REVIEW ARTICLE

Automated biological sulphate reduction: a review on mathematical models, monitoring and bioprocess control

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One sentence summary: Sulphide is the end product of biological sulphate reduction. Novel bioprocesses that use it to precipitate and recover heavy metals from groundwater, wastewaters and process water have been developed in the last two decades. Bioprocess control combining mathematical modelling with process monitoring is instrumental for the further optimisation of these sulphate reduction based bioreactor systems.

ABSTRACT

In the sulphate-reducing process, bioprocess control can be used to regulate the competition between microbial groups, to optimize the input of the electron donor and/or to maximize or minimize the production of sulphide. As shown in this review, modelling and monitoring are important tools in the development and application of a bioprocess control strategy. Pre-eminent literature on modelling, monitoring and control of sulphate-reducing processes is reviewed. This paper firstly reviews existing mathematical models for sulphate reduction, focusing on models for biofilms, microbial competition, inhibition and bioreactor dynamics. Secondly, a summary of process monitoring strategies is presented. Special attention is given to in situ sensors for sulphate, sulphide and electron donor concentrations as well as for biomass activity and composition. Finally, the state of the art of the bioprocess control strategies in biological sulphate reduction processes is overviewed.

Keywords: bioreactor; sulphate-reducing bacteria; wastewater; modelling; sensor; control

INTRODUCTION

Biological sulphate reduction for wastewater treatment

Sulphate and other sulphur compounds are present in fresh water from geological origin or from the release of industrial activities. The production of edible oil, tannery, food processing, fermentation, coal mining and paper/pulp processing are industrial activities that emit elevated concentrations of sulphur compounds (Shin, Oh and Bae 1996). In addition, elevated sulphate concentrations in fresh water bodies can be caused by seawater intrusion. In the absence of oxygen and nitrate, sulphate reduction by sulphate-reducing microorganisms causes an increase in hydrogen sulphide concentration, which is toxic and causes an unpleasant smell and corrosion problems (Sawyer, McCarty...
and Parkin 2003). Hydrogen sulphide is fatally toxic to humans, causing death within 30 minutes at gaseous concentrations of 800–1000 mg L⁻¹, and instant death at higher concentrations (Speece 1996). Therefore, it is important to desulphurize industrial wastewater prior to its discharge to the fresh water bodies. There are several methods for the removal of sulphur compounds from wastewater, including membrane filtration and chemical methods, which are expensive, and require a post-treatment of the brine. For high-strength sulphate-containing wastewaters, biological sulphate removal is a cost-effective alternative (Lens et al. 1998).

Biological sulphate reduction is performed by a group of anaerobic bacteria, called sulphate-reducing bacteria (SRB). These bacteria are classified into two subgroups: autotrophic and heterotrophic SRB. Heterotrophic SRB (HSRB) use organic matter as the substrate, whereas autotrophic SRB (ASRB) use CO₂ as carbon source and H₂ as an electron donor (Liamleam and Annachhatre 2007). Biological, anaerobic reduction of sulphate has been successfully applied for the treatment of sulphate-contaminated wastewater from industries on a larger scale for many years, as it offers the possibility of an efficient treatment with low operation costs using various organic and easily utilizable carbon sources (Liamleam and Annachhatre 2007). The end product is hydrogen sulphide (H₂S). Hence, this technological approach is very suitable for the treatment of waste streams containing dissolved metals. The metals can be precipitated simultaneously with the produced H₂S and removed as stable metal sulphide precipitates (Lewis 2010).

Wastewaters from industries deficient in dissolved organic matter need to be supplemented with electron donors for the SRB. Electron donors most commonly used are ethanol, lactate, formate, methanol, hydrogen, synthesis gas (80% H₂ and 20% CO₂) and CO. The application of the various electron donors for sulphate removal from various types of wastewater has been extensively reviewed (Liamleam and Annachhatre 2007), with ethanol and hydrogen being the most commonly used electron donors in industrial applications. Lactate, in terms of energy and biomass yield, is reported as the best-suited carbon source (Postgate 1984; Koydon 2004), as many species of sulphate reducers can use it (Liamleam and Annachhatre 2007). Acetate is a key intermediate in the breakdown of organic substances in anaerobic processes and has also been used as an electron donor in the sulphate reduction process (Lens et al. 2002). However, acetate is less suitable for high rate sulphate reduction processes, as some species of SRB cannot completely oxidize acetate and acetate utilization becomes the rate-limiting step, even with excess sulphate levels.

Several bioreactor designs have been developed and applied successfully for biological sulphate reduction (Kaksonen and Puhakka 2007). These include batch reactors, sequencing batch reactors, continuously stirred tank reactors, anaerobic contact processes, anaerobic baffled reactors, anaerobic filters, fluidized bed reactors (upflow and downflow), gas-lift reactors, upflow anaerobic sludge blanket reactors, anaerobic hybrid reactors and membrane bioreactors.

Bioreactor modelling for sulphate reduction processes

The performance of bioreactors can be evaluated either experimentally (empirical approach) or by simulation (modelling approach). The former is rather time and resource consuming due to the many experiments that are often needed and could possibly lead to irreversible modification of the biological processes under study. Making new experimental designs based on mathematical model simulations can reduce the number of required experiments to make predictions, whilst improving the effectiveness of the results. Hence, using a modelling approach to predict current and probable future events, whilst reducing the number of experiments, is an attractive way to get insight in the bioprocesses or to design bioreactors. The key problem when addressing bioreactor modelling is to find an appropriate model structure with reliable model parameters.

Modelling bioreactor performance typically starts from a well-established theory of the processes that occur in the bioreactor. The bioreactor model is usually expressed in terms of non-linear differential equations, forcing a very detailed understanding of the processes going on in the bioreactor (Ryhiner, Heinzle and Dunn 1993; Dunn et al. 2005). Modelling is a crucial tool to identify the variables that significantly influence the system response and to give direction when establishing design criteria. In addition, a reactor model helps to identify possible causes for system malfunctioning or failure as well as in devising remedial measures (Kalyuzhnyi et al. 1998). Mathematical models, aimed at simulating the biochemical processes prevailing in the bioreactors, always need to be coupled to experimental studies in order to obtain calibrated and validated models to give decisive answers. Depending on the complexity and goals (see the next section), the validated models can subsequently be used to (1) address laboratory experimental procedures, (2) enhance the design and operation of the treatment systems or (3) optimize the bioreactor process performance (Esposito, Lens and Pirozzi 2009).

Bioprocess control

Validated mathematical models are of great help for the development of advanced bioprocess control. In the sulphate-reducing process, bioprocess control can be used to regulate the competition between microbial groups, to optimize the input of the electron donor and/or to maximize or minimize the production of sulphide. The latter is of great use for heavy metal recovery applications (Veeken et al. 2003). Bioprocess control also facilitates strategies for the management of the biocatalytic environment. In addition, control is necessary to induce the (micro)organisms to produce substances in economically important amounts (Dunn et al. 2005). Anaerobic systems often show instabilities that may be caused either by toxic substances or overloading, which in turn may cause an irreversible collapse of the bioreactor. For this to be overcome, the process needs to be controlled to allow prolonged stable operation. Process optimization is closely linked with control. For example, the objectives of optimal control may be to maximize productivity, final concentration, yield or to minimize effluent concentrations and energy costs (Dunn et al. 2005).

Significant progress has been made with the control of anaerobic systems, mostly methanogenic bioreactors (Pind et al. 2003). However, little research has been reported on the control of sulphate-reducing bioreactors. Mathematical models reviewed in the sections below are the starting point for the development of such control strategies.

MODELS FOR BIOLOGICAL SULPHATE REDUCTION PROCESSES

Mathematical models are important tools in the understanding and optimization of the performance of biotechnological processes. The Monod model is commonly used to describe the kinetics of bacterial metabolism. The Monod model has
been widely accepted, and offers mathematical simplicity. In this model, the bacterial growth rate (μj) is related to the concentration of the limiting substrate (Si):

$$\mu_j = \tilde{\mu}_j \frac{S}{K_{s,i} + S}.$$  \hspace{1cm} (1)

where $\tilde{\mu}_j$ is the maximum specific growth rate for biomass j and $K_{s,i}$ is the affinity constant of biomass j with respect to substrate i.

The Contois model is another very common model to describe microbial cell growth and substrate uptake kinetics. In this model, $K_{s,i}$ is considered to be dependent on the biomass concentration (Xj), thus

$$\mu_j = \tilde{\mu}_j \frac{S}{K_{s,i}X_j + S}.$$  \hspace{1cm} (2)

The maintenance energy requirement to explain the often observed decrease of yield at relatively low growth rates can be described by the Pirt equation:

$$\frac{\nu_j}{X_j} = \mu_j \frac{1}{X_{s,i}} + m_u.$$  \hspace{1cm} (3)

where $\nu_j$ is the substrate utilization rate, $m_u$ is the maintenance coefficient, $X_{s,i}$ is the bacterial yield coefficient and $\mu_j$ is given by equations (1) and (2) or other kinetic models. The specific growth rate models described are used accordingly in the models reviewed below.

Comprehensive mathematical modelling of anaerobic processes is rather complex, as it involves complex dynamics of biological, chemical and physical subsystems with many interconnections between them. This section reviews models for biological sulphate reduction found in the literature. To help guide the readers selecting the most appropriate model, a summary of the main characteristics of the models discussed below can be found in Tables 1-4. Four families of models have been distinguished, describing (a) anaerobic biofilms and granules, (b) microbial competition, (c) inhibition and (d) bioreactor dynamics.

**Anaerobic biofilm and granule models**

Biological sulphate reduction in anaerobic fixed growth reactors has been investigated extensively at lab scale. In particular, it was pointed out that the composition of the microbial community influences the performance and stability of the overall biological sulphate-reducing process (Celis et al. 2008). Modelling biofilms can help to further understand the dynamics of the microbial community, mass transport of substrates and their microbial conversion in the biofilm.

Mass transfer limitation of sulphate in UASB granules was studied theoretically by calculating the steady-state sulphate microprofiles using a reference set of parameters obtained from experimental work (Overmeire, Lens and Verstraete 1994). The model calculations showed that sulphate reduction can be limited in the UASB granules by mass transfer of sulphate into the granule (Fig. 1). The parameters that mostly affected the diffusion of substrate in the granules were the sulphate concentration, the maximum sulphate utilization rate, the granular size and the effective diffusion coefficient. To reach these conclusions, the authors proposed a second-order differential equation that expresses the steady-state mass balance for sulphate
<table>
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<tr>
<th>Goal</th>
<th>Substrate</th>
<th>Sludge feed</th>
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<tr>
<td>Self-oscillating coexistence</td>
<td>Acetate</td>
<td>–</td>
<td>SRB MA</td>
<td>Moser and Monod kinetics</td>
<td>Non-steady state</td>
<td>sulphide and pH</td>
<td>Chemostat</td>
<td>√</td>
<td>√</td>
<td>Vavilin et al. (1994)</td>
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<tr>
<td>Dynamic competition for substrate</td>
<td>Volatile fatty acids</td>
<td>UASB granules</td>
<td>SRB MA</td>
<td>Monod kinetics</td>
<td>Non-steady state</td>
<td>X</td>
<td>Chemostat</td>
<td>√</td>
<td>x</td>
<td>Oyekola, Harrison and van Hille (2012)</td>
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<tr>
<td>Competition for substrate</td>
<td>Lactate</td>
<td>facultative pond treating sewage</td>
<td>SRB LF</td>
<td>Pirt, Monod and Contois kinetics</td>
<td>Non-steady state</td>
<td>X</td>
<td>Chemostat</td>
<td>√</td>
<td>x</td>
<td>Oyekola, Harrison and van Hille (2012)</td>
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</tbody>
</table>
| Coexistence and competition in biofilm | Lactate                    | –           | SRB MA
SRB AC
MA | Monod kinetics | Non-steady state | X | – | – | – | Mattei et al. (2014) |
| Competition for substrate              | Methanol                   | –           | SRB MA           | Monod kinetics | Non-steady state | X | EGSB | √          | x         | Spanjers et al. (2002)       |
| Competition for multiple substrate     | Sucrose, propionate, acetate | Anaerobic granular sludge | SRB MA | Dual substrate Monod kinetics | Steady state | First order kinetic | UASB | √          | √         | Kalyuzhnyi and Fedorovich (1998) |
| Effect of operational conditions on the bacterial competition | H₂ and CO₂ | – | HSRB ASRB HB MA | Monod kinetics | Non-steady state | X | Gas lift | √          | √         | Frunzo et al. (2012)         |

*a calibrated with parameter values obtained from the literature; √: positive; X: negative; –: information not available.
Table 3. Characteristics of selected inhibition models.

<table>
<thead>
<tr>
<th>Processes Involved</th>
<th>Microbial groups</th>
<th>Substrate</th>
<th>Sludge feed</th>
<th>Hydrodynamics</th>
<th>Inhibition</th>
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<tr>
<td>Lactate</td>
<td>Acetate</td>
<td>Lactate</td>
<td>Lactate</td>
<td>Non-steady</td>
<td>Monod kinetics</td>
<td>Non-steady</td>
<td>Polyomial fit</td>
<td>x</td>
<td>Okabe et al. (1995)</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Anaerobic</td>
<td>Ethanol</td>
<td>Ethanol</td>
<td>Non-steady</td>
<td>Monod kinetics</td>
<td>Non-steady</td>
<td>Polyomial fit</td>
<td>x</td>
<td>Moose et al. (1990)</td>
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<tr>
<td>Sulphide inhibition</td>
<td>Desulfovibrio</td>
<td>Sulphide</td>
<td>Sulphide</td>
<td>Non-steady</td>
<td>Monod kinetics</td>
<td>Non-steady</td>
<td>Polyomial fit</td>
<td>x</td>
<td>Ris et al. (1990)</td>
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<tr>
<td>Acetate inhibition</td>
<td>Anaerobic</td>
<td>Acetate</td>
<td>Acetate</td>
<td>Non-steady</td>
<td>Monod kinetics</td>
<td>Non-steady</td>
<td>Polyomial fit</td>
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<td>Desulfovibrio</td>
<td>Sulphide</td>
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<td>x</td>
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<td>Non-steady</td>
<td>Polyomial fit</td>
<td>x</td>
<td>Ris et al. (1990)</td>
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</table>

\[
\frac{1}{L^2} \frac{d}{dr} \left( D_s L^2 \frac{dS_{SO_4}^-}{dr} \right) = \frac{\hat{v}_{SO_4}^-}{k_{a1} + \hat{v}_{SO_4}^-} X_{SRB}
\]

\[
\frac{dS_{SO_4}^-}{dL} = 0
\]

\[
D_s \left( \frac{dS_{SO_4}^-}{dL} \right) = k_1 \left( S_{SO_4}^- - \hat{v}_{SO_4}^- \right)
\]

where \( L \) is the distance normal to the granular surface with \( L = 0 \) the centre of the granule and \( L_R \) the radius; \( D_s \) is the effective diffusion coefficient of the sulphate for transport in the granule; \( \hat{v}_{SO_4}^- \) is the maximum specific sulphate utilization rate (kgSO\(_4\)\(^{-2}\)-kg\(^{-1}\)-VSS\(^{-1}\)); \( k_1 \) is the mass transport coefficient of sulphate in the stagnant liquid film (m\(^2\)-kg\(^{-2}\)-s\(^{-1}\)) \((f\text{-fluid and gr-granule})\).

A mathematical model that incorporates the mechanisms of diffusion mass transport and Monod kinetics, similar to equation (4–6), but now under non-steady-state conditions was developed for an anaerobic fixed biofilm reactor for phenol and sulphate removal (Lin and Lee 2001). The model was validated with data from a pilot-scale column reactor. Batch tests were also conducted with the goal of determining the biokinetic coefficients used in the model. The model predictions agreed well for the non-steady state, but were not so successful under steady-state conditions. Most likely, the authors did not include the effect of higher shear loss in thicker biofilms, which would have resulted in a higher suspended biomass concentration and therefore increasing total COD effluent concentrations. A sensitivity analysis of this process was performed, which showed that operational parameters such as the hydraulic retention time and initial phenol concentration have a strong effect on the process efficiency (Lin and Wu 2011). In addition, the results showed sensitivity to kinetic parameters (yield coefficient of phenol-utilizing bacteria, Monod maximum specific utilization rate of phenol and phenol-utilizing bacteria decay rate) and biofilm parameters (biofilm density of phenol-utilizing bacteria and initial biofilm thickness).

A one-dimensional, multispecies biofilm model, which includes dual-substrate Monod kinetics and which describes the coexistence of denitrifiers and sulphate reducers in a H\(_2\)-fed membrane biofilm reactor is described by Tang et al. (2013). The model was calibrated and validated with experimental chemical and biological data. The authors assumed a steady-state mass balance at any point in the biofilm and used Fick’s law to describe the diffusivity. The model predicted that the onset of sulphate reduction occurred only when the nitrate concentration at the fibre outer surface was low enough, leading to an equal growth rate for denitrifiers and sulphate reducers, i.e. the lower the concentration of nitrate the higher the SRB activity.

The biological, chemical and physical processes occurring in a sulphate-reducing biofilm under dynamic conditions in an anaerobic fixed growth reactor were theoretically evaluated in the model of D’Acunto et al. (2011). A convection-diffusion model was developed. The convection term governs the biofilm...
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<th>Substrate</th>
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<th>Bioreactor</th>
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<td>Bioreactor model with simple biological system structure</td>
<td>Acetic acid, methanol, formic acid</td>
<td>Mixed culture methanogenic and sulphate-reducing reactors</td>
<td>SRB MA</td>
<td>Monod kinetics</td>
<td>Steady-state and batch spike experiments</td>
<td>X</td>
<td>Chemostat</td>
<td>✓</td>
<td>Gupta et al. (1994b)</td>
<td></td>
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<tr>
<td>Define reactor’s volume for given efficiency</td>
<td>H₂ and CO₂</td>
<td>–</td>
<td>1A.HB/HSRB 1B HB/HSRB/MA</td>
<td>Monod kinetics</td>
<td>Steady state X</td>
<td>Gas lift</td>
<td>✓ ✓ ✓*</td>
<td>Esposito, Lens and Pirozzi (2009)</td>
<td></td>
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</tr>
<tr>
<td>Plug flow model</td>
<td>Acetate Propionate Butyrate Hydrogen</td>
<td>–</td>
<td>FE AC MA SRB</td>
<td>Monod kinetics and dual substrate Monod kinetics</td>
<td>Steady state First order kinetics</td>
<td>UASB</td>
<td>✓ ✓ ✓*</td>
<td>Kalyuzhnyi et al. (1998b)</td>
<td></td>
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</tr>
<tr>
<td>Plug flow model</td>
<td>Ethanol UASB granules</td>
<td>SRB SRB¹ AC</td>
<td>Dual substrate Monod kinetics</td>
<td>Steady state X</td>
<td>HAIB</td>
<td>✓ ✓</td>
<td></td>
<td>Rodriguez et al. (2011)</td>
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<tr>
<td>Inclusion of sulphate reduction in AD model</td>
<td>Primary sewage sludge</td>
<td>–</td>
<td>SRB</td>
<td>Monod kinetics</td>
<td>Steady state Sulphide and pH</td>
<td>UASB</td>
<td>✓ ✓</td>
<td>Poinapen and Ekama (2010)</td>
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<tr>
<td>Performance enhancement</td>
<td>Ethanol</td>
<td>Mixed culture of SRB</td>
<td>SRB</td>
<td>Dual substrate Monod kinetics</td>
<td>Non-steady state Non-competitive Liquid-solid fluidized bed</td>
<td>✓ ✓</td>
<td>Nagpal et al. (2000)</td>
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*model 1A validated in the work of Frunzo et al. (2012).
*calibrated with parameter values obtained from the literature; ✓: Positive; X: negative; --: information not available.
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Figure 1. Influence of the bulk sulphate concentration, $S$, (left panel) and the effective diffusion coefficient, $D_e$, (right panel) on steady-state sulphate concentration profiles in a granule (adapted from Overmeire, Lens and Verstraete 1994).

growth and the diffusion term was used for the substrate gradient throughout the biofilm. Three microbial groups were taken into account in this model: complete oxidizing SRB (SRB$^{(c)}$), incomplete oxidizing SRB (SRB$^{(I)}$) and acetate degraders (AcD) and three reaction components were considered (substrates and products): sulphate, lactate and acetate. The model was applied to simulate the effect of different COD/SO$_4^{2-}$ ratios (Fig. 2) and to predict the reactor performance with respect to the volume fraction of bacterial species and substrate diffusion trends in the biofilm as a function of time.

Microbial competition

Sulphate reduction and methanogenesis are both involved in the final step of the degradation of organic matter in anaerobic environments (Fig. 3). Several microbial groups, fermenters (FE), acetogens (AC) and methanogenic archaea (MA) can use the same substrates as SRB, and therefore compete for it. In the anaerobic digestion process, SRB can compete with AC for volatile fatty acids and ethanol or with MA for acetate and hydrogen. Several factors can affect the outcome of this competition: COD/SO$_4^{2-}$ ratio, type of seed sludge, sludge retention time, hydrogen sulphide inhibition, pH and nutrient limitation (Lens et al. 1998). This section overviews several models describing this competition.

Vavilin et al. (1994) simulated anaerobic degradation of organic matter by using a previously developed model that described the self-oscillating coexistence of MA and SRB (Vavilin et al. 1993). They calibrated and validated the model on the experimental data of Parkin et al. (1990), where anaerobic chemostats were maintained at changing acetate/sulphate influent concentrations. The authors concluded that both methanogenesis and sulphate reduction ceased when the COD/SO$_4^{2-}$ ratio was below 10/1. In this model, the Monod function (equation 1) was modified, adding two terms considering the pH and the sulphide concentration as inhibitory factors of the sulphate-reducing process. The model simulations showed that pH and free hydrogen sulphide were the main factors for the system failure. The H$_2$S concentration acts as a trigger stimulating the positive feedback loop between an increase in acetate and sulphate concentrations and a decrease in the pH level through the activity of the SRB and MB. Interestingly, simulations showed that this feedback loop induced an oscillating coexistence between the two microbial groups (Fig. 4). The modified function can be simplified if only one of the inhibitors has an effect on the process. Torner-Morales and Buitrón (2010) considered only pH to cause an inhibitory effect, and thus simplified the model by excluding sulphide inhibition from the equation. After calibration and validation, the model resulted in a predominance of incomplete oxidation of lactate over its complete oxidation.

Fomichev and Vavilin (1997) simplified the model of Vavilin et al. (1994) and created a reduced model of self-oscillating dynamics in an anaerobic system with sulphate reduction. Again, the authors calibrated the model with the experiments of Parkin et al. (1990). This reduced model is based on the competition between two microbial groups, MA and SRB, for the same substrate.
(acetate), product inhibition and pH influence on this inhibition. The authors concluded that, using this model, the oscillating phenomenon is due to hydrogen sulphide inhibition to both MA and SRB biomass growth and the influence of the pH on the equilibrium between ionized and non-ionized sulphide.

Similarly, the competition for acetate was studied by dynamic simulations of acetate utilizing SRB and MA in a UASB reactor treating volatile fatty acids (Omil et al. 1998). The simulations confirmed the long-term competition between the acetotrophs. The main factors affecting the time required for acetate utilizing SRB to outcompete MA were pH (Fig. 5), SRT and the size of the SRB population in the inoculum, under the assumption of a completely mixed high rate anaerobic reactor, with the sludge retention time (SRT) independent from the hydraulic retention time (HRT).

When lactate is used as electron donor, the competition between lactate oxidizers, such as SRB, and lactate fermenters (Fig. 3) must be addressed in order to optimize its dosage. Kinetic properties of these pathways were determined and used to simulate the competition among the microbial species involved in anaerobic lactate degradation (Oyekola, Harrison and van Hille 2012). The model was calibrated and validated on experimental data of laboratory-scale chemostat cultures at different residence times and sulphate concentrations. The kinetic constants for lactate fermentation and lactate oxidation were calculated using the Monod (equation 1), Contois (equation 2) and Chen and Hashimoto kinetic expressions. The model also included the relationship between the kinetics of bacterial growth and lactate utilization rate ($r_L$) described by the Pirt equation (3). On the basis of these simulations, the authors concluded that lactate oxidizers compete more efficiently with lactate fermenters for the carbon source at lactate concentrations below 5 g L$^{-1}$ and sulphide concentrations above 0.5 g L$^{-1}$.

Using a dual-substrate Monod kinetics and previously described numerical method (D’Acunto et al. 2011), a model was developed to assess the microbial coexistence and competition between SRB, SRB, AC and MA growing on lactate (Mattei et al. 2014). The latter was simulated at various COD/SO$_4^{2-}$ ratios and
Figure 5. Effect of pH on the evolution of the SRB and MA population (adapted from Omil et al. 1998).

showed that, although SRB are the most abundant throughout the biofilm for all simulations, the AC seem to occupy a greater area of the biofilm at higher COD/SO$_4^{2-}$ ratios since a higher COD load results in higher lactate concentrations throughout the biofilm thickness.

A simple model that can describe the competition between MA and SRB in a bioreactor fed with methanol was developed and calibrated on experimental data (Spanjers, Weijma and Abusam 2002). The model was based on growth kinetics of hydrogen-consuming MA and SRB and methanol-oxidizing AC. It describes three processes: growth of AC, SRB and MA, and four state variables: the methanol, hydrogen, sulphate and methane concentration. The conversion rates are assumed to be a function of the substrate concentration according to Monod kinetics (equation 1). However, the model did not give a good fit between the simulated and experimental values for the methane production rate. The authors hypothesized that acetate formation from methanol is an important step in the overall process and should have been included in the model.

When the substrate concentration is below a certain value, i.e. threshold substrate concentration, no substrate consumption occurs, even after a long incubation period. The following modification of the Monod kinetic expression (equation 1) that also takes into account the substrate threshold concentration ($S_t$) observed in bacterial growth has been proposed (Ribes, Keesman and Spanjers 2004):

\[
\mu(S) = \mu_{max} \frac{S_t - S}{K_S + S_t - S} f(F),
\]

where $f$ and $F$ are two sigmoid functions. $f$ was added to avoid negative values of the specific growth rate when $S_t$ is below but very close to $S$, and $F$ has still a certain non-zero value. $F$ was added to smoothly lead the Monod function to zero at a certain value of $S_t$ below $S_t$.

This kinetic expression was applied to an anaerobic model (Ribes, Keesman and Spanjers 2004) to simulate the competition for H$_2$ between MA and SRB in a thermophilic methanol-fed bioreactor. Using this model, the mathematical instabilities around the substrate threshold concentration that were observed when using the conventional kinetic model were avoided, and thus more accurate estimations of the biological process behaviour at very low substrate concentrations were obtained.

Wastewaters often contain multiple substrates. Thus, it is important to understand their effect on the competition between SRB and MA. The latter was included in a model of sulphate-fed ideally mixed anaerobic reactors developed by Kalyuzhnyi and Fedorovich (1998). Four substrates were considered: sucrose, propionate, acetate and sulphate. The model was calibrated with data from laboratory studies. Subsequently, it was used to determine the effect of several factors on the outcome of the competition. The model was able to describe the steady-state performance of the reactor and the increase of total COD converted by the SRB relative to that converted by the MA under the different HRTs imposed. The specific growth rates were determined with the same equations of Kalyuzhnyi and Fedorovich (1997). The material balances, however, were slightly different because of the different hydrodynamics. The gas volumetric flow rate ($G$) from the reactor and the mass transfer rate to the gas phase ($M_l$) are given by

\[
G = \sum_i (M_i V_i) V, \quad M_l = k_i a \left( \frac{S^i_t - P}{K_{H_i}} \right),
\]

where $V_g$ is the specific volume of gas; $k_i a$ is the mass transfer coefficient; $P$ is the partial pressure; $K_{H_i}$ is the Henry constant; $*$ stands for undissociated.

The material balance for the influent substrates (sucrose, propionate, acetate and sulphate) can be written as

\[
\frac{d}{dt} S_i = D (S^i_t - S_i) + n_i,
\]

where $D$ is the dilution rate (HRT$^{-1}$).

The behaviour of each bacterial group in the reactor can be described as

\[
\frac{d}{dt} X_j = \mu_j X_j - \frac{(1 - ER_j) X_j}{HRT} - bX_j,
\]

where $b$ is the decay coefficient and ER$_j$ is the efficiency of retention of bacterial group $j$.

Frunzo et al. (2012) presented a mathematical model that was able to simulate the biological, chemical and physical processes prevailing in a biological sulphate reduction gas-lift reactor under dynamic conditions. The model considers the kinetics of microbial growth and decay. In particular, the model takes five groups of bacteria into account, i.e. HSRB, ASRB, homoacetogenic bacteria (HB), MA and AcD, and six components (substrates and products), i.e. H$_2$, SO$_4^{2-}$, CO$_2$, acetate, H$_2$S and inert material. The mass balance equations for both liquid and
gas phases and both substrates and bacterial groups were

\[
\frac{d[S]_i}{dt} = \frac{Q}{V} (\{S\}_i^{in} - \{S\}_i) - k_{a_s} (\{S\}_i - \{S^\ast\}_i) + \sum_{j=1,...,N} \rho_{ij} v_j
\]

where \([S]_i^{in}\) and \([S]_i\) are the molar concentration of the specific gas in the digester influent and the aqueous phase, respectively; \(Q\) is the total effluent gas flow rate; \(V\) is the gas phase volume; \(\beta\) is the thickening ratio in the sedimentation tank; and \(R\) is the ratio between the sludge recycle flow rate and the influent flow rate.

The model was calibrated and validated using an experimental study (Esposito et al. 2003). It adequately simulates the bioconversion processes and predicts properly the effects of the variations of the operational conditions on the bacterial competition in the gas-lift reactor, which can be subsequently used for process optimization and control.

**Inhibition**

In addition to the structure of the microbial community and microbial competition, inhibition may have a strong effect on biochemical processes by decreasing the conversion or growth rate affecting the overall performance. Inhibition can be induced by substrate or product concentrations. Commonly, inhibition increases with an increase in the inhibitor concentration, leading to a gradual decrease in the specific substrate utilization rate. Therefore, it is important to understand the inhibition kinetics.

The sulphide product inhibition of *Desulfovibrio desulfuricans* in batch experiments was modelled (Okabe et al. 1995) using a simplified Monod model (equation 1), assuming \(S_{\text{g}} \gg S_{\text{aq}}\). In the batch experiments, the inhibition coefficient, \(k_{i,k}\) for the maximum specific growth rate was determined to be 251 mgS L\(^{-1}\). Since there was no significant change in lactate utilization rate below 437 mgS L\(^{-1}\) and the only varying parameter was the g of cells produced, the authors were able to use the cell yield (\(\rho_{\text{cell}}\)) to represent inhibition. In the chemostat experiments, at pH 7.0, the cell yield was halved at a sulphide concentration of approximately 250 mgS L\(^{-1}\), which was very close to the \(k_{i,k}\) determined in the batch experiments. Thus, the non-competitive inhibition model adequately described sulphide inhibition of *D. desulfuricans* in the batch experiment. The authors showed that it is crucial to distinguish between sulphide inhibition of cell yield (growth) and the activity (lactate utilization rate). In their study, the calculated maintenance coefficient increased at total sulphide concentrations above 200 mgS L\(^{-1}\), thus leading to a decrease of the cell yield but not affecting lactate consumption.

When high biomass concentrations are present in the bioreactor, one might consider using a modified Contois model (Moosa, Nemati and Harrison 2002):

\[
\mu_j = \left( \hat{\mu}_j \frac{S}{K_{i,j} + S} - b \right) \frac{X}{Y_{X/S}}
\]

The latter was used by Moosa and Harrison (2006) to determine the microbial growth parameters under different pH conditions which altered the sulphide speciation in an acetate-fed mixed population of SRB treating acid mine drainage. Their results showed a constant \(K_s\) for the tested pH values (6.0–7.8). However, the \(\hat{\mu}_j\) was lower when H\(_2\)S was the dominant sulphide species (low pH) than at higher pH where dissociated sulphide species dominated. Thus, the volumetric sulphate reduction rate and specific growth rate of SRB correlate inversely to the concentration of undissociated H\(_2\)S, i.e. at lower pH (<7.0). In fact, the inhibition of the sulphate conversion at pH 7.8 was observed when the sulphide concentrations exceeded 750 mgL\(^{-1}\), which is higher than the value of 250 mgL\(^{-1}\) (at pH 7.0) observed in the work of Okabe et al. (1995). Thus, it is likely that the increase in bacterial activity with increasing pH is due to the lower concentrations of undissociated H\(_2\)S observed at the higher pH values.

Reis et al. (1990) proposed an empirical model, which involves the kinetics of acetate inhibition on lactate-fed SRB in the absence of hydrogen sulphide. The authors concluded that the undissociated acid is inhibitory for the SRB at pH 5.8–7.0. The model gave a good fit to the experimental data and predicted a concentration for the undissociated acetic acid of 54 mgL\(^{-1}\) (\(= K_{AcH}\)) that leads to a 50% inhibition. The same authors extended their work and proposed an empirical model to describe the concomitant inhibition of hydrogen sulphide and acetic acid on lactate-fed SRB (Reis et al. 1992). The inhibitory concentration for hydrogen sulphide for the specific growth rate of SRB obtained from this equation was 547 mgL\(^{-1}\). The inhibition kinetics of hydrogen sulphide were described mathematically using a non-competitive inhibition model similar to equation (14). The study also showed that when the undissociated acetic acid concentration is high, the effect of hydrogen sulphide is no longer relevant. On the other hand, when the sulphide concentration is high, an increase in acetic acid concentration leads to a significant decrease of the specific growth rate of SRB.

Biological sulphate reduction is an increasing popular method for the treatment of acid mine drainage and wastewaters from metal processing, mining and petrochemical industries which contain high concentrations of both heavy metals and sulphate. Thus, it is important to also study the inhibition of sulphate reducers by heavy metals. A mathematical model for cadmium removal by precipitation with biogenic sulphides produced by a single bacterium species, *D. alaskensis* GSR, was developed taking into account the inhibition of hydrogen sulphide and Cd\(^{2+}\) on bacterial growth (López-Pérez et al. 2013). A modified Levenspiel inhibition model was used and a high correlation (0.99) was obtained between simulation and experimental results. It predicted inhibitory effects of Cd\(^{2+}\) for microbial growth at concentrations above 190 mgL\(^{-1}\):

\[
\frac{dX}{dt} = v_i \left( 1 - \frac{H_2S}{K_{i,H_2S}} \right)^{\frac{S_{\text{SO}_4}}{S_{\text{SO}_4} + S_{\text{SO}_4}^{\ast}}} \left[ \frac{S_{\text{Cd}^{2+}}}{k_{i,\text{Cd}^{2+}} + S_{\text{Cd}^{2+}}} \right]^{\frac{S_{\text{Cd}^{2+}}}{k_{i,\text{Cd}^{2+}} + S_{\text{Cd}^{2+}}}^{\ast}} \times X_{\text{Lactate}}^{\ast} - bX_{\text{Lactate}}^{\ast}
\]

where \(k_{i,\text{Cd}^{2+}}\) is the inhibition constant for Cd\(^{2+}\), \(\alpha\) is the exponential term for Luong model, \(\eta\) is the exponential term for Moser model and \(\epsilon\) is the exponential term for the lactate concentration.

Gonzalez-Silva et al. (2009) studied the inhibition of the specific substrate utilization rate of ethanol-fed anaerobic granular sludge by iron, cadmium and sulphide using batch tests. For this purpose, the authors used the Monod equation (1) to
determine the kinetic parameters ($k_1$ and $K_i$) and fitted the Andrews-Noack (Andrews 1968) non-competitive inhibition model (equation 16) to calculate the inhibition constant, $K_i$. At pH 6.2–6.6, the IC$_{50}$, total sulphide and IC$_{50}$,H$_2$S were 397 and 291 mgS L$^{-1}$, respectively. These results again support the importance to take into account the chemical speciation of H$_2$S. For Fe$^{2+}$ and Cd$^{2+}$, the inhibition occurred at concentrations above 467 and 60 mg L$^{-1}$, respectively. The Andrews-Noack non-competitive inhibition model for substrate inhibition can be considered as a multiplicative Monod model (Andrews 1968):

$$\mu_j = \frac{\mu_j S}{(K_{S,j} + S_i) K_{I,j}} I_i \delta_K, \quad \text{(16)}$$

where $I_i$ is the inhibitor concentration.

Most wastewaters contain nitrate in addition to sulphate. It is thus important to understand how to optimize the process of simultaneous or sequential reduction of both terminal electron acceptors. To understand the effects of one another, Xu et al. (2014) developed a model to simulate the co-reduction of nitrate and sulphate. For this model, the authors used Monod kinetics (equation 1) incorporated with a competitive inhibition modifier to predict the effects of the anions on the nitrate and sulphate reduction rates. Although the authors verified simultaneous removal of nitrate and sulphate, the sulphate reduction rate was retarded with 56% in the presence of nitrate.

**Bioreactor dynamics**

The models described so far aimed at predicting or stimulating the microbial performance. Modelling can also be used as a tool to develop scaling-up criteria, i.e. if the substrate removal is established as the model input, design models give the reactor size as an output. This is of great importance when designing a full-scale reactor. In this section, such models are discussed based on the distinction between (i) continuous stirred tank and (ii) plug flow reactors.

**Continuous stirred tank reactors**

Gupta et al. (1994b) developed the first design model for sulphate-fed anaerobic reactors. The model described the complex chemistry involved in anaerobic digestion of organic matter incorporating the various buffer systems, acid-base and liquid–gas equilibria (carbon dioxide, hydrogen sulphide, ammonia, methane, nitrogen and water vapour), ionic interactions and metal precipitation. The overall mass balance equations of the various components include liquid as well as gas phase concentrations in order to accurately predict the effluent gas production rate and composition. The model was calibrated and validated with previous experiments where three different substrates were used (acetic acid, methanol and formic acid) and operating under two different conditions (methanogenic and sulphate reducing). Iron was added to precipitate the sulphide produced (Gupta et al. 1994a).

The model was able to predict the reactor performance fairly well for both steady-state and batch experiments under ideally mixed conditions (Fig. 6). The general mass balance for a specific component in a CSTR is given by

$$\text{net rate of accumulation} = \text{rate in} - \text{rate out} \pm \text{rate of reaction}. \quad \text{(17)}$$

The mass balance equation for the biomass assuming constant volume of the reactor’s liquid was also incorporated into the model to calculate the amount of substrate converted into biomass. This equation is needed to close the substrate mass balance

$$V \frac{dX_i}{dt} = Q(X_{i,in} - X_i) + V(Y_{i,ol} + X_i - bX_i). \quad \text{(18)}$$

where $V$ is the liquid phase volume and $Q$ is the influent flow rate.

A more complex structure of the biological subsystem for the description of the dynamic and steady-state behaviour of an anaerobic digester for the treatment of high-strength sulphate wastewaters was developed by Knobel and Lewis (2002). The model applicable for a number of carbon sources (both simple and complex) and for different microbial groups accounted for inhibition by pH, sulphide, hydrogen and fatty acids and was valid for a number of reactor types. A first-order model was used to describe the hydrolysis rate, and the Monod model (equation 1) was used for the specific biomass growth rate. Competitive, non-competitive and uncompetitive inhibition models were taken into consideration for unionized fatty acids or sulphide. The inhibitory effects of a too high or too low pH were also accounted for.

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**Figure 6.** Comparison of the model simulation with results from the batch spike experiments with acetate (A) and methanol (B) as electron donor. In A: filled circles—experimental, dashes—model simulation and in B: open squares—experimental methanol, double dashes—model simulation methanol; filled circles—experimental methane, dashes—model simulation methane (adapted from Gupta et al. 1994b).
The model was shown to be capable of predicting a number of different scenarios, including the time-dependent sulphate and COD concentrations in molasses-fed packed bed and UASB reactors (Fig. 7). Also the dynamic sulphate conversion rate in a gas-lift reactor fed with hydrogen and carbon dioxide was well fitted. The calibration of the model was done with data from the literature.

The Anaerobic Digestion Model No.1 (ADM1) (Batstone et al. 2002) is a structured model that includes disintegration and hydrolysis, acidogenesis, acetogenesis and methanogenesis as the steps in anaerobic biodegradation. Additional blocks describing the sulphate reduction processes were later included by Fedorovich, Lens and Kalyuzhnyi (2003). The revised model was applied to describe a long-term experiment on sulphate reduction with volatile fatty acids as the substrate in an upflow anaerobic sludge bed reactor and was able to predict the outcome of the competition among AC, MA and SRB for these substrates. The model was validated on an experimental study which considered different operating regimes of granular sludge bed reactors with effluent recycle. The kinetics of sulphate reduction processes were introduced following the principles of ADM1 taking into account both the electron donor $S_e$ (organic substrate or hydrogen) and electron acceptor $S_r$ (SO$_4^{2-}$) concentration in an extended Monod model:

$$
\mu_j = \frac{\hat{\mu}_j S_r}{K_{S_r} + S_r} \exp \left[ - \frac{[H_2S]}{0.60056K_{H_2S}} \right] \left( \frac{S_{SO_4^{2-}}}{K_{S_o} + S_{SO_4^{2-}}} \right).
$$

Similarly, Poinapen and Ekama (2010) extended an anaerobic digestion model (Sötemann et al. 2005) by adding the biological, chemical and physical processes associated to biological sulphate reduction. For this purpose, the authors considered Monod kinetics with concomitant inhibition by undissociated H$_2$S and pH. A more stable inhibition function (equation 20) was used instead of a first-order inhibition function. The model was successfully validated with experimental results obtained from UASB reactors fed with several COD/SO$_4^{2-}$ ratios at different temperatures.

$$
\mu_j = \hat{\mu}_j S \frac{F \left( \text{pH} \right)}{K_i + S} \exp \left[ - \frac{[H_2S]}{0.60056K_{H_2S}} \right] \left( \frac{S_{SO_4^{2-}}}{K_{S_o} + S_{SO_4^{2-}}} \right).
$$

A model for sulphate reduction in a liquid–solid fluidized bed reactor was developed by Nagpal et al. (2000) with the aim to identify the limiting factors and design modifications, allowing enhancement of SRB growth. The model was calibrated and validated and showed a good match between the simulation results and experimental data using ethanol as electron donor. Two substrates (sulphate and ethanol) and two products (acetate and sulphide) were considered as well as the inhibition of the SRB growth rate by ethanol and acetic acid. The model took into consideration both the biomass attached to the beads and the biomass in the liquid phase. The model suggested that a significant increase in the sulphate reduction capacity of the system is possible by increasing the volume of the bed relative to the total liquid volume of the fluidized bed reactor.

Three steady-state mathematical models for the design of H$_2$/CO$_2$-fed gas-lift reactors, aiming at sulphate reduction, were developed by Esposito, Lens and Pirozzi (2009). The proposed models gave the reactor volume required for an assigned sulphate removal efficiency. The simulations performed showed that the size of these reactors highly depends on the number and type of trophic groups present in the sludge. Thus, knowledge on the microbial groups present is crucial to obtain the required volume properly. Their model 1A takes into account two groups of heterogenic bacteria (HB and HSRB), three substrates (H$_2$, SO$_4^{2-}$ and acetate) and two products (acetate and sulphide). Model 1B considers the same assumptions as Model 1A, but it is based on the hypothesis that besides HSRB and HB, also MA may grow on H$_2$/CO$_2$ with CH$_4$ as the end product, whereas acetoclastic methanogens do not grow in the reactor. Model 2 is based on the hypothesis that ASRB is the dominant microbial group in the reactor and considers the same assumptions as the previous models. Thus, model 2 takes into account one group of bacteria (ASRB), two substrates (H$_2$ and SO$_4^{2-}$) and one product (H$_2$S). The steady-state design model 1B (Esposito, Lens and Pirozzi 2009) was validated in the work of Frunzo et al. (2012) presented in the previous section ‘Microbial Competition’.

**Plug-flow reactors**

In plug flow reactor configurations with axial dispersion and reactions, the spatial distribution of any component $N$ in the liquid phase can be written by the following equation:

$$
\frac{\partial}{\partial z} N(z, t) = \frac{\partial}{\partial z} \left[ D_N(z, t) \frac{\partial}{\partial z} N(z, t) \right] - \frac{\partial}{\partial z} \left[ W(z, t) N(z, t) \right] + q(z, t) - M(z, t)
$$
with the first term on the right-hand side of the equation characterizing the degree of mixing by gas induced dispersion; \( D_c \) represents the axial dispersion coefficient, the second term of the equation determining a convective part of mass transfer in the flow direction; \( W \) represents the superficial velocity, the third and fourth elements represent the net biological production/consumption rate and transfer rate from the liquid to gas phase for the component \( N \), respectively.

The behaviour of a bacterial group in a plug flow system can be described as

\[
\frac{\partial}{\partial t} X_j (z, t) = \frac{\partial}{\partial z} \left[ D_{a,j} (z, t) \frac{\partial}{\partial z} X_j (z, t) \right] - \frac{\partial}{\partial z} \left[ W_j (z, t) X_j (z, t) \right] + (\mu_j - b) X_j (z, t). \tag{22}
\]

The first attempt to develop a model for the concentration gradients on substrates, intermediates, products and bacteria in sulphate-fed UASB reactors was undertaken by Kalyuzhnyi and Fedorovich (1997). The approach was generalized, which resulted in the development of the dispersed plug flow model of sulphate-fed UASB reactors (Kalyuzhnyi et al. 1998). The model was calibrated and validated with experimental studies of UASB reactors with acetate, propionate and sucrose as COD source. It adequately described the experimental data on the functioning of UASB reactors both during the start up with almost non-sulphate adapted seed sludge and during the stage when mature granular sulphidogenic sludge had been formed. It includes fermenters, AC, SRB and MA. Thus, the model could be used for maximization of the sulphide yield and model-based process control. The model includes four blocks: (1) kinetics, described by a non-competitive inhibition model; (2) physico-chemical parameters; (3) hydrodynamics; and (4) mass balances for gas, soluble substrates and bacterial groups.

A model similar to Kalyuzhnyi et al. (1998) (equation 22) composed by eight partial differential equations using single- and dual-substrate Monod-type kinetics for biomass growth rate was developed to simulate the processes in a horizontal-flow anaerobic immobilized biomass (HAIB) bioreactor (Rodriguez et al. 2011). It considered that the concentrations of substrates and products were subjected to both the plug flow hydrodynamics and metabolic reactions. The model comprised AC, SRB\(^{(c)}\) and SRB\(^{(s)}\) as microbial groups and ethanol as the initial carbon source and presented good agreement with the data.

**Kinetic parameters**

Not only the choice of a suitable mathematical model structure is important but also the choice of the kinetic parameters to be used in any mathematical model is of crucial importance to obtain accurate and valid results. Values of kinetic parameters are initially determined experimentally and can be better calibrated to obtain the best fit (Keisman 2011). This calibration can be done using several performance measures (Janssen and Heuberger 1995) which aim at finding the best value that minimizes the difference between experimental and simulated data. Tables 5–8 overview the kinetic values used in the previously described models. It should be noted that the coefficient of variation is significantly high for each group of kinetic parameters used in all models reported, even for the same substrate. This is probably due to a high variability in the experimental conditions under which the parameters were estimated. Thus, careful attention must be given when choosing the parameter values to be used.

**Evaluation on models for biological sulphate reduction processes**

Several models for biological sulphate reduction have been developed in the last few years with different objectives, such as understanding biofilm dynamics, microbial competition and inhibition as well as design of bioreactors (Tables 1–4). In particular, these models are of great importance to understand and test hypotheses of the processes taking place in biofilms on microscale, the population dynamics, to optimize operational performance and to design bioreactor controllers. Despite the great complexity of anaerobic sulphate-reducing processes, the feasibility to describe their essential characteristics and dynamics seems evident as already done in the models of Table 4. Processes such as substrate degradation and accumulation, microbial interaction and growth can have great impact on process control systems (see the section ‘Control of biological sulphide production’). Thus, more knowledge and information is required to fully understand such processes in order to accomplish more complete and accurate models. See for instance Klok et al. (2012, 2013) for the introduction of physiologically based kinetic models for bacterial sulphide oxidation. The models developed are very specific in their nature, and thus not simple to be adapted to a full-scale application. Thus, it is advisable to develop a more generic model, such as the ADM, comprising the different processes encountered in previous sections for sulphate reduction bioreactors so that the gained knowledge can be easily transferred to others.

The choice of model is directly dependent on the defined goals and underlying processes. If several substrates are used and thus microbial competition is expected, one might consider using a similar approach to Fedorovich, Lens and Kalyuzhnyi (2003) or Poinapen and Ekama (2010). When several microbial groups and limited substrates are present, it is advisable to use models similar to Esposito, Lens and Pirozzi (2009) or Frunzo et al. (2012). Inhibition can sometimes play a big role in such systems and if so, metal inhibition (Gonzalez-Silva et al. 2009), acetate and/or sulphide (Reis et al. 1992; Moosa and Harrison 2006) should be included in the model.

On the other hand, if the model is to be used in a control system (see the section ‘Control of anaerobic sulphate reduction processes’), for more or less time-invariant processes, i.e. dynamic systems with constant rate coefficients, then it is advisable to reduce its complexity to a minimum so that it is able to simulate and predict the response of the bioreactor to different events on a short time scale. It is important to note, that in general, the more variables to control the more complex the model should be as a result of the interactions between the variables. In such cases, it is common to make gross simplifying assumptions, which may be eliminated or improved as knowledge increases. Critical judgement must be used in order to minimize the errors associated to these simplifications. Therefore, the theoretical assumptions, choice of model parameters and accuracy of the numerical solution method are crucial to obtain valid models (Dunn et al. 2005). Moreover, sensitivity analysis and cross-validation techniques, as in Keisman (2011), will help to find invalid assumptions and incorrect descriptions of subprocesses. In addition, one might consider using ANN or other types of black box models (see the section Adaptive control of biological sulphate reduction) which require less prior information on structure and interaction between variables when compared to mechanistic models.
Table 5. Kinetic parameters for SRB with acetate as the substrate.

<table>
<thead>
<tr>
<th>Microbial groups</th>
<th>Substrate</th>
<th>$K_{S,SO_4^2^-}$ (g SO$_4^{2-}$/L)</th>
<th>$K_{s,i}$ (g L$^{-1}$)</th>
<th>$Y_{XS}$ (g biomass g$^{-1}$ substrate)</th>
<th>$K_{v,i}$ (g L$^{-1}$)</th>
<th>$\mu$ (d$^{-1}$)</th>
<th>$\nu$ (d$^{-1}$)</th>
<th>$b$ (d$^{-1}$)</th>
<th>Kinetics</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRB</td>
<td>Acetate</td>
<td>0.012</td>
<td>0.13</td>
<td></td>
<td></td>
<td>0.64</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Moser and Monod</td>
</tr>
<tr>
<td>SRB</td>
<td>Acetate</td>
<td>0.00084</td>
<td>0.05 ± 0.015</td>
<td></td>
<td></td>
<td>0.36$^a$</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Monod</td>
</tr>
<tr>
<td>SRB</td>
<td>Acetate</td>
<td>0.13</td>
<td>–</td>
<td></td>
<td>$K_{H,LS} = 0.0336/0.07$</td>
<td>–</td>
<td>8</td>
<td>0.03</td>
<td>–</td>
<td>Single Monod</td>
</tr>
<tr>
<td>SRB</td>
<td>Acetate</td>
<td>0.033</td>
<td>0.055</td>
<td></td>
<td>0.612</td>
<td>–</td>
<td>0.0275</td>
<td>–</td>
<td>–</td>
<td>Single and dual Monod</td>
</tr>
<tr>
<td>SRB</td>
<td>Acetate</td>
<td>0.0192</td>
<td>0.024</td>
<td>$K_{H,LS} = 0.285$</td>
<td>0.51</td>
<td>–</td>
<td>0.025</td>
<td>–</td>
<td>–</td>
<td>Dual Monod</td>
</tr>
<tr>
<td>SRB</td>
<td>Acetate</td>
<td>0.0096</td>
<td>0.22</td>
<td>$K_{H,LS} = 0.26554$</td>
<td>–</td>
<td>0.24282</td>
<td>0.015</td>
<td>–</td>
<td>–</td>
<td>Dual Monod</td>
</tr>
<tr>
<td>SRB</td>
<td>Acetate</td>
<td>–</td>
<td>0.131</td>
<td></td>
<td>1.416</td>
<td>–</td>
<td>0.816</td>
<td>–</td>
<td>–</td>
<td>Modified Contois</td>
</tr>
<tr>
<td>HSRB</td>
<td>Acetate</td>
<td>0.00045</td>
<td>0.015</td>
<td></td>
<td>4.9</td>
<td>–</td>
<td>0.04</td>
<td>–</td>
<td>–</td>
<td>Dual Monod</td>
</tr>
<tr>
<td>SRB</td>
<td>Acetate</td>
<td>0.01069</td>
<td>0.00975</td>
<td></td>
<td>9.89$^b$</td>
<td>0.0158</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Dual Monod</td>
</tr>
<tr>
<td>SRB(C)</td>
<td>Acetate</td>
<td>0.51</td>
<td>0.61</td>
<td></td>
<td>0.025</td>
<td>–</td>
<td>0.022</td>
<td>–</td>
<td>–</td>
<td>Dual Monod</td>
</tr>
<tr>
<td>Cv</td>
<td></td>
<td>1.881</td>
<td>1.444</td>
<td></td>
<td>0.556</td>
<td>1.343</td>
<td>2.254</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

$^a$expressed as g L$^{-1}$ d$^{-1}$; $^b$expressed as g g$^{-1}$ VSS d$^{-1}$; Cv: coefficient of variation; minimum and maximum in italic.
### Table 6. Kinetic parameters for SRB with lactate as the substrate.

<table>
<thead>
<tr>
<th>Microbial groups</th>
<th>Substrate (i)</th>
<th>$K_{S,SO_4}$ (g SO$_4^{2-}$/L$^{-1}$)</th>
<th>$K_{S,i}$ (g L$^{-1}$)</th>
<th>$Y_{X/S}$ (g biomass/g i substrate)</th>
<th>$K_{LX}$ (g L$^{-1}$)</th>
<th>$\mu$ (d$^{-1}$)</th>
<th>$v$ (d$^{-1}$)</th>
<th>$b$ (d$^{-1}$)</th>
<th>Kinetics</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRB</td>
<td>Lactate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Polynomial fit</td>
<td>Reis et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>Lactate</td>
<td>–</td>
<td>–</td>
<td>0.1489 (SO$_4^{2-}$)</td>
<td>$K_A = 0.054$</td>
<td>3.288</td>
<td>–</td>
<td>–</td>
<td>Monod</td>
<td>Reis et al. (1992)</td>
</tr>
<tr>
<td>Desulfovibrio desulfuricans</td>
<td>Lactate</td>
<td>–</td>
<td>0.00235</td>
<td>0.036</td>
<td>$K_{H,S} = 0.251$</td>
<td>7.92</td>
<td>–</td>
<td>–</td>
<td>Single Monod</td>
<td>Okabe et al. (1995)</td>
</tr>
<tr>
<td>SRBc</td>
<td>Lactate</td>
<td>0.0036</td>
<td>0.1427</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Dual Monod</td>
<td>Torner-Morales and Buitrón (2010)</td>
</tr>
<tr>
<td>SRB</td>
<td>Lactate</td>
<td>0.00045</td>
<td>0.015</td>
<td>0.12</td>
<td>–</td>
<td>4.9</td>
<td>–</td>
<td>0.04</td>
<td>Single and dual Monod</td>
<td>D’Acunto et al. (2011)</td>
</tr>
<tr>
<td>SRBc</td>
<td>Lactate</td>
<td>0.00045</td>
<td>0.015</td>
<td>0.12</td>
<td>–</td>
<td>4.9</td>
<td>–</td>
<td>0.04</td>
<td>Dual Monod</td>
<td>Mattei et al. (2014)</td>
</tr>
<tr>
<td>SRBI</td>
<td>Lactate</td>
<td>–</td>
<td>0.60</td>
<td>–</td>
<td>–</td>
<td>4.8</td>
<td>–</td>
<td>–</td>
<td>Contois</td>
<td>Oyekola, Harrison and van Hille (2012)</td>
</tr>
<tr>
<td>Desulfovibrio desulfuricans</td>
<td>Lactate</td>
<td>–</td>
<td>18.5</td>
<td>0.57</td>
<td>$K_{H,S} = 0.680 \pm 0.1$</td>
<td>3.12</td>
<td>–</td>
<td>0.192</td>
<td>Levenspiel inhibition model</td>
<td>López-Pérez et al. (2013)</td>
</tr>
<tr>
<td>Desulfovibrio alaskenis 6SR</td>
<td>Lactate</td>
<td>0.93</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.352$^a$</td>
<td>–</td>
<td>–</td>
<td>Single Monod</td>
<td>Xu et al. (2014)</td>
</tr>
<tr>
<td>Mixed microbial biomass</td>
<td>Lactate</td>
<td>0.93</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.352$^a$</td>
<td>–</td>
<td>–</td>
<td>Single Monod</td>
<td>Xu et al. (2014)</td>
</tr>
</tbody>
</table>

$^a$expressed as g SO$_4^{2-}$/g VSS d$^{-1}$. Cv: coefficient of variation; minimum and maximum in italic.
This review describes chemical sensors, microsensors and biosensors used or that may be used for the monitoring of sulphate reduction processes. In the first part, sensors for chemical analysis of the microbial activity are reviewed. The second part focuses on how the sensor measurements can be combined with molecular techniques to determine both the activity and microbial ecology in bioreactors where sulphate reduction occurs. Many traditional laboratory-based analytical techniques are commonly used to measure crucial parameters for monitoring sulphate reduction processes due to their great reproducibility and precision. However, these techniques are mostly offline, time consuming and require extensive manual handling. In the past decades, much effort and research has been put in the development of real-time monitoring equipment. An overview of these real-time monitoring techniques to measure sulphide, electron donors, sulphate and biomass composition is given below.

In situ sulphide sensors

Dissolved sulphide measurements are very important in sulphate reduction processes, because sulphide is the end product of the process. Hence, dissolved sulphide measurements are frequently used to measure the efficiency of the process. The classical offline methods for sulphide measurements, e.g. methylene blue, Cord-Ruwisch or other iodometric methods (Cord-Ruwisch 1985; APHA 1995) are rather time consuming and require elaborate sample handling. To overcome this, ion-selective electrodes (ISE) or similar electrode types (such as the Ag/Ag2S) have been developed. ISE are usually chosen for routine applications due to the fact that they have many advantages over other methods for ion concentration determination. These include analysis speed, portability, no sample destruction and wide measuring range. In ISE, selectivity is introduced by the receptor molecules (or ionophores) which are usually immobilized in a polymeric membrane matrix. The receptor molecule attributes selectivity to the sensor by its strong and selective interactions with the target analyte (Morigi et al. 2001). From the measured activity of the free sulphide ions with an ISE, the analytical concentration of the total dissolved sulphide (TDS) can also be calculated if the protonation constants of the sulphide ion (Ki and K2) and the pH of the sample solution are known:

$$S^{2−} = \frac{TDS}{1 + \left(\frac{\text{pH}−8}{\text{pK}_1}\right)^2 + \left(\frac{\text{pH}−9}{\text{pK}_2}\right)^2}.$$  

(Grootscholten, Keessman and Lens (2008) used equation (23) to estimate simultaneously the sulphide and metal concentrations in a precipitation reactor using an online estimation algorithm, also called a software sensor. The zinc concentration and precipitation rate in the CSTR were estimated based on the pH and pS (which measures the activity of the $S^{2−}$ species and is defined as $−\log[S^{2−}]$) in the reactor.

Table 9 summarizes the characteristics of sensors that measure sulphide concentrations, such as concentration range, pH range and tested interfering compounds. Frevert and Galster (1978) suggested a combined pH glass and sulphide electrode measuring system for the direct determination of the total sulphide concentration in solution. However, this system was only developed for pH < 5, while the pH of the sulphide containing natural waters and sewage is usually higher, i.e. pH > 7. Guter-
man, Ben-Yaakov and Abeliovich (1983) and Thôt and Solymosi...
Table 8. Kinetic parameters for SRB with butyrate, propionate, ethanol and formate as the substrates.

<table>
<thead>
<tr>
<th>Microbial groups</th>
<th>Substrate (i)</th>
<th>( K_{s,i} ) (g SO(_4^{2-}) L(^{-1}))</th>
<th>( K_{s,i} ) (g L(^{-1}))</th>
<th>( Y_{X/S} ) (g biomass g(^{-1}) substrate)</th>
<th>( K_{i,X} ) (g L(^{-1}))</th>
<th>( \mu ) (d(^{-1}))</th>
<th>( v ) (d(^{-1}))</th>
<th>( b ) (d(^{-1}))</th>
<th>Kinetics</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRB</td>
<td>Butyrate</td>
<td>0.01</td>
<td>0.099</td>
<td>0.03</td>
<td>( K_{H_2S} = 0.4 )</td>
<td>0.22</td>
<td>–</td>
<td>0.035</td>
<td>Single and dual Monod</td>
<td>Kalyuzhnyi et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>Butyrate</td>
<td>0.02016</td>
<td>0.10</td>
<td>0.0329</td>
<td>( K_{H_2S} = 0.27642 )</td>
<td>–</td>
<td>0.45073</td>
<td>0.01</td>
<td>Dual Monod</td>
<td>Fedorovich, Lens and Kalyuzhnyi (2003)</td>
</tr>
<tr>
<td>SRB</td>
<td>Propionate</td>
<td>0.0074</td>
<td>0.295</td>
<td>0.035</td>
<td>( K_{H_2S} = 0.285 )</td>
<td>0.81</td>
<td>–</td>
<td>0.018</td>
<td>Dual Monod</td>
<td>Kalyuzhnyi and Fedorovich (1998)</td>
</tr>
<tr>
<td>SRB</td>
<td>Propionate</td>
<td>0.019</td>
<td>0.015</td>
<td>0.03</td>
<td>( K_{H_2S} = 0.22 )</td>
<td>0.29</td>
<td>–</td>
<td>0.035</td>
<td>Single and dual Monod</td>
<td>Kalyuzhnyi et al. (1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Poinapen and Ekama (2010)</td>
</tr>
<tr>
<td>SRB</td>
<td>Propionate</td>
<td>0.0192</td>
<td>0.11</td>
<td>0.0329</td>
<td>( K_{H_2S} = 0.27642 )</td>
<td>–</td>
<td>0.41454</td>
<td>0.01</td>
<td>Dual Monod</td>
<td>Fedorovich, Lens and Kalyuzhnyi (2003)</td>
</tr>
<tr>
<td>Cv (Propionate)</td>
<td></td>
<td>0.362</td>
<td>0.830</td>
<td>0.063</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.496</td>
<td></td>
</tr>
<tr>
<td>SRB</td>
<td>Ethanol</td>
<td>0.816</td>
<td>0.207</td>
<td>0.0052(^b)</td>
<td>( K_{EtOH} = 80.5 )</td>
<td>( K_{Ac} = 7.08 )</td>
<td>0.312</td>
<td>–</td>
<td>Dual Monod</td>
<td>Nagpal et al. (2000)</td>
</tr>
<tr>
<td>SRB</td>
<td>Ethanol</td>
<td>–</td>
<td>0.18</td>
<td>–</td>
<td>( K_{Pe-} = 0.709 )</td>
<td>–</td>
<td>0.25(^c)</td>
<td>–</td>
<td>Single Monod and non-competitive inhibition</td>
<td>Gonzalez-Silva et al. (2009)</td>
</tr>
<tr>
<td>SRB</td>
<td></td>
<td>0.091</td>
<td>0.0026</td>
<td>0.076</td>
<td>–</td>
<td>0.019</td>
<td>–</td>
<td>0.01</td>
<td>Dual Monod</td>
<td>Rodriguez et al. (2011)</td>
</tr>
<tr>
<td>Cv (Ethanol)</td>
<td></td>
<td>0.698</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRB</td>
<td>Formate</td>
<td>–</td>
<td>–</td>
<td>0.08 ± 0.02</td>
<td>–</td>
<td>–</td>
<td>1.98(^a)</td>
<td>–</td>
<td>Monod</td>
<td>Gupta et al. (1994b)</td>
</tr>
<tr>
<td>SRB</td>
<td>Sulphate</td>
<td>1.34 × 10(^{-4})</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.73 × 10(^{-5})</td>
<td>–</td>
<td>Monod with molecular diffusion</td>
<td>Overmeire, Lens and Verstraete (1994)</td>
</tr>
</tbody>
</table>

\(^a\)expressed as g L\(^{-1}\) d\(^{-1}\); \(^b\)expressed as g protein g SO\(_4^{2-}\) d\(^{-1}\); \(^c\)expressed as g g\(^{-1}\) VSS d\(^{-1}\); \(^d\)expressed as g SO\(_4^{2-}\) g\(^{-1}\) VSS s\(^{-1}\); 

Cv: coefficient of variation (minimum and maximum in italic).
Table 9. Characteristics of selected electrodes for sulphide measurements.

<table>
<thead>
<tr>
<th>Measuring principle</th>
<th>Concentration range</th>
<th>Application</th>
<th>pH</th>
<th>Interfering anions</th>
<th>Non-interfering anions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined pH glass and ISE</td>
<td>–</td>
<td>–</td>
<td>&lt; 5.0</td>
<td>–</td>
<td>–</td>
<td>Frevert and Galster (1978)</td>
</tr>
<tr>
<td>Microprocessor interface for ISE</td>
<td>$10^{-5}$ to $10^{-3}$ M</td>
<td>–</td>
<td>7.5-11.5</td>
<td>–</td>
<td>–</td>
<td>Guterman, Ben-Yaakov and Abeliovich (1983)</td>
</tr>
<tr>
<td>Microprocessor interface for ISE</td>
<td>$10^{-5}$ to $10^{-3}$ M</td>
<td>–</td>
<td>9.0-12.0</td>
<td>–</td>
<td>–</td>
<td>Thöt and Solymosi (1988)</td>
</tr>
<tr>
<td>ISE-glass electrode pair or an Ag/Ag₂S electrode-glass electrode system</td>
<td>$10^{-12}$ to $10^{-2}$ M</td>
<td>Total sulphide concentration in sewage waters</td>
<td>3.0-11.4</td>
<td>–</td>
<td>–</td>
<td>Schmidt et al. (1994)</td>
</tr>
<tr>
<td>PVC membrane with surfactant modified clinoptilolite zeolite</td>
<td>$10^{-7}$ and $10^{-1}$ M</td>
<td>Potentiometric titration of $\text{S}^{2-}$ with $\text{Zn}^{2+}$, $\text{Cu}^{2+}$; direct determination of $\text{S}^{2-}$ in waste water samples</td>
<td>3.0-10.0</td>
<td>$\text{NO}_3^-$, $\text{ClO}_3^-$, $\text{Cl}^-$, $\text{Br}^-$, $\text{CH}_3\text{COO}^-$, $\text{SO}_4^{2-}$, $\text{I}^-$, $\text{CN}^-$, $\text{CO}_3^{2-}$, $\text{PO}_4^{3-}$, $\text{AsO}_4^{3-}$, $\text{ClO}_4^-$</td>
<td>Nezamzadeh-Ejhieh et al. (2012a)</td>
<td></td>
</tr>
<tr>
<td>Chalcogenic glass chemical sensor</td>
<td>–</td>
<td>–</td>
<td>5.0-11.0</td>
<td>$\text{CO}_3^{2-}$</td>
<td>$\text{Cl}^-$, $\text{NO}_3^-$, $\text{SO}_4^{2-}$</td>
<td>Miloshova, Baltes and Bychkov (2003)</td>
</tr>
<tr>
<td>Redox electrode</td>
<td>–</td>
<td>Sulphide oxidation control</td>
<td>8.0</td>
<td>–</td>
<td>–</td>
<td>Janssen et al. (1998)</td>
</tr>
<tr>
<td>Microelectrode</td>
<td>$10^{-3}$ to $10^{-6}$ M $\text{H}_2\text{S}$</td>
<td>Understanding microzonation and dynamics of sulphide oxidation and sulphate reduction in aerobic biofilms from trickling filters</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Kühl and Jørgensen (1992)</td>
</tr>
<tr>
<td>Microelectrode based on the amperometric measuring principle</td>
<td>1 to &gt;1000 $\mu$m $\text{H}_2\text{S}$</td>
<td>Understanding sulphate reduction and sulphide oxidation in acidic sediments</td>
<td>&lt; 9.0</td>
<td>–</td>
<td>–</td>
<td>Kühl et al. (1998)</td>
</tr>
</tbody>
</table>

– information not available
(1988) developed an appropriate microprocessor interface for the pH ranges of 7.5–11.5 and 9–12, respectively, using a sulphide ISE as sensor. In the work of Guterman, Ben-Yaakov and Abeleiovich (1983), the authors were able to measure sulphide in the concentration range of $10^{-5}$–$10^{-1}$ M. To determine the total sulphide concentration for sewage waters in the pH range 3–11.4, an electrochemical method was tested using a potentiometric cell, which contained either a sulphide ISE–glass electrode pair or an Ag/Ag$_2$S electrode–glass electrode system (Schmidt, Marton and Hlavay 1994). Both gave good results for the measurement of the TDS, in a sulphide concentration range of $10^{-12}$–$10^{-2}$ M. Villa-Gomez et al. (2014) showed that an Ag$_2$S pS electrode could be used to continuously monitor online the sulphide concentration in sulphate-reducing bioreactors and can thus be used to develop a strategy for sulphide control (see the section ‘Control of biological sulphide production’).

Recently, an electrode with a modified PVC membrane with surfactant modified clinoptilolite zeolite was applied successfully for the measurement of sulphide in wastewater samples (Nezamzadeh-Ejhieh et al. 2012a). It showed a good response for sulphide concentrations between $10^{-7}$ and $10^{-1}$ M with a detection limit of $6.6 \times 10^{-8}$ M. It also showed good performance for the pH range 3–10. Other types of sensors are the chalcoalgenic glass chemical sensors for S$^2$− and dissolved H$_2$S. These are good for the detection in a broad pH range (5–11) and exhibit better sensitivity, enhanced selectivity (no notable effect in the presence of Cl$^−$, NO$_3$− and SO$_4^{2−}$) as well as response stability at neutral pH compared to commercial sulphide ion sensors (Miloshova, Baltes and Bychkov 2003). The sulphide concentration range is dependent on the glass membrane composition. However, the authors did not disclose the details due to patent-related issues.

As an alternative method to measure dissolved sulphide concentrations in sulphide-oxidising bioreactor systems, a redox electrode was proposed (Janssen et al. 1998). The redox potential is mainly determined by the sulphide concentration since it has a high standard exchange current density with the platinum electrode surface. Thus, by maintaining a particular redox set-point value, the reactor becomes ‘sulphide-stat’. In contrast with the previously discussed sensors, the redox potential reading depends less on pH fluctuations of the solution.

At neutral pH a great part of the amount of sulphide will be present as H$_2$S, which is easily transferred into the gaseous form. The measurement of the latter can be done by numerous sensors. This has been extensively reviewed in the work of Pandey, Kim and Tang (2012). One example of a H$_2$S sensor is the wireless electronic nose system (WENS). Electronic noses usually consist of an array of sensors for chemical detection, a data acquisition system and a mechanism for pattern recognition, such as neural networks or neuro-fuzzy networks. WENS showed good performance in the concentration range of 0.15–1.5 ppm H$_2$S (Cho et al. 2008). The sensor elements and the electronics are integrated in a chip, thus increasing the sensitivity and decreasing the measurement time (0.2 s), which is very important for automatic control. Thus, WENS seems to be an attractive option for real-time monitoring of H$_2$S at the sub-ppm concentration range. However, these sensor arrays and/or electronic noses are still at the development stage, and more research on their application in process control is ongoing.

The quantification of sulphate reduction rates within biofilms was only possible after performing mass balance calculations or by tracer techniques in which biofilms were growing on metal surfaces that react with the produced H$_2$S (Kühl and Jørgensen 1992). The development of microsensors, which can monitor the processes within biofilms, was a great improvement in this field. Kühl and Jørgensen (1992) successfully used oxygen, pH and sulphide microelectrodes to study the microzonation and dynamics of oxygen respiration, sulphide oxidation and sulphate reduction at high spatial resolution in aerobic biofilms collected from a trickling filter. The calibration curves for the sulphide microelectrodes exhibited a log linear response for $10^{-3}$–$10^{-6}$ M H$_2$S. The electrode response times depend on the H$_2$S concentration and varied from $<1$ min for the highest concentration up to 10–15 min for the lowest.

The construction and use of well-functioning Ag/Ag$_2$S electrodes can, however, be problematic (Kühl et al. 1998) due to e.g. non-ideal responses, signal drift and very long response times at low sulphide levels. The very high pKs of the sulphide system may also prevent the application of such electrodes in acidic environments, where S$^2$− is practically inexistent. Kühl et al. (1998) optimized a H$_2$S microsensor based on the amperometric measuring principle. The microsensor allowed to obtain a microprofile of H$_2$S in an acidic lake sediment with a flocculant surface layer several cm thick (Fig. 8). Its application was demonstrated for sulphate reduction and sulphide oxidation studies in acidic sediments. The microsensor exhibited a fast (0.2–0.5 s) and linear response over a concentration range of 1 to $>1000$ $\mu$M H$_2$S at pH 4.6 and relatively low SO$_4^{2−}$ concentrations. This microsensor also showed good results for neutral and moderate alkaline (pH $<9$) biofilms and sediments. The microelectrode was used to measure the H$_2$S concentration and to quantify the microbial sulphate reduction activity in a process that removes uranium (U(VI)) (Beyenal et al. 2004).

**In situ sensors for electron donor concentrations**

The real-time measurement of electron donors the SRB use is very helpful when automated control of the process is wanted. Indirect measurements of most of the electron donors (lactate, volatile fatty acids) use the chemical oxygen demand (COD). In recent years, the development of rapid and environmentally friendly methods for the COD determination has attracted more attention. Most of these new methods were based on
in the pH range 3.0–7.0 and assisted in the potentiometric titration of sulphate and barium ions. The response of an ISE with an imidazole derivative (Li et al. 1999) was closely related to the pH of the solution. A linear response was obtained, at pH 3, in the concentration range of 3.2 × 10⁻⁵ to 0.5 M and was applied for the determination of sulphate in pharmaceutical samples. A zwitterionic bis(guanidium) ionophore bearing a dihydricloride analogue ISE was investigated and showed a Nernstian behaviour in a concentration range of 10⁻⁶–10⁻² M in the presence of Cl⁻ concentrations below 10⁻³ M (Fibbioli et al. 2000). On the other hand, a tris(2-aminoethylamine) derivative ISE (Berrocal et al. 2000) showed a Nernstian response for higher sulphate concentrations (10⁻⁵–10⁻¹ M).

An ISE based on the dispersion of hydrotalcites into a poly(dimethylsiloxane) membrane had linear response in the sulphate range of 4.0 × 10⁻⁵–4.0 × 10⁻² M, which was constant over a pH range 4.0–7.0 (Morigi et al. 2001). The ISE was successfully applied in the sulphate determination in commercial mineral waters. A PVC-membrane ISE based on 2,5-diphenyl-1,2,4,5-tetraaza-bicyclo[2.2.1]heptane as a neutral carrier revealed a linear response for sulphate concentrations ranging from 9.0 × 10⁻⁶ to 1.0 × 10⁻¹ at a pH of 4.0 (Shamsipur et al. 2002). A derivative of pyrillium perchlorate was also used as a neutral carrier for a PVC-membrane ISE. The range of detection was slightly less, 1.0 × 10⁻⁶ to 1.0 × 10⁻² M, but it was less sensitive to pH changes, working in a pH range 4.0–9.0 (Ganjali et al. 2002). This sensor was applied as an indicator electrode in the potentiometric titration of sulphate and barium ions in aqueous solutions with varying anions (Table 10) and for the indirect determination of the zinc concentration in zinc sulphate tablets.

An electrode with a surfactant-modified zeolite carbon paste was applied for the potentiometric determination of sulphate (Nezamzadeh-Ejhieh et al. 2012b). It showed a good nernstian response for concentrations between 2.0 × 10⁻⁹ and 3.1 × 10⁻³ M and with constant nernstian response for pH 4–10. The electrode was applied to determine sulphate concentrations in a pharmaceutical zinc sulphate capsule (78.53 ± 1.53 mg SO₄²⁻/mg capsule⁻¹) and as an indicator electrode in the potentiometric titration of sulphate. Other neutral carriers used were zincphthalocyanine (Ganjali et al. 2003), 1,3,5-triphenylpyrillium perchlorate (Ganjali et al. 2004) and 2-amino-6-(tbutyl)-4-(pyridine-2-yl)pyrimidine(dichloride)palladium(II) (Mizani and Rajabi 2014). These three carriers presented good linear responses for sulphate concentrations between 1.0 × 10⁻⁶ and 1.0 × 10⁻² M (pH 2.0–7.0), 6.3 × 10⁻⁶ to 1.0 × 10⁻¹ (pH 2.5–9.5) and 5.0 × 10⁻¹ to 4.0 × 10⁻⁷ (pH 2.9–9.5), respectively.

A biosensor for sulphate was developed using Thiobacillus ferroxidans strain 15 for the measurement of SO₄²⁻ in acid rain (pH range 2–3) (Sasaki et al. 1997). In this microbial sensor, the bacteria used oxidize Fe(II) in the presence of sulphate. The sulphate concentration is calculated based on the decrease of current at the microbial electrode induced by the oxidation of Fe(II) to Fe(III) and simultaneous consumption of dissolved oxygen. The biosensor showed linear responses between 4 and 200 μM SO₄²⁻. However, nitrate was an interfering substance for this sensor, which also showed poor stability.

**Sensors for biomass composition**

The microsensors described in the section ‘In situ sulphide sensors’ can be coupled to molecular techniques to get more insight in the processes prevailing in a biofilm. This combination was done for the first time by Ramsing, Küll and Jørgensen (1993) to study SRB in trickling filter biofilm treating municipal
Table 10. Characteristics of selected electrodes for sulphate measurements.

<table>
<thead>
<tr>
<th>Measuring principle</th>
<th>SO$_4^{2-}$ concentration range (M)</th>
<th>pH range</th>
<th>Interfering anions$^a$</th>
<th>Non interfering anions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisthiourea ionophore ISE</td>
<td>$10^{-6}$ to $10^{-2}$</td>
<td>7.0</td>
<td>Br$^-$, NO$_3^-$, NO$_2^-$, SCN$^-$</td>
<td>Cl$^-$, CH$_3$COO$^-$, SO$_4^{2-}$</td>
<td>Nishizawa et al. (1998)</td>
</tr>
<tr>
<td>Bisthiourea ionophore ISE</td>
<td>$3.0 \times 10^{-7}$ to 1.0 $\times 10^{-1}$</td>
<td>3.0-9.0</td>
<td>–</td>
<td>F$^-$, Br$^-$, I$^-$, Cl$^-$, NO$_3^-$, SCN$^-$, ClO$_4^-$, I$_3^-$, CN$^-$, HPO$_4^{2-}$, CH$_3$COO$^-$, Citrate, NO$_2^-$, CO$_2^{3-}$, ClO$_4^-$</td>
<td>Firouzabadi et al. (2013)</td>
</tr>
<tr>
<td>Derivative of imidazole ISE</td>
<td>$3.2 \times 10^{-5}$ to 0.5</td>
<td>3.0</td>
<td>Br$^-$, NO$_3^-$, NO$_2^-$</td>
<td>Cl$^-$, CH$_3$COO$^-$</td>
<td>Li et al. (1999)</td>
</tr>
<tr>
<td>Zwitterionic bis(guanidinium) ISE</td>
<td>$10^{-6}$ to $10^{-2}$</td>
<td>–</td>
<td>Br$^-$, I$^-$, NO$_3^-$, NO$_2^-$, ClO$_4^-$, CO$_2^{3-}$</td>
<td>Cl$^-$, CH$_3$COO$^-$</td>
<td>Flibioli et al. (2000)</td>
</tr>
<tr>
<td>Tris(2-aminoethylamine) derivative</td>
<td>$10^{-3}$ to $10^{-1}$ M</td>
<td>–</td>
<td>–</td>
<td>Br$^-$, I$^-$, Cl$^-$, NO$_3^-$, SCN$^-$, ClO$_4^-$, SO$_4^{2-}$</td>
<td>Berrocal et al. (2000)</td>
</tr>
<tr>
<td>Schiff base complex of Zn (II) ISE</td>
<td>$5.0 \times 10^{-5}$ to 1.0 $\times 10^{-1}$</td>
<td>3.0-7.0</td>
<td>–</td>
<td>Br$^-$, I$^-$, Cl$^-$, NO$_3^-$, NO$_2^-$, CN$^-$, SCN$^-$, ClO$_4^-$, CH$_3$COO$^-$, SO$_4^{2-}$, CO$_2^{3-}$</td>
<td>Shamsipur et al. (2001)</td>
</tr>
<tr>
<td>Dispersion of hydrotalcites ISE</td>
<td>$4.0 \times 10^{-6}$ to 4.0 $\times 10^{-2}$</td>
<td>4.0-7.0</td>
<td>NO$_3^-$, SCN$^-$</td>
<td>Br$^-$, Cl$^-$, CH$_3$COO$^-$, H$_3$PO$_4$</td>
<td>Morigi et al. (2001)</td>
</tr>
<tr>
<td>2,5-Diphenyl-1,2,4,5-tetrazabicyclo[2.2.1]heptane ISE</td>
<td>$9.0 \times 10^{-6}$ to 1.0 $\times 10^{-1}$</td>
<td>4.0</td>
<td>–</td>
<td>Br$^-$, I$^-$, Cl$^-$, NO$_3^-$, NO$_2^-$, CN$^-$, SCN$^-$, ClO$_4^-$, CH$_3$COO$^-$, SO$_4^{2-}$, CO$_2^{3-}$</td>
<td>Shamsipur et al. (2002)</td>
</tr>
<tr>
<td>Derivative of pyrillium perchlorate ISE</td>
<td>$1.0 \times 10^{-6}$ to 1.0 $\times 10^{-2}$</td>
<td>4.0-9.0</td>
<td>SCN$^-$</td>
<td>Br$^-$, I$^-$, Cl$^-$, NO$_3^-$, NO$_2^-$, CN$^-$, ClO$_4^-$, SO$_4^{2-}$, CO$_2^{3-}$</td>
<td>Ganjali et al. (2002)</td>
</tr>
<tr>
<td>Zinc-phthalocyanine ISE</td>
<td>$1.0 \times 10^{-6}$ to 1.0 $\times 10^{-2}$</td>
<td>2.0-7.0</td>
<td>–</td>
<td>Br$^-$, Cl$^-$, NO$_3^-$, NO$_2^-$, SCN$^-$, ClO$_4^-$, CH$_3$COO$^-$, SO$_4^{2-}$, CO$_2^{3-}$, H$_2$PO$_4^-$</td>
<td>Ganjali et al. (2003)</td>
</tr>
<tr>
<td>1,3,5-Triphenylpyrillium perchlorate ISE</td>
<td>$6.3 \times 10^{-6}$ to 1.0 $\times 10^{-1}$</td>
<td>2.5-9.5</td>
<td>–</td>
<td>I$^-$, Cl$^-$, NO$_3^-$, NO$_2^-$, CN$^-$, SCN$^-$, ClO$_4^-$, CH$_3$COO$^-$, SO$_4^{2-}$, H$_2$PO$_4^-$</td>
<td>Ganjali et al. (2004)</td>
</tr>
<tr>
<td>2-Amino-6-(tbutyl)-4-(pyridine-2-yl)pyrimidine)(dichloride)palladium(II)</td>
<td>$5.0 \times 10^{-1}$ to 4.0 $\times 10^{-7}$</td>
<td>2.9-9.5</td>
<td>–</td>
<td>I$^-$, Cl$^-$, NO$_3^-$, NO$_2^-$, SCN$^-$, ClO$_4^-$, CH$_3$COO$^-$, SO$_4^{2-}$, HPO$_4^-$, Br$^-$, F$^-$, Tartrate$^{2-}$</td>
<td>Mizani and Rajabi (2014)</td>
</tr>
<tr>
<td>Microbial sensor using Thiobacillus ferrooxidans</td>
<td>$4 \times 10^{-6}$ to 2.0 $\times 10^{-4}$</td>
<td>2.0-3.0</td>
<td>Cl$^-$ ($\approx$8.97 mM); NO$_3^-$ ($\approx$200 µM)</td>
<td>–</td>
<td>Sasaki et al. (1997)</td>
</tr>
</tbody>
</table>

$^a$Anions were considered to be interfering when the selectivity coefficient was equal or higher to 1; – information not available.
Figure 9. Sulphide and methane microsensor profiles (lines) and activity values (bars) in methanogenic - sulphidogenic (A) and methanogenic (B) aggregates in the presence (white) or absence (black) of sulphate. The aggregate surface is at distance of 0 mm, the centre of the aggregates is at a distance of ca. 0.9 mm (Santegoeds et al. 1999).

wastewater. Gradients of O$_2$, H$_2$S and pH were measured with microelectrodes and the distribution of SRB was determined by specific oligonucleotide probes. This approach of using microelectrodes together with specific oligonucleotide probes proved fruitful in that it was possible to relate the distribution of bacteria to their chemical microenvironment at a spatial resolution of below 100 μm.

Analysing the transients of sulphate reduction, using microsensors, and the successive changes in the composition of microbial species using molecular techniques (PCR, DGGE) were studied in a multispecies aerobic bacterial biofilm (Santegoeds et al. 1998). The goal of this study was to determine how the species composition is related to the activity in a biofilm with microenvironments changing gradually. However, the molecular techniques used were not sufficient to accurately predict the microbial population changes in this complex environment. Other molecular techniques (DGGE, PCR and FISH) in combination with microsensors for H$_2$S and CH$_4$ were used to study the activity distribution in anaerobic aggregates and the population structure (Santegoeds et al. 1999). The microsensors and molecular techniques used provided direct information about sulphate reduction and methanogenesis in UASB aggregates (Fig. 9). The data obtained on the community structure could then be related to the metabolic function of the respective populations. Microsensors with a high spatial resolution were used to measure O$_2$ and H$_2$S profiles and to localize aerobic respiration and sulphate reduction activities within the biofilm (Kühl et al. 1998). The molecular techniques used included DGGE and PCR-amplified 16S ribosomal DNA fragments to determine the microbial complexity in the biofilm in an acidic lake sediment. The researchers were able to follow the development of the microbial community and to detect several SRB groups in complex biofilms with several species. However, the techniques also showed some limitations such as the inability to quantify the activity and the difficulty in identifying the specific molecular probes for the species present.

The techniques for biomass and activity characterization mentioned above have the disadvantage of being invasive, destructive and do not give information in real time. Some progress has been made to quantify microbial activity with online monitoring. An increasing trend towards the development of impedimetric biosensors is observed. Impedimetric biosensors have been fabricated to study biomolecular reactions (Oliveira et al. 2008) as well as specific recognitions of proteins (Bogomolova et al. 2009), lectins (La Belle et al. 2007), antibodies (Rezaei et al. 2009) or nucleic acids (Hu et al. 2011). For SRB detection, rapid and non-labelled impedimetric biosensors were developed based on agglutination reactions (Wan, Zhang and Hou 2009), antibody recognition platforms on 3D Ni foam substrates (Wan et al. 2010), self-polymerized polydopamine films (Wan et al. 2011b), RGSs-CS nanocomposite films (Wan et al. 2011a) and on bioimprinted films (Qi, Wan and Zhang 2013). An electrochemical SRB
et al. 2010). An in situ methodology based on covalently bound redox indicators can be used for determining when sulphate-reducing conditions exist in environmental samples. Sulphide coupled well to the cresyl violet immobilized redox indicator in the concentration range of 1–100 mM total sulphide and the pH range of 6–8. Thionine, the indicator with the highest potential (actual potential measured by the electrode), reacts rapidly with sulphide at levels well below 1 mM (Jones and Ingle 2005). The amplification of responses of vancomycin-functionalised magnetic nanoparticles, using a quartz crystal microbalance under an external magnetic field, gave good results to detect and quantify SRB (Wan, Zhang and Hou 2010a). Potentiometric stripping analysis was used for the selective detection of D. caledoinensis (Wan, Zhang and Hou 2010b).

Another easily measurable parameter to quantify the microbial activity is conductivity. The metabolism of bacteria can cause an increase in the conductivity of the culture medium due to the generation of charged, mobile metabolites such as organic acids and the decomposition of large molecules into smaller ones. The changes in the conductivity can then be correlated with bacterial activity and be used to enumerate bacteria. Lyew and Sheppard (2001) used conductivity measurements to measure the SRB activity for the treatment of acid mine drainage. They concluded that the latter is more sensitive for the assessment of SRB activity than pH or the oxidation-reduction potential.

**Evaluation on ‘Process monitoring’**

Online monitoring of substrates, products and possible intermediates leads to increased knowledge on the process, and thus more accurate models and controller applications are possible. Although there has been great advance in the development of sensors, there are still few reports on their application on continuous sulphate-reducing bioreactors. A successful example was the use of a solid-state Ag2S ISE assisting in a control strategy design for biological sulphide production in bioreactors (Villa-Gomez et al. 2014). Several online sensors have been developed for the measurement of crucial variables in the sulphate reduction processes. The development of these sensors is bringing researchers one step closer to a better understanding of the process and to validate models developed, for example, for the dynamics inside biofilms (Mašić et al. 2010). These type of sensors seem to be a promising alternative to the traditional methods of monitoring chemical substances and microbial populations. However, more research is needed for the optimization of the discussed techniques in order to minimize the interference with other wastewater contaminants and minimize response times so that they can be utilized in control strategies for continuous bioreactors treating wastewater.

Some sensors are already commercially available, but many of them are still underdeveloped. Especially for sulphate, for which there is still no published research on (micro) sensors tested in wastewater. The latter would be very interesting to use in control of the sulphate load to a bioreactor or to experimentally study the substrate diffusion in biofilms.

Overall, no sensor has presented optimal overall performance, and thus the choice must be directly related to the characteristics of the process to be monitored, i.e. concentration range, pH, interfering compounds (Tables 9 and 10), cost, ease of use, placement of the sensors, response time, reliability, accuracy and detection limit (Bourgeois, Burgess and Stuetz 2001). However, as shown in this review, promising research is being done in the development and optimization of these online measuring devices, and thus allowing further optimization in the monitoring step of bioprocess controllers.

**CONTROL OF ANAEROBIC SULPHATE REDUCTION PROCESSES**

The design of advanced bioprocess control strategies is highly related to the available models and sensors. With the development of models and in situ sensors for online monitoring, it is now possible to develop high-performance control strategies to control biological systems such as sulphate-reducing bioreactors. This section reviews the existing (Table 11) and potential control strategies for sulphate-reducing bioreactor systems.

Large progress has been made in recent years in the control of anaerobic digestion processes. In these processes, the controlled variables are usually the process intermediates such as the volatile fatty acid concentration, pH, bicarbonate alkalinity or gas concentration flow rates (Pind et al. 2003). However, the number of experimental applications of control approaches in sulphate-reducing bioreactors is still scarce. Mathematical models, presented in the section ‘Models for biological sulphate reduction processes’, can serve as support for the design of control strategies. When selecting a control strategy one must take into account the unique characteristics of the process to be controlled. In the studies presented in this section, single input–single output feedback control is used. Feedback control (Fig. 10) starts by measuring the variable to be controlled and comparing it to the set point on a set-point trajectory, defined by the user. It then uses the difference between these two values to determine which action to be taken by the controller that will then change the manipulated variable (Dunn et al. 2005).

The sections below review control strategies utilized for different sulphate reduction and anaerobic digestion processes. The first section will focus on the control of chemical/biological sulphide addition, the second section on the control of biological sulphide production and the third will evaluate the use of adaptive controllers for sulphate reduction bioprocesses.

**Control of sulphide addition**

Simple control strategies can be chosen when the process to be controlled presents itself with low complexity. In these cases, a commonly used controller is the so-called proportional-integral (PI) controller. A PI controller has two adjustable parameters, the controller gain, $K_c$, and the integral time, $\tau_I$. These parameters can be obtained by using different tuning methodologies. More information on these tuning methodologies can be found in the literature (Dunn et al. 2005).

Selective metal precipitation with sulphide has been shown possible by applying a combination of a pH and pH electrode, and controlling the addition of chemical sulphide using a PI control strategy to achieve the stoichiometric addition of sulphide entering a precipitation reactor (Veeken et al. 2003; Esposito et al. 2006; Sampaio et al. 2009, 2010). In the work of Veeken et al. (2003), experiments were performed in batch and continuous systems with synthetic wastewater containing Cd, Cu, Ni, Pb and Zn. The heavy metals were successfully removed to concentrations <0.05 mg L$^{-1}$ at pH 6.0 by sulphide precipitation, while maintaining the total sulphide concentration < 0.02 mg L$^{-1}$. During precipitation, the pH electrode gave a unique response for each heavy metal. The latter was directly related to the solubility product of the corresponding metal sulphide. Thus, the metals
Table 11. Control strategies for sulphate reduction processes.

<table>
<thead>
<tr>
<th>Controlled variable</th>
<th>Manipulated variable</th>
<th>Controller type</th>
<th>System</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical sulphide concentration</td>
<td>Sulphide buffer flow</td>
<td>PI</td>
<td>Selective heavy metal precipitation (Cu-Zn and Pb-Zn)</td>
<td>Veeken et al. (2003)</td>
</tr>
<tr>
<td>Biogenic sulphide concentration</td>
<td>Sulphide buffer flow</td>
<td>PI</td>
<td>Zinc precipitation</td>
<td>König et al. (2006)</td>
</tr>
<tr>
<td>Chemical and biogenic sulphide concentration</td>
<td>Sulphide buffer flow</td>
<td>PI</td>
<td>Zinc precipitation</td>
<td>Esposito et al. (2006)</td>
</tr>
<tr>
<td>Chemical sulphide concentration</td>
<td>Sulphide buffer flow</td>
<td>PI</td>
<td>Copper and Zinc selective precipitation</td>
<td>Sampaio et al. (2009)</td>
</tr>
<tr>
<td>Chemical sulphide concentration</td>
<td>Sulphide buffer flow</td>
<td>PI</td>
<td>Zinc and Nickel selective precipitation</td>
<td>Sampaio et al. (2010)</td>
</tr>
<tr>
<td>Biogenic sulphide production</td>
<td>Organic loading rate</td>
<td>PID</td>
<td>Sulphate reduction in an Inversed fluidized bed reactor</td>
<td>Villa-Gomez et al. (2014)</td>
</tr>
</tbody>
</table>

Figure 10. Feedback control loop for simple process control of sulphate reduction with: \( r \) is the input, \( \varepsilon \) is the error, \( c \) is the manipulated variable and \( y \) is the feedback.

in mixtures of Cu-Zn (CSTR) and Pb-Zn (batch reactor) were selectively precipitated from solution at pH 6.0 by control of the pS at different levels. At pH 6.0, the pS values for Cu, Pb and Zn were 39.0, 30.0 and 24.0, respectively. This resulted in the production of pure metal sulphide sludges that could possibly be reused.

In a similar work, Sampaio et al. (2009) measured the process variable in the reactor and manipulated the sulphide flow using a feedback control (Fig. 10). The reactor was run at constant metal and sulphide flows and, at a sudden point, the sulphide flow was increased to another constant value. The pS electrode response to this step change can then be used to calculate the PI controller parameters \( K_c \) and \( \tau_I \). Consequently, it was possible to continuously and selectively precipitate Cu with chemical sulphide to concentrations below 0.3 ppb from water containing around 600 ppm of both Cu and Zn in a CSTR at pH 3 and pS 25. The Cu was recovered with a purity of around 100%, whereas the total soluble sulphide concentration was below 0.02 ppb even with increasing input concentrations (Fig. 11). Later, Sampaio et al. (2010), using a similar strategy, showed the selective removal of Zn in a CSTR at pH 5 and pS 18 from an aqueous mixture of Zn/Ni with a purity of 99%. The current design of the pS electrode appeared to be incompatible with the NiS precipitation process at pH 4–6 due to interferences of the precipitates with the pS electrode.

Figure 11. Continuous selective precipitation of Cu from Zn controlled at pS 25 and pH 3 (Sampaio et al. 2009) with \( Q_{sulph} \) sulphide flow.

The PI control strategy was adapted and successfully used to control biogenic sulphide entering a precipitator reactor with only metal precipitation taking place (König et al. 2006). Similarly, the Cohen Coon method (Stephanopoulos 1984) was used to estimate the control parameters based on experimental step response data, i.e. based on the sulphide potential response to a stepwise variation of the buffer flow. The pS was controlled at 15 and the pH at 5.87 ± 0.55. The pS/pH control system was able, using a PI controller, to bring the sulphide concentration to the
desired value within acceptable margins regarding the optimal removal of zinc. Esposito et al. (2006) assessed the performance of a zinc sulphide precipitation process using a PI control algorithm to control the pH and sulphide (both chemical and biogenic) concentration using pH and pS electrodes. A residual zinc concentration of 0.07 mg L\(^{-1}\) was obtained from the precipitation of zinc sulphide at pS 15 from a 3 g L\(^{-1}\) Zn\(^{2+}\) influent for both sulphide sources. However, at pS 10 and 20 the ZnS precipitation efficiency decreased when using the biogenic instead of the chemical sulphide, which was related to the presence of other substances present in the sulphate-reducing bioreactor where the sulphide was produced.

**Control of biological sulphide production**

For metal removal and recovery processes, the required amount of sulphide to be produced by SRB depends on the composition of the wastewater to be treated, i.e. its metal concentration. Steering the sulphide production towards this required stoichiometric amount in bioreactors is highly relevant to avoid H\(_2\)S overproduction, which increases operational costs and may impose a sulphide removal post-treatment step (Villa-Gomez et al. 2014). To control the production of sulphide in a bioreactor is more complex since the production itself must be considered in the control strategy. This strategy must take into account the amount of substrate added, and thus an additional control parameter is required to overcome the lag time between substrate dosing, substrate bioconversion and release of the desired product (S\(^{2-}\)) (Villa-Gomez et al. 2014).

In practice, PI control is sufficient in most systems. However, when fast changes are anticipated in the process, a derivative (D)-action may be added to smooth the controller response by predicting the error in the immediate future (Dunn et al. 2005). In addition, if the D-action is used, typically a first-order filter is added to make the controller strictly proper, i.e. to increase the stability of the controller. The proportional-integral-derivative (PID) controller has been successfully used in anaerobic bioreactors, partly due to the derivative control parameter used to overcome the lag caused by the time required for substrate degradation prior to being used or transformed to the desired product (Dunn et al. 2005; Jagadeesh and Sudhaker 2010). In addition to the controller gain and the integral time, the PID controller has an extra adjustable parameter called the derivative time \(\tau_d\). More detailed information on PID controller can be found in the literature (Dunn et al. 2005).

Different tuning strategies, using a pS electrode, were evaluated to control the production of biological sulphide in an inverse fluidized bed reactor performing biological sulphate reduction. The bioreactor was run on automated operation using the LabView software version 2009\(^{\text{®}}\) (Villa-Gomez et al. 2014). Step changes in the organic loading rate (OLR) were applied either by changing the HRT or the concentration of lactate in the influent (Fig. 12). The pS output values resulting from both control strategies were used to determine the PID parameters. As a result, the sulphide concentration in these bioreactors is likely to be controlled by the variation of the lactate concentration or of the HRT depending on the desired outcome of the sulphide concentration. A variation of the lactate concentration should be applied if an increase in the sulphide concentration is desired, and a change in the HRT should be applied if the sulphide concentration is to be decreased. This was a crucial first step in showing that a control strategy for sulphide production towards a desired concentration is possible, but more research is needed to turn this into practical applications. The critical factors when controlling biological sulphide production are the delays in response time, the time variant response and a high control gain (Villa-Gomez et al. 2014). Thus, it is crucial to fully understand the metabolic pathways in the sulphate-reducing biomass in

Figure 12. Step responses of the pS electrode response for a change in (a) COD\(_{in}\) and (b) HRT (Villa-Gomez et al. 2014).
Adaptive control of biological sulphate reduction

A well-tuned controller has parameters adapted to the dynamic properties of the specific process in order to have a fast and stable control system. If the process dynamic properties vary without the controller being retuned, the control system may become unstable or may become sluggish. The problems encountered with time varying process dynamics can be solved by tuning the controller in the most critical operation (conservative tuning) to guarantee the stability of the control when the process operates in a different operation point. However, if the tuning is too conservative the tracking speed is reduced, giving more sluggish control (Dunn et al. 2005). Another option to solving these problems is to use adaptive tuning, in which the controller parameters are varied along with variations of the process dynamics, so that the performance of the control system is maintained or optimized at any operation point. In such cases, a model for the process is necessary if the controller is to adapt to changing conditions. Typically, adaptive optimal control seeks for a maximum in a performance index function (Heinzle, Dunn and Ryhiner 1993) and requires a model that accounts for changing process conditions (Fig. 13; section 'Models for biological sulphate reduction processes').

To our knowledge, there are still no studies reported on adaptive control for biological sulphate reduction systems. However, there are reports on adaptive control strategies designed for anaerobic digestion methanogenic processes. A few examples of these successful applications of adaptive control to anaerobic digestion processes are given below. These may be used as a starting point in the development of strategies to control biological sulphate reduction processes.

The feed flow in an anaerobic digestion process was controlled in order to maintain the optimum production of methane and organic acids (Heinzle, Dunn and Ryhiner 1993). A mechanistic simulation model appeared to be very useful in the design and testing of the adaptive controller. Once the parameters in the controller were determined, it was tested and an acceptable control of the one-stage bioreactor was obtained. The decrease of the flow rate by the controller corresponded to keeping the OLR of the reactor constant. Similarly, Steyer et al. (1999) designed a control strategy using the feed flow as the manipulated variable and biogas flow rate and pH as controlled variables. This control strategy, which was based on rather simple and reliable sensors that are widely used in industrial applications, was able to automatically monitor an anaerobic digestion process and to prevent overloading. In addition, it was able to adapt its parameters to any change in the influent concentration, and thus to force the process to reach its maximum treatment ability.

Adaptive feedback control has been shown experimentally to be an appropriate tool to compensate for model uncertainties. Mailleret, Bernard and Steyer (2004) proposed such a controller for a single substrate/single biomass model under general assumptions about the growth rate functions using the substrate concentration as a set point. Similarly, Dimitrova and Krastanov (2011) aimed at stabilizing a four-dimensional nonlinear dynamic system, modelling the anaerobic degradation of organic wastes with methane production. A non-linear feedback adaptive controller was proposed under general assumptions on the growth rate functions. The latter was able to stabilize...
asymptotically the dynamic system towards the unknown optimal (maximal) methane production.

In a different work, the acidity excess was avoided by maintaining a constant OLR in laboratory-scale upflow anaerobic filters for the treatment of dairy and coffee effluent (Johnson, Wheatley and Fell 1995). Turbidity and conductivity were shown to be able to represent dissolved and suspended organic load. Thus, an adaptive feedback control model, based on the online determination of COD and gas production, was used to automatically vary the influent pumping rate and so avoid any imbalances and instability.

When systems involve highly complex and not fully understood processes, it becomes difficult to develop a mechanistic model which describes the system to its full extent. If the model cannot predict the effects of short timescale events, adaptive control will become less efficient. In these cases, utilizing black box models such as artificial neural networks (ANN) are a suitable alternative (Zupan and Gasteiger 1993, 1999; Holubar et al. 2002 and references therein). The latter do not require prior knowledge about the structure and relationships that exist between important variables. In addition, they are adaptable to system changes, in a short time scale, due to their learning abilities (Zupan and Gasteiger 1999).

ANN have been applied with some success to anaerobic digestion systems (Wilcox et al. 1994; Holubar et al. 2002; Strik et al. 2005). Strik et al. (2005) successfully modelled the concentration of H₂S and NH₃ in biogas with ANN for the anaerobic digestion of flour type W480 and peptone from casein (25:1) in a CSTR. The authors concluded that the developed ANN was suitable in predictive control tools. Atasoy, Babar and Sahinkaya (2013) predicted the performance of fluidized bed reactors treating acid mine drainage using a designed, trained and validated ANN. Feed and effluent pH, feed sulphate, metal, COD concentration as well as operation time were used as input parameters. As output parameters, the effluent sulphate, COD, alkalinity and sulphide concentrations were taken. The ANN gave good agreement with experimental data showing the possibility to model complex systems without fully understanding the interactions within the various microbial groups present. Thus, ANN are also an attractive option to be applied to foresee and control the production of H₂S during various anaerobic processes.

Evaluation on ‘Control of anaerobic sulphate reduction processes’

For the full-scale application of sulphur cycle-based biotechnologies, it is of crucial importance to design and implement efficient control strategies to optimize the microorganisms growth and competition, to control inhibitory factors and/or to optimize the production of products for secondary processes, e.g. heavy metal precipitation with sulphide.

The number of full-scale applications of biocontrol approaches in sulphate-reducing bioreactors is, however, still scarce. Work has been done on the comparison of different strategies to manipulate the production of biological sulphide (Villa-Gomez et al. 2014), which should be the starting point for the development of a control strategy. The latter should be accustomed to each specific case and adapted to the dynamic conditions of the biological sulphate reduction process by using adaptive control. One of the biggest obstacles in modelling and the design of control strategies relies on how to monitor the process variables. One way to overcome this gap may be to develop control strategies based on simple and available online measurements and on general assumptions of the processes or the use of so-called software sensors (Bastin and Dochain 1990; Keesman 2002). The use of a complex model, i.e. with simplifications to a minimum, which can simulate well the dynamic processes in the bioreactor, is advisable to design and analyze control strategies before implementation as it can reduce significantly the experimentation time (Heinzle, Dunn and Ryhiner 1993).

CONCLUSION

The development and application of a strategy for automated control of sulphate reduction bioprocesses in bioreactors is not an easy process as it should comprise and understand the complex dynamics of the process at hand. To attain a high controller performance for anaerobic biological processes, such as sulphate reduction, it is of crucial importance to develop models capable of simulating the chemical, physical and biological processes prevailing in the bioreactor and to correctly choose a sensor for online monitoring of the critical variables. There are very few reports on application of online sensors on biological sulphate-reducing bioreactors. Thus, more research on sensor development and application is highly recommended as sensors are probably the biggest bottleneck when developing an automated sulphate reduction process.

In summary, to achieve automated sulphate reduction the following steps must be followed: (1) define the control variables, e.g. sulphide production, microbial competition control and inhibition minimization; (2) choose appropriate sensors, with additional software sensors, for online monitoring for substrates, products and/or intermediates; (3) develop or adapt model to current needs with complexity level depending on its purpose (high complexity for developing control strategies or low complexity for control application); and (4) combine the previous steps to develop an adaptive control strategy (examples given in this review). With the ongoing research in modelling and in the development of sensors shown in this review, it becomes more attainable to design efficient control strategies for the automated biological sulphate reduction processes.

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