Simulation of Sulphate Reduction by SRB in AMD Systems using AQUASIM

Enoch A. Akinpelu, Elvis Fosso-Kankeu, Frans Waanders and Seteno K. Ntwampe

***Abstract*—** This study presents the modelling of biological sulphate reduction in a heavy-metal laden acid mine drainage with high strength sulphate concentration. A simple dynamic model was utilised for the simulation of the biological sulphate reduction process in a continuously stirred tank reactor. The model developed described substrate utilisation via parameter estimation albeit, low description of biomass formed was obtained. The absolute relative sensitivity analysis of the parameters showed that the maximum specific growth rate of the biomass and the saturation constant were significant.

***Keywords*—** Acid mine drainage; Aquasim; substrate utilisation, simulation; sulphate reducing bacteria

I. Introduction

The process of sulphide mineralisation can lead to voluminous of tailings especially in developing nations where mining plays a major role in the economy. The process generates heavy metal-laden acid mine drainage (AMD) with high sulphate concentration due to the weathering and biological oxidation of sulphide containing tailings, including mine waste [1-8]. Traditionally, AMD treatment involves the addition of lime to raise pH and subsequently precipitate associated heavy metal hydroxides [9]. The other methods for treating AMD includes, chemical precipitation, membrane-filtration, floatation, ion-exchange, reverse osmosis, electrochemical and coagulation-floatation, amongst others [1, 10, 11]. However, these methods are expensive and often require a post-treatment facility for the brine generated. Biological sulphate removal is a cost effective alternative for high strength sulphate containing wastewater such as AMD.

Biological sulphate reduction is performed by diverse groups of sulphate reducing bacteria (SRB) that use sulphate as a terminal electron acceptor. There are reports on the successful application of SRB in high strength sulphate industrial wastewater using various organic and easily utilisable carbon sources with low operational cost [12]. SRB are well known for heavy metal precipitation as metal sulphides under anaerobic conditions. This process reduces sulphate to sulphide, and raise the pH of the AMD being remediated.

Bioreactor configuration is one of the factors that influence the performance of the SRB in wastewater treatment. Several bioreactor designs such as batch reactors, continuously stirred tank reactors, anaerobic filters, gas-lift reactors, fluidised bed reactors and membrane reactors, among others have been developed and harnessed successfully for biological sulphate reduction [13]. The performance of the bioreactor can be appraised either through an empirical approach which requires several experiments or by simulation. The latter involves the use of mathematical model simulation software for experimental designs in order to improve the reliability of the experimental results. Modelling approach has the advantage of being able to predict both current and future events while reducing the number of experiments to be completed.

AQUASIM is a tool for the identification and simulation of aquatic systems. AQUASIM program can be used to simulate systems such as mixed reactors, biofilm reactors, advective-diffusive reactors, saturated soil column, river system, lake system and sediment system [14]. Therefore, the presented work attempts to model substrate utilisation and SRB proliferation in AMD during sulphate reduction using AQUASIM v2.1.

II. Methodology

A. Sample collection, isolation and growth media

Samples of acid mine drainage collected were used to explore the presence of sulphate reducing bacteria in a Postgate isolation media as described in Akinpelu et al [15].

B. Batch experiment

Experiments were carried out in a 1 L continuously stirred tank reactors (CSTRs) under anaerobic conditions. The bioreactor operated for 21 days at 35°C and pH of 7 contained 800 mL Postgate isolation media inoculated with 100 mL of inoculum. Samples (70% v/v) were drawn weekly and replaced with fresh Postgate isolation medium. To minimize methanogesis, sodium bromoethane sulphonate (3.2 g/L) was added to the bioreactor during enrichment (21 days). Subsequently, acid mine drainage (100 mL) was added to the bioreactor, operated in a continuous mode for 7 days and

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sampled at predetermined intervals. The microbial growth was observed in a GENESYS™ 10S UV-Vis spectrophotometer (Thermo Fisher Scientific™, Waltham, MA, USA) based on optical density at a wavelength of 600 nm. Control experiments were not inoculated with SRB. All measurements were in triplicate.

C. Model description

Mathematical models are essential tools for the interpretation and optimisation of biological processes. The Monod model is commonly used to describe the kinetics of microbial metabolism due to its mathematical simplicity. This model has been used for description of both methanogenesis and sulphate reduction in a CSTR under anaerobic conditions [16]. The model was able to predict the steady-state and batch spike experimental data considerably well without considering pH modulation and sulphide inhibition. The microbial specific growth rate (μ) is related to the concentration of the limiting substrate (S) as shown in Eq. (1):

$$\mu = \frac{rmax\, S}{K + S} \tag{1}$$

where *rmax* is the maximum specific growth rate of the biomass and K is the Monod saturation constant.

In addition, Kalyuzhnyi et al [17] also modelled the growth of sulphate reducing bacteria (SRB) using Monod kinetics, albeit, taking into account pH fluctuation and undissociated H_2S inhibition. The undissociated H_2S was expressed as a first order for the SRB and the specific growth rate formulated as in Eq. (2):

$$\mu = rmax \frac{[S]F(pH)}{K_s + S}\left[1 - \left(\frac{[H_2S]}{K_I}\right)\right]\left(\frac{[SO_4^{2-}]}{K_n + [SO_4^{2-}]}\right) \tag{2}$$

Where F(pH) is the pH inhibition function, S is the organic substrate concentration, K_I is the inhibition constant of undissociated hydrogen sulphide, K_n is the Monod saturation constant for sulphate, and K_s is the Monod saturation constant for the organic substrate.

Furthermore, previous studies have shown that when microorganisms are grown in an environment with more than a substrate, the kinetics of the microbial growth can either be interactive or non-interactive. When the microbial specific growth rate is limited by only one substrate, the model is considered non-interactive. Meanwhile, in the interactive model, many substrates affect the overall microbial specific growth rate, and one substrate can also affect the uptake of other essential substrates. When the uptake of one substrate does not affect the uptake of the other substrate, a model can be established by a product of individual substrate utilisation as in Eq. (3) [18]:

$$\mu = rmax \frac{S_1}{K_1 + S_1}\frac{S_2}{K_2 + S_2} \tag{3}$$

Where S_1 and S_2 are concentrations of substrates 1 and 2, respectively, with K_1 and K_2 being saturation constants of substrates 1 and 2, respectively.

D. Biomass growth stoichiometry

For lactate degrading SRB, biomass yield and specific growth rate parameters can be estimated from the thermodynamics of microbial growth based on catabolic and anabolic stoichiometric reactions [19, 20]. During microbial growth, a portion of the substrate is used up during catabolism where hydrogen sulphide is precipitated as in Eq. (4) [21]:

$$C_3H_6O_{3(aq)} + \frac{1}{2}H_2SO_{4(aq)} \rightarrow C_2H_4O_{2(aq)} + \frac{1}{2}H_2S_{(g)} + CO_{2(aq)} + H_2O_{(l)} \tag{4}$$

For the anabolic process, assuming molecular composition of SRB biomass as $C_5H_7O_2N$ [22], the anabolic reaction can be expressed as Eq. (5):

$$\frac{5}{3}C_3H_6O_{3(aq)} + NH_{3(aq)} \rightarrow C_5H_7O_2N + 3H_2O_{(l)} \tag{5}$$

The overall stoichiometric description of SRB growth when lactate is used as a carbon source is a combination of equations (4) and (5) as highlighted in Eq. (6):

$$\frac{8}{3}C_3H_6O_{3(aq)} + \frac{1}{2}H_2SO_{4(aq)} + NH_{3(aq)} \rightarrow C_5H_7O_2N + C_2H_4O_{2(aq)} + \frac{1}{2}H_2S_{(g)} + CO_{2(aq)} + 4H_2O_{(l)} \tag{6}$$

E. Model processes and components

The modelling and simulation were done in the AQUASIM v2.1 tool [14]. The experimental results of *rmax* and K were 0.30 per day and 1.7 mg/l, respectively, which are closely related to those reported by Gupta et al [16] for biological sulphate reduction using the Monod's kinetic model. As a result, the Monod's kinetic model was used with the following input components:

- Lactate and biomass are state variables;
- Kinetic constants; *rmax* and K were 0.36 per day and 0.84 mg/l, respectively from Gupta et al [16];
- Stoichiometric matrix is as defined in Eq. (6);
- Experimental values of lactate utilisation and biomass formation were real list variables;
- Initial lactate concentration (*C_Aini*) was 10 mg/l;
- A mixed reactor compartment with a constant volume of 1 L was used;
- The system was simulated stepwise with 0.1 intervals and 70 steps;
- Sensitivity analysis was done using the absolute relative sensitivity function for substrate and biomass concentration with *rmax*, K and initial lactate concentration being constant;
- Parameter estimation was done using the secant method with a maximum of 100 iterations; and
- Inhibitory effects of heavy metals and sulphite,

including pH fluctuations were considered to be minimal.

III. Results And Discussion

A. Substrate utilization and biomass formation

The model was calibrated using data from a continuous reactor mode operation of 7 days. During this stage, the CSTR was fed with 100 mL AMD containing 8080 mg SO_4^{2-} /L which was gradually reduced to 1195 mg SO_4^{2-} /L at the end of the 7th day of reactor operation. The initial values of constant variables were analogous to those obtained in Guptal et al [16] for substrate (lactate) consumption and biomass formation over time during sulphate reduction. The profile in Fig. 1 shows the exponential decrease in substrate concentration while there was an exponential increase in biomass formed over time.

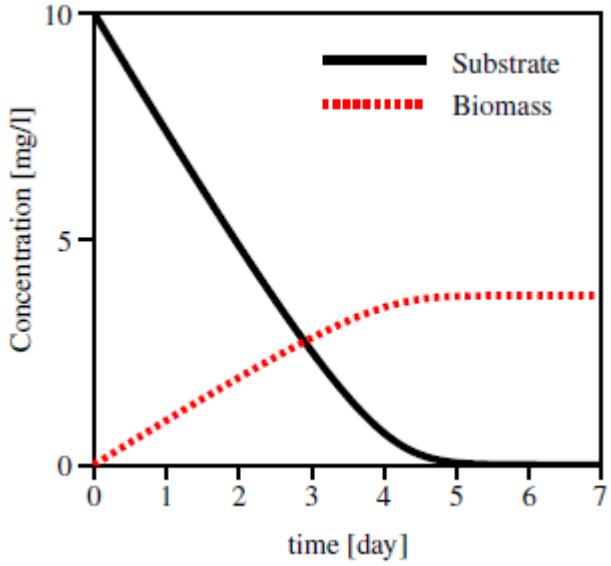

Fig. 1. Substrate uptake and biomass formation profile

B. Model simulation and parameter estimation

The simulation of experimental values with the model in Fig. 2A shows that the initial parameter values were undescriptive of both substrate utilisation and biomass formation. Although, Guptal et al [16] reported sufficient correlation between experimental data and model prediction, albeit, their simulation was for a system not as rich in heavy metal as in the AMD used in this study. Similarly, the high sulphate concentration (8080 mg SO_4^{2-}/L) in the AMD sample would have contributed to the disparity unlike 4.16 mg/L in Guptal et al [16] report. Parameter estimation of the initial parameters: *rmax* and *K* were estimated using secant method minimised the standard deviations, confirming the suitability of the stoichiometric matrix as well as the kinetic parameters used see – Fig. 2B. Iterations (n=12) were required to reach convergence of the parameters estimated. The value of *rmax* increased from 0.36 to 0.59 per day and that of *K* increased from 0.84 to 8.4 mg/L. The deviation between the model and experimental data decreased from 684.5 to 44.13. The estimated correlation matrix showed a large correlation between parameters for substrate consumption whereas there was a lower correlation between the parameters for biomass formed. The variation may be explained by the sensitivity functions of the state variables with respect to constant parameters used (rmax, *K*,).

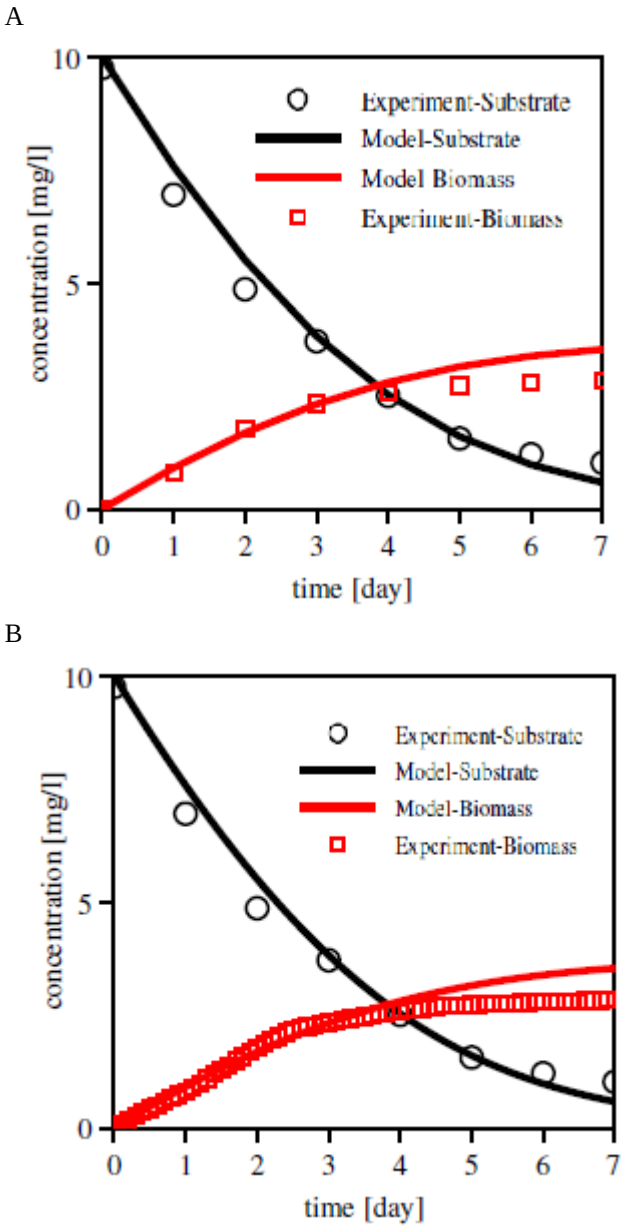

Fig. 2. Comparison of model simulation and experimental data before (A) and after (B) parameter estimation

C. Sensitivity analysis

The absolute relative sensitivity functions were performed based on two scenarios. First, the initial substrate concentration was assumed to be constant – Figure 3. The sensitivity of substrate to *C_Aini* has its maximum at time of zero, and decreases exponentially – Figure 3A. Meanwhile, with respect to *rmax*, the sensitivity of substrate increases from zero to a maximum and then decreases again to zero. An indication that the substrate concentration decreases with increasing *rmax*,

which is peculiar behaviour of the absolute value of the sensitivity function [23]. The sensitivity of substrate with respect to *K* is similar in shape to changes in *rmax* with different sign and magnitude, which make the parameters (*rmax* and *K*) non-identifiable from measured substrate data. The sensitivity of biomass has its minimum at time of zero for all the three parameters – Figure 3B. For *C_Aini*, the biomass sensitivity increases exponentially, while for *rmax* and *K*, sensitivity functions are alike in shape but differs in sign and magnitude, any change induced by one parameter can be compensated by an appropriate change in the other parameter. Only the parameter *C_Aini* is identifiable from measured data of both substrate and biomass.

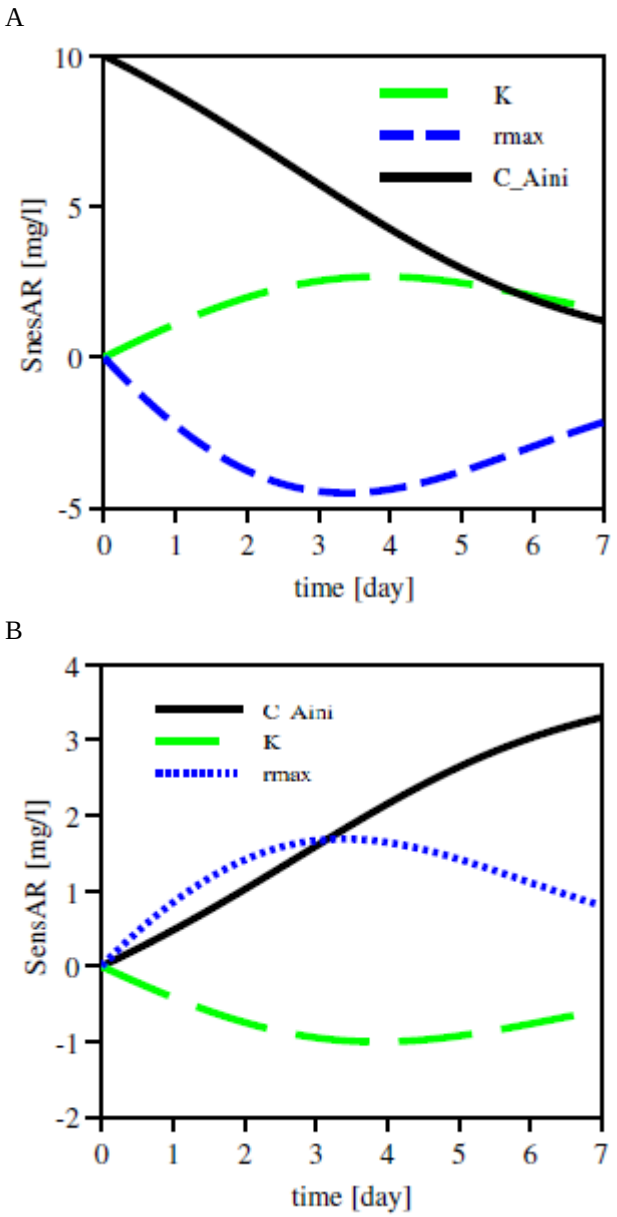

Fig. 3 Sensitive function for substrate (A) and Biomass (B) at constant initial substrate condition

Secondly, taking initial substrate conditions in the reactor compartment as real list experimental values – Figure 4. The plot shows that both substrate and biomass are insensitive to initial substrate concentration (*C_Aini*). The dependence of substrate and biomass on other parameters (*rmax*, *K*) are different. The sensitivity functions of both substrate and biomass with respect to rmax and *K* are similar in shape with different sign and magnitude. This implies that any change in *rmax* can be compensated by appropriate change in *K*, which makes the two parameters non-identifiable from measured data of substrate and biomass. This is because the concentration of substrate and biomass are not larger than the half saturation constant *K*.

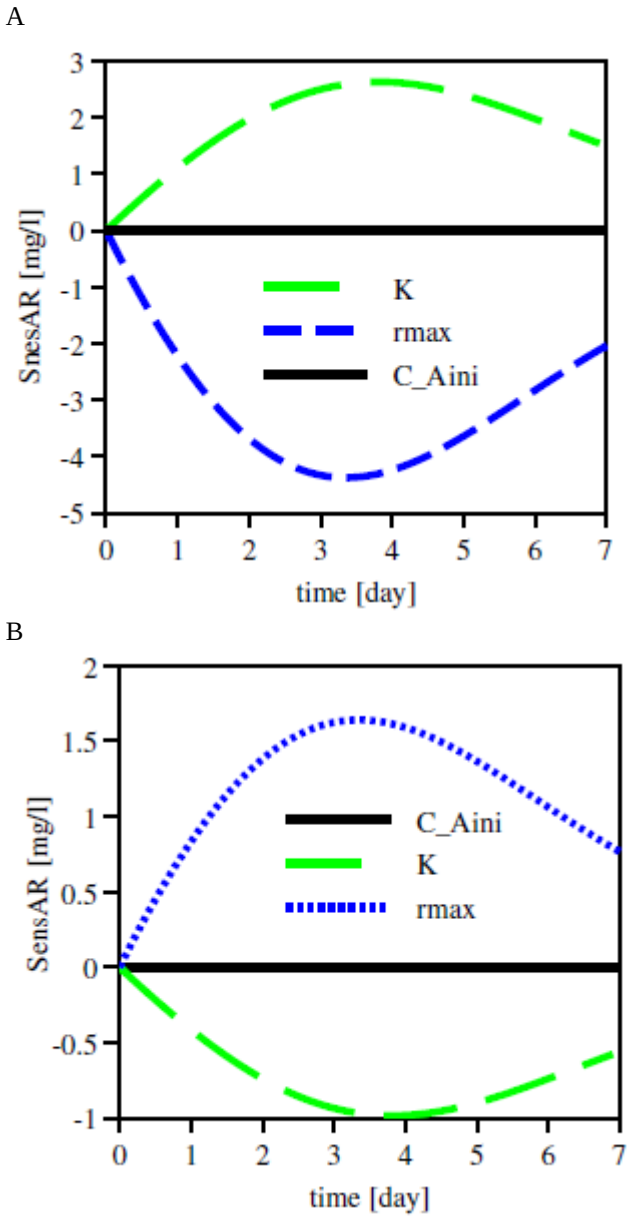

Fig. 4 Sensitive functions for substrate (A) and Biomass (B) at real list experimental values as initial conditions

IV. Conclusion

The simulated model was a better fit for substrate utilisation model while it was rudimentary in describing biomass formation. This can be attributed to effect of inhibitors and

associated heavy metals the AMD used which were not considered for the model. Modification of kinetic parameters in the model increased the correlation between the model and the experimental values. The absolute relative sensitivity function indicated that the saturation constant and the maximum biomass growth were the most significant parameters in the model. Therefore, this study forms the basis for further model development in biological remediation of AMD. In addition, modification of kinetic expressions to incorporate the role of inhibitors and heavy metals must be considered.

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