Optimising Brewery-Wastewater-Supported Acid Mine Drainage Treatment vis-à-vis Response Surface Methodology and Artificial Neural Network

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Keywords: acid mine drainage; artificial neural network; brewing wastewater; response surface methodology; sulphate reduction; optimisation

1. Introduction

Of the numerous wastewaters from different food and beverage industries, malting and brewing wastewaters are especially nutrient-rich. In South Africa alone, the brewing industry's capacity is more than 3.1 billion litres of beer per annum [1], which culminates in an industry which is potable-water-intensive with up to 10 litres of water being used to produce 1 litre of beer [2]. The composition and characteristics of brewery wastewater (BW) vary, albeit with high concentrations of crude protein (up to 754 mg/L), BOD_5 (up to 3980 mg/L), COD_{total} (up to 8926 mg/L), and total nitrogen (up to 1305 mg/L) with minute traces of heavy metals such as lead, nickel, iron, manganese,

and copper; all of these are suitable for effective biomass growth and to support other beneficial biological processes [3,4]. Some of these nutrients are from extracts of spent biowaste, as for 1 hectolitre (hl) of beer produced, approximately 20 kg of spent bio-waste in the form of grains, excess yeast, and hops ends up in the wastewater. This is equivalent to 85% of all by-products; hence, 15% of the bio-waste is attributed to surplus hops and yeast [5]. Overall, malting and beer racking have been determined to contribute the largest COD_{total} (up to 3000 mg/L) to BW [6]. Therefore, the disposal of such nutrient-rich wastewater into municipal wastewater treatment plants (MWTPs) increases nutrient loading, which can negatively affect the operation of such works. It is advisable to repurpose such wastewater as a bio-waste-type medium for the remediation of other environmental challenges, while others prefer that the water be treated—an undertaking which can result in additional costs to plant operations. Redirecting such wastewater to low-performance and passive processes as a medium could provide a sustainable mitigation strategy for the bio-waste-containing wastewater while providing for an environmentally benign approach to remediate significant environmental pollution [7–9]. A recent example was when BW was used as a fertiliser treatment for crop production, resulting in yields that resembled those under inorganic fertiliser [10]. This means that BW can provide an alternative sustainable nutrient source to passively support biological and environmentally benign processes in other industries while providing remedial action for other unrelated brewery operation challenges. It was further suggested that BW, when at ambient temperature, has high biodegradability; however, this assertion was made for aerobic conditions [6].

South Africa is a semi-arid country with limited water resources, with increasingly reduced rainfall and number of rainy days during autumn, a season associated with high rainfall [11]; current groundwater-sourced acid mine drainage (AMD) water production is high due to previous mining activity, with an example being the 2500 m^3 AMD/day draining into the West Rand Goldfields [12]. Instead of disposing of the BW into MWTPs, it can be redirected to support the passive treatment of AMD for the effective removal of sulphates. Current technologies and research studies in the nexus of BW and AMD are largely focusing on (1) the use of sludge containing sulphate-reducing bacteria treating BW for the anaerobic treatment of AMD in bioreactors [13] and (2) mine tailing treatment in bioreactors supported by anaerobic sludge generated using BW [14], with no studies focusing on the BW being used directly to support a passive system for the treatment of AMD—in particular, sulphate reduction using bacteria supported on BW while remediating AMD for water recovery to be used elsewhere, of which the primary step is sulphate removal. Furthermore, optimising such treatment systems adapted for local conditions, i.e., taking into account the unique characteristics of the AMD in South Africa, has never been attempted. For biological system model development, artificial neural networks (ANNs) and response surface methodology (RSM) have been reported as effective tools for process optimisation [15,16]. Therefore, an artificial neural network and a Box–Behnken design RSM were used to determine the better predictor of the performance of an anaerobic bioreactor designed specifically to treat South African AMD using BW as the primary carbon source in the Postgate medium. Furthermore, the novelty of using BW lies in the fact that it is usually discarded and not used for AMD remediation.

2. Materials and Methods

2.1. Chemical Reagents

Postgate medium B, herein referred to as Postgate medium, constitutes monopotassium phosphate (0.5 g/L), ammonium chloride (1.0 g/L), sodium sulphate (1.0 g/L), calcium chloride dihydrate (0.1 g/L), magnesium sulfate (2.0 g/L), yeast extract (1.0 g/L), ascorbic acid (0.1 g/L), thioglycolic acid (0.1 g/L), ferrous sulphate heptahydrate (0.5 g/L), sodium chloride (26 g/L), and sodium lactate (5 mL). The medium pH was 7–7.5. Sodium bromoethane sulphonate (98%, Merck, Modderfontein, South Africa) and sodium DL-lactate solution (60% *w*/*w*, Sigma-Aldrich, Modderfontein, South Africa) were used as received from the suppliers.

2.2. Bacterial Inoculum

The AMD sample was collected using a previously described procedure from a mining facility in South Africa [17]. The sulphate-reducing consortium was activated using selective modified Postgate isolation medium for the propagation of sulphate-reducing bacteria (SRB) at 35 °C and pH of 7 ± 0.2 in an anaerobic reactor. The constituents of the modified Postgate medium were as described earlier [17]. The black-grey colour of the medium signified positive growth of the sulphate-reducing consortium. The experiments were conducted in triplicate.

2.3. Carbon Source Limiting Growth Test

A sterile bioreactor containing 100 mL AMD (8080 mg SO_4^{2-}/L) was inoculated with 20% (*v*/*v*) SRB consortium in the Postgate medium. The characteristics of the AMD were as described in Akinpelu et al. [17]; i.e., the AMD had an E^0 value of 229.5 mV, which is high, a fairly acidic pH of 2.98, and electrical conductivity of 7.84 mS/cm. SRB growth was observed on both the lactate (L) and BW, used as carbon sources at a feed rate of 0.05 g/L h in the Postgate medium. To minimise methanogenic activities, sodium bromoethane sulphonate (3.2 g/L) was added to the bioreactors. The bioreactors were incubated anaerobically at 35 °C, pH of 7 ± 0.2, in a rotary ZHICHENG shaker (model ZHYWY-1102) at 160 rpm (ZHICHENG Analytical Instruments Manufacturing Co. Ltd, Shanghai, China). The bioreactors were purged with nitrogen gas to displace the dissolved oxygen and then sealed with Parafilm tape to sustain the anaerobic conditions. Bioreactors without inoculum served as a control. The BW, collected from a beer plant in South Africa, contained protein (3.3 ± 0.01 mg/mL), glucose (5 ± 0.02 mg/mL), fat (2.6 ± 0.03 mg/mL), lactose (5 ± 0.02 mg/mL), maltose (5 ± 0.03 mg/mL), sucrose (5 ± 0.02 mg/mL), fructose (5 ± 0.03 mg/mL), and dry matter (4.2 ± 0.02 mg/mL), as measured using a high-performance liquid chromatograph (HPLC, Agilent 1290 Infinity) (Chemetrix Export (Pty) Ltd, Johannesburg, South Africa); equipped with a 300 m × 7.8 mm Aminex HPX-97H column. The HPLC columns used isocratic conditions with water as the mobile phase at 0.6 mL/min and temperature as the main variable for control of the resolution (60–70 °C), according to the manufacturer's manual for the separation of carbohydrates. Samples were taken at predetermined intervals for turbidity measurement in a GENESYSTM 10S UV/Visible spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at a wavelength of 600 nm. All procedures were performed in triplicate. Experimental errors were estimated as the standard deviation of a set of data ($n = 3$).

2.4. Experimental Set-Up

The experiments were done in 250 mL anaerobic bioreactors (35 °C, 160 rpm, N_2 purged) and were initiated with 20% (*v*/*v*) inoculum in 100 mL Postgate medium for 48 h. Sodium bromoethane sulphonate (3.2 g/L) was added to the bioreactor to minimise methanogenic activity. Subsequently, 50 mL of AMD (8080 mg SO_4^{2-}/L) was added, and the bioreactor was operated for 72 h at various pH, COD/SO_4^{2-}, and BW levels specified by the Box–Behnken design in Table 1. All bioreactors, with or without inoculum, were conditioned to the various specified pre-set testing variables, and the pH of the cultures was adjusted using 0.1 M HCl (Lasec SA, Cape Town, South Africa); or 0.1 M NaOH (Lasec SA, Cape Town, South Africa); accordingly. The sulphate removal efficiency was estimated using Equation (1):

$$\text{Removal (\%)} = [(C_i - C_f)/C_i)] \times 100, \quad (1)$$

where C_i and C_f are concentrations of sulphate (mg/L) in the initial and treated AMD, respectively.

Table 1. Experimental design variables.

Variable	Code	Unit	Coded Factor Level		
			1	**0**	**−1**
pH	A	-	7	5	3
COD/SO_4^{2-}	B	-	3	1.75	0.5
Brewing wastewater	C	mg/L	500	350	200

2.5. Design of Experiment—Box–Behnken

To optimise the process and investigate the dynamics among the key process parameters for biological sulphate reduction, RSM was used. It has been previously used in optimising numerous environmental processes, having been determined to be superior in the extended optimisation of such processes [18]. RSM is a collection of statistical methods for the design of an experiment involving several variables, leading to peak system performance at minimal cost to establish the optimum response [19,20]. Amongst the numerous RSM designs, there is the Box–Behnken design (BBD), which is an efficient quadratic design where variable combinations are at the lower, centre and higher levels, usually coded as −1, 0, and +1, respectively, to give a minimum number of experimental runs. The centre points are used to calculate the experimental deviations [21,22]. Three operational parameters (pH, COD/SO_4^{2-}, and BW concentration) were designated as input variables, and the sulphate removal efficiency after 120 h was the dependent parameter. The ranges of the variables (pH and COD/SO_4^{2-}) were determined based on the optimum values reported for most biological sulphate-reducing systems [19,23]. The concentration of brewing wastewater was chosen based on the exponential phase in substrate limitation of the SRB consortium. Design-Expert® software version 12 (Stat-Ease Inc., Minneapolis, MN, USA, 2019) was used for the design of experiments (DoE) and data analysis. Seventeen points, comprising 5 centre points and 12 factorial points, were examined, and bioreactors without inoculum served as controls at various specified conditions (see Table 2). A COD meter and Multiparameter Bench Photometer HI 83,099 (Hanna Instruments Inc., Woonsocket, RI, USA) were used to measure both the COD and sulphate concentration in the AMD samples. This was done via a photometric chemical procedure that is based on the absorption of a compound using special subminiature tungsten lamps and a narrow bandwidth from a specific chemical reaction between the sample and reagents, so that high performance and reliable results can be guaranteed. Based on the Lambert–Beer Law, the molar concentration of the sample can be estimated. All reagents were of analytical grade. The COD and sulphate were measured using Hanna's reagents HI 93754C-25 and HI 93751-01, respectively. All experiments were performed in triplicate, and the mean values of experimental data were fitted into the following polynomial quadratic model (Y):

$$Y = \beta_o + \sum \beta_i x_i + \sum \beta_{ii} x_{ii}^2 + \sum \beta_{ij} x_i x_j + \varepsilon, \quad (2)$$

where x_i, x_j, and x_{ii} are independent coded factors; β_o is the offset term; β_i, β_{ii}, and β_{ij} are linear, square, and interaction effects, respectively; and ε is the error.

Table 2. Design matrix with actual and predicted response values via response surface methodology (RSM) and artificial neural network (ANN).

Run	Variables			Sulphate Removal (%)		
	A	B	C	Actual	RSM Predicted	ANN Predicted
1	**1**	**1**	**0**	**77.80**	**80.56**	**79.27**
2	−1	−1	0	30.70	27.94	29.40
3	0	1	−1	68.80	68.43	68.81
4	0	1	1	61.60	64.25	60.02
5	1	0	1	84.60	79.19	84.94
6	1	0	−1	87.90	85.51	87.58
7	**−1**	**0**	**−1**	**25.70**	**31.11**	**25.63**
8	**0**	**0**	**0**	**51.62**	**51.62**	**51.95**
9	0	0	0	51.62	51.62	51.93
10	−1	1	0	42.90	37.86	42.95
11	0	−1	1	63.50	63.88	63.21
12	0	0	0	51.62	51.62	51.93
13	0	0	0	51.62	51.62	51.93
14	−1	0	1	35.60	37.99	37.19

Table 2. *Cont.*

Run	Variables			Sulphate Removal (%)		
	A	B	C	Actual	RSM Predicted	ANN Predicted
15	1	−1	0	75.80	80.84	75.82
16	0	0	0	51.62	51.62	51.93
17	0	−1	−1	61.80	59.15	62.08

ANN training set: normal numbers, ANN testing set: bold numbers.

2.6. Artificial Neural Network (ANN) Analysis

Neural Power (CPC-X Software, version 2.5, Carnegie, PA, USA, 2019) was used for the neural network analysis in this study. This software is a Windows-based package that supports several types of training algorithms. The optimum artificial neural network conditions reported by Betiku and Taiwo [16] were used to predict the percentage of sulphate reduction in the treatment of South African AMD. The ANN training algorithm chosen in the present study was a multilayer full feedforward (MFFF) connection with an incremental backpropagation network and hyperbolic tangent (tanh) as the transfer function. The ANN framework consisted of three input layers, three hidden neurons, and one output layer, forming a 3-3-1 topology (Figure 1). After several trials, an optimal network topology, with only one hidden layer, three hidden neurons with tanh as the transfer function, and an output layer with Linear as the transfer function, was iteratively used in training the network. The neural network was trained until the root-mean-square error (RMSE) was less than 0.0001, with the average correlation coefficient (R^2) and the average determination coefficient (DC) tending towards unity (1). Other ANN parameters were chosen as the standard settings of the software. In this study, data generated from the BBD (Table 1) were deployed in ANN model development. Experimental data sets from the Box–Behnken design (BBD) were split into training and testing sets. The data were divided in a ratio of 1 to 5 with training sets comprising 14 data sets (70%) and testing sets (30%) comprising 3 data sets, making a total of 17 experimental runs. The details of all other conditions and techniques used in the ANN analysis for prediction and optimisation in bioprocessing have been reported in detail elsewhere [16].

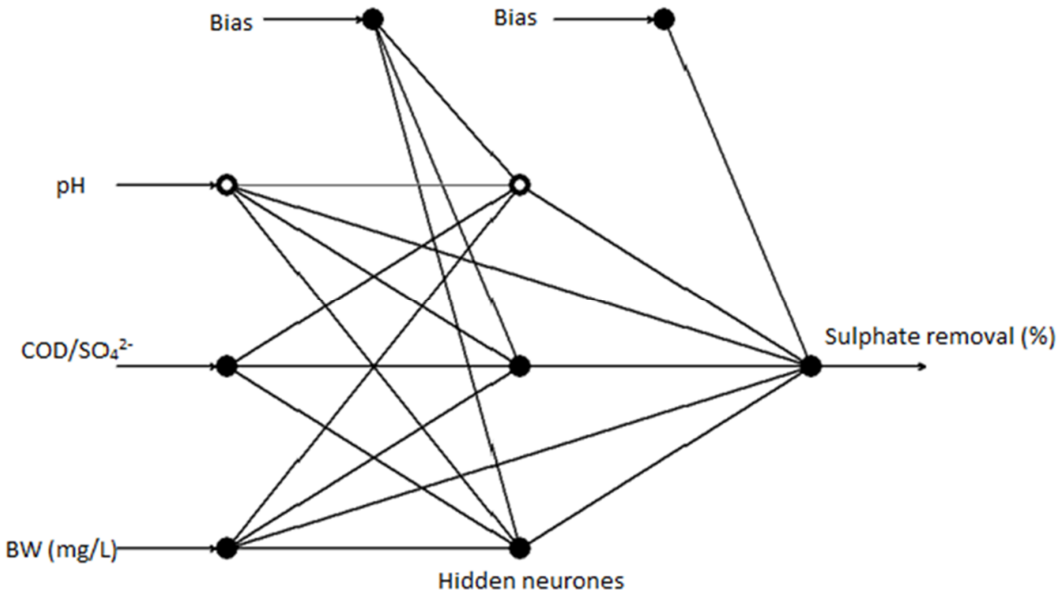


Figure 1. Artificial neural network (ANN) architecture for the estimation of sulphate reduction in the treatment of acid mine drainage (AMD) using brewing wastewater (BW) as carbon source.

2.7. Appraisal of Artificial Neural Network Predictability

To measure the model fit for predictability in the ANN, it was necessary to do a controlled training for estimation of the ANN output error between the actual and predicted outputs. Equations (3)–(6) show the predictive measures used for the model evaluation: the mean squared error (MSE), root mean square error (RMSE), R^2, and absolute average deviation (AAD) [24]. The predicted and actual responses are denoted y_i and y_{di}, respectively. The number of the experimental runs is n, and y_m is the average of the experimental values. The network having minimum errors (MSE, RMSE, AAD) and R^2 closest to unity was taken as the best neural network model [25–27].

$$\mathrm{MSE} = \frac{1}{\mathrm{n}}\sum_{\mathrm{i}=1}^{\mathrm{n}}\left(\mathrm{y_i} - \mathrm{y_{di}}\right)^2 \tag{3}$$

$$\mathrm{RMSE} = \mathrm{MSE}^{1/2} \tag{4}$$

$$AAD = \left\{\left[\sum_{i=1}^{n}\left(\left|y_i - y_{di}\right| / y_{di}\right)\right] / n\right\} \tag{5}$$

$$R^2 = 1 - \sum_{i=1}^{n}\left(\frac{\left(y_i - y_{di}\right)^2}{\left(y_{di} - y_m\right)^2}\right) \tag{6}$$

3. Results and Discussion

3.1. Effect of Carbon Substrate Limitation on the SRB Consortium

The SRB consortium's growth increased with increasing substrate concentration up to a saturation point of 0.3 g/L for both lactate and BW used as a carbon source. Nevertheless, the maximum biomass growth in BW was greater than the growth in lactate (Figure 2). Although previous reports have shown that lactate shows superior biomass growth when compared to other carbon sources, the scenario in this study can be attributed to the several readily available reducible sugars in the BW for sustained microbial growth [19,28]. Hence, BW was assessed as being important for its interaction and ability in combination with other factors and environmental conditions in the optimisation of sulphate removal in AMD.

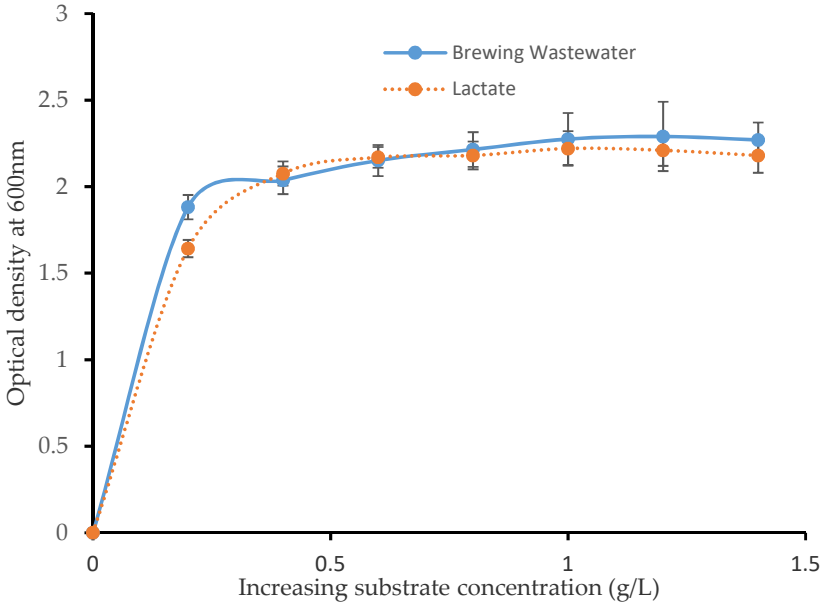


Figure 2. Effect of carbon substrate limitation on the sulphate-reducing bacteria (SRB) consortium's growth.

3.2. RSM Modelling: Box–Behnken Design

The result of individual factors and their interactive effects on the biological sulphate removal is given in Table 2. The responses demonstrated random variations in the results measured that indicated the effect of operational parameters on the SRB consortium's metabolic activity and, perhaps, process instability. The highest sulphate removal was observed at a factorial point (Run 6), at pH of 7, COD/SO_4^{2-} of 1.75, and BW of 200 mg/L, where sulphate removal of 87.9% from an initial sulphate concentration of 8080 mg SO_4^{2-}/L was observed 72 h after introducing the AMD. The residual sulphate concentration of 978 mg SO_4^{2-}/L was well below the discharge standard (1500 mg SO_4^{2-}/L) for South Africa. This is also within the range reported for sulphate reduction by diverse carbon sources in Postgate medium, including cheese whey supplemented with calcite tailings, which resulted in 80% sulphate removal [23,29]. Most of the peak performance (removal percent > 75%) corresponds to a neutral pH, and the BW concentration was within the exponential phase of the consortium's growth, as shown in Figure 2. Studies have shown that optimum sulphate reduction can only be achieved when the pH is >5 in an anaerobic environment [23,29]. Since the pH criterion was met, it created amiable conditions for SRB activity, allowing consumption of the carbon source, i.e., BW and sulphate reduction. However, some runs (2, 7, 10, and 14) displayed extremely low sulphate reduction, a confirmation of slow microbial activity as a result of low-pH conditions.

Statistical analysis of the data was performed using analysis of variance (ANOVA) to assess the significance of each variable in the model developed (Table 3). The quadratic polynomial model of sulphate removal in terms of coded values was as follows:

$$Y = 51.62 + 23.9A + 2.41B + 0.14C - 0.15A^2 + 5.33B^2 + 6.89C^2 - 2.55AB - 3.3AC - 2.23BC \quad (7)$$

where A, B, and C are coded values for pH, COD/SO_4^{2-}, and brewing wastewater concentration, respectively.

Table 3. ANOVA of the sulphate removal response quadratic model.

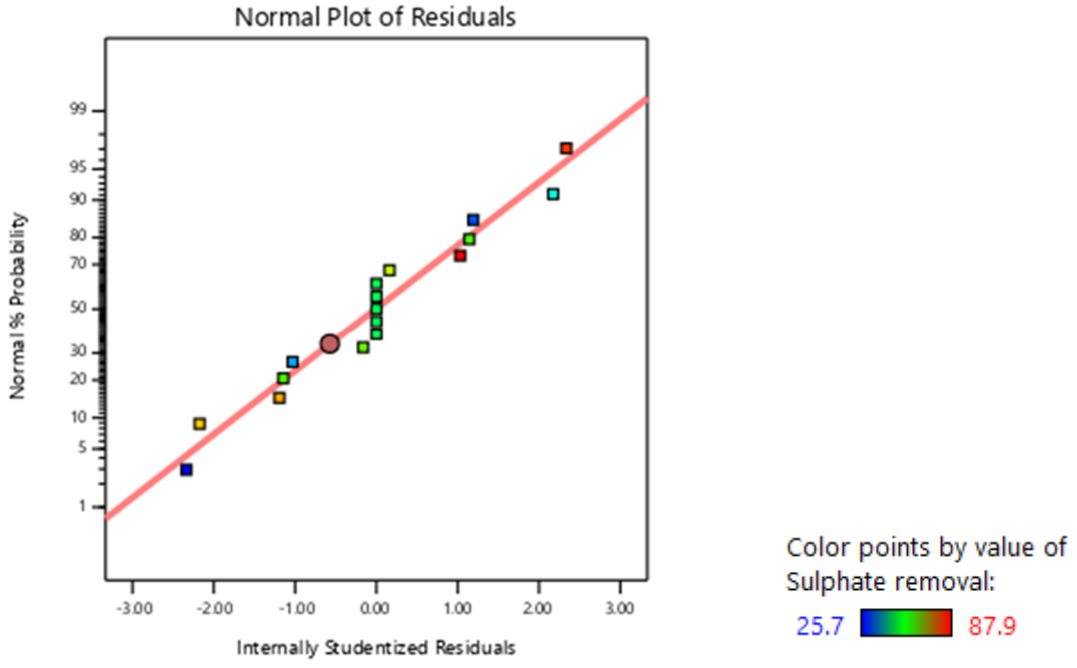
Source	Sum of Squares	df	Mean Square	*F*-Value	*p*-Value	
Model	5048.90	9	560.99	26.12	0.0001	significant
A—pH	4569.68	1	4569.68	212.78	<0.0001	significant
B—COD/SO_4	46.56	1	46.56	2.17	0.1844	
C—BW	0.1512	1	0.1512	0.0070	0.9355	
AB	26.01	1	26.01	1.21	0.3075	
AC	43.56	1	43.56	2.03	0.1974	
BC	19.80	1	19.80	0.9221	0.3689	
A^2	0.0916	1	0.0916	0.0043	0.9498	
B^2	119.50	1	119.50	5.56	0.0504	
C^2	204.99	1	204.99	9.55	0.0176	significant
Residual	150.33	7	21.48			
Lack of Fit	150.33	3	50.11			
Pure Error	0.0000	4	0.0000			
Cor Total	5199.23	16				

The R^2 (0.9711), adjusted R^2 (0.9339), predicted R^2 (0.5374), and adequate precision (16.199), including the low standard deviation (4.63). "Prob > F" of less than 0.05 indicated model terms that were significant, with any values greater than 0.1 indicating model terms that were not significant.

Similarly, the coefficient of interaction was estimated from the mean of the two confidence levels. Since the predicted R^2 was not close to the adjusted R^2, a model reduction was considered, i.e., removal of terms with *p*-values of >0.1, to improve the model to the following:

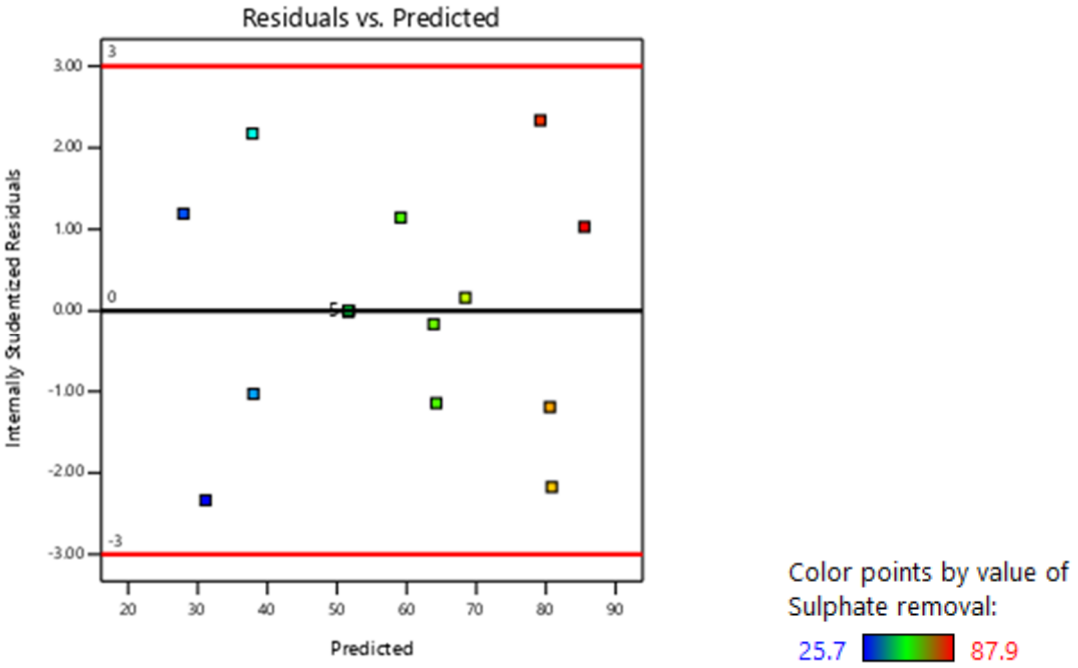
$$Y = 51.62 + 23.9A + 5.33B^2 + 6.89C^2. \quad (8)$$

The *p*-value and the *F*-value of 26.12 implied that the model was significantly relevant, and there was only a 0.01% probability that an *F*-value this large could occur due to noise. The R^2 further showed the precision of the fitted model to the data, as a model with $R^2 > 0.8$ is considered to be well fitted to the experimental data [30]. In this study, the R^2 of 0.9711 implies that 97% of variations in the predicted and actual values are clarified by the model. This indicated the applicability and accuracy of the model for forecasting biological sulphate removal using BW. The adequate precision ratio of 16.199 is a sufficient signal that can be used to explore the design space in this model. The non-significance of the *F*-value of lack of fit further supported the suitability of the model. The diagnostic investigation of the model showed a normal distribution of errors, shown in Figure 3a, with an average value of zero. Similarly, a study of the residual showed evenly distributed scatters above and below the horizontal axis, validating the sufficiency of the model (Figure 3b). Also, the plot of predicted values against the actual experimental value showed clustering of points around the diagonal line, an indication of the high correlation and robustness of the model (Figure 3c). To validate the model, a plot of the standard error in sulphate removal as a function of the pH and COD/SO_4^{2-} is shown in Figure 3d. The shape of the standard error plot was well fitted on the design points, as well as presenting symmetrical shapes and circular contours around the centre point, signifying ideal conditions. The standard error value around the centre point was 0.5 and increased away from the optimisation point.



(**a**)

Figure 3. *Cont.*



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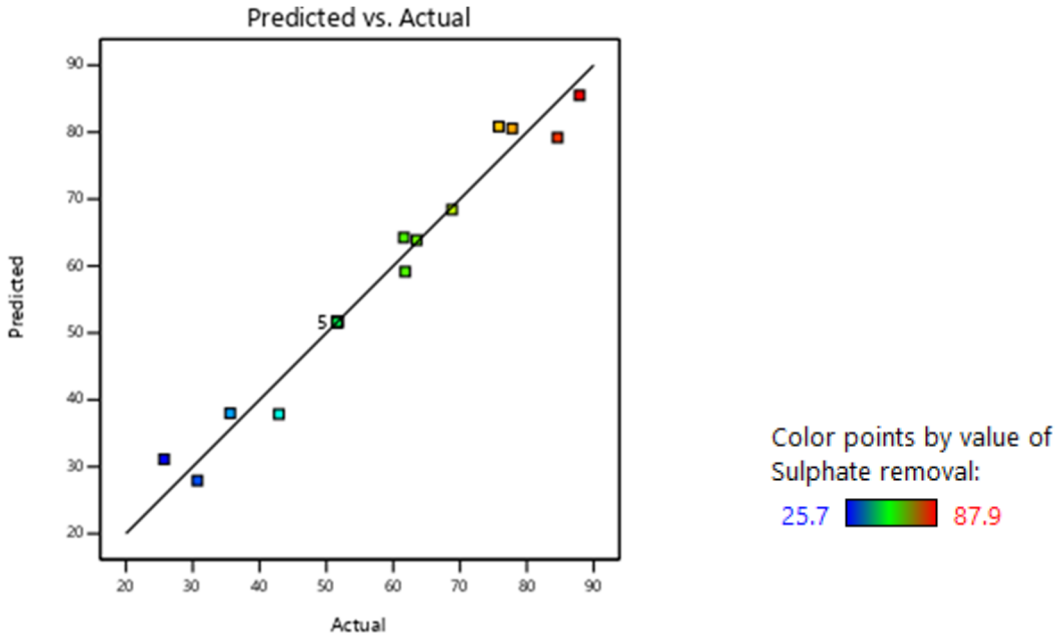

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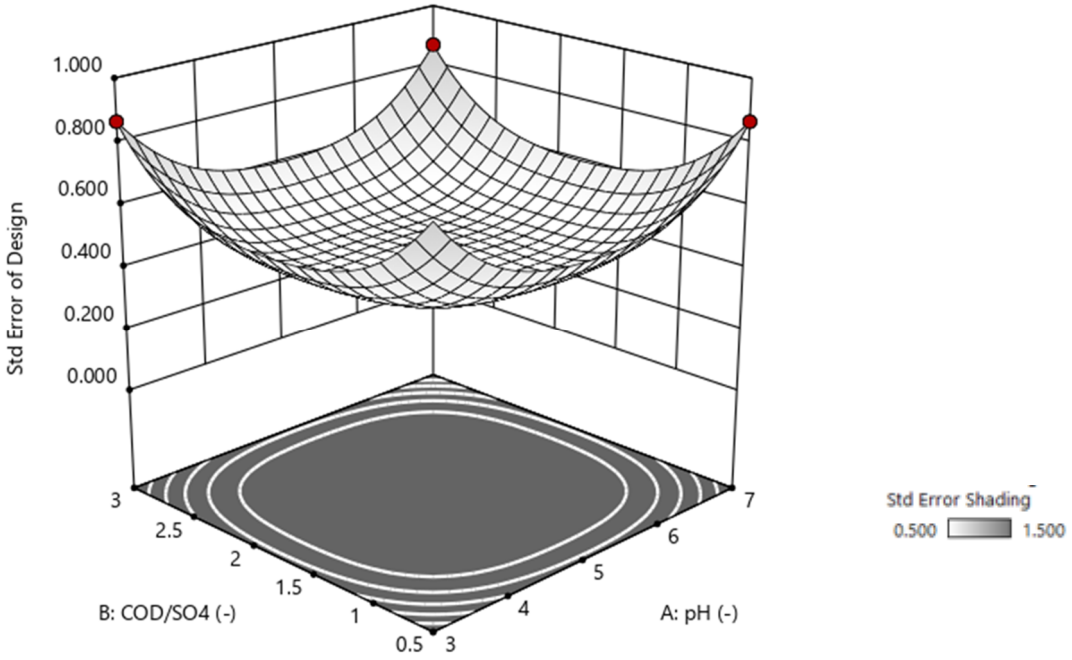
Figure 3. *Cont*.



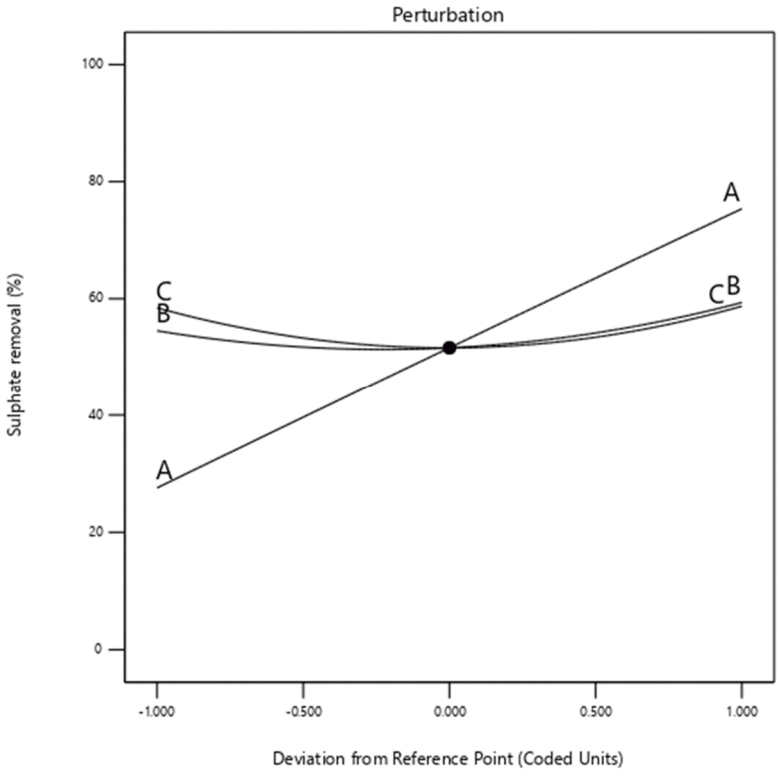
(**d**)

Figure 3. (**a**) Normal probability plot of residuals, (**b**) residuals vs. predicted, (**c**) predicted vs. actual output of the model, and (**d**) 3-D standard error plot for sulphate removal.

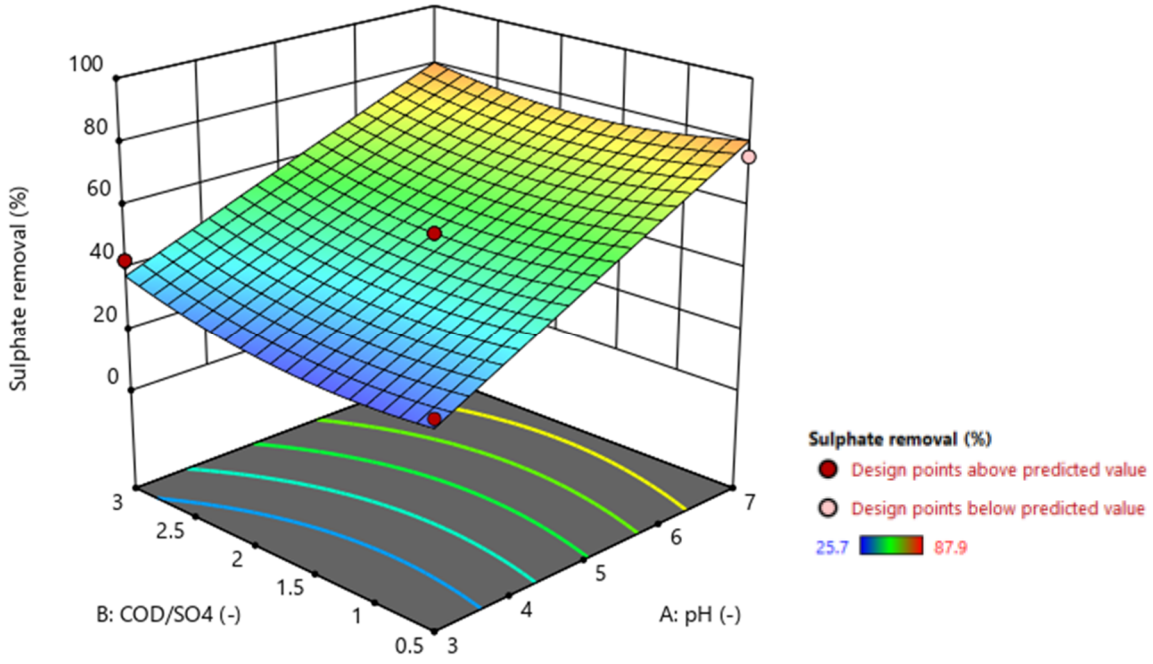
3.3. Graphical Representation of the Model

The effects of changes in all the three variables on sulphate removal are shown in the perturbation diagram (Figure 4a). The effect of Parameter A on sulphate removal was shown to be close to linear and deviate farther from the centre point, though predictably. The influence of Parameters B and C is non-linear, and their values are similar and of the same order of magnitude; however, the effect of Parameter C is more notable in lower values. Besides this, for ease of clarification of these results and the forecasting of optimal conditions, 3-D plots of the system response were analysed by comparing any two factors while keeping the third factor constant, which allowed for the analysis of any interactive effects the three independent factors have on the system's response. For the pairs involving pH, the sulphate removal increased with increasing pH value, while for the two other factors, sulphate removal increased away from the centre point (Figure 4b–d). The pair pH and BW at constant COD/SO_4^{2-} resulted in the highest sulphate removal.

In certain instances, RSM was determined to have a lesser prediction ability of biologics, with ANN models presenting higher predictive coefficient of determination and minor RMSE and mean absolute deviation (MAD), thus having a higher model resolution than RSM [31]. Therefore, ANN analysis of sulphate reduction is warranted, particularly if BW in AMD remediation is to be used on an industrial scale.

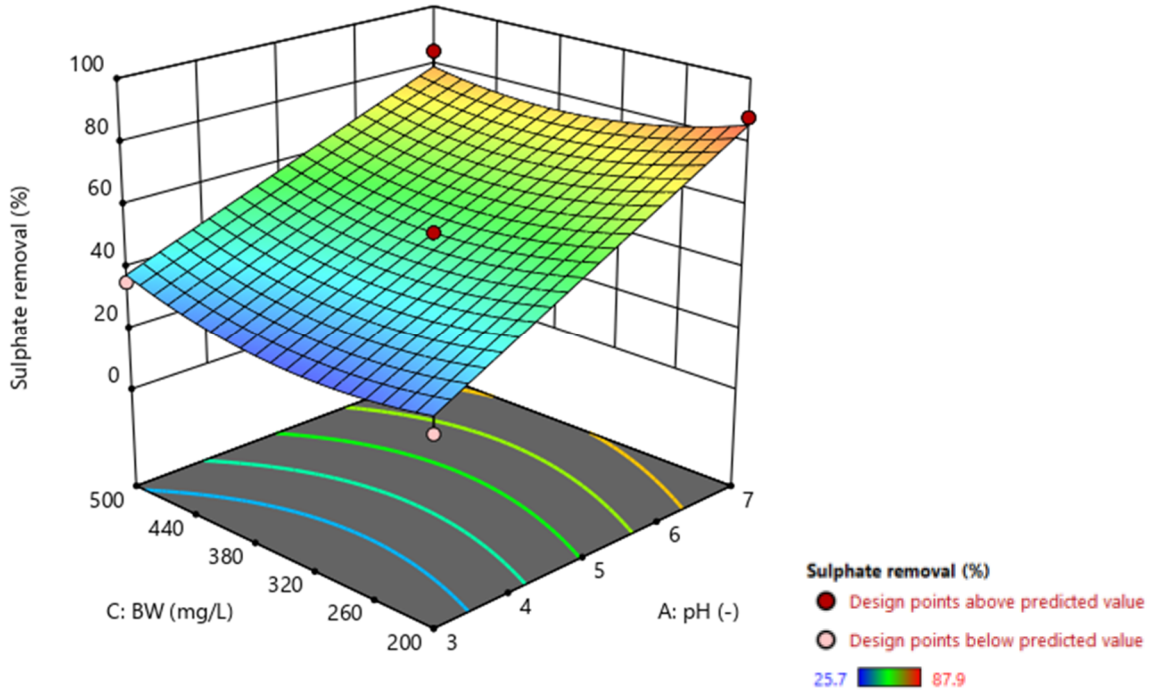


(**a**)

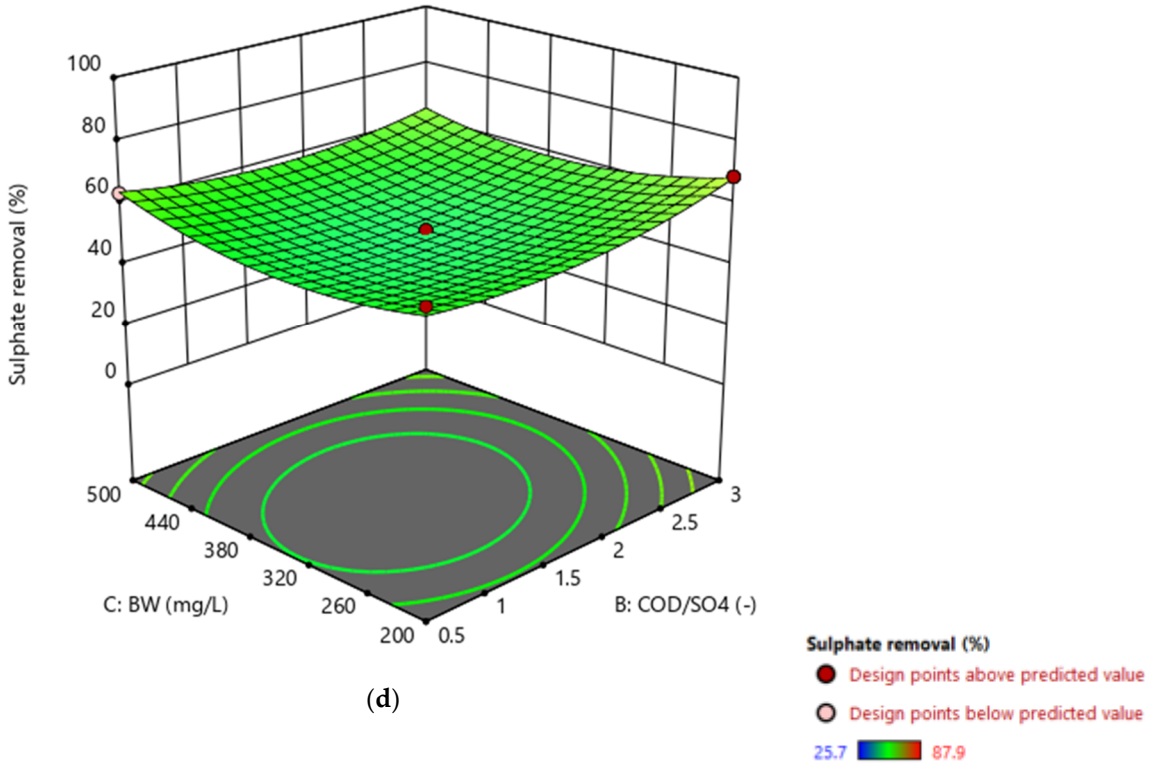


(**b**)

Figure 4. *Cont.*



(**c**)



(**d**)

Figure 4. Perturbation of factors as a function of coded values (**a**) and 3-D plots (**b**–**d**) showing the effect of independent variables on sulphate removal.

3.4. Artificial Neural Network Analysis of Sulphate Reduction Using Brewery Wastewater

The multilayer full feedforward (MFFF) interaction with an incremental backpropagation network and hyperbolic tangent (tanh) as the transfer function used for the ANN modelling proved to be effective, with the coefficient of determination being close to unity. ANN analysis showed that the

percentages of controlled variable contributions for sulphate removal in the treatment of the South African AMD were 44.3, 32.09, and 23.61%, for BW, pH, and COD/SO_4^{2-}, respectively. This shows that BW was the most impactful variable in the experimental studies. Table 4 shows that all the error measurements (MSE, RMSE, and AAD) for both the training and testing datasets were less than 1, while R^2 for the model fitness neared unity for both datasets. A comparison of the actual and predicted values showed the competency of the ANN in the prediction of unknown data [16] (Figure 5). This suggests that the model generated by the ANN can sufficiently explain the relationship between the operating parameters and sulphate reduction.

Table 4. ANN model predictive measurements for model fit in the training and testing sets.

ANN Model Predictive Tool	Training Set	Testing Set
MSE	0.53	0.76
RMSE	0.73	0.87
AAD	0.011	0.0093
R^2	0.99	0.99

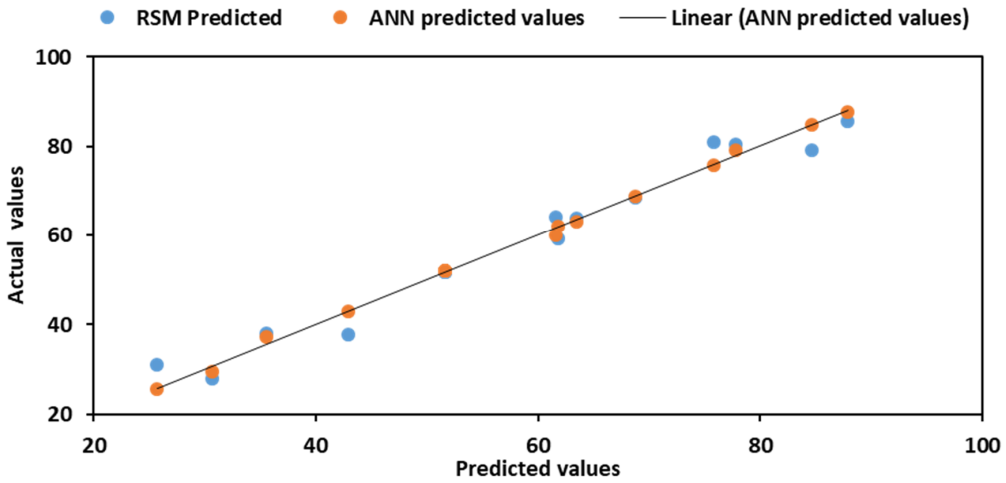


Figure 5. Parity plots of RSM and ANN predictions for sulphate removal in the treatment of South African acid mine drainage.

3.5. Optimum Comparison of RSM and ANN

For RSM, numerical analysis in Design-Expert® version 12 software was deployed for the optimisation of the sulphate removal. Independent variables COD/SO_4^{2-} and BW were set within range, with pH being set between 5 and 7 for a maximum outcome, i.e., sulphate removal. Design-Expert® generated a set of 89 solutions that matched the criteria from most to least desirable. The optimum point with maximum sulphate reduction of 91.59% was found at a pH of 6.99 (which is within the South African discharge standards), COD/SO_4^{2-} of 2.874, and BW concentration of 200.24 mg/L. The experiment conducted at this optimum culminated in the reduction of the sulphate concentration by 90%.

Table 5 shows the statistical tool used in model evaluation between the actual and predictive data sets for both RSM and ANN. Although both models performed well with respect to the R^2 and AAD values, with stable responses, the ANN-based approach was superior in terms of data estimation and fitting when MSE and RMSE were considered. This was also confirmed by the parity plots between the predicted and actual values (Figure 5). Genetic optimisation (GA) was used as the optimal algorithm in the ANN analysis, while RSM optimisation was carried out as embedded in the BBD design. The optimal results, presented also in Table 5, show about a 2% difference in the

percentage of sulphate reduction in RSM when compared to the ANN. However, it is worth noting that there was a 2% decrease in the optimal value of COD/SO_4^{2-} in the ANN optimisation variables.

Table 5. Statistical measurement of the RSM and ANN data sets for model fitting.

Statistical Tool	RSM Whole Data Set	ANN Whole Data Set	Optimisation Variable	RSM	ANN
MSE	8.84	0.57	Sulphate reduction (%)	91.59	89.56
RMSE	2.97	0.75	pH	6.99	6.99
AAD	0.046	0.011	COD/SO_4^{2-}	2.87	0.50
R^2	0.97	0.99	BW (mg/L)	200.24	200.31

3.6. Overall Effect of Individual Parameters

The effect of pH can be seen in both Table 3 and Figure 4. An increase in pH increased the sulphate removal efficiency, and the maximum removal rate was observed at pH around 7. This aligns with the available data in the literature showing that most SRB grow optimally at pH values between 6 and 8 [29,32], as a reduced level of removal was observed in low pH. This may be attributed to acidotolerant and acidophilic SRB that have been reported [33–35], which made it possible to treat AMD without prior neutralisation. Similarly, at low pH, there are more protons than at neutral pH, which causes diffusion pressure on the cell membrane due to higher Gibbs free energy at low pH during sulphate reduction; hence, growth can be achieved at low pH [36]. However, a higher retention time is required to achieve substantial sulphate removal at low pH.

The COD/SO_4^{2-} plays a major role in the oxidation–reduction reactions during sulphate removal. The SRB oxidise the BW, and the released electron is used to reduce the sulphate. Hence, the number of electrons transferred between the two reactions determines the sulphate removal efficiency [37]. In this study, the sulphate removal increased away from the centre point for both COD/SO_4^{2-} and BW. Although there are reports that have indicated that COD/SO_4^{2-} should not be greater than 2.72 to prevent methanogenic activity [38,39], sodium bromoethane sulphonate was added to the reactor, and the COD/SO_4^{2-} corresponding to optimum sulphate reduction in this study was 1.75. Since the carbon source determines bacterial proliferation, irrespective of the carbon source selected, the minimum amount of organic compound needed for reducing sulphate should be a little more than the theoretical stoichiometric ratio since a portion of the energy dissipates into microbial maintenance and growth [40]. The BW amount (carbon source) for optimum sulphate reduction in this study was 200 mg/L.

4. Conclusions

The SRB consortium growth on BW containing Postgate medium showed that BW is a suitable carbon source that can be deployed in a system designed for environmental remediation of AMD and the recovery of such water for other purposes. The analysis of response from Box–Behnken RSM showed that pH is the most significant factor, while ANN analysis indicated carbon source (BW) as the most significant factor. The optimised conditions for BBD in RSM were pH of 6.99, COD/SO_4^{2-} of 2.87, and BW concentration of 200.24 mg/L with sulphate reduction of 91.58%, while those for ANN were pH of 6.99, COD/SO_4^{2-} of 0.50, and BW concentration of 200.31 with sulphate reduction of 89.56%. The performance of both models was very good with respect to the R^2 and AAD values, with stable responses; nonetheless, the ANN-based approach was superior in terms of data estimation and fitting. The residual sulphate concentration (680 mg SO_4^{2-}/L) of the optimised conditions is within the discharge limit in South Africa. This study recognises that BW could function as a suitable feedstock in biological sulphate reduction in South Africa and in semi-arid regions with high BW production and AMD challenges.

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References

1. SAB. South African Breweries Heritage 2020. Available online: http://www.sab.co.za/heritage (accessed on 30 July 2020).
2. Seluy, L.G.; Isla, M.A. A process to treat high-strength brewery wastewater via ethanol recovery and vinasse fermentation. *Ind. Eng. Chem. Res.* **2014**, *53*, 17043–17050. [CrossRef]
3. Ma, L.; Liang, J.; Liu, Y.; Zhang, Y.; Ma, P.; Pan, Z.; Jiang, W. Production of a bioflocculant from *Enterobacter* sp. P3 using brewery wastewater as substrate and its application in fracturing flowback water treatment. *Environ. Sci. Pollut. Res.* **2020**, *27*, 18242–18253. [CrossRef] [PubMed]
4. Hultberg, M.; Bodin, H. Fungi-based treatment of brewery wastewater—Biomass production and nutrient reduction. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 4791–4798. [CrossRef] [PubMed]
5. Nassary, E.K.; Nasolwa, E.R. Unravelling disposal benefits derived from underutilized brewing spent products in Tanzania. *J. Environ. Manag.* **2019**, *242*, 430–439. [CrossRef]
6. Karlović, A.; Jurić, A.; Ćorić, N.; Habschied, K.; Krstanović, V.; Mastanjević, K. By-Products in the malting and brewing industries—Re-usage possibilities. *Fermentation* **2020**, *6*, 82. [CrossRef]
7. Pham, T.-L.; Bui, M.H. Removal of nutrients from fertilizer plant wastewater using *Scenedesmus* sp.: Formation of bioflocculation and enhancement of removal efficiency. *J. Chem.* **2020**, *2020*, 8094272.C. [CrossRef]
8. Hawrot-Paw, M.; Koniuszy, A.; Gałczyńska, M.; Zając, G.; Szyszlak-Bargłowicz, J. Production of microalgal biomass using aquaculture wastewater as growth medium. *Water* **2020**, *12*, 106. [CrossRef]
9. Simate, G.S.; Cluett, J.; Iyuke, S.E.; Musapatika, E.T.; Ndlovu, S.; Walubita, L.F.; Alvarez, A.E. The treatment of brewery wastewater for reuse: State of the art. *Desalination* **2011**, *273*, 235–247. [CrossRef]
10. Riera-Vila, I.; Anderson, N.O.; Hodge, C.F.; Rogers, M. Anaerobically-digested brewery wastewater as a nutrient solution for substrate-based food production. *Horticulture* **2019**, *5*, 43. [CrossRef]
11. Nicholson, S.E.; Funk, C.; Fink, A.H. Rainfall over the African continent from the 19th through the 21st century. *Glob. Planet Chang.* **2018**, *165*, 114–127. [CrossRef]
12. Auditor-General SA. *Report of the Auditor-General to Parliament on a Performance Audit of the Rehabilitation of Abandoned Mines at the Department of Minerals and Energy*; Auditor-General: Pretoria, South Africa, 2009.
13. La, H.-J.; Kim, K.-H.; Quan, Z.-X.; Cho, Y.-G.; Lee, S.-T. Enhancement of sulfate reduction activity using granular sludge in anaerobic treatment of acid mine drainage. *Biotechnol. Lett.* **2003**, *25*, 503–508. [CrossRef] [PubMed]
14. Valenzuela, E.I.; García-Figueroa, A.C.; Amábilis-Sosa, L.E.; Molina-Freaner, F.E.; Pat-Espadas, A.M. Stabilization of potentially toxic elements contained in mine waste: A microbiological approach for the environmental management of mine tailings. *J. Environ. Manag.* **2020**, *270*, 110873. [CrossRef] [PubMed]
15. Sivamani, S.; Selvakumar, S.; Rajendran, K.; Muthusamy, S. Artificial neural network-genetic algorithm-based optimization of biodiesel production from *Simarouba glauca*. *Biofuels* **2019**, *10*, 393–401. [CrossRef]
16. Betiku, E.; Taiwo, A.E. Modeling and optimization of bioethanol production from breadfruit starch hydrolyzate vis-à-vis response surface methodology and artificial neural network. *Renew. Energy* **2015**, *74*, 87–94. [CrossRef]
17. Akinpelu, E.A.; Fosso-Kankeu, E.; Waanders, F.; Angadam, J.O.; Ntwampe, S.K.O. Diversity and Performance of sulphate-reducing bacteria in acid mine drainage remediation systems. In *Frontiers in Water-Energy-Nexus—Nature-Based Solutions, Advanced Technologies and Best Practices for Environmental Sustainability*; Naddeo, V., Balakrishnan, M., Choo, K.-H., Eds.; Springer: Cham, Switzerland, 2020; pp. 121–123.

18. Kozik, V.; Barbusinski, K.; Thomas, M.; Sroda, A.; Jampilek, J.; Sochanik, A.; Smolinski, A.; Bak, A. Taguchi method and Response Surface Methodology in the treatment of highly contaminated tannery wastewater using commercial potassium ferrate. *Materials* **2019**, *12*, 3784. [CrossRef] [PubMed]
19. Najib, T.; Solgi, M.; Farazmand, A.; Heydarian, S.M.; Nasernejad, B. Optimization of sulfate removal by sulfate reducing bacteria using response surface methodology and heavy metal removal in a sulfidogenic UASB reactor. *J. Environ. Chem. Eng.* **2017**, *5*, 3256–3265. [CrossRef]
20. Akinpelu, E.A.; Ntwampe, S.K.O.; Mekuto, L.; Tombo, E.F.I. Optimizing the bioremediation of free cyanide containing wastewater by *Fusarium oxysporum* grown on beetroot waste using response surface methodology. In *Lecture Notes in Engineering and Computer Science, Proceedings of the World Congress on Engineering and Computer Science, San Francisco, CA, USA, 22–24 October 2019*; Ao, S.I., Douglas, C., Grundfest, W.S., Eds.; Newswood Limited: San Francisco, CA, USA, 2016; pp. 664–670.
21. Mona, S.; Kaushik, A.; Kaushik, C.P. Biosorption of chromium(VI) by spent cyanobacterial biomass from a hydrogen fermentor using Box-Behnken model. *Int. Biodeterior. Biodegrad.* **2011**, *65*, 656–663. [CrossRef]
22. Montgomery, D.C. *Design and Analysis of Experiments*; John Wiley & Sons: Hoboken, NJ, USA, 2008.
23. Martins, M.; Faleiro, M.L.; Barros, R.J.; Veríssimo, A.R.; Costa, M.C. Biological sulphate reduction using food industry wastes as carbon sources. *Biodegradation* **2009**, *20*, 559–567. [CrossRef]
24. Ghaffari, A.; Abdollahi, H.; Khoshayand, M.R.; Bozchalooi, I.S.; Dadgar, A.; Rafiee-Tehrani, M. Performance comparison of neural network training algorithms in modeling of bimodal drug delivery. *Int. J. Pharm.* **2006**, *327*, 126–138. [CrossRef]
25. Taiwo, A.E.; Madzimbamuto, T.N.; Ojumu, T.V. Optimization of corn steep liquor dosage and other fermentation parameters for ethanol production by *Saccharomyces cerevisiae* type 1 and anchor instant yeast. *Energies* **2018**, *11*, 1740. [CrossRef]
26. Sin, H.N.; Yusof, S.; Hamid, N.S.A.; Rahman, R.A. Optimization of enzymatic clarification of sapodilla juice using response surface methodology. *J. Food Eng.* **2006**, *73*, 313–319. [CrossRef]
27. Nath, A.; Chattopadhyay, P.K. Optimization of oven toasting for improving crispness and other quality attributes of ready to eat potato-soy snack using response surface methodology. *J. Food. Eng.* **2007**, *80*, 1282–1292. [CrossRef]
28. White, C.; Gadd, G.M. A comparison of carbon/energy and complex nitrogen sources for bacterial sulphate-reduction: Potential applications to bioprecipitation of toxic metals as sulphides. *J. Ind. Microbiol.* **1996**, *17*, 116–123. [CrossRef] [PubMed]
29. Tan, L.C.; Papirio, S.; Luongo, V.; Nancharaiah, Y.V.; Cennamo, P.; Esposito, G.; van Hullebusch, E.D.; Lens, P.N.L. Comparative performance of anaerobic attached biofilm and granular sludge reactors for the treatment of model mine drainage wastewater containing selenate, sulfate and nickel. *Chem. Eng. J.* **2018**, *345*, 545–555. [CrossRef]
30. Weijma, J.; Bots, E.A.A.; Tandlinger, G.; Stams, A.J.M.; Pol, L.W.H.; Lettinga, G. Optimisation of sulphate reduction in a methanol-fed thermophilic bioreactor. *Water Res.* **2002**, *36*, 1825–1833. [CrossRef]
31. Shafi, J.; Sun, Z.; Ji, M.; Gu, Z.; Ahmad, W. ANN and RSM based modelling for optimization of cell dry mass of *Bacillus* sp. strain B67 and its antifungal activity against *Botrytis cinerea*. *Biotechnol. Biotechnol. Equip.* **2018**, *32*, 58–68. [CrossRef]
32. Sánchez-Andrea, I.; Sanz, J.L.; Bijmans, M.F.M.; Stams, A.J.M. Sulfate reduction at low pH to remediate acid mine drainage. *J. Hazard. Mater.* **2014**, *269*, 98–109. [CrossRef]
33. Senko, J.M.; Zhang, G.; McDonough, J.T.; Bruns, M.A.; Burgos, W.D. Metal Reduction at Low pH by a *Desulfosporosinus* species: Implications for the biological treatment of acidic mine drainage. *Geomicrobiol. J.* **2009**, *26*, 71–82. [CrossRef]
34. Sánchez-Andrea, I.; Stams, A.J.M.; Amils, R.; Sanz, J.L. Enrichment and isolation of acidophilic sulfate-reducing bacteria from Tinto River sediments. *Environ. Microbiol. Rep.* **2013**, *5*, 672–678. [CrossRef]
35. Alazard, D.; Joseph, M.; Battaglia-Brunet, F.; Cayol, J.-L.; Ollivier, B. *Desulfosporosinus acidiphilus* sp. nov.: A moderately acidophilic sulfate-reducing bacterium isolated from acid mining drainage sediments. *Extremophiles* **2010**, *14*, 305–312. [CrossRef]
36. Bijmans, M.F.M.; Dopson, M.; Peeters, T.W.T.; Lens, P.N.L.; Buisman, C.J.N. Sulfate reduction at pH 5 in a high-rate membrane bioreactor: Reactor performance and microbial community analyses. *J. Microbiol. Biotechnol.* **2009**, *19*, 698–708. [CrossRef] [PubMed]

37. Mohan, S.V.; Rao, N.C.; Prasad, K.K.; Sarma, P.N. Bioaugmentation of an anaerobic sequencing batch biofilm reactor (AnSBBR) with immobilized sulphate reducing bacteria (SRB) for the treatment of sulphate bearing chemical wastewater. *Process Biochem.* **2005**, *40*, 2849–2857. [CrossRef]
38. Nagpal, S.; Chuichulcherm, S.; Peeva, L.; Livingston, A. Microbial sulfate reduction in a liquid–solid fluidized bed reactor. *Biotechnol. Bioeng.* **2000**, *70*, 370–380. [CrossRef]
39. Choi, E.; Rim, J.M. Competition and inhibition of sulfate reducers and methane producers in anaerobic treatment. *Water Sci. Technol.* **1991**, *23*, 1259–1264. [CrossRef]
40. Kousi, P.; Remoundaki, E.; Hatzikioseyian, A.; Battaglia-Brunet, F.; Joulian, C.; Kousteni, V.; Tsezos, M. Metal precipitation in an ethanol-fed, fixed-bed sulphate-reducing bioreactor. *J. Hazard. Mater.* **2011**, *189*, 677–684. [CrossRef]