

## Technical note

# The formulation of synthetic domestic wastewater sludge medium to study anaerobic biological treatment of acid mine drainage in the laboratory

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### ABSTRACT

Requirements for successful biological treatment of acid mine drainage (AMD) rely on the reduction of sulphates by microorganisms using a suitable organic carbon source. Various carbon sources, such as domestic wastewater sludge, have previously been used in the semi-passive biological treatment of AMD. Domestic wastewater sludge is however highly variable in its composition, making laboratory experimentation difficult. Synthetic medium was therefore formulated based on the chemical oxygen demand (COD) and the biological degradable organic matter (BOD) of domestic wastewater sludge. Four synthetic media compositions were formulated consisting of different ratios of meat extract, vegetable extract, sodium chloride, potassium phosphate, urea, ammonium chloride, iron sulphate, magnesium sulphate and glucose. The media composition with BOD and COD measurements closest to that of anaerobic domestic wastewater sludge was selected for further studies. The combination of AMD to synthetic wastewater sludge in 3 ratios was determined for COD and sulphate reduction in bioreactors over a period of 90 d. The highest reduction of 86.76% in COD and 99.22% in sulphate content were obtained in a 1:1 AMD: synthetic domestic wastewater sludge (SDWWS) ratio that calculated to a COD/sulphate ratio of 3.

**Keywords:** acid mine drainage, synthetic domestic wastewater sludge, sulphates, COD

## INTRODUCTION

Industrial and mine wastewater is acidic in nature that contains sulphur, pyrite and other heavy metals and is generally referred to as acid mine drainage (AMD) (Geremias et al., 2003). AMD is formed during biological and chemical oxidation of the sulphur-containing compounds in the effluent to sulphate, when exposed to dissolved oxygen, water and micro-organisms (Nordstrom and Alpers, 1999; Benner et al., 2000; Baker and Banfield, 2003; Johnson and Hallberg, 2003). AMD is regarded as an environmental pollutant that may negatively impact environmental (Peplow and Edmonds, 2005; Lee et al., 2010) and human health (Keller et al., 2005).

The maximum sulphate level allowed in industrial effluent, in South Africa, is 600 mg/L (DWAf, 1996). However, AMD may contain sulphate concentrations as high as 30 000 mg/L (Poinapen et al., 2009). Treatment of AMD to reduce the sulphate concentrations and neutralise the pH before release into the environment is essential. AMD can be treated in anaerobic bioreactors that rely on sulphate-reducing bacteria (SRB) (Garcia et al., 2001; Kappler and Dahl, 2001; Burns et al., 2012; Sánchez-Andrea et al., 2012). SRB use inorganic sulphate as a terminal electron acceptor obtained by oxidation of carbon sources and the reduction of sulphate or molecular hydrogen to hydrogen sulphide (LeGall and Fauque, 1988; Garcia et al., 2001).

A prerequisite for AMD treatment using bacteria relies on a suitable organic substrate, a sulphate-reducing bacterial consortium and anaerobic conditions, where the sulphate in the

system is reduced and the alkalinity increased to neutralise the AMD. A parameter used in biological sulphate reduction is the COD to sulphate ratio. A ratio of 0.67 indicates sufficient sulphate available for complete reduction of organic material (Vela et al., 2002). Therefore the challenge is to find a suitable inexpensive and sustainable carbon source for adequate reduction of sulphates (Santamaria et al., 2014). The co-treatment of AMD and municipal wastewater has become a treatment option of interest as the simultaneous treatment of municipal wastewater and AMD allows a reduction in treatment costs (Strosnider et al., 2011a; Strosnider et al., 2011b; Strosnider et al., 2013). A 1:1 ratio of AMD and sewage also showed a significant decrease in acidity, organic matter, nutrients, iron and manganese concentrations, and complete removal of pathogens (Neto et al., 2010). The chemical composition of domestic waste varies (Al-Salem, 1987; Mohammed et al., 2012) and representative synthetic domestic sludge does not exist (Hiraishi et al., 1998; Mazumder, 2010). The aim of this study was to formulate a synthetic domestic wastewater sludge to study anaerobic biological treatment of AMD in laboratory studies. The efficiency of the synthetic formula was evaluated by determining sulphate and COD reduction.

## MATERIALS AND METHODS

### Formulation of synthetic domestic wastewater sludge media

For the formulation of the synthetic anaerobic domestic wastewater sludge (SDWWS), only the nutritional value of the anaerobic domestic wastewater sludge was of interest and not the specific chemical composition itself, hence the exclusion of most trace metals (Stover et al., 1976; Alloway and Jackson, 1991). The chemical oxygen demand (COD), biological oxygen

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demand (BOD), sulphate concentration and pH determined for anaerobic domestic wastewater sludge were used as the nutrient parameters, as described below.

### Chemical analyses of anaerobic domestic wastewater sludge

Anaerobic domestic wastewater sludge was obtained from the anaerobic digester tank at the Pniel wastewater treatment plant situated on the outskirts of Stellenbosch by collecting samples in 5 L plastic containers. These containers were kept at room temperature (22°C) until chemical analyses were conducted within 24 h. The COD and sulphate concentrations were determined by using the Merck Spectroquant Pharo 300 and cell test kits according to the recommended protocol. A BOD 16S kit from Oxitop was used to determine the BOD and pH was determined by using a digital pH meter (PCTestr 35 Multi-Parameter).

### Composition of the synthetic domestic wastewater sludge media

Vegetable extract (Sigma-Aldrich (Pty) Ltd., Johannesburg, South Africa) and meat extract (Sigma-Aldrich) served as the basis of the synthetic media as it incorporates the protein, carbohydrate and fat content. The rest of the components included sodium chloride (Sigma-Aldrich), potassium phosphate (Sigma-Aldrich), urea (Sigma-Aldrich), ammonium chloride (Sigma-Aldrich), iron sulphate (Sigma-Aldrich), magnesium sulphate (Sigma-Aldrich) and glucose (Sigma-Aldrich) (Table 1). Four different ratios of the mentioned components were prepared and chemical analyses including COD, BOD, sulphate concentrations and pH were performed as described earlier. The medium that compared best to the chemical analysis of SDWWS was selected for further optimisation. The optimised SDWWS media were then used for further studies.

### Determining the optimal acid mine drainage to synthetic anaerobic domestic wastewater sludge ratio

#### Experimental design for the anaerobic treatment of AMD

Sterile medical drip bags (1 L) (Stelmed, Stellenbosch, South Africa) served as small anaerobic bioreactors. Acid mine drainage sampled from an Exxaro coal mine was couriered overnight in 5 L plastic containers and stored at room temperature (20–21°C) until use. Three ratios of AMD and the selected SDWWS (as

described earlier) were prepared to a final volume of 900 mL in the bioreactors and the pH adjusted to 7.5 with 5 mM NaOH solution where needed (Table 2). The bioreactors were then incubated upright in a dimly-lit enclosed environment at room temperature (20–21°C) for 90 d. Incubation periods in the co-treatment of AMD and domestic wastewater or sludge vary between 40 days and 300 days depending on the experimental set-up (Pulles and Heath, 2009; Strosnider et al., 2011c; Hughes and Gray, 2013) (Fig. 1). Mixtures of AMD and sterile distilled water in the ratios of 1:1, 1:2 and 2:1 served as experimental controls. Two trials were run in triplicate.

From here on the 1:2 ratio will be referred to as Ratio 1, the 1:1 ratio referred to as Ratio 2 and the 2:1 as Ratio 3.

### Microbial inoculum used in the bioreactors

Anaerobic domestic wastewater sludge obtained from the anaerobic digester tank at the Pniel wastewater treatment plant was used as microbial inoculum. Samples were collected in 5 L containers and left overnight at 21°C. Thereafter the bioreactors containing the SDWWS:AMD ratios (Table 2) were inoculated with 10 mL domestic wastewater sludge.



Figure 1

The bioreactors containing different ratios of AMD and SDWWS on Day 0 of incubation

Component	Medium 1	Medium 2	Medium 3	Medium 4
Meat extract (g/L)	20	0.6	10	40
Vegetable extract (g/L)	35	0.06	10	20
Sodium chloride (g/L)	1	0.2	0.2	1.3
Potassium phosphate (g/L)	1	0.1	1	1
Urea (g/L)	8	0.1	10	10
Ammonium chloride (g/L)	1	0.5	0	0
Iron sulphate (g/L)	1	0.1	1	1
Magnesium sulphate (g/L)	1	0.1	1	1
Glucose (g/L)	0	0.5	5	3

Ratio	Composition		
	dH <sub>2</sub> O (mL)	AMD (mL)	SDWWS (mL)
AMD control 1:2	300	600	0
AMD control 1:1	450	450	0
AMD control 2:1	600	300	0
Ratio 1 1:2	0	600	300
Ratio 2 1:1	0	450	450
Ratio 3 2:1	0	300	600
Medium control 1:2	300	0	600
Medium control 1:1	450	0	450
Medium control 2:1	600	0	300

## Chemical analyses of the different ratios of synthetic anaerobic domestic wastewater sludge to acid mine drainage

The COD and sulphate concentrations of the different ratios of SDWWS to AMD were determined on Days 1 and 90 of the trials as described earlier.

## RESULTS AND DISCUSSION

### Formulation of synthetic anaerobic domestic wastewater sludge

The chemical analyses of the four SDWWS media are indicated in Table 3. The COD and BOD of Medium 3 were 2 600 mg/L and 330 mg/L, respectively, and compared best to the COD (3 650 mg/L) and BOD (320 mg/L) of anaerobic domestic wastewater sludge. The concentrations of components in Medium 3 were further optimised by increasing the concentration of meat extract and decreasing the concentrations of vegetable extract, sodium chloride, magnesium sulphate, potassium phosphate, iron sulphate, urea and glucose (Table 4). The COD of the optimised synthetic SDWWS medium was 3 646 mg/L, the BOD was 317 mg/L and the pH 6.9. The synthetic anaerobic domestic wastewater sludge was therefore standardised and thereby excluded the potential variability that could be found when anaerobic domestic wastewater sludge samples are collected at wastewater plants (Snaird et al., 1997; Boon et al., 2002; Juretschko et al., 2002; Henze, 2008; Abbas et al., 2011).

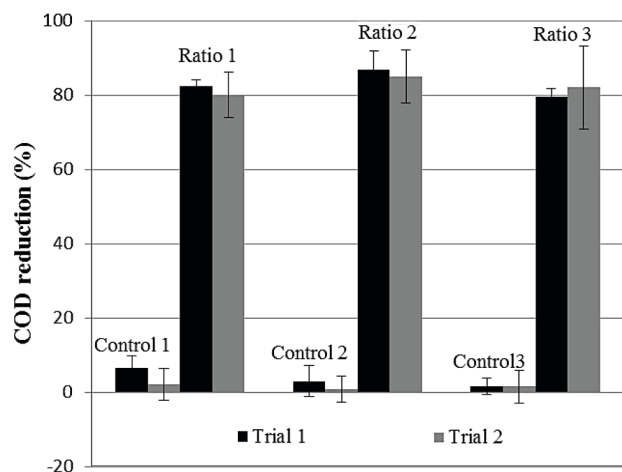
### Chemical analyses of the different ratios of synthetic anaerobic domestic wastewater sludge to acid mine drainage

The COD/sulphate ratios of the three different AMD:SDWWS ratio mixtures (1:2; 1:1; 2:1) were calculated as 1.5, 3 and 4. The COD of all of the controls decreased between 0.83% and 3.06% (Figs 2 and 3). The media control values are not indicated in the

Sample	COD (mg/L)	BOD	pH
Anaerobic domestic wastewater sludge sample	3 650	320	6.9
Medium 1	15 500	3 500	7.0
Medium 2	1 900	200	7.1
Medium 3	2 600	330	6.8
Medium 4	17 000	3 600	6.5

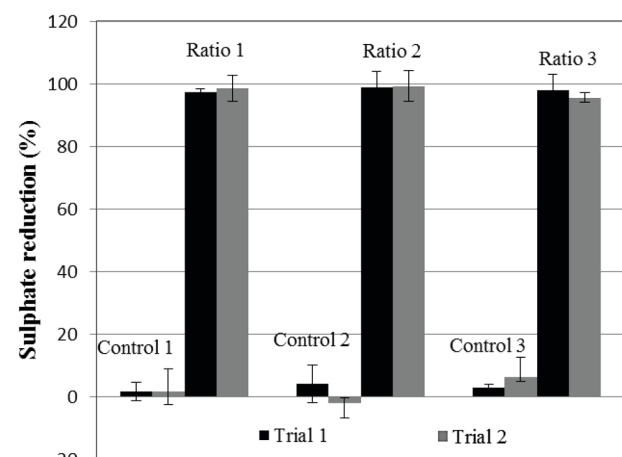
Component	Mass mg/L
Meat extract	2182
Vegetable extract	218
Sodium chloride	72.7
Magnesium sulphate	182
Potassium phosphate	145
Iron sulphate	36
Glucose	182

graphs. A decrease of between 0% and 6.25% in sulphate content in the controls can possibly be attributed to the bacterial oxidation of iron, forming an oxyhydroxysulfate of iron with sulphate as structural component (Bigham et al., 1990). The highest reduction in COD (86.76%) and sulphate content (99.22%) was obtained in Ratio 2, although reductions in both COD and sulphate levels in Ratio 1 and Ratio 3 were similar (Figs 2 and 3). Therefore it can be concluded that COD/sulphate ratios of 1.5 to 4 in biological treatment of AMD with wastewater sludge are adequate for sulphate reduction. These results were confirmed by Deng and Lin (2013) who treated AMD and municipal waste (MW) in different ratios in a two-stage process by first mixing the two wastes followed by anaerobic biological treatment. More than 80% COD and sulphate was removed at COD/sulphate ratios of 0.6 to 5.4. Poinapen and co-workers (2009) investigated the use of upflow anaerobic sludge bed reactors with sewage as carbon source. The trial was conducted at 35°C and resulted in a sulphate reduction of  $\geq 83\%$  (from 1 500 mg/L to  $\leq 250$  mg/L) with a 14 h retention time, compared to the reduction of  $\geq 99\%$  (from 500 mg/L to  $\leq 7$  mg/L) in this study (Figs 2 and 3).



**Figure 2**

The average percentage reduction in COD after the 90 d incubation period for Trials 1 and 2 for the three ratios and AMD controls



**Figure 3**

The average percentage reduction in sulphates after the 90 d incubation period for Trials 1 and 2 for the three ratios and AMD controls

## CONCLUSION

A synthetic media was formulated to simulate the COD and BOD values of domestic wastewater sludge as a carbon source for the anaerobic treatment of AMD in batch reactors. The COD and sulphate content of the AMD were reduced by 86% and 99% by bioreactors containing a 1:1 AMD:SDWWS ratio or a COD/sulphate ratio of 3, and these results could be repeated in a second trial. The synthetic media will be used in future AMD studies to assess sulphate reduction under different parameters.

Small volumes of AMD and domestic wastewater sludge were treated per bioreactor in this study. The results obtained may differ in the treatment of larger volumes of wastewater. This should also be verified in future studies by up-scaling the process to determine the efficiency of the SDWWS and AMD combination in a bioreactor for COD and sulphate reduction.

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