinetic model were changed in comparison to kinetic parameters obtained from lab-scale [3]. Of course, no substrate gradients were predicted. Both the above mentioned findings imply the conclusion that, not the models of fluid dynamics but the models of microbial kinetics are the decisive factor for successful model integration at the present state of the art. A second aspect supporting this conclusion is the situation in the later stage of the fermentation, where a tendency of increasing glucose concentrations was observed (Figs. 4A and 5A). This phenomenon cannot be explained by any fluid dynamic model, but only by using a more detailed description of microbial kinetics (for example with an increasing saturation constant).

The used set of kinetic data is not the optimal one, because the data were obtained from experiments limited in their nature and amount. However, it is consistent with the existing knowledge of microbial kinetics. The primary goal of this study was to apply one set of kinetic data in connection with different flow models on real fermentation varying in scale and process strategy. Fig. 8 shows a simulation of the third fermentation, where multi-parameter optimisation of the kinetic model was performed (qS_{MAX} and qO_{MAX}, respectively, see Fig. 3). The description of all measured variables is rather good. This may imply the conclusion that if reliable kinetic data are obtained from complex lab-scale or scale-down experiments, this kind of integrated model is flexible enough to describe such a phenomenon as they were observed in a large-scale fermentation.

6. Conclusions

Three large-scale fermentations of *E. coli* were modelled using an integration of a fluid dynamics model based on CMA and a microbial kinetic model including glucose overflow metabolism. One set of kinetic data obtained from a lab-scale reactor and modified by the results of scale-down experiments simulating substrate oscillations was applied to simulate three large-scale fermentations.

Biomass concentrations were predicted rather well. Acetate and glucose were predicted on a qualitative level. The kinetic model did not cope with a peculiar increase of glucose concentrations at the final stage of two fermentations. The integrated model allows for an analysis of undesirable acetate production either as a result of glucose overfeeding or as a result of insufficient mixing.

The decisive factor for a successful integration of fluid dynamics and microbial kinetics at present is the model of microbial kinetics. Its complexity has to be increased to utilise the information provided by existing flow models such as CMA and CFD.

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